NUTRIENT PROFILING, FUNCTIONAL FOOD COMPOUNDS AND ANTIOXIDANT PROPERTIES OF SOME UNDERUTILIZED FRUITS OF MIZORAM

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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SUBMITTED

In partial fulfillment of the requirement of the degree of Doctor of Philosophy in Horticulture, Aromatic and Medicinal Plants of Mizoram University, Aizawl.

Mizoram University, Aizawl

(A Central University under the Act of Parliament)
Department of Horticulture, Aromatic & Medicinal Plants
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CERTIFICATE

This is to certify that **Ms Rody Ngurthankhumi** has prepared a Thesis under My supervision on the topic "Nutrient Profiling, Functional Food Compounds and Antioxidant Properties of Some Underutilized Fruits of Mizoram" in partial fulfillment for the award of the Degree of Doctor of Philosophy (PhD) in the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl.

This thesis has been the outcome of her original work and it does not form a part of other thesis submitted for the award of any other degrees.

She is duly permitted to submit the Thesis.

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Mizoram University

OCTOBER, 2024

I **Rody Ngurthankhumi**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

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List of abbreviations and symbols

Acronym/symbol	Full form/meaning
ºB	:Degree Brix
ºC	:Degree Celcius
%	:Percentage
/	:Per
g^{-1}	:Per gram
µg	:Microgram
g	:Gram
5FU	:5-fluorouracil
AAS	:Atomic Absorption Spectrometer
ABTS	:2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
AD	:Alzheimer's disease
AIF	:Apoptosis-inducing factor
ANOVA	:Analysis of Variance
AOAC	:Association of Official Analytical Collaboration
AO/EB	:Acridine Orange/Ethidium bromide
ASA	:Ascorbic Acid
Al	:Aluminium
Ar	:Argon
ATP	:Adenosine triphosphate
B	:Boron
Bcl-2	:B-cell lymphoma-2
Bax	:Bcl-2 Associated X-protein
Bid	:BH3-interacting domain death agonist
Bcl-XL	:B-cell lymphoma-extra large
Ca	:Calcium
CAT	:Catalase
cm	:Centimetre
Cr	:Chromium
Cu	:Copper
Co	:Cobalt
DMC	:Dry Matter Content
DNA	:DNA
DNase	:Deoxyribonuclease
DMRT	:Duncan's Multiple Range Test
et al	:and others
etc.	:and other similar things
FRAP	:Ferric-reducing antioxidant power
F	:Fluorine
Fe	:Iron
Gpx	:Glutathione Peroxidase
GC–MS	:Gas Chromatography–Mass Spectrometry
GSH	:Glutathione-s-transferase
GST	:Glutathione
H2SO4	:Sulphuric acid

HCN	:Hydrogen cyanide
HNO3	:Nitric acid
HClO4	:Perchloric acid
I	:Iodine
IC50	:Half maximal inhibitory concentration
IBS	:Irritable Bowel Syndrome
ISFR	:Indian State Forest Report
kcal	:Kilocalories
LC-HRMS	:Liquid Chromatography-High Resolution Mass Spectrometry
LPO	:Lipid peroxidation
Li	:Lithium
MDA	:Malondialdehyde
Mn	:Manganese
Mo	:Molybdenum
MTT	:3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
mRNA	:Messenger Ribonucleic acid
Ni	:Nickel
pH	:Potential Of Hydrogen
PT	:Permeability transition
PCA	:Principal component analysis
PC	:Principal Component
qRT-PCR	:Quantitative reverse transcription polymerase chain reaction
RNA	:Ribonucleic acid
RDA	:Recommended Dietary Allowance
ROS	:Reactive oxygen species
R.T	:Retention Time
SOD	:Superoxide dismutase
SEM	:Structural Equation Modeling
SD	:Standard deviation
Se	:Selenium
Si	:Silicon
Sn	:Tin
TPTZ-Fe	:2,4,6-tripyridyl-s-triazine
TSS	:Total Soluble Solids
UPGMA	:Unweighted pair group method with arithmetic mean
Zn	:Zinc

List of Photo plates

Chapter I

INTRODUCTION

Introduction

Nutritional status among individuals pertains to the state of health in relation to nutrient intake and utilisation. Maintaining optimal nutritional status is vital for overall health, as it can affect energy levels, immune function, and disease prevention. Unfortunately, global nutritional status presents a concerning trend, with malnutrition impacting millions of individuals worldwide, leading to numerous health issues and contributing to the disease burden (Narayan *et al.,* 2019). It is crucial for individuals to be mindful of their nutritional intake and make informed choices to ensure their daily nutrient requirements are met for optimal health. "Ensuring adequate nourishment for all" is a clear and prominent goal for developing countries worldwide. Inadequate consumption of balanced nutrients can result in various disorders, including malnutrition, which is closely associated with poverty and has global repercussions. This condition stems from an unbalanced diet where certain nutrients are deficient or consumed improperly. $1/3^{rd}$ of the population in the world is affected by malnutrition, underscoring its substantial impact (Nordin *et al.,* 2013).

India accounts for nearly one-third of the global population of malnourished children. (Mansuri and Latif, 2023). Despite India's significant economic growth over the past decade, the nutritional status of its population has yet to improve sufficiently. Recent research indicates that although India has doubled its economic growth rate in the past ten years compared to the previous decade, it still owns the largest share of the world’s undernourished population (Shahnawaz and Singh, 2014). The Global Hunger Index 2018 positioned India at 103rd among 119 nations, highlighting the concerning state of undernutrition.

Thus, incorporating diverse fruits, vegetables, whole grains, and lean proteins into one's diet can help ensure a well-rounded nutritional profile (Cena and Calder, 2020). To mitigate the impact of chronic illnesses and improve the health outcomes of vulnerable populations, it is imperative to take action against the root

causes of inadequate nutrition and assist people and families in making better choices (Gropper, 2023).

In relation to this issue, it is vital to emphasise that vegetables and fruits are fundamental elements of our daily diet. Fruits have long served as a natural and nutritious source of sustenance, playing a pivotal role in promoting human health (Hazarika *et al.*, 2012). They supply essential vitamins and minerals, carbohydrates, proteins, fibres, organic acids, and bioactive compounds such as secondary metabolites. Inadequate consumption of fruits has been associated with various health related issues. On the other hand, regularly ingesting fruits and their by-products decreases the manifestation of various diseases including cardiovascular illnesses and cancer. (Hall *et al.*, 2009). Integrating a diverse range of fruits and vegetables into one's diet might assist in attaining a comprehensive diet by supplying essential elements that could otherwise be deficient.

Fruits are renowned for their nutritional advantages, characterised by their low calorie and fat content, as well as their abundance of vital elements such as vitamins, minerals and dietary fibres (Karasawa and Mohan, 2018). A fruit-rich diet may significantly enhance overall health by supplying vital elements such as phytochemicals, fibres, vitamins, and minerals (Shi *et al.*, 2022). These bioactive compounds, which include alkaloids, glycosides, flavonoids, saponins, and tannins derived from fruits, have been demonstrated to possess numerous beneficial health effects and are known for their protective and restorative properties, including anti-carcinogenic, cardio-protective, anti-inflammatory, neuro-protective, anti-allergic, gastro-protective, anti-obesity, and anti-diabetic effects (Sultana *et al.*, 2023).

In recent years, there has been an increasing trend to take non-conventional plant species as potential sources of alternative foods and medicines. The presence of low concentrations of bioactive compounds within these plants has been demonstrated to exert positive effects on human health, leading to a burgeoning scientific focus in this area (Abdulhadi *et al.*, 2023). Scientific inquiries have revealed that interventions in dietary patterns and lifestyle choices can delay the onset and mitigate the severity of age-related diseases (Drețcanu *et al.*, 2022). As a result,

attention has been resurgent towards health-promoting plant varieties and their derivatives, particularly in fruit-bearing trees exhibiting nutraceutical potential. Among these, underutilized fruits are of particular interest due to their capacity to facilitate improvements in human health, characterised by anti-inflammatory, anti-microbial, metabolic syndrome, and anti-cancer properties (Al-Soufi *et al.,* 2022).

Underutilized fruits refer to those that are not extensively utilised, have limited availability, or are specific to certain regions. They lack a coordinated cultivation framework nor prepared using established techniques. These fruits typically experience seasonal demand and face low marketability due to external appearances or poor storability (Kumari *et al.,* 2024). These fruits are rich in bioactive compounds, making them potential sources for nutraceutical development. Despite their potential, much of the biodiversity of underutilized fruits remains undocumented (Kell, 2009). They are often referred to as uncommon or hidden hunger fruits. The nutritional benefits of underutilized fruits depend on factors such as nutrient profiles, bioactive phytochemicals, organoleptic attributes, disease preventive and therapeutic properties. These fruits possess significant bioactive components that have the potential to provide health advantages and may be used to produce nutraceuticals (Nezbedova *et al.,* 2021). In tropical horticulture, exotic local fruits are valuable for various compounds, including secondary metabolites with antioxidant, anticancer, anti-inflammatory activities, and β-carotenes and carotenoids. Therefore, focusing on underutilized fruits may contribute to the food and nutritional security of minority groups and enhance the livelihood of marginalised populations in remote and hilly terrains by exploring their cultivation and commercial potential (Berni *et al.,* 2019).

The diverse geographical features of India have a wide range of flora and fauna. India is a major producer of fruit crops, boasting over 150 fruit species. Tropical fruits have been a critical component of traditional medical systems viz. Siddha, Ayurveda, and Unani (Donno *et al.,* 2018). Despite India contributing 10.9 % of global fruit production, numerous underutilized fruit crops are yet to be commercially explored. Many of these fruits are already extinct because of the introduction of high-yielding varieties of economically important fruits. These

underutilized fruits typically refer to fruits that are not prioritised as horticultural crops and are not commercially available (Deb *et al.,* 2019).

India, particularly in the eastern himalayan region of the Indo-Burma biodiversity hotspot, holds significant importance in relation to species diversity and endemism. The northeastern part of India, rich in genetic variability and diversity of horticultural crops, is considered a critical biodiversity hotspot. This region is also recognised for its ethnic diversity and traditional culture (Bora *et al.,* 2024).

The Northeastern regions of India own a rich diversity of wild edible fruits, thanks to the varied topography, altitude, soil, and climate. Many of these fruits are found in natural forests or homestead gardens. This region also boasts one of the highest forest coverage percentages in the country, with Mizoram having the highest forest cover percentage at 84.53% (ISFR 2021). These fruits provide essential nutrients, including vitamins, minerals, carbohydrates, proteins, and fats, and have historically been important for fulfilling the nutritional requirements of rural tribal communities. The tribal population in the northeastern states has been consuming these fruits for generations without fully understanding their nutritive and medicinal value (Pachuau and Dutta, 2020; Talang *et al.*, 2023). Despite the availability of several underutilized fruits, pressure on land due to increasing population and prevailing shifting cultivation in these regions has led to their under-exploitation. Due to a lack of awareness of their potential, many underutilized fruits remain untapped. Therefore, there is a pressing need for the scientific community to explore the genetic resources of these rare plants. Analysing these fruits' biochemical, nutritional, antioxidant, and nutraceutical properties could pave the way for the development of new food and health products by the food and pharmaceutical industries (Chakravarty *et al.,* 2016).

Mizoram, located in northeastern India, has around 25 distinct tribes with unique cultures, languages, and traditions. This region boasts a rich biodiversity, particularly in underutilized plant species, including various fruits (Sibiya *et al.,* 2021). Renowned for its abundant phyto diversity, Mizoram is a treasure trove for

several fruits grown in nature without commercial cultivation. The genetic diversity among the Mizo people, influenced by their diverse cultural practices, languages, and geographical location, has significantly enriched the flora and expanded the list of underutilized indigenous fruits, further contributing to the existing genetic resources. Abiotic stresses, seasonal availability, low yield, and the absence of cultivation practices have limited the domestication and commercialisation of certain fruits, relegating them to underutilized status, primarily valued for nutrition and sporadically for their ethnomedicinal properties. Despite various ecological challenges, a few fruits have piqued local interest, likely due to their ready accessibility or palatable taste, and are supporting some commercial activity (Welch, 2022). The people in Mizoram primarily rely on traditional food sources, mainly green plants such as leaves, stems, and flowers, as well as wild edibles and certain fruits. Despite the abundance of fruits, they are rarely consumed due to their poor taste, as people prefer widely grown fruits. Throughout history, local tribes have used wild fruits and other plant parts, such as leaves, barks, stems, and tubers, to prepare traditional foods, a practice sustained mainly among the elders (Oyenihi *et al.*, 2022). Incorporating indigenous plants known for their nutritional and antioxidant properties is a safe and effective approach. Mizoram, with its diverse ethnic minority population, harbours a wide variety of unique and nutritionally valuable wild and underutilized fruits, making it an ideal location for researching the nutrient profiling, nutraceutical, functional food compounds, and antioxidant properties of such fruits (Ralte *et al.*, 2021). Moreover, cultivating and consuming underutilized fruits can significantly benefit the local economy and environment. By promoting the growth and utilisation of these fruits, communities in Mizoram stand to gain from increased agricultural diversity and economic opportunities. Furthermore, given the fruits' adaptability to local climate and soil conditions, their cultivation can support sustainable farming practices and environmental conservation efforts.

Functional food components consist of essential nutrients such as vitamins, minerals, healthy fats, and fiber, helping to defend against deficiencies. These food components also include bioactive compounds like antioxidants, probiotics, and phytochemicals, which can play a role in reducing the risk of chronic diseases

(Gropper 2023). Probiotics and dietary fiber in functional foods support gut health, thereby improving digestion and nutrient absorption. Including various functional foods can contribute to overall well-being and health by providing a wide range of beneficial nutrients. Bioactive compounds such as antioxidants, polyphenols, and flavonoids offer protective health benefits, potentially lowering the risk of degenerative diseases and supporting overall well-being. Research indicates that phytochemicals may contribute to the prevention of chronic diseases, thus enhancing public health. There is growing evidence that underscores the significance of these micronutrients for human health (Vasco *et al.,* 2008; Veer and Singh, 2019).

Fruits serve as a significant source of dietary antioxidants (Liu, 2003; Kolar *et al*., 2011), highlighting the need for increased consumption in contemporary diets (Hall *et al*., 2009). The overall antioxidant activity of fruits is primarily due to the additive and synergistic effects of intrinsic phytochemicals, particularly phenolic compounds (Liu, 2003; Cartea *et al*., 2011). Numerous natural antioxidants, particularly flavonoids, demonstrate a range of biological effects, such as antibacterial, antiviral, anti-inflammatory, anti-allergic, anti-thrombotic, and vasodilatory actions (Cook and Sammon, 1996). Antioxidant activity is a crucial characteristic essential for a healthy life. This property underlies numerous biological functions, including anti-mutagenicity, anti-carcinogenicity, and anti-ageing (Cook and Samman, 1996; Huang *et al*., 1992). Beyond their antioxidant properties, phytochemicals provide additional unique beneficial toxicological and pharmacological effects in humans. Therefore, assessing the free radical scavenging activity and phytochemical composition of fruits reveals the potential health benefits associated with their consumption (Liu, 2003; Pisoschi *et al*., 2009; Vera de Rosso, 2013). Natural sources of antioxidants, including fruits and vegetables, are more beneficial to health compared to synthetic alternatives or supplements (Liu, 2003). The presence of various phytochemicals, including phenolics, thiols, carotenoids, anthocyanins, and tocopherol, has been proposed to provide chemopreventive (Dragsted *et al*., 1993) and cardioprotective (Vita, 2005) effects, in addition to safeguarding the human body from oxidative damage caused by free radicals (Halliwell, 1997). Antioxidants play a significant role in deciding the pharmaceutical

effect of plants and making them potent drugs against chronic diseases. These antioxidants are taken in our diet from plant compounds, which is a rapid and straightforward method. (Robinson *et al*., 2017)

Cancer is a prominent contributor to mortality rates and a significant obstacle to improving life expectancy worldwide. Cancer is an assortment of disorders marked by various genetic and cellular abnormalities that lead to unrestrained cell proliferation, invasion, and progression (Hanahan & Weinberg, 2000). According to 2019 estimates from the World Health Organisation (WHO), cancer is recognised as the main or additional cause of death for individuals under 70 in 112 of 183 countries. According to GLOBOCON 2020, data were obtained from 185 countries encompassing 36 different forms of malignancies; approximately 19.3 million new cancer diagnoses and 10 million cancer-related deaths occurred worldwide (Deo et al., 2022). Although all types of cancer pose a tremendous health fatality, among them, lung cancer is a highly fatal form of cancer that has a significant impact on both rates of morbidity and mortality (Ellis *et al.,* 2015). The elevated mortality rate and unfavourable prognosis are attributed to challenges in early diagnosis and the significant capacity for local invasion and distant metastasis. Lung cancer accounted for 1.6 million deaths globally in relation to all cancer-related fatalities (Torre *et al.,* 2015). The A549 cell line comprises hypotriploid alveolar basal epithelial cells. The establishment of this cell line was first accomplished by Giard *et al*. (1973) by the extraction and cultivation of lung carcinoma tissue from the excised tumour of a 58-year-old Caucasian man. These cells proliferate as a single layer in a laboratory setting and are often used to evaluate the anticancer effects of various plant extracts in vitro (Kumar *et al.,* 2017).

The recognition of natural products as potential anti-cancer agents began in the 1950s at the U.S. National Cancer Institute, which initiated a large-scale screening program utilising leukemic mice. Subsequently, in 1985, an in vitro screening panel was established, comprising over 60 distinct human tumour cell lines. Over the past fifty years, the majority of novel clinical applications of plant secondary metabolites and their derivatives have focused on cancer treatment (Asma

et al., 2022). Plants and natural products remain a significant reservoir of anti-cancer substances because of their safety, effectiveness and reduced adverse effects. Since the beginning of ancient medicine, plant-derived chemical substances have been used to treat human ailments. Over the last three decades, there has been a growing interest in natural products which possess the potential as innovative agents for the prevention and treatment of cancer (Newman, 2008; Newman *et al.*, 2003). There have been reports of over 3000 plants from around the world possessing properties that may be effective against cancer. On a global scale, using plant-derived products for cancer treatment ranges from 10% to 40%, increasing to 50% among Asian patients (Solowey *et al.*, 2014). The current clinical application includes four primary categories of plant-derived anticancer agents: *Catharanthus* alkaloids, epipodophyllotoxins, taxanes, and camptothecins. Catharanthus alkaloids and various semi-synthetic derivatives induce metaphase arrest, inhibiting mitosis through binding to tubulin specifically, which leads to its de-polymerization (Okouneva *et al*., 2003). Vinblastine and vincristine were isolated from *Catharanthus roseus* (L.), G. Don (Apocynaceae), previously known as Vincarosea L, and have been utilised in clinical settings for over 40 years (Jacobs *et al*., 2004). Epipodophyllotoxins interact with tubulin, leading to DNA strand breaks in the G2 phase of the cell cycle through the irreversible inhibition of DNA topoisomerase II (Damayanthi and Lown, 1998). Podophyllotoxin was extracted from the resin of *Podophyllum peltatum* L. (Berberidaceae) (Gordaliza *et al*., 2004). Due to its high toxicity in mice, derivatives were developed, with etoposide being the first podophyllotoxin-derived drug approved for clinical use (Damayanthi and Lown, 1998).

The available information on the nutrient composition, functional food components, antioxidants and anticancer properties of commonly consumed fruits are widely reported. However, very little data is accessible regarding the above aspects of underutilized fruits in the country. These fruits may contain substantial levels of phytochemicals and distinct compounds that confer health benefits. Their level of antioxidant capacity could potentially surpass that of the more extensively studied fruits. In light of this background information, the current investigation aims

to explore the nutrient composition, functional food components, and antioxidant properties of selected wild edible fruits in Mizoram, North-East India.

The proposed research aimed to comprehensively analyse the state's nutrient composition, functional food components, and anticancerous and antioxidant properties of certain underutilized fruits. This study will significantly enhance our understanding of these fruits and assist their genetic resource management and mainstreaming. The findings will provide valuable insights for researchers to pursue more in-depth studies on these fruits and aid policymakers in developing targeted development programs for this area.

Taking into account the background information mentioned above, the current study aimed to address the following objectives:

1.2 Objectives

1. To analyse the nutrient composition of selected underutilized fruits.
2. To analyse the functional food components of the selected underutilized fruits.
3. To evaluate the anti-cancerous and antioxidant properties of the selected fruits.

Chapter II

REVIEW OF LITERATURE

Review of Literature

The discovery of wild fruits in remote areas offers valuable sustenance and economic opportunities for indigenous communities, yet these fruits are often overlooked and underexploited. There is significant economic potential in these fruits and opportunities for sustainable harvesting and commercialization within local communities in Mizoram, north-east India. This research emphasizes the need to conserve and promote the consumption of underutilised fruits for both nutritional and economic purposes in Mizoram NE India.

This literature review covers various aspects of investigating underutilized fruit species from different parts of India and the world.

2.1 Nutrient composition of selected underutilized fruits

Sundriyal and Sundriyal (2001) conducted a study analysing the nutritional values of 27 commonly consumed wild edible plants in the Sikkim Himalaya. Of the 27 plant species examined, 22 were found to have edible fruits, while five had edible leaves and shoots. The crude fibre content exhibited significant variation among different species, with values ranging from 2.15% to 39.90%. The total soluble salts ranged from 4.66% to 21.0%, and the vitamin C content varied from 6 to 286 mg per 100 g. The carbohydrate content in the fruits of various wild edibles exhibited significant variation, ranging from 32.00% to 88.00%. The reducing sugar content varied between 1.25% and 12.42%, whereas the total sugar content ranged from 2.10% to 25.09%. The lignin content ranged from 9.05% to 39.51%, hemicellulose content from 25.63% to 55.71%, and cellulose content from 9.57% to 33.19% across various species.

Seal (2011) analysed the nutritional values of few wild edible fruits from Meghalaya and explored their significance in ethnobotany. According to his findings, fruits are a significant source of fats, protein, carbohydrates, and calories. Wild fruits were rich in potassium, followed by calcium and sodium. They are also a great source of micronutrients like zinc, iron, copper, manganese, and chromium.

Nandal and Bhardwaj (2014) investigated the role of underutilised fruits in enhancing the nutritional and economic status of tribal communities. The use of underutilised horticultural crops can potentially mitigate social issues including health and nutrition insecurity, poverty, and unemployment. Investigating lesser-known fruit crops is essential for meeting the nutritional requirements of marginalised communities, especially those facing economic challenges. Underutilised fruits, nuts, and vegetables provide a rich source of carbohydrates, fats, proteins, energy, vitamins, and minerals, as well as dietary fibre. Consequently, they possess the nutritional potential to effectively address and manage a range of health conditions, such as kwashiorkor, marasmus, night blindness, diabetes, anaemia, hypertension, cancer, and hidden hunger. Consuming seasonal, locally sourced, and affordable fruits is acknowledged as a means to sustain health and nutritional security.

Vino and Harshita (2016) examined the lesser-known fruits in India and found that these fruits are not only affordable but also packed with essential nutrients. The local tribes have been utilising these plants for their medicinal and therapeutic benefits, effectively treating various ailments. In traditional Indian medicine and ayurveda, multiple fruits, seeds, and plant leaves are utilised as healing foods which possess significant nutritional and medicinal properties. Thus, they have an impressive ability to thrive in challenging climates.

In an investigation conducted by Cheema *et al*. (2017), they examined the nutritional value of specific underutilised fruits from the Terai region of Uttarakhand, India. The protein content was elevated in *Solanum nigrum*, *Broussonetia papyrifera*, and *Aegle marmelos.* Simultaneously, *Terminalia bellerica, Ficus racemosa*, and *Artocarpus heterophyllus* exhibited high lipid content. The observed range of reducing sugars was 1.1% to 68.2%, whereas non-reducing sugars varied from 0% to 6.4%. The analysis of vitamins indicates that *Emblica officinalis* and *Pithecellobium dulce* possess elevated ascorbic acid levels, while *Broussonetia papyrifera* and *Ficus palmata* exhibit significant riboflavin content. Additionally, *Artocarpus lakoocha* and *Solanum nigrum* were noted for their high thiamine concentrations.

Rajalakshmi *et al.* (2017) analysed the nutritional parameters of three wild edible fruits. These fruits, *namely Syzygium jambos, Trema orientalis,* and *Rubus ellipticus*, were collected from the natural strands of the Nilgiris in Tamil Nadu. The total ash content of *Rubus ellipticus* was 4.02 ± 0.16 %. *Trema orientalis* had the highest levels of total carbohydrates, fats, fibre, and vitamin A, while *Syzygium jambos* had higher protein content and vitamin C.

Amadi *et al*. (2018) investigated the nutrient and phytochemical composition of various parts of the jackfruit (*Artocarpus heterophyllus*). The study demonstrated that jackfruit seeds contained significantly elevated levels of fat (4.29 ± 0.12 %), crude protein (10.09 ± 0.11 %), and carbohydrate (7.89 ± 0.13 %). The jackfruit leaves exhibited significantly elevated levels of crude fibre (4.91 ± 0.06 %) and ash (2.53 ± 0.06 %). The analysis of micronutrient composition indicates that the potassium content in jackfruit pulp was significantly higher at 0.33 mg per 100 g. The vitamin C content was significantly elevated at 2.1 mg 100 g^{-1}, while the zinc content was also notably higher at 9.28 mg 100 g^{-1}. The amount of calcium in the leaves was higher at 0.52 mg 100 g^{-1}. The manganese concentration was 12.75 mg 100 g^{-1}, while the iron concentration was 59.50 mg 100 g^{-1}.

Acharya (2018) conducted the biochemical profiling of *Citrus macroptera*, an underutilized citrus species in north-eastern India. The analysis revealed the fruit's nutrient composition, including carbohydrates (40.5 g 100 g^{-1}), crude fibre (1.4 g 100 g^{-1}), ascorbic acid (50 mg 100 g^{-1}), total carotene (14 µg 100 g^{-1}), anthocyanin (10 mg 100 g^{-1}), flavonoids (0.30 mg 100 g^{-1}), total phenolic content (0.10 mg 100 g^{-1}), and total protein (1.5 g 100 g^{-1}).

A study by Raj (2018) assessed the food and nutritional potential of the wild edible fruit *Alangium salviifolium ssp sundanum* (Miq.) Bloemp, utilised by the Kani tribe in the Southern Western Ghats. The findings indicated that the fruits possess a high moisture content of 83.9 ± 1.49 % and a carbohydrate content of 11.67 %. The fruit contains 62.46 mg of ascorbic acid, 0.71 mg of vitamin E 100 g^{-1}, and has an energy value of 81 kcal 100 g^{-1}. The research indicated that the fruit contains a significant amount of anthocyanin, quantified at 157.37 mg 100 g^{-1}.

Bhutia *et al.* (2018) analysed the chemical composition of various wild edible fruits of Sikkim. Their study revealed that *Machilus edulis* had highest fat content 21.5 ± 0.02%. *Prunus cerasoides* contained highest protein (10.0 ± 0.6%). *Castanopsis hystrix* was the least acidic (0.5 %), whereas *Elaeocarpus sikkimensis* was the highest (4.2 %). *Diploknema butyracea* had the maximum TSS (18 ^{O}Brix), while *Machilus edulis* had the lowest (3.55 ^{O}Brix).

In a study by Islam *et al.* (2018) in Tripura, they analysed six types of monkey jackfruit (*Artocarpus lakoocha* Roxb.). The biochemical analysis showed that Type-5 had the highest moisture content (87.11 %), Type-6 had highest TSS (20.20 ^{O}Brix), with TSS/acid ratio of 23.22 and lowest acidity 0.87%. Additionally, Type-4 exhibited the highest content of ascorbic acid (182.04 mg 100 g^{-1}).

The proximate study carried out by Kabra and Baghel (2018) revealed that *Myrica esculenta* fruit had greater levels of moisture content, carbohydrates and crude lipids with values of 72.33 ± 0.23%, 4.93 ± 0.06%, and 78.03 ± 0.14% respectively. In contrast, *Myrica esculenta* leaves had lower levels of moisture content, carbohydrates and crude lipids with values of 3.54 ± 0.11%, 1.38 ± 0.54%, and 46.19 ± 0.21%, respectively. The leaves had greater levels of total ash, crude fibre, and crude protein, with values of 8.3 ± 0.28%, 22.45 ± 110.17%, and 10.55 ± 0.22%, respectively. In comparison, the fruit had lower levels of total ash, crude fibre, and crude protein, with values of 2.18 ± 0.02%, 5.22 ± 0.08%, and 9.62 ± 0.03% respectively. Therefore, the research concluded that the leaves and fruits of *Myrica esculenta* have the potential to be a beneficial nutraceutical supplement and a cost-effective source of critical nutrients for the human diet.

Pandey *et al.* (2018a) conducted the nutritional composition of *Eleaocarpus sikkimnesis* and *Baccaurea sapida*. The study found that *Eleaocarpus sikkimnesis* has a greater calorific value (389.56 ±3.29 kcal 100 g^{-1}) compared to *Baccaurea sapida* (377.44 ± 3.26 kcal 100 g^{-1}). The dry matter content of *Eleaocarpus sikkimnesis* was found to be 31.33 ± 0.40 %, while the crude protein content was 6.93 ± 0.03% and the carbohydrate content was 88.17 ± 0.80 %. *Baccaurea sapida* exhibited greater levels of ash content (3.59 ± 0.72 %), fibre (3.60 ± 0.03 %), and crude fat (1.24 ± 0.09 %).

Pandey *et al*. (2018b) analysed the nutritional composition and phytochemical content of two lesser-known fruits, *Spondias axillaris* and *Eriolobus indica*, originating from Sikkim Himalaya region. The fruit of *Spondias axillaris* exhibited elevated levels of dry matter (21.40 ± 0.43 %), crude protein (6.31 ± 0.58 %), and crude fat (2.10 ± 0.28 %) in comparison to *Eriolobus indica*. *Eriolobus indica* exhibited elevated levels of fibre (4.57 ± 0.06 %), total carbohydrate (89.06 ± 0.52 %), and energy value (393.14 ± 1.96 kcal 100 g^{-1}).

Rana *et al.* (2018) conducted a research to assess the nutritional value of five wild edible fruits viz, *Rubus ellipticus, Rubus paniculatus, Benthamidia capitata, Coriaria nepalensis,* and *Pyracantha crenulate*. The research revealed that *Pyracantha crenulata* and *Coriaria nepalensis* had the greatest carbohydrates (80.71 g 100 g^{-1}) and protein (10.49 g 100 g^{-1}). The most significant values for fat, sugar, and energy content were observed in *Rubus paniculatus*, with 4.56 g of fat, 27.95 g of sugar, and 373.28 Cal 100 g^{-1}, respectively. The research found that these wild fruits have high nutritional content and they might be utilised as supplemental food in mountain regions and should be encouraged to protect and improve their genetic variety.

Soe and Lin (2018) examined the nutritional composition of *Dillenia indica* L fruit. Their study revealed that the fruit is a good source of protein (1.58%), fat (8.00%), fibre (29.32%), carbohydrate (43.23%), moisture (12.16%), and ash (5.71%). The ripe fruit was found to have an ascorbic content of 19.81 mg 100 g^{-1} of fresh sample.

Surya *et al.* (2018) conducted a nutritional study of five distinct wild *Rubus* species. The findings indicate that *Rubus fraxinifolius* has the greatest concentration of total carbohydrate (11.48%), sugar (5.05 g 100 g^{-1} of fruits), and calorie content (45.92 calories). The raw fibre content of *Rubus chrysophyllus* was the highest (8.43 %). The ripening fruit of *Rubus fraxinifolius* (stage II) had the largest amount of vitamin C (83.65 mg 100 g^{-1}). Conversely, *Rubus rosifolius* reported maximum content of vitamin C (54.30 mg 100 g^{-1}).

Loukrakpam *et al.* (2019) analysed the nutritional and phytonutrient composition of *Rhus semialata*. The fruit had protein (8.13 %), fat (16.70 %), ash (2.86 %), total dietary fibre (44.91 %), and carbohydrate (24.29 %).

Sibiya *et al.* (2020) investigated the mineral composition of several indigenous wild fruits from southern Africa. The study identified substantial levels of potassium and calcium in the majority of fruits, with concentrations varying from 522 to 14,289 mg/kg. *Cordyla africana* is distinguished among fruits for its remarkable mineral content. Various fruits, such as *Dovyalis longispina*, *Manilkara mochisia*, *Garcinia livingstonei*, and *Syzygium guineense*, exhibit significant levels of vitamin A.

Abifarin *et al.* (2021) analysed the nutritional composition and antinutrient content of *Heteromorpha arborescens* (Spreng.) Cham. & Schltdl leaves. The study revealed that the leaves comprised several constituents: total ash (8.5 %), crude fat (4.92 %), moisture (8.41 %), crude protein (15.74 %), crude fibre (21.48 %), carbohydrates (40.95 %), and an energy value of 271.04 kcal 100 g^{-1}. The leaves of *H. arborescens* exhibited elevated levels of potassium, calcium, and iron. Substantial amounts of Mg, Mn, Na, P, Cu, and Zn were identified. It contains substantial levels of vitamins A, C, and E. Antinutrients such as phytate, oxalate, saponin, and alkaloids were found in non-toxic concentrations, with the exception of saponin, which was identified at a moderately elevated level. The findings indicate that the leaves of *H. arborescens* are rich in nutrients and may serve as a beneficial component of daily nutrient consumption.

Bayang *et al.* (2021) examined the distinctions in physical properties, nutritional content, and bioactive components between dried and fresh wild edible fruits across twenty-three species from the northern region of Cameroon. A significant difference was observed among twenty-eight fruits. *Ziziphus mauritiana* and *Ziziphus spinachristi* exhibited the highest dry matter content among both dry and fresh fruits, recording values of 99.39 g 100 g^{-1} and 56.78 g 100 g^{-1}, respectively. The dry and fresh forms of *Phoenix reclinatum* demonstrated markedly higher carbohydrate content, measuring 52.34 g GE 100 g^{-1} and 60.95 g GE 100 g^{-1}, respectively, compared to the other fruits. *Balanites eagyptiaca* had significantly

higher protein value of 165.98 mg 100-g among dry fruits, whereas *Phoenix reclinatum* had a lower amount of protein 124.17 mg 100 g^{-1} among fresh fruits. In addition, dried fruits exhibited greater energy content (ranging from 141.08 to 244.50 Kcal/100g) compared to fresh fruits (ranging from 39.70 to 307.48 Kcal/100g), with the exception of *Phoenix reclinatum* and *Hyphaene thebaica*. The current research demonstrates that the wild edible fruits available in the Far North region of Cameroon serve as excellent novel sources of bioactive and nutritional components. Therefore, their regular intake may be used to combat malnutrition, maintain good health, and avoid a range of illnesses. Moreover, they may serve as novel food constituents for the aim of food composition.

Peduruhewa *et al.* (2021) reviewed the potential of underutilized wild edible plants found in Sri Lanka as a food source for the future. Their studies indicated that underutilized wild food plants have significant nutritional value. These plants provide a great reservoir of protein, fat, fibre, vitamins, carbohydrates, minerals, and antioxidants. In addition to its nutritional properties, several scientific research conducted worldwide have established this substance's therapeutic and pharmacological significance. Underutilized species of wild food plants exhibited constant productivity even in the face of unfavourable circumstances and limited resources. Hence, it is essential to focus on fostering the cultivation, processing, and pharmaceutical manufacturing of underutilized wild food plants.

Meena *et al*. (2022) investigated 19 underutilised fruits that exhibit resilience to climate conditions in arid and semi-arid regions of India. The crops encompass lasora, Indian gooseberry, bael, khejri, karonda, wood apple, custard apple, jharber, kair, tamarind, Jamun, mahua, pilu, chironji, manila tamarind, mulberry, Indian jujube, timroo and khirni. The researchers concluded that these underutilized fruits possess excellent nutritional properties, containing a high concentration of phytochemicals and exhibiting medicinal value.

Kamdem Bemmo *et al*. (2023) studied the nutritional value, physicochemical properties and antioxidant properties of the pulp and seeds of jackfruit (*Artocarpus heterophyllus*) from the eastern forests of Cameroon. The results indicated that the

pulp of jackfruit exhibited moisture content of 89.85 % ± 0.49, which was significantly greater than that of the seeds at 60.07 % ± 0.12. The jackfruit pulp pH was (5 ± 0 ˚B) which was significantly lower compared to the seeds (6 ± 0 ˚B). Jackfruit pulp exhibited higher content of carbohydrates (54.39 % ± 0.47) compared to the seeds (49.01 % ± 0.43). The protein content (18.35% ± 0.04) was lower for jackfruit pulp, when compared to the seeds (21.66 % ± 0.31). The jackfruit pulp exhibited maximum mineral contents of K (848 mg/100 g ± 10.34) and Na (69.53 mg/100 g ± 0.12). In contrast, the seeds contained maximum levels of Ca (132 mg/100 g ± 9.42), Mg (43.73 mg/100 g ± 9.12), and P (101.51 mg/100 g ± 4.02). All extracts contained antioxidant compounds, specifically phenols and flavonoids, as well as antioxidant activities. In conclusion, jackfruit has the potential to enhance the nutritional status of populations in eastern Cameroon.

Kanfon *et al.* (2023) studied the ethnobotanical and nutritional significance of the pulps, leaves, seeds, and kernels of *Tamarindus indica* L. Their study revealed that pulp of *Tamarindus indica* L. contains 60.99 g of sugars, 4.05 g of raw ash, 18.40 g of tartaric acid, and 6.12 g of total fibre in 100 g. *Tamarindus indica* L. seeds contain significant amounts of protein (20.21g), potassium (217.04mg), calcium (45.33mg), magnesium (31.32mg), and iron (14.28mg). These parts of fruits possess a well-balanced composition of α-amino acids and secondary metabolites.

Angami *et al*. (2024) evaluated the nutritional value and antinutritional compositions of wild edible fruits from the eastern himalayas. *Elaeagnus umbellata* exhibited a high concentration of soluble solids (24.50 ± 0.41ºBrix), free fatty acids (67.79 ± 1.75 mg KOH g^{-1}), and vitamin A (136.22 ± 1.44 mg 100 g^{-1}). *Terminalia chebula* exhibited significant reducing sugars (8.38 ± 0.07%) and total flavonoids (445.20 ± 1.18 mg 100 g^{-1}). *Spondias pinnata* exhibited elevated levels of total carbohydrates (12.51 ± 0.22%) and vitamin C (74.16 ± 5.33 mg per 100 g). *Castanopsis hystrix* exhibited highest starch content (1764.84 ± 8.85 mg 100 g–1) and cellulose (711.62 ± 7.68 mg 100 g–1), while *Machilus edulis* demonstrated a significant fat content (36.44 ± 1.23%). *Canarium strictum* exhibited the highest concentration of phenols among the studied plants, measuring 902.00 ± 5.72 mg per 100 g. *Terminalia chebula* exhibited the highest tannin content (1059.33

± 17.46 mg 100 g^{-1}), while *Streblus asper* showed the highest alkaloid concentration (274.27 ± 6.31 mg 100 g^{-1}). *Castanopsis hystrix* recorded the highest cyanogen level (31.04 ± 1.28 mg 100 g^{-1}), and *Viburnum foetidum* had the highest phytic acid content (10.64 ± 0.30 mg 100 g^{-1}). The results indicate that all analysed fruit species display both nutritional and antinutritional properties, rendering them potentially beneficial for the nutraceutical and pharmaceutical industries.

2.2 Functional food components of the selected underutilized fruits

Khomdram *et al*. (2014) found that *Phyllanthus emblica* exhibited the highest concentration of ascorbic acid, (375.68 ± 110.6 mg 100 g^{-1}). *Spondias pinnata* exhibited (86.16 ±11.04 mg 100 g^{-1}), while *Citrus phouheiri* displayed the lowest concentration (36.33 ±6.56 mg 100 g^{-1}). The ascorbic acid levels in these fruits exhibited a strong correlation with their IC_{50} values, with correlation coefficients (r) of -0.940, -0.924, and -0.915, respectively. *Elaeagnus pyriformis, Elaeocarpus floribundus, Prunus armeniaca, Punica granatum* and *Prunus persica* demonstrated substantial levels of ascorbic acid, which correlated with their IC_{50} values. The correlation coefficients (r values) were -0.896, -0.864, -0.726, -0.726, -0.703, and -0.704, respectively. This suggests a correlation between antioxidant activities and ascorbic acid content.

Fungo *et al.* (2015) examined the nutritional and bioactive component levels in *Trichoscypha abut, Baillonella toxisperma,* and *Pentaclethra macrophylla* from Cameroon. They reported that *Trichoscypha abut* was a valuable reservoir of bioactive substances, including flavonoids, polyphenols, proanthocyanins, vitamin C, and total oxalates. *P. macrophylla* had high concentration of total fat, protein, fibre, and some beneficial components, including vitamin E and proanthocyanins. *B. toxisperma* possessed a significant amount of carbohydrates, potassium, and calcium.

Mann *et al*. (2015) investigated the bio-protective properties of the underutilised *Myrica esculenta* Buch.–Ham. ex D. Don fruit from Meghalaya. The study demonstrated that the fruit had notable concentrations of phenolic, flavonoid, and flavonol compounds of 26.21±0.1 GAE µg/mg, 38.00±0.5 RE µg/mg, and 122.75±0.1 RE µg/mg, respectively. The MeOH extract demonstrated a dose-dependent radical scavenging activity, achieving maximum inhibition of 91.91±0.2%

for DPPH and 82.57±2.9% for ABTS. The GC/MS analysis identified multiple compounds, such as 4H-Pyran-4-one, pentadecanoic acid, 2-furancarboxaldehyde, phytol, and hexadecanoic acid. The compounds demonstrate potential antimicrobial and antioxidant properties. LC-MS data reveals the presence of ferulic and gallic acid, indicating their potential role in the compound's anticancer properties. The MeOH extract of Soh-phie fruits may serve as a valuable resource for the food industry, functioning as a natural antioxidant and preservative.

In another study, Vino and Harshita (2016) examined the potential of underutilized fruits in India. Their findings suggest that these fruits are affordable and packed with essential nutrients. It is widely acknowledged that these plants possess medicinal and therapeutic properties, which indigenous communities have utilised to treat a range of ailments. In Indian traditional medicine and ayurveda, various plants' fruits, seeds, and leaves are utilised for their healing properties. Minor fruits, often called 'less known fruits' or 'underutilized fruit', possess significant nutritional and medicinal properties. Thriving under challenging weather conditions, these organisms demonstrate remarkable adaptability and tolerance. These underutilised fruits have the potential to contribute to sustainable agriculture.

Bakar *et al.* (2016) investigated the biological activities and phytochemical composition of selected wild berries. The study found that *Rubus alpestris* demonstrated the highest total phenolic content at 24.25 ± 0.1 mg gallic acid equivalent (GAE)/g and carotenoid content at 21.86 ± 0.63 mg β-carotene equivalents (BC)/g. *Rubus alpestris* exhibited the highest DPPH scavenging and FRAP activities. *Rubus moluccanus* exhibits the highest total flavonoid content at 18.17±0.20 mg catechin equivalents (CE)/g and anthocyanin content at 36.96±0.39 mg cyanidin-3-glucoside equivalents (c-3-gE)/g. The extracts of *Rubus moluccanus* and *Rubus alpestris* demonstrated a mild inhibitory effect on *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Salmonella enteritidis* in antibacterial assays. The extracts demonstrated anticholinesterase activity between 23% and 26%. The GC-MS analysis identified at least 12, 21, and 7 unique organic compounds in the 80% methanol extracts of *Rubus alpestris, Rubus moluccanus,* and *Rubus*

fraxinifolius respectively. These compounds may contribute to the observed bioactivity.

Cheema *et al.* (2017) examined the nutritional value of 25 commonly consumed wild and underutilised fruits from the Terai region of Uttarakhand, India. The protein content was higher in *Broussonetia papyrifera*, *Solanum nigrum,* and *Aegle marmelos,* whereas higher lipid content was observed in *Ficus racemosa, Terminalia bellerica,* and *Artocarpus heterophyllus*. The concentration of reducing sugars varied from 1.1% to 68.2%, while non-reducing sugars ranged from 0% to 6.4%. Analysis of vitamins indicated elevated levels of ascorbic acid in *Emblica officinalis* and *Pithecellobium dulce*, significant riboflavin concentrations in *Broussonetia papyrifera* and *Ficus palmata* and notable thiamine content in *Artocarpus lakoocha* and *Solanum nigrum*. The phenolic content ranged from 32.9 to 2662.4 mg per 100 g, flavonoids from 1.2 to 93.8 mg per 100 g, and carotenoids from 0 to 85.7 mg per 100 g. The phenolic content was found to range from 32.9 to 2662.4 mg per 100 g, flavonoids from 1.2 to 93.8 mg per 100 g, and total carotenoids from 0.11 to 85.7 mg per 100 g.

Seal *et al.* (2017) researched the water-soluble vitamins in five different wild edible fruits. These fruits, namely *Docynia indica, Elaeagnus latifolia, Elaeagnus pyriformis, Flemingia vestita*, and *Myrica esculenta*, are commonly consumed in the North-eastern region of India by the tribal people. The experimental findings revealed that the vitamin C content varied significantly across various fruits, ranging from 37.31 ± 0.10 to 95.54 ± 3.33 mg 100 g^{-1} dry matter. The B1 content in *Elaeagnus latifolia* was found to be high (1.20 ± 0.007 mg 100 g^{-1}). *Elaeagnus pyriformis* contains a substantial amount of B3 (70.75 ± 0.002 mg 100 g^{-1}). *Docynia indica* contained a significant amount of B2 (0.525 ± 0.004 mg 100 g^{-1}), while *Myrica esculenta* had a notable amount of B9 (5.36 ± 0.03 mg 100 g^{-1}).

Amadi *et al*. (2018) investigated the nutrient and phytochemical composition of the pulp, seeds, and leaves of Jackfruit (*Artocarpus heterophyllus*). Jackfruit pulp exhibited the lowest concentrations of phytic acid, oxalate, alkaloids, tannin, and flavonoid, measured at 6.14, 3.69, 7.88, 0.03, and 3.91 mg per 100 g, respectively. Jackfruit seeds exhibited elevated levels of phytic acid (8.11 mg 100 g-1) and oxalate

(5.53-13 mg 100 g-1), whereas the leaves contained higher concentrations of alkaloids (7.88 mg 100 g-1), tannins (0.06 mg 100 g-1), and flavonoids (2.03 mg 100 g-1). This study demonstrated that jackfruit pulp, seeds, and leaves possess a high nutrient content. Phytochemicals present in jackfruit pulp, seeds, and leaves contribute to health improvement, particularly in combating non-communicable diseases.

Bhutia *et al.* (2018) analysed the chemical makeup of several wild edible fruits in Sikkim. *Machilus edulis* have the greatest carotenoid concentration (62.9 ± 0.5 mg 100 g^{-1}) and the highest chlorophyll level (2.29 ± 0.06 mg 100 g^{-1}). Ascorbic acid content was highest in *Spondias axillaris* at 32.0 ± 4.0 mg 100 g^{-1}, whereas *Rubus ellipticus* had a significant amount of anthocyanin (3.8 ± 0.05 mg 100 g^{-1}).

Pandey *et al*. (2018b) analyzed the nutritional composition and phytochemical content of two lesser-known fruits, *Spondias axillaris* and *Eriolobus indica*, obtained from the Sikkim Himalaya region. *Spondias axillaris* exhibited higher levels of phytochemicals, specifically total phenol (71.83 ± 0.76 mg GAE g^{-1}) and ascorbic acid (34.54 ± 0.99 mg 100 g^{-1}). In contrast, *Eriolobus indica* exhibited the highest total flavonoid concentration (17.48 ± 0.31 mg QE g^{-1}).

Loukrakpam *et al.* (2019) examined the phytonutrient content of *Rhus semialata*. The fruit exhibited 21.26 mg of Vitamin C 100 g^{-1}, 314 mgs of folates, 628 mg of carotenoids, and 2.94 mg of α-tocopherol 100 g^{-1} of sample. The protein included 31.43 mg of essential amino acids 100 g^{-1}. The fruit mainly consisted of linoleic acid as its principal fatty acid. The fruit showed a 7.5 mg/mL of IC_{50} in the DPPH test and 1226 mmol $FeSO_4$ equivalent/100 g in the FRAP assay.

Veljkovic *et al*. (2019) investigated the antioxidant and anticancer properties of extracts derived from the leaves and fruits of the wild raspberry (*Rubus idaeus* L.). The total phenolic content varied from 59.68 to 96.83 mg GA g^{-1} in leaf extracts and from 24.29 to 38.71 mg GA g^{-1} in fruit extracts. A population in east Serbia were found to have the maximum concentrations of tannins and anthocyanins in leaf extracts 1.27 mg mL^{-1} and 9.00 mg mL^{-1}, respectively.

Bayang *et al.* (2021) conducted a study on the differences in physical qualities, bioactive components and nutritional value between twenty-three dried and

fresh wild edible fruit species from the north area of Cameroon. They found a substantial ($p < 0.01$) variance among twenty-eight fruits. The bioactive elements lutein, α-carotene, β-carotene, and vitamin C were found to be high in dry fruits, with respective levels of 123.65 mg 100 g^{-1}, 74.43 mg 100 g^{-1}, 72.88 mg 100 g^{-1}, and 780.16 mg 100 g^{-1}. On the other hand, fresh fruits exhibited high concentrations of lycopene (70.61 mg 100 g^{-1}) and vitamin A activity (0.54 μg RAE 100 g^{-1}). High levels of bioactive nutrients are a significant signal of good bioactive qualities, which may aid in protecting the body against illnesses. Their research demonstrates that untamed edible fruits serve as promising novel sources of nutritional and bioactive constituents. Therefore, their regular intake may be used to combat malnutrition, maintain good health, and avoid a range of illnesses. Moreover, they may serve as novel food components for the aim of food composition.

In another study, Borgohain *et al.* (2022) reviewed the selected unconventional fruits found in the upper Brahmaputra valley, Assam. viz. Heloch (*Antidesma acidum*), Bon-pitha (*Chrysophyllum roxburghii*), Bilombi-tenga (*Averrhoa bilimbi*), Korja-tenga (*Carissa carandas*), and Thereju (*Prunus jenkinsii*), are abundant in various macro and micronutrients. Phytochemical analyses of different components revealed the existence of phenols, flavonoids, terpenes, and other associated chemicals. These underutilised fruits have several traditional uses, especially in folkloric medicine, although they are not well recognised in the pharmaceutical industry. Furthermore, due to insufficient public awareness, several non-traditional fruits are on the brink of extinction. Therefore, more scientific research is required to preserve and enhance their worth.

Rawat *et al.* (2023) researched the changes in the physicochemical, nutritional, phytochemical composition, and antioxidant potential of the fruits *Mahonia nepaulensis* during the ripening process. Significant and sequential changes were observed in the nutritive phytochemicals, including riboflavin, lycopene, β-carotene, and total carotenoids, as well as in the antioxidant potential of the species throughout various ripening stages. The concentrations of phenolic, flavonoid, and flavonol compounds initially decreased and subsequently increased consistently throughout the ripening process. In contrast, tannin content reduced as the fruit

ripened. The acidified methanolic extract exhibited higher levels of flavonoid, flavonol, total phenolic, tannin contents, and antioxidant activity than the methanolic extract. These fruits contain a significant amount of glycosides rather than aglycones. The presence of abundant and beneficial plant compounds, strong antioxidant properties, carefully selected extraction solvent, and optimal harvesting stage in *Mahonia nepalensis* berries may be used as alternative sources of nutrition, prospective nutraceuticals, and effective post-harvest management.

Pan *et al*. (2023) investigated the fruit quality and volatile compounds of Liuyuezao pummelo. The study revealed that it contained elevated levels of organic acids (12.01 mg/g), phenols (669.01 mg/L), and vitamin C (75.73 mg/100 mL), along with superior antioxidant capacity (77.65 mg/100 mL) relative to other pummelo varieties. Nonetheless, it exhibited reduced concentrations of soluble sugars (62.85 mg/g), carotenoids (0.25 mg/L), and flavonoids (46.3 mg/L). Furthermore, it exhibited a reduced quantity of fruit volatiles (49) and a significantly lower content (7.63) compared to Guanxi, Sanhong, and Hongrou. The flavour profile of Liuyuezao, characterised by a balance of sweetness and moderate sourness, is attributed to its relatively high levels of fructose (20.6 mg/g) and organic acids. The subtle fragrance of pummelo is due to its minimal concentration of volatile compounds.

In another research, Bachheti *et al.* (2023) reviewed the bioactive elements and health-promoting chemicals found in underutilized fruits from the northern Himalayas of India. They revealed that these fruits, derived from wild and underutilized plants, had significant nutritional value. They serve as significant sources of fat, protein, carbohydrates, macronutrients, and micronutrients. These fruits contain various phytochemical substances, such as flavonoids, terpenoids, glycosides, saponins, tannins, and alkaloids. These chemicals have several uses and treat numerous health issues in humans and cattle. Their phytochemical components also showed properties such as reducing fever, relieving pain and inflammation, preventing cancer, fighting against microorganisms, combating malaria, and reducing sensitivity to pain. These fruits, which are not used to their full potential, can provide dietary supplements, various phytochemical components, and minerals. Their

inherent components have significant antioxidant, antibacterial, antidiabetic, and anti-inflammatory properties. Neglected fruits can also provide carbohydrate, fat, vitamins, and fibre. The nutritional profiles of these fruits are sometimes superior to those of their exotic and native counterparts. A substantial number of these fruits are necessary for the industry, which poses a barrier to their utilisation.

Angami *et al*. (2024) investigated the bioactive compounds and functional dietary compositions in various wild edible fruits from the eastern Himalayas. Research indicates that *Elaeagnus umbellata* contains a significant concentration of vitamin A (136.22 ± 1.44 mg 100 g^{-1}). *Terminalia chebula* exhibited a substantial concentration of total flavonoids (445.20 ± 1.18 mg 100 g–1), while *Spondias pinnata* demonstrated elevated levels of total carbohydrates (12.51 ± 0.22%) and vitamin C (74.16 ± 5.33 mg 100 g–1). *Castanopsis hystrix* exhibited the highest starch content (1764.84 ± 8.85 mg 100 g^{-1}). The *Phoebe cooperiana* exhibited the highest chlorophyll concentration, *Rhus semialata* demonstrated the greatest carotenoid content, and *Prunus nepalensis* showed the highest anthocyanin level. *Artocarpus lakoocha* (84.58 ± 2.38%) and *Spondias pinnata* (84.09 ± 0.62%) were recognised as significant sources of antioxidants. The results indicate that all analysed fruit species display both nutritional and antinutritional properties, rendering them potentially beneficial for the nutraceutical and pharmaceutical industries.

2.3. Anti-cancerous properties of the underutilized fruits.

Solowey *et al*. (2014) assessed the anticancer properties of medicinal plants, specifically *Artemesia monosperma*, *Urtica membranacea*,and *Origanum dayi*. The study revealed that three complete plant extracts (obtained via ethanol extraction) from *Artemesia monosperma* (Asteraceae), *Urtica membranacea* (Urticaceae), and *Origanum dayi* post (Labiatae) exhibited dose- and time-dependent cytotoxic effects on various human tumour cell lines and primary cultures derived from patient biopsies. These extracts specifically targeted tumour cells while leaving healthy human cells unaffected. Cell death was attributed to apoptosis. Plant extract (*Urtica membranacea*) demonstrated significant anticancer effects, effectively inhibiting

tumour progression in a mouse model having breast adenocarcinoma. This study indicates that whole plant extracts may serve as potential anticancer agents.

Hsieh *et al*. (2016) investigated the anticancer properties of *Kalanchoe tubiflora* extract against human lung cancer cells through both in vitro and in vivo methods. The research demonstrated that the water-soluble fraction of *Kalanchoe tubiflora* (KT-W) caused cell cycle arrest and senescence in A549 cells. Through 2-dimensional PAGE, the researchers examined protein expression levels post-KT-W treatment and identified alterations in proteins associated with energy metabolism and senescence. In vivo experiments indicated that KT-W significantly inhibited tumour growth in A549-xenografted nude mice. The findings indicate that KT-W may serve as a potential antitumor agent by inducing cell cycle arrest and senescence.

El-Hallouty *et al*. (2015) investigated the in vitro anticancer activity of specific Egyptian plant extracts against multiple human cancer cell lines. A total of 20 plants from 5 distinct families were collected in Egypt. Among the 25 methanol extracts evaluated, 7 exhibited potential cytotoxic activities against cancer cells. The extract derived from the leaves of *Harpophyllum caffrum* exhibited significant efficacy across all cancer cell lines, with an IC50 range of 21-29 μg/ml and a selectivity index (SI) of 4.5 specifically against the MCF-7 breast cancer cell line. Furthermore, it demonstrated selectivity indices of 3.3 against HepG2, A549, and HCT-116, respectively.

The study carried out by Beg *et al*. in 2016 examined *Ziziphus*'s in vitro anticancer activity. The researchers used MTT assay to detect the cytotoxic effects of the fruit's methanolic extract from three *Ziziphus* varieties: *Z. nummularia, Z. mauritiana*, and *Z. jujube*. The phytochemical profiling of the fruit's methanolic extract revealed the presence of various phytochemicals. The study observed that the wild variety of *Ziziphus* exhibited significant cytotoxicity in HeLa cells (cervical carcinoma cells). At the same time, *Z. mauritiana* also showed cytotoxic effects on cancer cells, highlighting the extract plant potential in cancer treatment.

Tuzcu *et al*. (2017) examined the anticancer properties of extracts obtained from the leaves and roots of the wild edible plant *Eremurus spectabilis*. In PC-3 cells

treated with *E. spectabilis*, there was an increase in the expression of Bax and caspase-3 genes, whereas the expression of the Bcl-2 gene decreased. This indicates that the apoptosis of PC-3 cells was induced through the mitochondrial pathway, in contrast to the control group. The research findings indicate the efficacy of E. spectabilis as an anticancer agent targeting prostate cancer cells.

Wu *et al*. (2018) conducted a study examining the anti-cancer properties of polysaccharides derived from *Glehnia littoralis* on the A549 human lung cancer cell line. The findings from the MTT test demonstrated that the polysaccharide significantly inhibited the growth of A549 cells, with effects contingent upon the duration of exposure and the dosage administered. PGL demonstrated inhibitory effects on the migration of A549 cells in the Transwell migration assay. The results from flow cytometry analysis and Hochst 3342 staining of apoptotic cells indicate that PGL can promote apoptosis and induce cell cycle arrest in A549 cells. The immunofluorescence experiment demonstrated that PGL reduces the expression of proliferating cell nuclear antigen (PCNA). The findings indicated that PGL exhibited a significant anticancer effect by inhibiting the migration and proliferation of A549 cells while enhancing cell death. Their discovery may function as a novel natural anticancer agent for lung cancer treatment and could be significant for the development of supplements and pharmaceuticals.

Robinson *et al*. (2017) examined the cytotoxic activity of *Tecoma stans* in relation to lung cancer cells (A549) in their experiment. The research investigated cytotoxic activity using the MTT assay, demonstrating a relationship between extract concentration and cell death rate. At a concentration of 100µg/mL, there was a significant increase in cytotoxic activity, leading to a 99% inhibition of cell growth.

Biswas *et al*. (2018) conducted a study to examine the potential anticancer effects of *Asparagus racemosus* root extracts on non-small cell lung cancer A549 cells. The research indicated that the methanol extract demonstrated a notable cytotoxic effect, with an IC_{50} value of 100.5 µg/ml, in contrast to the chloroform:methanol extract, which had an IC_{50} value of 136.5 µg/ml. The cells exhibited a marked alteration in morphology, and their migratory capacity was

considerably diminished after treatment with the root extracts. The findings indicate that extracts from *Asparagus racemosus* root can induce cytotoxic effects, alter cell morphology, and inhibit growth in A549 cells. Consequently, it may be further investigated as a pharmaceutical option for treating NSCLC patients in the future.

Bukke *et al*. (2018) examined the effects of extracts from the heartwood and leaves of *Caesalpinia sappan* L. on MCF7 and A549 cell lines. The findings indicated that Brazilin A, a natural bioactive compound derived from the heartwood of *Caesalpinia sappan* L., has the potential to induce apoptosis in MCF-7 breast cancer cells. Further evidence of therapeutic potential was shown through the docking of the Brazilin A molecule with the BCL-2 protein, recognised for its role in inhibiting apoptosis. The docking was conducted utilising AutoDock tools.

Veljkovic *et al*. (2019) investigated the anticancer properties of leaf and fruit extracts from the wild raspberry (*Rubus idaeus* L.). Both leaf and fruit extracts demonstrated significant efficacy against *Escherichia coli* (ATCC 8739). The research indicated that the leaf extracts exhibited anticancer activity, with IC_{50} values of 162.38 μg mL-1 at 24 hours and 95.69 μg mL-1 at 48 hours. Wild raspberry leaf and fruit extracts contain various secondary metabolites that contribute to their notable antioxidant, antimicrobial, and anticancer properties.

In an investigation carried out by Damodaran *et al*. (2019) they reported the chemical composition of *Calotropis gigantea* leaf extract and its potential effects on cancer cells. The extract was found to contain compounds including steroids, alkaloids, terpenoids, flavonoids, tannins, and phenols. The research concentrated on the cytotoxic effects of the extract on MCF7, HeLa, and A549 cancer cell lines. The extract demonstrated notable cytotoxic effects on HeLa, MCF7, and A549 cancer cell lines in vitro. The research indicates that Calotropis gigantea may serve as a viable candidate for cancer therapy, particularly targeting cervical, breast, and lung cancer cell lines.

Kumar *et al*. (2019) examined the anticancer properties of five plant species: *Moringa oleifera, Calotropis procera, Basela alba, Millettia pinnata, and Euphorbia*

neriifolia. Cytotoxicity assessments indicated that the chloroform and ethyl acetate extracts of *Millettia pinnata* exhibited comparatively greater cytotoxic effects on certain cell types. Phytochemical analysis utilising UPLCESI–MS/MS revealed the presence of β-sitosterol, lanceolatin B, karanjin, and stigmasterol in the chloroform and ethyl acetate extracts of Millettia pinnata. The findings indicate the potential of *Millettia pinnata* extracts as anticancer, antioxidant, and antimicrobial agents.

Shendge *et al*. (2020) conducted a study examining the anti-inflammatory properties of *Terminalia chebula* fruit and its ability to inhibit the proliferation of lung and breast carcinoma cells by regulating the Bax/Bcl-2 and caspase-cascade pathways. Their research reported that *Terminalia chebula* fruit modulates the Bax/Bcl-2 and caspase-cascade pathways associated with cell death. The cytotoxic effects were demonstrated on A549 cells (IC50 – 359.06 ± 20.04 μg/ml) and MCF-7 cells (IC50 – 61.02 ± 5.55 μg/ml). The flow cytometer analysis revealed an increase in the sub G1 population and apoptotic cells, as indicated by cell cycle analysis and annexin-V-FLUOS staining. Confocal microscopy demonstrated DNA fragmentation in both cell lines following TCME treatment. The administration of *Terminalia chebula* activates apoptosis-related caspase-cascade pathways in both cell lines. TCME therapy applied to RAW 264.7 cells effectively regulated nitrite and TNF-α production, as well as the levels of iNOS and COX-2, and the translocation of NF-κB protein, thereby demonstrating anti-inflammatory effects. The HPLC analysis confirmed the presence of bioactive phytocompounds in TCME. The findings collectively indicate the significant anti-cancer and anti-inflammatory properties of *Terminalia chebula* fruit.

Sairi *et al*. (2020) conducted an experiment to examine the effects of *Donkioporiella mellea* on MRC5 and A549 cell lines. The findings indicated that the survival rates of MRC5 cells were significantly greater than those of A549 cells when subjected to varying concentrations of polypore extracts. This study elucidates the potential cytotoxic effects and anticancer properties of *Donkioporiella mellea*. The survival performance of MRC5 cells was notably high when subjected to hot and cold aqueous extracts. The cytotoxicity activities of the cold aqueous extract were

significantly greater than those of the hot aqueous extract. The inhibitory concentration (IC_{50}) values for the cold and hot aqueous extracts were found to be 414.29 µg/ml and greater than 1000 µg/ml, respectively. The cell lines exhibited necrotic characteristics upon treatment with tamoxifen as a control. The findings suggest that *Donkioporiella mellea* possesses significant pharmacological properties that warrant further investigation as a potential bioresource for human consumption. This discovery may encourage additional research in mycology and nutraceutical studies.

Gibbert *et al*. (2021) investigated the anticancer properties of *Syzygium cumini* (L.) and *Syzygium malaccense* (L.). Their findings indicated that these fruits demonstrate antiproliferative activity against lung carcinoma cells. The maximum concentration evaluated (2 mg/mL) resulted in nearly 80% inhibition of cell proliferation. Furthermore, the evaluation of *S. cumini* in HEK-293 demonstrated that all tested concentrations exhibited cell viability exceeding that of the positive control. In summary, the advantageous nutritional properties of both *S. cumini and S. malaccense* position them as potential nutraceuticals in complementary therapies.

Madhavan (2021) conducted an investigation on the phytochemical analysis and anticancer properties of an ethanolic extract from *Azadirachta indica*. The research examined the impact on the A549 human lung cancer cell line. The study indicated that the ethanolic extract of *Azadirachta indica* exhibited notable anticancer activity against the A549 cell line, with efficacy contingent upon the dosage administered. The treatment of A549 cells with *Azadirachta indica* ethanolic extract for varying durations (6, 12, 24, and 36 hours) resulted in effective growth control. A study indicated that the ethanolic extract of *Azadirachta indica* exhibited a notable inhibition rate of 68% against A549 lung cancer cells at a concentration of 500µg/ml. At a concentration of 100 µg/ml, the minimum inhibition observed was 12%. The ethanolic extract of *Azadirachta indica* exhibited a notable inhibition of 68% against A549 lung cancer cells at a concentration of 500 µg/ml. At a concentration of 50 µg/ml, the observed minimum inhibition was 12%.

Nghakliana *et al*. (2021) examined the potential anticancer effects of *Elaeagnus caudata* (Schltdl) on Type-II human lung adenocarcinoma A549 cells. The research examined the impact of the aqueous extract of the plant, particularly its capacity to trigger caspase-mediated apoptotic cell death. The effect of *Elaeagnus caudata* on A549 cells was found to be contingent upon the dosage and duration of exposure. *Elaeagnus caudata* was found to enhance antioxidant levels and activities while decreasing lipid peroxidation (LPO) in A549 cells. This study investigates the impact of *Elaeagnus caudata* treatment on A549 cells, highlighting its potential anti-cancer effects via the induction of DNA damage and enhanced caspase-6 activity.

Calderón-Montaño *et al*. (2021) assessed the anticancer potential of multiple plant extracts sourced from western Andalusia, Spain. A total of 65 extracts from 45 distinct plant species were examined, leading to an intriguing observation. An extract from the leaves of *Tetraclinis articulata* (Vahl) Mast (Cupressaceae) demonstrated notable cytotoxicity (IC_{50} = 0.37±0.03 g/mL) and selectivity (selectivity index = 378.3) against lung cancer cells. Cisplatin, 5-fluorouracil, and an extract from the leaves of *Taxus baccata* L. (Taxaceae) exhibited reduced cytotoxicity and selectivity in comparison. The extracts from *Cascabela thevetia* (L.) Lippold (Apocynaceae), *Frangula alnus* Mill. (Rhamnaceae), *Iberis ciliata* subsp. contracta (Pers.) Moreno (Brassicaceae), *Juniperus macrocarpa* Sm (Cupressaceae), and *Pancratium maritimum* L. (Amaryllidaceae) demonstrated notable selective cytotoxicity, indicated by a selectivity index exceeding 10. Active extracts were tested on various cancer cell lines derived from different tissues. This study identifies plants that may serve as potential sources of natural compounds with selective toxicity against cancer cells.

Kausar *et al*. (2021) assessed the antimicrobial and anticancer properties of specific medicinal plants indigenous to the Himalayas in Pakistan. The study concentrated on the analysis of *Prunus cornuta* and *Quercus semicarpifolia* species. The preliminary phytochemical screening identified the presence of alkaloids, tannins, saponins, flavonoids, glycosides, and quinones. The extracts from *Prunus cornuta* and *Quercus semicarpifolia* exhibited notable inhibition of breast (MDA-

MB-231) and lung carcinoma cells, with cell viabilities between 19% and 30% and 22% and 39%, respectively. Furthermore, *Q. semicarpifolia* demonstrated significant inhibition of gut cell line survival, with a range of 24% to 34%. The findings offer significant insights for the development of novel anticancer medicinal agents derived from extracts of Prunus cornuta and *Quercus semicarpifolia*.

2.4 Antioxidant properties of the underutilized fruits.

Loganayaki and Manian (2010) investigated the antioxidant properties of several underutilised fruits through various in vitro models. The evaluated fruits comprised *Syzygium cumini, Morus alba, Murraya koenigii, Opuntia dillenii, Carissa carandus, Kirganalia reticulata, Canthium parviflorum, Alangium lamarckii, Lantana camara, and Coccinia grandis.* The researchers employed established methodologies, including ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiozoline-6-sulfonic acid) diammonium salt (ABTS), hydroxyl radical (OH), nitric oxide radical (NO), superoxide radical (O2) scavenging, and metal chelating activities, to evaluate the antioxidant activity of the fruits. Significant levels of total phenolics, tannins, and flavonoids were observed in the fruit extracts. The extracts of *O. dillenii, M. koenigii, K. reticulata, L. camara, and M. alba* demonstrated significant activity in DPPH, ABTS, and FRAP assays. The antioxidant capacity of these fruits correlates significantly with their phenolic content. All extracts demonstrated significant scavenging activities against nitric oxide, superoxide, and hydroxyl radicals in a dose-dependent manner, particularly in comparison to the standard butylated hydroxyl anisole (BHA). Their research revealed that these frequently neglected fruits have considerable potential as important sources of natural antioxidants and nutraceutical compounds.

Maria do Socorro *et al*. (2010) studied the bioactive components and antioxidant properties of polyphenolic extracts from 18 fresh and dried native non-traditional fruits in Brazil. They used many techniques, including ABTS, DPPH, FRAP, and beta-carotene bleaching. The results indicated the possibility for using non-conventional tropical fruit species owing to their significant nutritious content

and antioxidant capability. The research highlighted the substantial antioxidant potential of *Malpighia emarginata* (acerola), *Myrciaria dubia* (camu-camu), and *Mouriri pusa* (puçá-preto).

Rawat *et al.* (2011) conducted a study on the antioxidant capabilities of the fruits of *Myrica esculenta*, a recognised wild edible in the Indian Himalayan area. A significant discrepancy existed in the total phenolic and flavonoid concentrations across different groups. The total phenolic content ranged from 1.78 to 2.51 mg of gallic acid equivalent per gramme of fresh weight (FW) of fruits. The total flavonoid concentration ranged from 1.31 to 1.59 mg quercetin equivalent per gramme of fresh weight. The efficacy of antioxidants was assessed by many methodologies, including radical scavenging and ferric-reducing antioxidant power (FRAP). The findings demonstrated a significant antioxidant capacity, significantly associated with the total phenolic and total flavonoid concentrations. Substantial variance in phenolic compounds, such as gallic acid, catechin, hydroxybenzoic acid, and ρ-coumaric acid, was detected across populations, as shown by high-performance liquid chromatography analysis. This research indicates that the consumption of *M. esculenta* fruits may possess antioxidant characteristics, possibly reducing the levels of free radicals in the body.

In an investigation carried out by Kolar *et al*. (2011) analysed the phytochemical composition and antioxidant capacity of three obscure fruits: *Averrhoea bilimbi, Muntingia calabura, and Artocarpus altilis.* The research examined the total phenolic and flavonoid concentrations, along with the antioxidant activity of these fruits, using several solvent systems including methanol, ethanol, acetone, and distilled water. The phenolic and flavonoid concentrations in *M. calabura* varied from 1.356 to 3.872 mg tannic acid equivalents per gramme of fresh weight (TAE/g fw) and 0.026 to 0.068 mg rutin equivalents per gramme of fresh weight (RE/g fw), respectively. The results for *A. bilimbi* varied from 0.581 to 1.334 mg TAE/g fw and 0.021 to 0.037 mg RE/g fw, while *A. altilis* exhibited ranges from 0.689 to 1.723 mg TAE/g fw and 0.013 to 0.043 mg RE/g fw.

Gordon *et al*. (2011) investigated the phenolic compounds and antioxidant potential of four underappreciated fruits indigenous to the Amazon area. Research indicated that jambolao (*Syzygium cumini*), Araca (*Psidium guineense*), muruci (*Byrsonima crassifolia*), and cutite (*Pouteria macrophylla*) are exceptional sources of hydrolysable tannins and flavonols. The researchers detected several flavanonols and proanthocyanidins in some fruits. The total oxidant scavenging capacity (TOSC) experiment evaluated antioxidant capability, indicating that cutite exhibited the highest antioxidant capacity, followed by jambolao, araca, and muruci.

Singh *et al.* (2012) analysed the phytochemicals and antioxidant activities of underutilised fruits from the Andaman Islands, India. Their results indicated that various fruits from a limited number of underutilised species, including *Averrhoa carambola L., Annona squamosa L., Dillenia indica L., Averrhoa bilimbi L., Annona muricata L., and Ficus racemose L.,* were identified. Their findings indicated that they had diverse antioxidant activity, spanning from 74.27% to 98.77%. The methanol extract of *M. glabra* exhibited the maximum antioxidant activity at 98.77%, with an inhibitory concentration (IC_{50}) of 262.46 mg/ml. The research demonstrated that methanol surpassed acetone and aqueous solvents in assessing antioxidant activity. The phytochemical examination of *M. glabra* indicated elevated concentrations of polyphenols (355.74 mg/100 g), anthocyanins (91.31 mg/100 g), carotenoids (109.16 mg/100 g), tannins (24.39 mg/100 g), and ascorbic acid (394.23 mg/100 g). *M. glabra* had the greatest carbohydrate content at 548 mg/100 g. The research identified a positive link between antioxidant activity and the levels of phenols, tannins, anthocyanins, and carotenoids, with correlation values (r2) of 0.846, 0.864, 0.915, and 0.806, respectively. The authors proposed that this knowledge may be beneficial for the bioprospecting of underutilised fruits from the Andaman Islands.

Romojaro *et al*. (2013) examined the nutritional and antioxidant qualities of numerous wild edible plants for their possible incorporation into contemporary diets. *Sanguisorba minor, Quercus ballota, and Sedum sediforme* had the greatest hydrophilic total antioxidant activity (H-TAA) and total phenolic content. *Asparagus*

acutifolius, Allium ampeloprasum, Foeniculum vulgare, and Malva sylvestris had elevated potassium concentrations. *Malva and Asparagus* were distinguished by their zinc level, but *Urtica urens* were characterised by a significant calcium concentration.

Song *et al.* (2018) performed research examining the antioxidant capabilities of Chinese wild *Passiflora foetida* fruits. The UPLC-Q-TOF-MSE investigation found 65 compounds, including 39 free phenolics, 14 insoluble glycoside-phenolics, and 22 insoluble ester-phenolics in both extractable and non-extractable phenolics. Moreover, non-extractable phenolics derived from alkali hydrolysis had considerable antioxidant activity, as shown by DPPH and ABTS radical scavenging assays. Their research indicates that *Passiflora foetida* fruits, rich in polyphenols, may provide significant nutraceutical advantages.

Veljkovic *et al*. (2019) investigated the antioxidant characteristics of leaf and fruit extracts from the wild raspberry (*Rubus idaeus* L.), assessing antioxidant activity by quantifying the extracts' scavenging capacity on DPPH. The leaf extracts exhibited greater antioxidant activity compared to the fruit extracts.

Kabra *et al*. (2019) examined the antioxidant activities of several solvent extracts obtained from the leaves of *Myrica esculenta* Buch.-Ham. ex. D. Don. The methanol extract of *Myrica esculenta* leaves shown the highest antioxidant activity in scavenging DPPH and ABTS radicals, with IC_{50} values of 39.29 µg/mL and 52.83 µg/mL, respectively. In contrast, the ethyl acetate and aqueous extracts had little antioxidant action. The methanol extract exhibited a phenolic content of 88.94±0.24 mg equivalent gallic acid (GAE)/g. The aqueous extract exhibited a reduced phenolic content of 62.38±0.14 GAE/g. The methanol extract exhibited the highest flavonoid content (67.44±0.14 mg quercetin equivalent (QE)/g). The aqueous extract had the lowest concentration (35.77 ±0.14 QE/g). The chemicals identified using LC-MS analysis included myricanol, gallic acid, epigallocatechin 3-O-gallate, myricanone, quercetin, β-sitosterol, palmitic acid, p-coumaric acid, n-octadecanol, n-pentadecanol, oleanolic acid, stigmasterol, cis-β-caryophyllene, n-hexadecanol,

lupeol, and myresculoside. Research demonstrates that the methanolic extract from the leaves of *Myrica esculenta* has significant antioxidant capabilities and may be a beneficial natural source of antioxidants and antimicrobials for the formulation of functional food items.

Bareh *et al*. (2021) examined the possible health advantages of *Prunus undulata* buch. Their study concentrated on the antioxidant, anti-inflammatory, and anti-diabetic effects of this plant. The research revealed that the methanol extract of *Prunus undulata* included many phytochemicals, such as carbohydrates, flavonoids, phenols, saponins, steroids, and triterpenoids. The methanol extracts of *Prunus undulata* Buch.-Ham. ex D. Don had significant antioxidant activity across all performed experiments. They were investigating the potential advantages of *Prunus undulata* Buch. -Ham. ex D Don's confirmation was also validated. This research concluded that the methanol extracts of *Prunus undulata* Buch.-Ham. ex D were analysed. Don leaves possess significant antioxidant, anti-inflammatory, and antidiabetic characteristics. The phytochemicals in the leaves of *Prunus undulata* Buch.-Ham. ex D Don may enhance its potential pharmacological action.

Biswas *et al*. (2022) investigated the antioxidant properties of wild edible fruits in Tripura, North-east India, revealing *Citrus macroptera* and *Flacourtia jangomas* Lour. Raeusch are abundant in vitamin C, with concentrations of 220.75 mg/100 g and 223.25 mg/100 g, respectively. The antioxidant activity of *Citrus macroptera* is very high, measuring 81.15 μmol/g. Furthermore, it has a notable metal chelating capacity (MCC) of 39.45 mg/mL. The total flavonoid content (TFC) of *Citrus macroptera* was significant, quantified at 36.78 mg QE/g. Their results provided a fundamental database on the nutritional makeup of these fruits and enhance public awareness of their importance in conserving the biodiversity of the forest region in Tripura.

Perera *et al*. (2022) investigated the nutritional composition and antioxidant properties of specific underutilised fruits cultivated in Sri Lanka, namely Maha Karamba (*Carissa carandas*), Màdan (*Syzygium cumini*), Ugurassa (*Flacourtia*

indica), Himbutu (*Salacia chinensis*), Barbados cherry (*Malpighia emarginata*), and Ceylon gooseberry (*Dovyalis hebecarpa*). The total phenolic content (TPC) of the fruits ranged from 6.8 ± 0.4 to 10.3 ± 0.3 mg of GAE g^{-1} of fruit. Barbados cherry had the greatest antioxidant activity (AOA) as determined by FRAP (0.022 ± 0.003 mM Fe 2+/g fruit) and the highest vanillin concentration (2.4 mg/kg). The greatest concentrations of potassium (434.60 ± 0.36 mg/kg), phosphorus (16.69 ± 0.46 mg/kg), and calcium (23.43 ± 0.45 mg/kg) were recorded in Uguressa. Màdan had the greatest concentrations of magnesium (13.25 ± 0.38 mg/kg), sodium (5.28 ± 0.30 mg/kg), iron (0.65 ± 0.12 mg/kg), and aluminium (1.15 ± 0.16 mg/kg).

Rymbai *et al*. (2023) performed an investigation on the biochemical and antioxidant properties of wild edible fruits in the eastern Himalayas, India. *Haematocarpus validus* had the highest observed levels of ascorbic acid (63.82 mg/100 g), total carotenoids (18.47 mg/100 g), and total monomeric anthocyanin (354.04 mg/100 g). *Docynia indica* had the greatest overall phenolic content (19.37 mg GAE/g), whilst *H. Validus* displayed the highest total flavonoid and flavanol content. The antioxidant activities of the fruits varied from 0.17 to 0.67 IC50 for DPPH activity and 3.59 to 13.82 mg AAE/g for FRAP. These fruits exhibited attractive colouration in both pulp and juice, offering promise for natural edible colour extraction in the food sector. Moreover, owing to their elevated market pricing, these fruits serve as significant sources of antioxidants and pigments, enhancing livelihood and nutritional security.

Yimer *et al*. (2023) investigated the antioxidant capacity of various wild edible plants in Southwest Ethiopia, noting that total phenol content ranged from 0.25 ± 0.06 mg GAE/g in *Dioscorea praehensilis* tuber to 35.73 ± 2.52 mg GAE/g in *Solanum nigrum* leaf, while total flavonoid content varied from 0.85 ± 0.03 to 11.25 ± 0.01 mg CE/g in *Dioscorea praehensilis* tuber and *Cleome gynandra* leaf. In the DPPH experiment, the antioxidant value varied from 50.09% in *Dioscorea praehensilis* tuber to 87.63% in *Solanum nigrum* leaf; conversely, in the FRAP assay, the value ranged from 49.16 ± 2.13 in *Dioscorea praehensilis* tuber to 188.12 ± 1.13 mM Fe2+/100 g in *Solanum nigrum* leaf.

A study conducted by Devi (2024) aimed to estimate the phytochemical components and antioxidant capacity of underutilised ethnobotanically significant wild edible fruits from Manipur. The phytochemical concentration was greatest in *Phyllanthus emblica*, which demonstrated greater antioxidant activity and vitamin C, although having lower tannin levels. *Spondias pinnata* had the greatest tannin concentration at 67.63 ± 0.97 mg/g. *Vangueria spinosa* demonstrated the lowest total phenolic and tannin concentrations. *P. emblica* had the greatest lowering capacity in both total in-vitro antioxidant activity (159.06 ± 4.10 AAE mg/g) and the FRAP assay (43.16 ± 0.05 AAE mg/g). Conversely, *Elaeocarpus floribundus* exhibited the lowest antioxidant activity in in-vitro assays, at 13.26 ± 0.10 AAE mg/g. The inhibitory percentages of the DPPH test ranked as follows: *Phyllanthus emblica* > *Spondias pinnata* > *Rhus semialata* > *Elaeocarpus floribundus* > *Microcos paniculata* > *Vangueria spinosa.* The fruit samples exhibited varied antioxidant activity, demonstrating a strong reaction to radicals. Their research emphasises the primary and secondary metabolites, together with the antioxidant characteristics of these neglected wild edible fruits, validating their medicinal significance and prospective applications.

Chapter III

MATERIALS AND METHODS

Materials and Method

This study, titled “Nutrient profiling, functional food compounds and antioxidant properties of some underutilised fruits of Mizoram,” was conducted at the Department of Horticulture Aromatic and Medicinal Plants, Mizoram University, Tanhril, Mizoram, from 2021 to 2023. The underutilised fruits were sourced from various districts in Mizoram. This chapter discusses the details regarding the selected fruit species, materials utilised, and methods employed during the investigation.

3.1 Geographical location of the experimental site

Various underutilised fruits are widely distributed in their wild forms across different regions of Mizoram. The experiment was carried out in seven districts of Mizoram: Aizawl, Kolasib, Lunglei, Serchhip, Champhai, Saitual, and Khawzawl, each representing distinct agro-climatic zones.

The laboratory analyses were conducted at the Department of Horticulture, Aromatics and Medicinal Plants (HAMP), Department of Biotechnology, and Department of Zoology at Mizoram University, as well as at the College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih, Aizawl. The geographical locations of the plant samples span various districts in Mizoram, Northeast India (Figure 3.2).

3.1.1 Mizoram

Mizoram covers an area of 21,081 square kilometres and is located between latitudes 21°56' N and 24°31' N, and longitudes 92°16' E and 93°26' E. The state is bordered internationally by Bangladesh to the west and Myanmar to the east and south. It also shares borders with the Indian states of Tripura to the northwest, Assam to the north, and Manipur to the northeast. The location is of considerable geographical and political significance, situated between Bangladesh and Myanmar. This hilly state features tropical and subtropical forest cover and is acknowledged as a biodiversity hotspot. The landscape is characterised by steep gorges and mountains orientated in a north-south direction. In winter, temperatures vary from 11°C to 21°C, whereas in

summer, they increase to a range of 20°C to 32°C. The monsoon season occurs from mid-May to mid-October, yielding an annual rainfall of around 250 mm (Figure 3.2).

3.1.1.1 Aizawl District

Aizawl district is located in the northern region of Mizoram, positioned between latitudes 4°25'16.04" N and 23°18'17.78" N, and longitudes 92°37'03.27" E and 93°11'45.69" E. Aizawl district encompasses a total geographical area of 3576.31 square kilometres, representing 16.96% of the state's overall geographical area. The region is bordered to the east by Champhai district and the state of Manipur, to the west by Mamit and Kolasib districts, to the north by Assam state, and to the south by Serchhip district.

3.1.1.2 Kolasib district

Kolasib district is located in the northern region of Mizoram, positioned between 24° 13' 52" N latitude and 92° 40' 34" E longitude, covering an area of 1382.51 km². The area is bordered to the north and northwest by the Hailakandi district of Assam, to the west by the Mamit district, to the south and east by the Aizawl district, and to the northeast by the Cachar district of Assam state.

3.1.1.3 Lunglei district

Lunglei District, the largest district in Mizoram, is bordered to the north by Mamit and Serchhip districts, to the south by Lawngtlai and Saiha districts, to the east by Myanmar, and to the west by Bangladesh. The town is the second largest after the capital, Aizawl, situated 165 km (102 miles) to the south of Aizawl. Lunglei is situated at coordinates 22.88°N latitude and 92.73°E longitude. The average elevation is 722 meters (2368 feet).

3.1.1.4 Serchhip district

Serchhip district is bordered to the north and northwest by Aizawl District, to the west and south by Lunglei District, to the southeast by Myanmar (Burma), and to the east by Champhai District. Geographically, Serchhip is situated between 23.3°N and 92.83°E. The average elevation is 888 meters (2913 feet).

3.1.1.5 Champhai district

The district is bordered to the north by the Churchandpur district of Manipur state, to the west by Aizawl and Serchhip districts, and to the south and east by Myanmar. The district covers an area of 3,185.83 km², with an average annual rainfall of 1,814 millimetres (71.4 inches).

3.1.1.6 Saitual district

The Saitual district was established by the Government of Mizoram on June 3, 2019, and is one of the eleven districts within the state of Mizoram, India. The district was delineated from the pre-existing administrative districts of Aizawl and Champhai. The Saitual district is situated at a latitude of 23°41'20.18″N and a longitude of 92°57'49.71″E. Saitual district is located in the northern region of Mizoram, adjacent to the neighbouring districts of Champhai and Aizawl. The district covers an area of 96,024 square kilometres. The distance from Saitual to Aizawl, the state capital, is 77 kilometres.

3.1.1.7 Khawzawl district

The district is bordered to the north by Serchhip, to the south by Lawngtlai, to the southeast by Saiha, and to the east by Myanmar. Khawzawl town serves as the administrative headquarters of the district. The distance from Khawzawl to Aizawl is 152 kilometres. The district covers an area of 1,089 km² and has an elevation of 1,187 m (3,894 ft).

3.2 Climatic condition

The higher elevations of Mizoram maintain consistently low temperatures, exhibiting cool conditions during the summer, whereas the lower regions experience warmer and more humid climates. Storms typically occur in March and April, preceding or coinciding with the summer season. The maximum average temperature in summer is 30˚C, whereas winter has a minimum average temperature of approximately 11˚C. Winter in Mizoram spans from November to February, succeeded by spring. In mid-April, storms signify the impending onset of summer. The temperature rises, resulting in a haze surrounding the hills. The interval from June to August is commonly designated as the rainy season. The climate reaches its optimal

temperate condition during the two months of autumn. The temperature during September and October ranges from 19 to 24˚C (Figure 3.3).

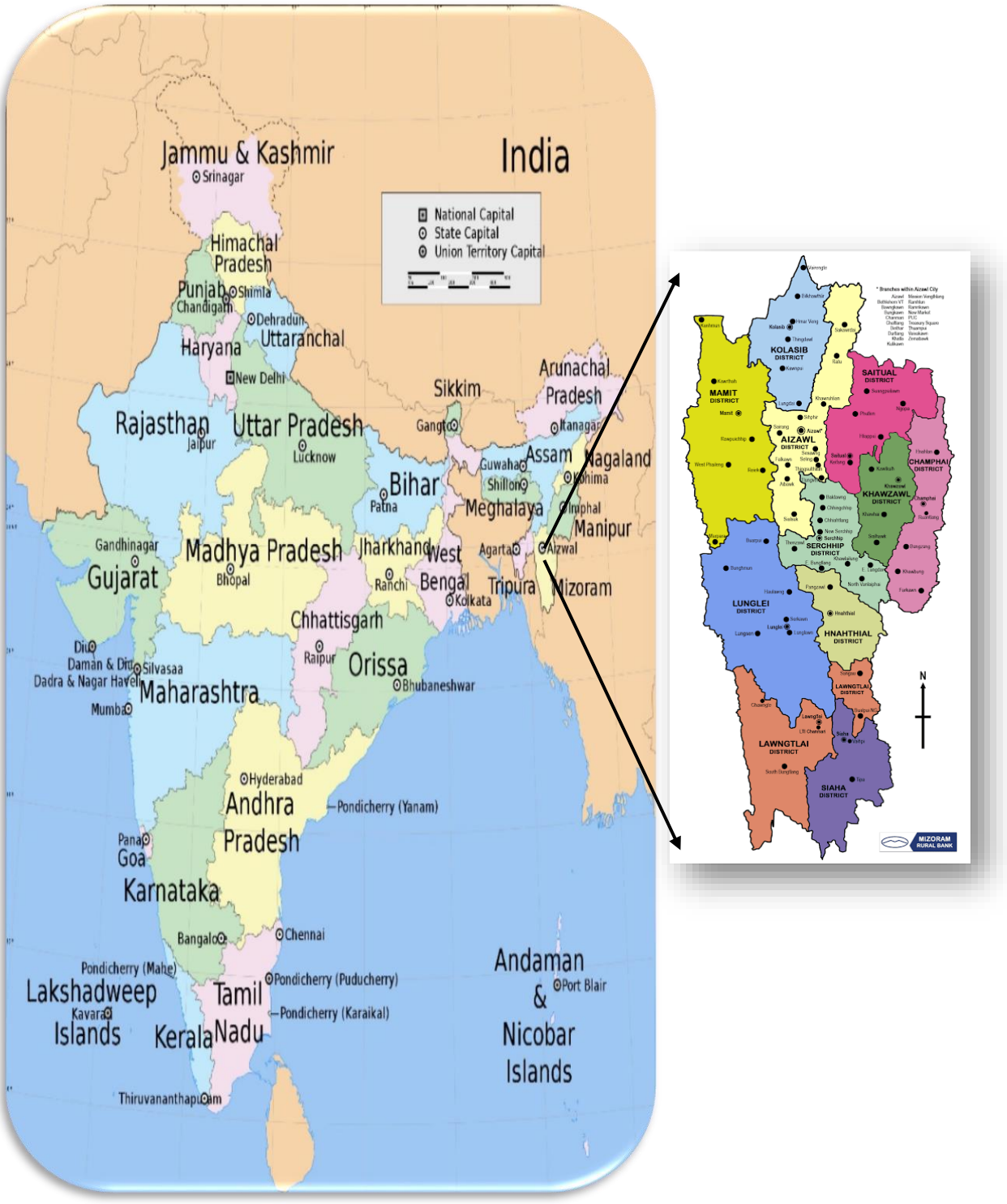


Figure 3.1 Map of India and Mizoram

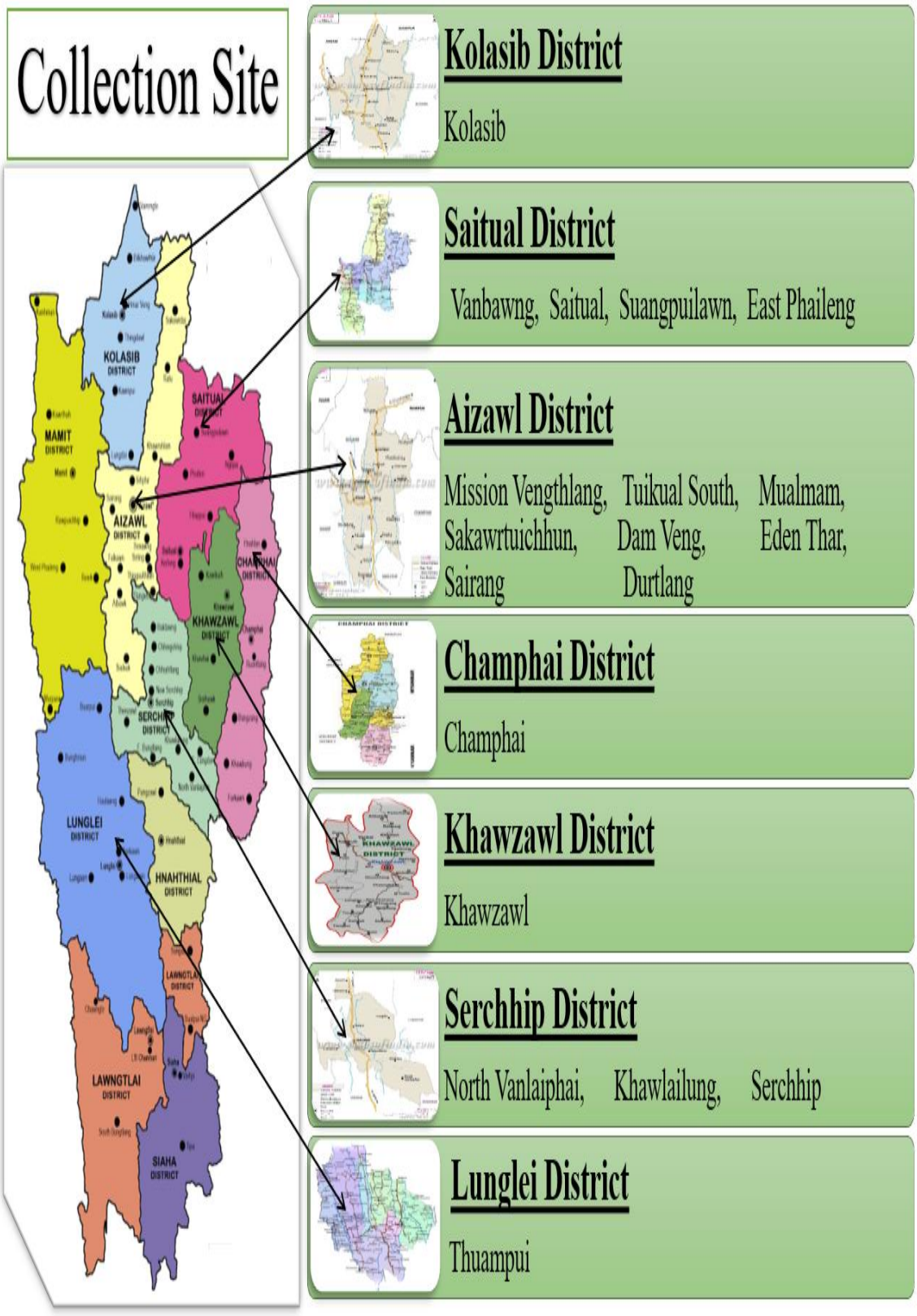


Figure 3.2 District-wise site for sample collection

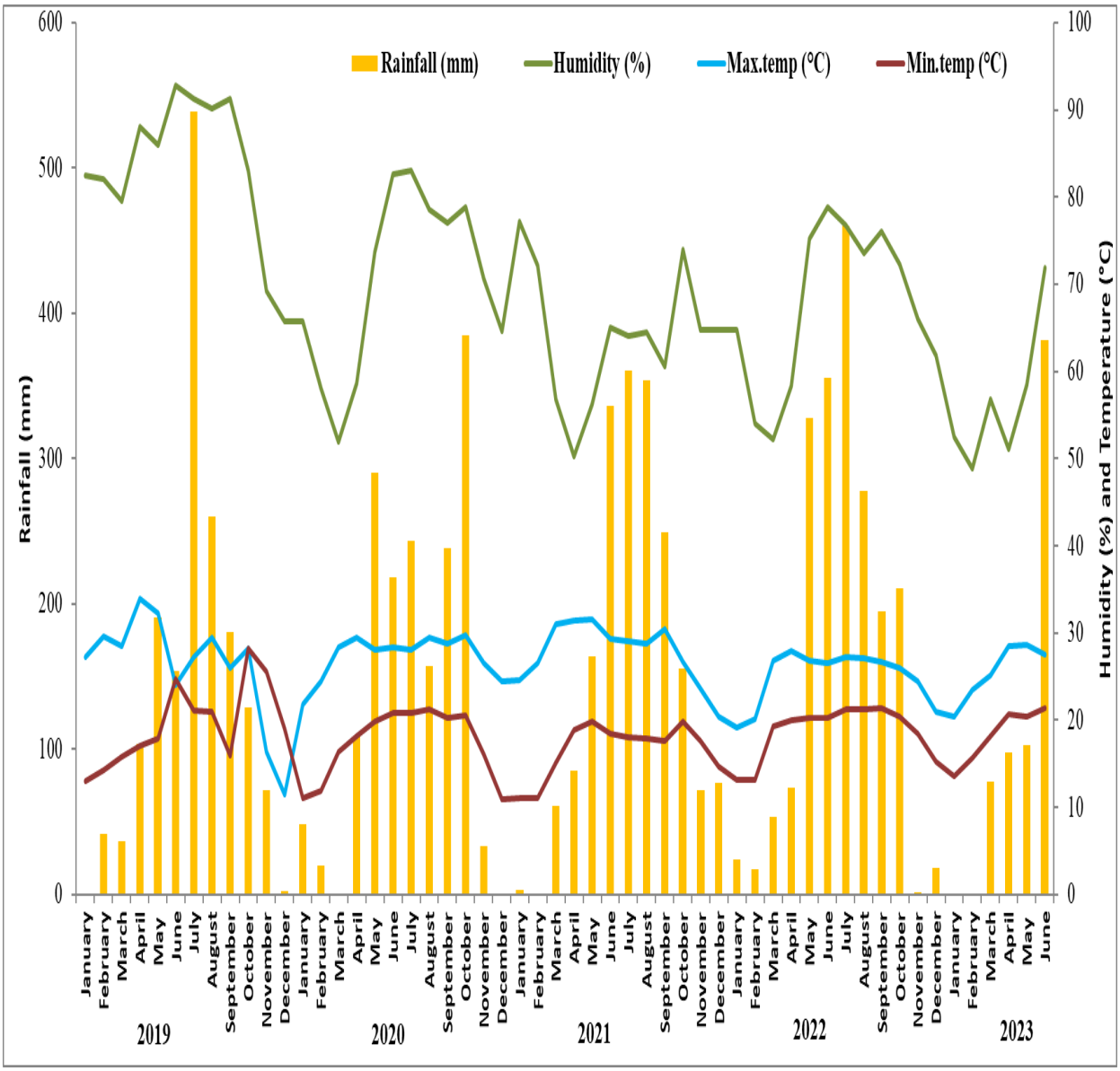


Figure 3.3 Rainfall and temperature of Mizoram during 2019-2023

3.3. EXPERIMENTAL MATERIALS

The experimental material for the current investigation consisted of 21 distinct underutilized fruits from Mizoram. The specimens were gathered from various regions of Mizoram. Table 3.1 provides a list of fruits, along with their common and botanical names.

3.3.1. Collection of underutilized fruit species for the study

Fruit samples were obtained from several sites in Mizoram according to their seasonal availability from the sites mentioned in Figure 3.2. A total of 21 underutilized fruit species (Table 3.1) were gathered from different districts of Mizoram and were taken as a replication of 3 where each replication consists of fruits from 3 different sites to ascertain minimization of errors in sampling. These species were represented by 15 families, with the Rosaceae family having the largest representation with 3 species. The Phyllanthaceae, Rutaceae, and Clusiaceae families each had 2 species, and so on (Fig. 3.4). The fruit samples were analysed at the Department of Horticulture Aromatic and Medicinal Plants Mizoram University Aizawl, Department of Biotechnology, and Department of Zoology at Mizoram University, as well as at the College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih, Aizawl, for the analysis of nutritional and biochemical characters along with their antioxidant and antinutritional properties.

Table 3.1 List of underutilized fruit species collected.

SL No	Scientific Name	Local Name	Family	Maturity	Parts Used
1.	***Antidesma bunius***	Queensland cherry, wild cherry, Bignay berry/Tuaitit	Phyllanthaceae	Fully Ripen	Fruits
2.	***Artocarpus heterophyllus***	Jackfruit/ Lamkhuang	Moraceae	Fully Ripen	Fruits
3.	***Carallia brachiata***	Kanthekera, karalli/Theiria	Rhizophoraceae	Fully Ripen	Fruits
4.	***Citrus grandis***	Pumelo/Sertawk	Rutaceae	Ripen	Fruits
5.	***Citrus jambhiri***	Rough Lemon/ Chawngbawla	Rutaceae	Mature Green	Fruits
6.	***Emblica officinalis***	Aonla/ Sunlu	Phyllanthaceae	Fully matured	Fruits
7.	***Embelia subcoriacea***	Poimuri Tenga/Tling	Myrsinaceae	Matured	Fruits
8.	***Elaeocarpus lanceifolius***	Saklong/ Kharuan	Elaeocarpaceae	Matured	Fruits
9.	***Garcinia kydia***	Tuaihabeh	Cluciaceae	Matured	Fruits
10.	***Garcinia xanthochymus***	False Mangosteen/ Tuaithleng	Cluciaceae	Fully Ripened	Fruits
11.	***Mahonia nepaulensis***	Nepal Berry/Pualeng	Beriberidaceae	Fully Ripened	Fruits
12.	***Morus nigra***	Blackberry/Theihmu	Moraceae	Fully Ripened	Fruits
13.	***Myrica esculenta***	Bayberry/ Keifang	Myricaceae	Fully Ripened	Fruits
14.	***Prunus jenkinsii***	Thereju/ Keihpui	Rosaceae	Fully Ripened	Fruits
15.	***Prunus undulata***	Laurel Cherry/ Theiarlung	Rosaceae	Fully Ripened	Fruits
16.	***Rubus treutleri***	Wild raspberry/Sialinuchhu	Rosaceae	Fully Ripened	Fruits
17.	***Spondias pinnata***	Wild Mango/Tawitaw	Anacardiaceae	Fully Ripened	Fruits
18.	***Syzygium cumini***	Jamun/ Lenhmui	Myrtaceae	Fully Ripened	Fruits
19.	***Tamarindus indica***	Tamarind/ Tengtere	Fabaceae	Matured Green	Fruits
20.	***Terminalia chebula***	Black Myrobalan/ Reraw	Combretaceae	Fully Matured	Fruits
21.	***Ziziphus mauritiana***	Wild Ber/ Borai	Rhamnaceae	Fully Matured	Fruits

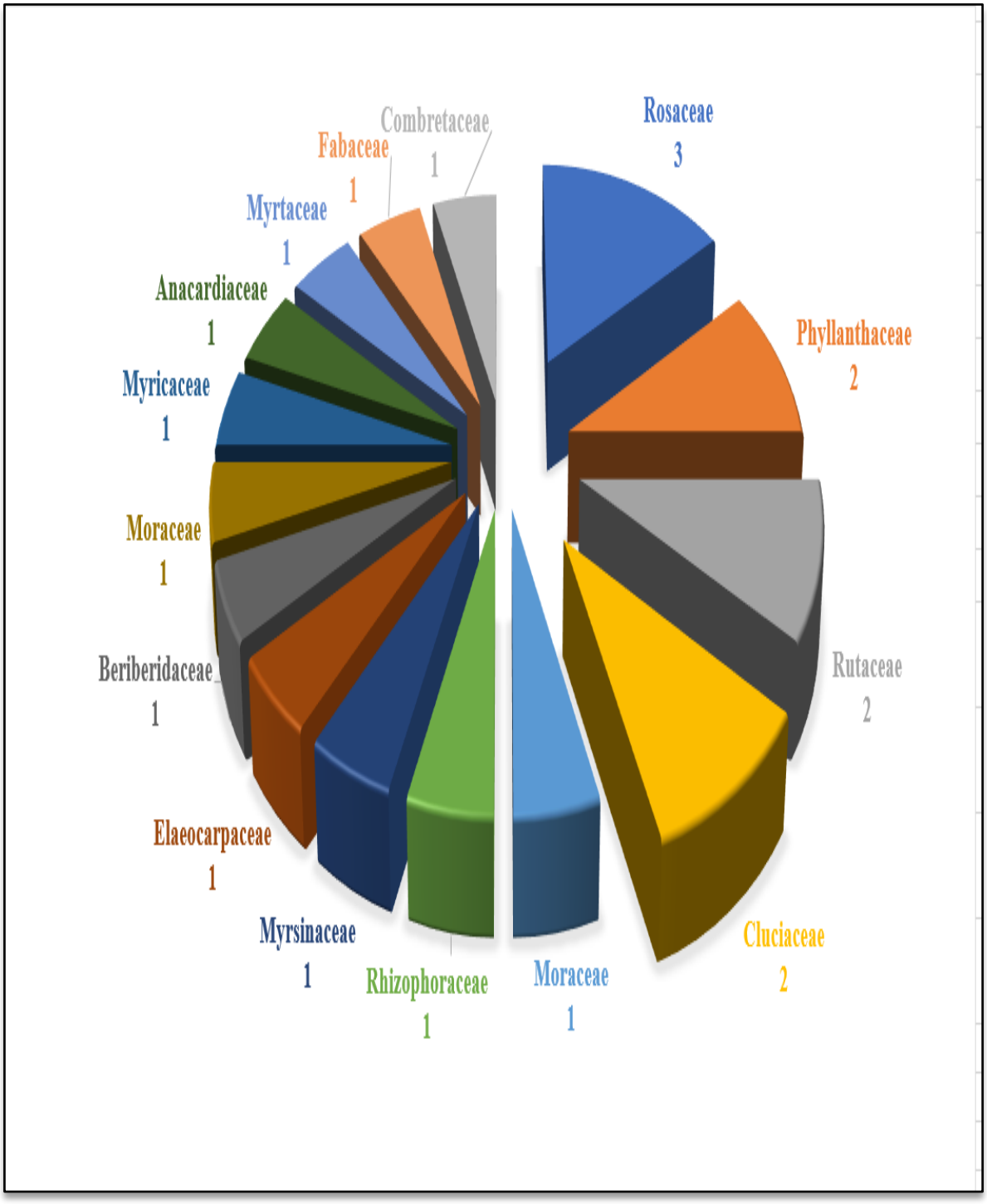


Fig. 3.4. Family-wise species representation of collected underutilized fruit species

3.3.2 Botanical description of selected underutilized fruit species

The underutilized fruit trees are distributed sporadically throughout many states, ranging from family gardens to marginal locations. Nevertheless, these fruits, which are often overlooked, are cultivated without adequate management practices, sometimes as part of a mixed crop with other forest trees or fruit crops.

3.3.2.1. *Antidesma bunius* L. Spreng.

Family: Euphorbiaceae
Common name: Bignay berries
Vernacular name: Ṭuaiṭit (Mizo)

The tree is an evergreen, dioecious species that may reach a height of up to 10 meters. It has a straight trunk and often branches out at the base. The leaves have an oval form and are leathery, measuring 20 cm in length and 7 cm in width. The inflorescence is located at the end or in the axil of the plant, and it is slender and spike-like or arranged in a raceme. It contains numerous flowers and is 6-20 cm in length. The fruit is a spherical or oval-shaped drupe, about 8-10 mm in diameter. It has a yellowish-red to blue-violet colour and contains a single seed. The fruit is arranged in clusters that resemble grapes and hang down. The seed is shaped like an oval oblong and is 6-8 mm in length and 4.5-5.5 mm in width. When consumed, ripe fruits may leave stains on the lips and fingers.

3.3.2.2 *Artocarpus heterophyllus* Lam

Family: Moraceae

Common name: Jackfruit

Vernacular name: Lamkhuang (Mizo)

Artocarpus heterophyllus is an evergreen tree that reaches a height of 15 to 20 m (50 to 70 feet) when fully grown. It is characterised by its enormous, rigid, and shiny green leaves, which are around 15 to 20 cm (6 to 8 inches) in length. The small unisexual blossoms are carried on compact clusters that arise straight from the main stem and branches. The jackfruit is the most sizable fruit that grows on trees, measuring up to 60 cm (about 2 ft) in length and weighing as much as 18 kg. The

object has an ellipsoidal shape and comprises several seed-containing flesh "bulbs" around a fibrous core, all of which is covered by a rough rind.

3.3.2.3 *Carallia brachiata* (Lour.) Merr.

Family: *Rhizophoraceae*

Common name: Freshwater mangrove

Vernacular name: *Theiria* (Mizo)

An evergreen tree that can grow up to 25 m tall. The bark is 10-12 mm thick, dark grey, corky, furrowed, and has prominent pustular-lenticellate patterns. When the bark is removed, the inner layer is pink. The petiole, which connects the leaf to the stem, is stout and glabrous, measuring 6-10 mm in length. The leaf blade is obovate or obovate-oblong in shape, measuring 4-11 cm long and 2-7 cm wide. The base of the leaf tapers or is acute, while the apex is either acute or obtuse. The leaf has an entire margin that is recurved, and it is thick and glossy. There are 6-10 pairs of slender, pinnate lateral nerves, with secondary laterals also present. The veins in between the lateral nerves form a reticulate pattern, which is not easily visible. The flowers are bisexual, cream-coloured, sessile, tiny, and arranged in trichotomous axillary branching cymes. The bracteoles are very small, and the calyx tube is bell-shaped with 5-8 ovate and pointed lobes that are arranged in a valvate manner. The petals, which are 5-8 in number, have a clawed base and are orbicular-cordate in shape with deeply lacerated margins. They are reddish in colour and are inserted on a crenulate disc. The disc itself is divided into 10-16 lobes. The stamens, also numbering 10-16, are inserted along with the petals on the disc. One of each pair of stamens is opposite to a sepal and is slightly longer than the other filament. The filaments are thread-like, and the anthers are small in size. The ovary is partially inferior and has 3-5 cells. Each cell contains 2 ovules. The style is subulate in shape, and the stigma is divided into 4 lobes. The fruit is a little drupe, about 5-6 mm in diameter. It is red in colour and has a filament-like appearance. The fruit contains a single seed, which is brilliant orange and has a somewhat kidney-shaped structure.

3.3.2.4 *Citrus grandis* Osbeck

Family: Rutaceae

Common name: Pummelo

Vernacular name: Sertawk (Mizo)

The tree has a maximum height of 5 to 15 m and has low, uneven branches. Plants that are reproduced by seeds have branches with lengthy spines measuring up to 5 cm in length. However, plants that are propagated through vegetative methods do not have these spines. The juvenile tertiary branches exhibit angularity and are often covered in fine hairs. The leaves have an obovate that is elliptical in shape. The blooms are yellow-white in colour and have a bisexual nature. They have a pleasant fragrance and grow either individually or in groups of 2 to 10 blossoms on the leaf axils. In tropical regions, the plant blooms biannually or triannually. The blooming period aligns with the periods of rapid shoot growth. The fruit has a rounded or pear-shaped (pyriform) appearance. The peel of this fruit is thick but soft, measuring between 1 to 4cm in thickness. The outer surface of the peel has a greenish-yellow to yellow colour and is adorned with green glands.

3.3.2.5 *Citrus jambhiri* Lush

Family: Rutaceae

Common name: Rough Lemon

Vernacular name: Chawngbawla, Serbawl (Mizo)

Citrus jambhiri is a tree of moderate to significant size, reaching a height of 3-6 meters. Branches are wide and possess pointed thorns. The leaves are little and have a pale green hue. The leaves have an oblong, elliptic-ovate form with serrated edges. Flowers are little in size, with a white top surface and a purple below surface. Fruits have a profound golden hue. The surface of the peel is coarse and has many sebaceous glands. The pulp has a light yellow colour and consists of 8 to 10 segments with a small number of seeds.

3.3.2.6 *Emblica officinalis* Gaertn

Family: Phyllanthaceae

Common name: Aonla, Indian gooseberry

Vernacular name: Sunhlu (Mizo)

The tree is of modest to moderate size, reaching a height of 1–8 m (3+1⁄2–26 feet). The bark displays a pattern of irregularly shaped spots or patches. The branchlets are covered in fine hairs (not smooth), measuring 10–20 cm (4–8 inches) in length, and often falling off in autumn. The leaves are simple, subsessile, and densely arranged along the branchlets. They are light green and have a similar appearance to pinnate leaves. The blooms have a greenish-yellow hue. The fruit has an almost spherical shape with a pale greenish-yellow colour. It seems smooth and firm and has six vertical lines or furrows. The diameter of the fruit may reach up to 26 millimetres (1 inch). Fruits weigh about 5.5 g (0.2 ounces), whereas farmed fruits have an average weight ranging from 28.4 g(1 ounce) to 56 g (2 ounces).

3.3.2.7 *Embelia subcoriacea* (C.B.Clarke) Mez

Family: Myrsinaceae

Common name: Poimur tenga

Vernacular name: Tling (Mizo)

Scandent or subscandent shrubs or climbers. The leaves are obovate, elliptic, or ovate-lanceolate in shape. They are subcoriaceous and have a smooth surface. The flowers grow in elongated clusters that emerge from older branches, and they have a light green colour. The fruits are spherical and become pinkish or reddish when they are fully mature. The flowering and fruiting period occurs from May to December.

3.3.2.8 *Elaeocarpus lanceifolius* Roxb

Family: Elaeocarpaceae

Common name: Lanceleaf Marble Tree

Vernacular name: Kharuan (Mizo)

The height of the tree ranges from 12 to 20 meters. The leaves are elliptic to narrowly elliptic, measuring 10-18 x 2.5-5cm. They have an acute to acuminate tip and an attenuate base. The underside of the leaves is either glabrous or thinly pubescent,

and they frequently have tiny blisters when dry. There are little glands in the axils of the lateral veins along the midribs underneath the leaves. The petioles are 1-2cm in length. The racemes are around 5-7 cm long and have around 10 flowers. The pedicels are approximately 0.5 cm in length. The sepals are lanceolate in shape, measuring 5-7 x 1.5-2 mm, and are either becoming hairless or covered with a fine layer of hair. The petals are white, triangular in shape, measuring 6-8 x 3-4 mm. They are sparsely covered with fine hairs on the back towards the base. The petals are split into 12-25 segments, reaching halfway towards the centre. The number of stamens is between 26 and 32, and they are 2.5 to 3.5mm in length. Each stamen has a little bristle at its tip. The ovary is tricarpellary. The fruit is shaped like an oval or ellipsoid and is 3-4 x 2-2.5 cm (5 x 3.5 cm when fresh). The stone of the fruit has three grooves, is heavily wrinkled, and contains a single seed in a single cell.

3.3.2.9 *Garcinia kydia* Roxb

Family: Cluciaceae

Common name: Kuji-thekera

Vernacular name: Tuaihabeh (Mizo)

Tropical evergreen forest, located at elevations below 600 m. The tree is tall, reaching a height of 25-40 feet, and has slender proportions. Its juvenile branchlets are dark in colour. The male flowers are briefly stalked and arranged in umbels, whereas the female flowers are sessile and solitary. The fruit is spherical and flattened, with 6-8 indentations towards the top. When fully ripe, it is yellow and has a shiny appearance.

3.3.2.10 *Garcinia xanthochymus* Hook. f.

Family: Clusiaceae

Common name: Himalayan Garcinia/False mangosteen/Sour mangosteen

Vernacular name: Tuaithleng (Mizo)

This is a moderately-sized evergreen tree that has a compact and slender crown. The leaves are dark green, measuring 20-40 cm in length and 5-8 cm in breadth. They have a thin oblong or oblong lanceolate shape and seem shiny. Flowers are unisexual.

The male flowers are located in the axils of the fallen leaves. The flower has 5 sepals, 5 petals, and 5 stamens. The Stiga is slanted at an angle. The fruit has a diameter of 4-6 cm and is sub-globose in shape. It is capped by persistent stigatic lobes and has a pointed apex. When mature, it becomes golden yellow.

3.3.2.11 *Mahonia nepaulensis* DC.

Family: Beriberidaceae

Common name: Nepal Berry

Vernacular name: Pualeng (Mizo)

The Nepal Barberry is a tall, upright evergreen shrub that may reach a height of 3 meters. It has strong, densely branching branches and huge, feather-like leaves that grow at the top of the stems. The flowers are yellow and are arranged in dense clusters of few or many spikes, each about 10-25 cm in length, located at the terminals of branches. The petals are around 6 mm in size and have a notched appearance. The leaves are oblong-lance shaped. The leaf of the plant is up to 40 cm and consists of 4-7 pairs of rigid, somewhat overlapping oval-shaped leaflets. These leaflets have sharp teeth along the edges and pointy tips. The biggest leaflets are found in the centre of the leaf and are 6-9 cm in length. The fruit is arranged in compact cylindrical clusters. The berries are spherical, with a pronounced bloom, and have a diameter of 8-9 mm. They are coloured purple-blue. The Nepal Barberry plant is native to the Himalayas and may be found in a range of elevations from 2000 to 2900 m. Its distribution extends from western Nepal to Bhutan and northeastern India, as well as the Southern Western Ghats region. Blooming period: October to April.

3.3.2.12 *Morus nigra* L.

Family: Moraceae

Common name: Blackberry, Black mulberry

Vernacular name: Keihfang (Mizo)

Morus nigra is a deciduous tree that reaches a height of 12 meters (39 feet) and a width of 15 meters (49 feet). The length of the leaves ranges from 10 to 20 cm (4 to 8 inches), and their width ranges from 6 to 10 cm (2 to 4 inches). On robust branches,

the leaves may grow up to 23 cm (9 inches) in length. The underside of the leaves is covered in fine hairs, while the top surface is rough due to extremely short, stiff hairs. The fruit is a conglomerate cluster of many little drupes that exhibit a dark purple, almost black hue when fully matured and possess a diameter of 2.5 cm (1 inch).

3.3.2.13 *Myrica esculenta* Buch.-Ham. ex D. Don

Family: Myricaeae

Common name: Bayberry

Vernacular name: Keihfang (Mizo)

The tree is of modest size, dioecious, and evergreen. The bark has a delicate and fragile texture. The leaves have a lanceolate and oval shape, and are almost entirely smooth or have serrated edges. The inflorescence is a kind of flowering structure that may be classified as a catkin, compound raceme, or axillary bearing. The pistillate blooms are tiny, without a stalk, occurring singly, and accompanied by bracts. Each male flower has around 12 stamens, each with a short filament. The fruit is a round, juicy drupe that is about 2.5 cm and has an ellipsoidal or ovoid shape. The fruit becomes a cheese colour when it ripens.

3.3.2.14 *Prunus jenkinsii* Hook.f,

Family: Rosaceae

Common name: Thereju

Vernacular name: Keihpui (Mizo)

The annual plants may reach a height of up to 70 cm. The stems are covered with hair, and they grow close to the ground, seeming thin and delicate. The leaves are compound, with 3-5 leaflets arranged in a digitate pattern. The stipules are around 1.5 cm long. The petioles are about 2-8 cm long. The leaf blade is approximately 0.6-2.4 x 0.3-1.2 cm and has an obovate shape. It tapers at the base and is rounded at the apex. The edges of the leaf blade are obtusely serrated. The underside of the leaf blade is sparsely covered in fine hairs, while the upper surface is smooth and hairless. The flowers are around 1-2 cm in length and are arranged in terminal cymose panicles. The petals of the flowers are yellow in colour. The achenes are around 1 mm in size, tiny,

ellipsoid-globose in shape, smooth, and have a wrinkled appearance. Flowers are of a white colour. The fruit is a small, ovoid or obovoid drupe, measuring less than one inch in size. Leaves undergo a process of becoming yellow before they fall, resulting in the presence of two different hues of leaves on the same tree.

3.3.2.15 ***Prunus undulata*** Buch.-Ham. ex D.Don

Family: Rosaceae

Common name: Wavy-Leaf Himalayan Cherry

Vernacular name: Theiarlung (Mizo)

The Wavy-Leaf Himalayan Cherry is a deciduous shrub or tree that typically reaches a height of 5-16 meters. The branchlets have a greyish brown to purplish brown colouration and lack hair, while also possessing little lenticels that are not easily distinguishable. The leaf-stalks of this plant are 5-10 mm long and do not have any hairs or nectaries. The leaf blade is elliptic to oblong-lanceshaped, measuring 6-15 x 3-5 cm. The texture is herbaceous to thinly leathery, and both surfaces are devoid of hairs. The lower surface typically features a pair of small flat nectaries located near the base, with the possibility of additional small nectaries arranged in multiple rows parallel to the midvein, particularly in the basal region of the leaf. The leaf's upper surface exhibits a shiny appearance. The base of the leaf is broadly wedge-shaped to somewhat rounded, and the margin is either entirely smooth or occasionally has a few teeth near the tip. The tip of the leaf tapers. There are 6-9 secondary veins on each side of the midvein, which spread out and arch. On the lower surface of the leaf, these veins are slightly raised. The flowers are arranged in racemes, either singly or in groups of 2-4 called fascicles. Each raceme is 5-10 cm long and contains 10-30 or more flowers. The flower stalks are 2-5 mm and do not have any hair. The sepal cup has a wide, bell-shaped structure and is devoid of hair on the exterior. The sepals are ovate-triangular in shape, without hair on the exterior, and have a blunt tip. The petals are yellowish white and have an elliptic to obovate shape, measuring 2-4 mm. The number of stamens ranges from 10 to 30, with a length of 3 to 4 millimetres. Style is of lesser length compared to stamens. The cherry is a purple black fruit that has an ovoid-spherical to ellipsoid shape, measuring 1-1.6 x 0.7-1.1 cm. It is smooth and does not

have any hair, with a pointy to blunt tip. The Wavy-Leaf Himalayan Cherry is distributed in Nepal, the Eastern Himalayas, Southeast China, and Southeast Asia. It is often found at elevations ranging from 500 to 3600 meters. Blossoming occurs from August to October.

3.3.2.16 *Rubus treutleri* Hook.f.

Family: Rosaceae

Common name: Wild raspberry

Vernacular name: Sialinuchhu (Mizo)

This plant is a scrambling or climbing shrub with long, thin branches. The branchlets have thick gland-tipped bristles and scattered sharp prickles. The leaves are suborbicular in shape. The dimensions of the leaves are 6-14 x 6-14cm. They are 3-5-lobed, with a cordate base and widely acute lobes. The leaves have serrated edges and are coated with fine hairs on the upper surface and soft hairs on the lower surface. The petioles are 3-6cm long and are densely covered with soft hairs, gland-tipped bristles, and scattered tiny prickles. The stipules are 2cm long and are split into 4-5 linear segments. The flowers are arranged in short axillary racemes, with 3-5 flowers per raceme. The bracts are pectinate. The calyx lobes are oval, measuring 12-15mm in length, and have 3-5 teeth at the tip. They are covered in fine hairs and have gland-tipped bristles and prickles. The petals are 10mm in size and are pink in colour. The fruit has a diameter of 1.5-2 cm and is composed of many drupelets. Flowering time is usually during summer, from June- August.

3.3.2.17 *Spondias pinnata* L.

Family: Anacardiaceae

Common name: Indian hog plum

Vernacular name: Tawitaw (Mizo)

The tree is of medium size, reaching a height of up to 10.5 m. It has a smooth bark and a straight trunk and emits a pleasant, fragrant, acidic odour. The tree is deciduous and lacks leaves throughout the winter months. The leaves have a glossy

appearance and are compound, with leaflets arranged in pairs of three. Flowers are diminutive, possessing either male or female reproductive structures, and have a colouration that ranges from greyish-white to light yellow. They are arranged in clusters on a long stem. The fruit is a drupe that is over one inch in length. It has an ovate, elliptic form and is acidic and fragrant. When mature, it turns greenish-yellow and grows in clusters.

3.3.2.18 *Syzygium cumini (L.) Skeels*

Family: Myrtaceae

Common name: Indian hog plum, Java plum

Vernacular name: Lenhmui (Mizo)

The tree is of moderate sizes, with a maximum height of 10.5 m The tree has a sleek outer layer and a vertical main stem, and releases a delightful, aromatic, acidic scent. The tree is deciduous and remains leafless throughout the winter months. The leaves have a lustrous aspect and are composed of many leaflets, organised in sets of three. Flowers are small in size, and they feature either male or female reproductive systems. Additionally, their colouration may vary from greyish-white to pale yellow. They are grouped together in clusters along an elongated stem. The fruit is a drupe that exceeds one inch in length. The object has a shape that is ovate and elliptic, and it has qualities of being acidic and aromatic. When fully developed, it has a greenish yellow hue and proliferates in clusters.

3.3.2.19 *Tamarindus indica L*

Family: Myrtaceae

Common name: Tamarind

Vernacular name: Tengtere (Mizo)

The trees reach a height of 20 m and have brown to brownish-black bark that is rough with vertical fissures. The branchlets are covered in warty growths and are covered in a layer of fine hair-like structures called tomentum. The leaves are paripinnate and alternating, with lateral stipules that are small and easily shed. The rachis is slender and measures 8-13 cm in length. It is smooth and lacks hair. The

leaflets are opposite each other, sessile (without a stalk), and do not have stipules. The leaflet blade is oblong, measuring 1.5-4 x 0.4-1.3 cm. The base of the leaflet is uneven, while the tip is blunt. The leaflet margin is smooth and the leaflet blade is smooth and papery. There are 10-15 pairs of lateral veins, which are slender and difficult to see. They form a pinnate pattern and loop near the edge, creating an intramarginal vein. The veins in between the lateral veins form a network that is difficult to see. The flowers are bisexual, measuring 1 cm in diameter. They are yellow with reddish-pink dots and are arranged in loose clusters at the end of the stem. The bracts and bracteoles are oval-shaped and coloured, but they fall off easily. The pedicels can grow up to 5 mm long. The calyx tube is narrow and shaped like a top, with a lining of disc. There are 4 lobes, which are similar in size and shape, and they overlap each other. There are 3 petals, with the outer one measuring 1 x 0.3 cm and rolled The fruit is a pod that is 10-15 x 1-2 cm. It has an oblong shape with a crustaceous fruit wall. The mesocarp is pulpy, while the endocarp is septate, leathery, and indehiscent. The fruit contains 3-8 or more seeds, which are obovoid-orbicular, compressed, and brown in colour.

3.3.2.20 *Terminalia chebula* Retz.

Family: Combretaceae

Common name: Chebulic myrobalan/ Hartaki/ Hur

Vernacular name: Reraw (Mizo)

The tree reaches a height of around 50-80 feet and has a circular crown with branches that spread out. The bark has a dark brown colouration and is characterised by the presence of longitudinal fissures. The leaves have an ovate and elliptical shape, and they possess two prominent glands located near the apex of the petiole. The flowers exhibit monoecious characteristics, displaying a dull white to yellow colouration and emitting a strong disagreeable scent. They are arranged in terminal spikes or short panicles. The fruit is a drupe measuring around 1-2 inches in size. The outer skin is adorned with five ribs. When fruit is unripe, it appears green, and when it is mature, it takes on a yellowish grey colour.

3.3.2.21 *Ziziphus mauritiana* Lam.

Family: Rhamnaceae

Common name: Ber

Vernacular name: Borai (Mizo)

Ziziphus mauritiana is a thorny, perennial shrub or tree that may reach a height of up to 15 meters. It has a trunk with a diameter of 40 cm or more, a wide crown, spines on its stipules, and many hanging branches. The leaves exhibit an alternating arrangement and are undivided, with three distinct basal veins and measuring between 2 and 7 centimetres in length. The blooms are small, unobtrusive, and have a yellow-green colour. The fruit is a consumable drupe, with a varied form and size, ranging from yellow-brown to red. The shape of the object might vary between oval, obovate, oblong, or round, with a length ranging from 2.5 to 6.25 cm, depending on the specific type. The flesh has a white colour and a crisp texture. The fruit's skin has a smooth, glossy, thin, and taut texture. It is mostly prevalent in tropical and sub-tropical climates.

3.3.3 Sample Preparation

The twenty-one (21) underutilised fruits were initially cleaned with an aqueous ethanol solution and subsequently rinsed with distilled water. This method was implemented to eliminate any impurities or particles present on the surface of the fruits. After washing, the fruits were dried through absorption using blotting techniques. The fruits were subsequently divided into smaller segments. The specimen underwent desiccation in a controlled atmosphere within an oven, with the temperature regulated between 50-60˚C. It was then ground into a fine powder and filtered using a 20 µm mesh. Subsequently, the specimens were stored in hermetically sealed containers to safeguard against external contamination. The samples underwent rotary evaporation with a Buchi R-300 to yield methanol extracts, subsequently stored in a tightly sealed container. Subsequently, the samples were analysed for evaluation.

3.4 Parameters Recorded

3.4.1 Fruit Nutrient Analysis

3.4.1.1 Proximate composition

3.4.1.1.1 Estimation of moisture content (%)

The moisture content of the fruits was assessed utilising the AOAC 2019 method. The fresh fruit samples were measured for their weight which was followed by the drying process in a hot air oven. Approximately 20 g of finely shredded fresh sample was placed in a clean, dried crucible with a cover and precisely weighed using an electronic balance. The samples were subjected to drying in an oven at 55-60°C until a constant weight was attained over two consecutive measurements. After drying, the crucible was cooled in a desiccator. The moisture content (MC) was determined as follows and presented as a percentage.

$$\text{Moisture Content (\%)} = \frac{W1-W2}{W1} \times 100$$

Where W1–fresh weight

W2–dried weight

3.4.1.1.2 Estimation of dry matter (%)

The dry matter content (DM) was determined using the following calculation and is presented as a percentage.

$$\text{Dry Matter (\%)} = 100 - \text{Moisture \%}$$

3.4.1.1.3 Estimation of total ash content (%)

The total ash content of the fruits was assessed according to the AOAC 2019 method. 2 g of dry fruit samples were placed in a pre-weighed silica crucible. The sample was subsequently decarbonised using a heater or flame until no smoke was produced. The silica crucible containing the decarbonised sample was subsequently placed in a muffle furnace and heated to 600°C for 2-3 hours until all black particles were eliminated. The crucible, together with the sample, was subsequently cooled in a

desiccator, and the final weight was recorded. The total ash content was determined as follows and expressed as a percentage.

$$\text{Total Ash (\%)} = \frac{(c-a)}{(b-a)} \times 100$$

Where,

a= Weight (g) of empty silica crucible

b= Weight (g) of silica crucible with dry sample

c= Weight (g) of silica crucible with ash

3.4.1.1.4 Estimation of crude fibre (%)

The crude fibre content was assessed using the method outlined by Maynard (1970). 2 g of the sample was homogenised in petroleum ether to extract fat. The dried sample (2 g) was placed in a gooch crucible and subjected to boiling in 20 mL of 0.26 N sulphuric acid for 30 minutes, utilising the fibra plus automatic fibre estimation system (Model FES 6). The sample underwent filtration within the system, followed by boiling the filtrate with 20 mL of NaOH solution for 30 minutes. The sample was subsequently filtered again, and the filtrate was washed with 1.25% sulphuric acid, 50 mL of distilled water, and 25 mL of alcohol. The gooch crucible and the final filtrate were subjected to drying for 2 hours at a temperature of 130°C. The filtrate was subsequently cooled in a desiccator, and its weight was recorded. The filtrate was ignited for 30 minutes at a temperature of 600 ± 15°C after weighing. The filtrate was subsequently cooled in a desiccator, and its weight was recorded. The crude fibre content was determined using the following formula:

% Crude fibre in fruit samples

$$= \frac{Loss\ in\ weight\ on\ ignition\ (w2-w1)-(w3-w1)}{weight\ of\ the\ sample} \times 100$$

3.4.1.1.5 Estimation of total carbohydrate (%)

The anthrone method, as outlined by Hedge and Hofreiter (1962), was employed to determine total carbohydrate content. A 100 mg sample was placed in a

boiling tube and hydrolysed in a water bath for three hours using 5 mL of 2.5 N HCl. The sample was allowed to cool to room temperature and was neutralised until effervescence ceased. The volume was adjusted to 100 ml, followed by centrifugation of the content, and collection of the supernatant. 1 mL aliquot was taken, and 4 mL of anthrone reagent was added. The substance was subjected to heating for eight minutes in a boiling water bath and subsequently cooled rapidly. The sample's dark green colour was measured against the blank solution at 630 nm utilising a UV-visible spectrophotometer. The total carbohydrate concentration in the sample was determined using the slope of the total carbohydrate standard curve. The total carbohydrate content (mg) was determined using the following formula:

Amount of carbohydrate in 100 mg of the sample = $\frac{mg\ of\ glucose}{Volume\ of\ test\ sample} \times 100$

3.4.1.1.6 Estimation of total protein (%)

A sample of 500 mg was ground with 5-10 mL of Tris buffer using a pestle and mortar. Subsequently, the mixture was centrifuged, and the supernatant was utilised for protein estimation.

Protein content was assessed utilising Lowry's method (1951). 0.1 mL and 0.2 mL of the extract were measured in a test tube, and the total volume was adjusted to 1 mL with distilled water. A tube containing 1 mL of distilled water was utilised as a blank. 5 mL of reagent C was added to each tube, including the blank, mixed thoroughly, and allowed to stand for 10 minutes. 0.5 mL of reagent D was added to each tube and incubated at room temperature in the dark for 30 minutes. The sample's blue colour intensity was measured against the blank solution at 660 nm with a UV-visible spectrophotometer. The total protein concentration in the sample was determined from the slope of the standard curve, which was constructed using 0.2-1 mL of the working standard.

The amount of protein was expressed as mg/g or 100g sample.

3.4.1.1.7 Estimation of total fat or crude fat (%)

A moisture-free sample weighing 5g was placed in a thimble, which was subsequently attached to a preweighed oil flask. The oil flask was integrated into the SOCS PLUS Six Place Automatic Solvent Extraction System (Model- SOCS PLUS SCS 6). Petroleum ether was transferred from the top of the condenser using a funnel fitted with cotton to minimise evaporative loss. The sample underwent extraction for a duration of 10 to 16 hours at a temperature of 100°C, followed by overnight oven drying and subsequent cooling in a desiccator. The weight of the oil flask was recorded post-cooling, and the fat percentage was calculated, as detailed below.

$$\text{Fat (\%)} = \frac{\boldsymbol{Weight\ of\ fat\ (a-b)}}{\boldsymbol{Weight\ of\ sample}} \times \mathbf{100}$$

Where,

a=Weight of oil flask after extraction

b= Weight of oil flask before extraction

3.4.1.1.8 Estimation of energy (kcal 100 g^{-1})

The energy value was calculated utilising the conversion factors specified by the Food and Agriculture Organisation (F.A.O.) in 2003.

Energy value (kcal/ 100g) = (% available carbohydrate × 4) + (% protein × 4) + (% fat x 9)]

3.4.1.1.9 Estimation of starch (mg100 g^{-1})

The starch content was assessed using the methodology established by Hedge *et al*. (1962). 500 mg of the sample was homogenised in 80% hot ethanol to extract the sugars. The mixture was then centrifuged, and the residue was collected. The residue underwent multiple washes with 30% hot ethanol until the washings no longer produced a colour reaction with the anthrone reagent. The residue was dried using a water bath. The residue was dissolved in 5 mL of water, followed by the addition of 6.5 mL of 52% perchloric acid. The mixture was then incubated at 0°C for 20 minutes.

The sample was centrifuged at 3000 rpm for 5 minutes, and the supernatant was subsequently collected. The extraction process was conducted multiple times with fresh perchloric acid, and the supernatant was adjusted to a final volume of 100 mL by combining the supernatants from each extraction iteration. 0.2 mL of the supernatant was pipetted, and the volume was adjusted to 1 mL with distilled water, followed by the addition of 4 mL of anthrone reagent to the sample. The content was heated in a boiling water bath for 8 minutes and subsequently cooled rapidly. The intensity of the dark green colour was measured against the blank solution at 630 nm using a UV-visible spectrophotometer. The starch content (mg) was determined using the following formula:

Starch (mg/100 g) = (Glucose present in the sample from the standard graph) X 0.9.

3.4.1.1.10 Estimation of total sugars (%)

The total sugars were estimated using an anthrone reagent method (Mckee, 1985). A 25 mg sample of fruit powder was dissolved in 10 mL of 80% ethanol as the solvent. The solution was centrifuged at 4000 rpm for a duration of 10 minutes. The supernatant was subsequently collected and adjusted to a final volume of 10 ml. 0.1 mL of extract was added to a solution comprising 4 mL of anthrone, which was prepared with concentrated sulphuric acid. The mixture was subsequently heated for 10 minutes in a water bath until it reached boiling temperature. Upon cooling to room temperature, the absorbance (A) at 625 nm was measured using a UV-visible spectrophotometer, utilising a reagent blank as the reference standard. The total sugars were quantified using a standard curve of glucose, created with known concentrations of glucose (ranging from 10 to 200 g/ml) in an anthrone reagent solution.

3.4.1.1.11 Estimation of reducing sugar (%)

The reduction of sugar content was assessed using the method outlined by Nelson (1944). Sugar extraction involved macerating 100 mg of the sample with 5 mL of hot 80% ethanol. The sample underwent centrifugation at 3000 rpm for a duration of 5 minutes. Water was introduced to facilitate the dissolution of sugars. An aliquot of 0.2 mL was diluted to a final volume of 2 mL using distilled water. One mL of alkaline copper tartrate reagent was added to each tube. The tube was immersed in

boiling water for a duration of 10 minutes. The tube was permitted to cool, and 1 mL of arsenomolybolic acid reagent was introduced to each tube; the final volume was adjusted to 10 mL with distilled water. The content underwent incubation for 10 minutes at ambient temperature. Following a 10-minute interval, the sample was analysed against the blank solution using a UV-visible spectrophotometer at a wavelength of 620 nm. The concentration of reducing sugar in the sample was determined using the slope of the reducing sugar standard curve. The percentage of reducing sugar was determined using the following formulas:

Absorbance corresponds to 0.1 mL of test = X mg glucose

$$10 \text{ mL contains} = \frac{X}{0.1} \times 10\ mg\ of\ glucose$$

= % of reducing sugar

3.4.1.1.12 Estimation of non-reducing sugar (%)

The disparity between the total sugar percentage and the reducing sugar percentage, when multiplied by a constant factor of 0.95, is indicative of the non-reducing sugar percentage (Dar *et al*., 2021).

Non-reducing sugar (%) = (Total sugar – Reducing sugar) x 0.95

3.4.1.1.13 Estimation of titratable acidity (%)

The titratable acidity of the fruit was evaluated through titration of the fruit juice using a 0.1 N NaOH solution, with phenolphthalein serving as the indicator to identify the endpoint, indicated by a pale pink colour. The titratable acidity was quantified as a percentage in relation to the concentration of citric acid.

$$\text{Titratable acidity (\%)} = \frac{Titre\ value \times Normality\ of\ alkali \times Equivalent\ weight\ of\ acid \times 100}{Volume/Weight\ of\ sample\ taken \times 1000}$$

3.4.1.1.14 Estimation of TSS (°Brix)

The total soluble solids (TSS) content of the fruit was quantified using a digital refractometer with a range of 0-85 °Brix. A small amount of fruit juice was applied to the calibrated prism surface, and the measurement obtained was documented.

3.4.1.1.15 Estimation of lignin (%)

Lignin was quantified utilising the AOAC 2019 methodology. The Acid Detergent Fibre was placed in a 100 mL beaker containing 25-50 mL of 72% sulphuric acid and 1 g of asbestos, and allowed to stand for 3 hours with periodic stirring using a glass rod. The acid underwent dilution with distilled water and was subsequently filtered using Whatman No. 1 filter paper. The residue was subjected to repeated washing to eliminate the acid, followed by drying at 100°C with the filter paper, and the weight was recorded after cooling in a desiccator. The filter paper was placed in a preweighed silica crucible and subsequently heated in a muffle furnace at 550°C for a duration of 3 hours. The crucible was cooled in a desiccator and weighed following the cooling process in the desiccator. The ash content was determined as outlined below.

$$\% \text{ ADL} = \frac{\boldsymbol{Weight}\ 72\%\ \mathbf{H_2SO_4}\ \boldsymbol{washed\ fibre - Ash}}{\boldsymbol{Weight\ of\ sample}} \times \mathbf{100}$$

3.4.1.1.16 Estimation of cellulose (mg 100 g^{-1})

The cellulose content was assessed utilising Updegroff's method (1969). Three mL of acetic/nitric reagent were added to one g of the sample and thoroughly mixed using a vortex mixer. Tubes were incubated in a water bath at 100 °C for 30 minutes. The sample was subjected to cooling and centrifugation at 3000 rpm for a duration of 15 minutes. The residue was collected and subsequently washed with distilled water. 10 mL of 67% sulphuric acid was added to the residue and allowed to stand for one hour. 1 mL of the diluted sample was further diluted to a total volume of 100 mL with distilled water. 10 mL of anthrone reagent was combined with 1 mL of the diluted sample and thoroughly mixed. The tubes were subjected to a boiling water bath for a duration of 10 minutes. The sample was cooled and measured against the blank solution using a UV-visible spectrophotometer at a wavelength of 630 nm. The

cellulose concentration in the sample was determined using the slope of the cellulose standard curve.

3.4.1.1.17 Estimation of hemicellulose (%)

The hemicellulose content was determined utilising the Goering and Vansoest (1970) method. 1 g of powdered sample was placed in a refluxing flask, followed by the addition of 10 mL of cold neutral detergent solution. 2 mL of decahydronaphthalene and 0.5 g of sodium sulfite were added simultaneously, followed by heating to boiling and refluxing for 60 minutes. The contents were filtered using a sintered glass crucible (G-2) via suction and subsequently washed with hot water, followed by two washes with acetone. The residue was placed in a crucible and subjected to drying at 100°C for 8 hours, followed by cooling in a desiccator and subsequent weighing. Hemicellulose content was calculated using the following formula

Hemicellulose= Neutral detergent fibre (NDF)- Acid Detergent fibre (ADF)

3.4.1.2 Estimation of Mineral Content

3.4.1.2.1 Atomic absorption Spectrophotometry (AAS) method.

Atomic absorption refers to a spectroscopic technique used to analyse the concentration of elements in a sample by measuring the absorption of light. This method is widely employed in various fields, including chemistry, environmental science, and metallurgy, for its sensitivity and accuracy in detecting trace elements. The spectrophotometry (AAS) instrument was employed for the estimation of minerals. 1 g of powdered dry plant sample was weighed and placed in a small beaker. 10 mL of a di-acid mixture was added to the solution and let it sit undisturbed for one night. Utilise controlled heating with a hot plate until the production of crimson NO_2 vapours diminishes and becomes colourless. The sample was subsequently transferred to a 50 mL flask and filtered using Whatman No. 1 filter paper. Measures were implemented to prevent contamination, and the solution was adjusted to the desired volume using distilled water. Minerals including Ca, Co, Cu, Fe, Mg, Mn, Na, K, and Zn were quantified from the prepared sample solution using Atomic Absorption Spectroscopy (A.A.S).

3.4.1.2.2 Estimation of Phosphorus (%)

Phosphorus was quantified using the method outlined by Koenig and Johnson (1942), which involved digesting the sample in a tri-acid mixture on a hot plate at a temperature of 180-200˚C until a moist, clear, and white residue remained. 30 mL of the aliquot was transferred to a 50 mL volumetric flask, and 10 mL of the vanadate-molybdate solution was diluted to 50 mL with distilled water. The sample was thoroughly mixed, and the absorbance was measured after 10 minutes at a wavelength of 420 nm. A blank was conducted concurrently without phosphorus. A standard curve was generated by plotting phosphorus concentration on the X-axis against percent transmission readings on the Y-axis.

$$\text{P content of sample (\%)} = \frac{\boldsymbol{A}}{\boldsymbol{100\,V}}$$

Where A stands for P concentration in µg against the sample reading, and V stands for volume of aliquot taken (ml) for colour development out of 100 mL acid digest made from 1 g sample.

3.4.1.2.3 Estimation of total nitrogen (%)

Nitrogen was quantified utilising the Micro-Kjeldahl method as outlined by AOAC 2019. A 0.5 mg sample was transferred to a 30 mL digestion flask. 1.9 g of potassium sulphate, 80 mg of mercuric oxide, and 2 mL of concentrated H2SO4 were added to the flask. Boiling chips were introduced, and the sample was digested until it reached a colourless state. The digested sample was cooled and diluted with a minimal amount of distilled water (free of ammonia) before being transferred to the distillation apparatus. A 100 mL conical flask containing 5 mL of boric acid and a few drops of mixed indicator was positioned beneath the condenser. 10 mL of sodium hydroxide–sodium thiosulphate solution was incorporated into the test solution. The sample underwent distillation, and the ammonia was collected using 20 mL of boric acid. The condenser tip was rinsed, and the sample was titrated with standard hydrochloric acid until a violet colour emerged. The blank was prepared using an equal volume of distilled water, and the titration volume was deducted from the sample volume. The nitrogen content was determined using the following formula:

$$\text{N (g/Kg)} = \frac{(\boldsymbol{ml\ HCL - ml\ blank})\boldsymbol{x\ Normality\ x\ 14.01}}{\boldsymbol{Weight\ (g)}}$$

3.4.2 Bioactive components of the fruits

3.4.2.1 Estimation of Phenols (mg 100 g^{-1})

The phenol content was assessed using the method outlined by Sagar *et al*. (2020). One g of the sample was homogenised in 10 mL of 80% ethanol. The sample was subjected to centrifugation at 10,000 rpm for a duration of 20 minutes. The supernatant was collected and subsequently evaporated to dryness. Five mL of water was added to dissolve the residue. An aliquot of 0.2 mL was taken and diluted to 3 mL with distilled water. Each tube received 0.5 mL of Folin-Ciocalteau reagent, followed by the addition of 2 mL of 20% Na2CO3 after a 3-minute interval. The tube was immersed in boiling water for one minute. The tube was permitted to cool. The sample was analysed in comparison to the blank solution, and the phenol content was determined using the slope of the phenol standard curve.

3.4.2.2 Estimation of Total Flavanoid (mg QE 100 g^{-1})

The total flavonoid content was assessed using the Aluminium chloride method, with quercetin serving as the standard (Sagar *et al*., 2020). 1 mL of the test sample and 4 mL of water were added to a 10 mL volumetric flask. After 5 minutes, add 0.3 mL of 5% sodium nitrite, followed by 0.3 mL of 10% AlCl3. Following a 6-minute incubation at room temperature, 1 mL of 1 M NaOH was introduced to the reaction mixture. The final volume was adjusted to 10 mL using distilled water. The absorbance of the sample was measured against the blank at 510 nm using a spectrophotometer, and the flavonoid content was determined from the slope of the phenol standard curve. The experiment was conducted three times to ensure precision, and the results were expressed as mean ± standard deviation regarding flavonoid content (Quercetin equivalent, QE) per g of dry weight.

3.4.2.3 Estimation of Chlorophyll (mg 100 g^{-1})

Chlorophyll content was determined by the method described by Nayak et.al. (2020). 0.5 g of fresh sample was taken and homogenized in tissue homogenizer with 10 mL of extractant solvent (80 % Acetone). The sample was centrifuged at 10000 rpm for 15 minutes at 4^0C and supernatant was collected. The sample was read in a UV-visible spectrophotometer at two different wavelengths 663 nm and 646 nm. The chlorophyll content was calculated by using the formulae:

$$\text{Chlorophyll a} = \{12.7(A663) - 2.69\ (A645)\} \times \frac{\boldsymbol{volume\ of\ sample}}{\boldsymbol{weight\ of\ sample\ (g) \times 1000}}$$

$$\text{Chlorophyll b} = \{22.9\ (A646) - 4.68)\ A663)\} \times \frac{\boldsymbol{volume\ of\ sample}}{\boldsymbol{weight\ of\ sample\ (g) \times 1000}}$$

$$\text{Total chlorophyll} = 17.76 \times (A646) + 7.34 \times (A663)$$

3.4.2.4 Estimation of Carotenoids (mg 100 g^{-1})

The carotenoid content was assessed using the methodology outlined by Nayak *et al*. (2020). A fresh sample weighing 0.5 g was homogenised using a tissue homogeniser with 10 mL of an extractant solvent consisting of 80% acetone. The sample underwent centrifugation at 10,000 rpm for 15 minutes at 4 °C, after which the supernatant was collected. The sample was analysed using a UV-visible spectrophotometer at a wavelength of 470 nm. The carotenoid content was determined using the following formula:

$$C_{x+c} = (1000A_{470} - 1.82C_a - 85.02C_b)/198$$

Where,

C_a – chlorophyll a

C_b – chlorophyll b

3.4.2.5 Estimation of anthocyanin (mg 100 g^{-1})

The anthocyanin content was assessed using the methodology outlined by Ranganna (1986). A sample of 1 g was mixed with 10 mL of ethanolic HCl, then transferred to a 100 mL volumetric flask and diluted to the mark. Refrigerate at 4°C overnight. Withdraw 0.2 mL from the volumetric flask and dilute to a final volume of 10 mL with ethanolic HCl. Measure and record the optical density (OD) of the filtrate at 535 nm.

Total OD per 100 g =

$$\frac{\boldsymbol{OD \times Volmade\ up\ of\ extract\ used\ for\ colour\ measurement \times total\ volume \times 100}}{\boldsymbol{ml.of\ extract\ used \times weight\ of\ sample}}$$

Total anthocyanin (mg/100 g) = $\frac{\boldsymbol{Total\ OD\ per\ 100\ g}}{\boldsymbol{98.2}}$

3.4.2.6 Estimation of Ascorbic acid (mg 100 g^{-1})

The ascorbic acid content was assessed using the method outlined by Ranganna (1986). A sample of 2 g was placed in a mortar, to which a small quantity of neutral glass powder was added. The mixture was subjected to grinding with an equal volume of 6% metaphosphoric acid. The mixture was subjected to centrifugation at 5000 rpm for 10 minutes, followed by filtration using Whatman No. 1 filter paper, and the resulting solution was collected in a volumetric flask. A 0.1 mL aliquot of the sample extract was diluted to 1.2 mL using 3% metaphosphoric acid, and the final volume was adjusted to 4 mL with distilled water. 0.4 mL of Folin-Ciocalteau was added to each tube and mixed thoroughly. The tubes underwent incubation for 10 minutes at room temperature, followed by centrifugation at 3000 rpm for 10 minutes. Following centrifugation, the supernatant was analysed against the blank solution using a UV-visible spectrophotometer at a wavelength of 760 nm. The concentration of ascorbic acid in the sample was determined using the slope of the ascorbic acid standard curve. The ascorbic acid concentration (mg/100 g) was determined using the following formula:

Vitamin C ($\frac{mg}{100g}$) = $\frac{\boldsymbol{Total\ volume\ of\ the\ sample \times Conc.of\ Vit.C \times 100 \times 1}}{\boldsymbol{Weight\ of\ sample \times Amount\ of\ sample \times 1000}}$

3.4.2.7 Estimation of vitamin E (Tocopherol) (mg 100 g^{-1})

The vitamin E content was assessed using the methodology outlined by Rosenberg (1992). A sample weighing 0.5 g was homogenised. Gradually introduce 10 mL of 0.1 N sulphuric acid and permit it to stand overnight. The material was subjected to filtration using Whatman No. 1 filter paper. Transfer 1.5 mL of tissue extract, standard, and water into three separate test tubes. 1.5 mL of ethanol was added to both the test and blank samples, while 1.5 mL of water was added to the standard, followed by centrifugation. Each tube received 1.5 mL of xylene, followed by mixing and centrifugation. 1 mL of the xylene layer was transferred to a separate tube, followed by the addition of one mL of 2,2'-dipyridyl reagent. Transfer 1.5 mL of the mixture into a cuvette and measure the test and standard absorbance at 460 nm, using the blank as a reference. 0.33 mL of ferric chloride was added to each blank test tube, and the supernatant was measured against the blank solution using a UV-visible spectrophotometer at 520 nm precisely 15 minutes later.

Amount of tocopherol in µg/g of tissue =

$$\frac{\textit{Reading of test at } 520\ nm - \textit{Reading of test at } 460\ nm \times 0.29 \times 15 \times \textit{Total volume of homogenate}}{\textit{Reading of standard at } 520nm \times \textit{Volume used} \times \textit{Weight of tissue}}$$

3.4.2.8 Estimation of Alkaloids (mg 100 g^{-1}AE)

The technique reported by Tambe and Bhambar (2014) was utilised to ascertain the total alkaloid content. The dried powder was macerated with a solution of 80% ethanol. The solvent was evaporated at 72°C to obtain a concentrated solution. After dissolving 1 mg of plant extract in dimethyl sulphoxide (DMSO), 1 mL of 2 N HCl was added. The mixture was then filtered and transferred to a separating funnel, where 5 mL of bromocresol green solution and an equal volume of phosphate buffer were introduced. The mixture was subjected to vigorous agitation with chloroform prior to being transferred into a 10 mL volumetric flask, where it was subsequently diluted to its final volume with chloroform. A series of reference standard solutions with different concentrations of atropine (20, 40, 60, 80, and 100 µg ml−1) were prepared. The transparent layer was collected, and absorbance was measured at a

wavelength of 470 nm using a UV-visible spectrophotometer, with the reagent blank serving as the reference.

3.4.2.9 Estimation of Saponins (mg 100 g^{-1} DE)

The total saponins were determined using the method described by Khatoon *et al*. (2022), which employs the vanillin-sulphuric acid colourimetric reaction. Several adjustments were made to the original procedure. 50µl of plant extract was mixed with 250µl of distilled water. A volume of approximately 250µl of vanillin reagent was subsequently introduced. A volume of 2.5 mL of sulphuric acid at a concentration of 72% was added to the mixture, followed by extensive agitation. The solution was incubated in a water bath at 60°C for 10 minutes. During the subsequent 10 minutes, the substance underwent a cooling process in water at a low temperature. Absorbance was measured at a wavelength of 544 nm. The data were quantified in diosgenin equivalents (mg DE/g extract) based on the standard curve utilising diosgenin as the standard.

3.4.3 Antinutrients Compounds

3.4.3.1 Estimation of Phytic Acid (mg 100 g^{-1})

The phytic acid content was assessed using the method outlined by Wheeler and Ferrel (1971). 1 g of finely ground sample was incubated with 50 mL of 3% trichloroacetic acid (TCA) for 30 minutes under continuous stirring. The sample underwent centrifugation, after which 10 mL of aliquot was transferred to a clean test tube, followed by the addition of 4 mL of $FeCl_3$ solution. The material was maintained in a hot water bath for a duration of 45 minutes. The aliquot underwent centrifugation for 5 minutes, after which the clear supernatant was meticulously decanted. The precipitate underwent washing with 20 mL of 3% TCA, was maintained in a boiling water bath for 10 minutes, and subsequently centrifuged for 15 minutes at 3000 rpm. Distilled water was used for repeated washing. The precipitate was dispersed in a few mL of water, followed by the addition of 3 mL of 1.5 N NaOH. The final volume was adjusted to 30 mL with distilled water, and the sample was heated for 30 minutes in a hot water bath. The sample underwent filtration using Whatman No.2 filter paper,

followed by washing the precipitate with 70 mL of hot water, after which the filtrate was discarded. The precipitate was dissolved in 40 mL of hot 3.2 N HNO3 within a conical flask. 5 mL of aliquot were transferred to a separate conical flask, and the volume was adjusted to 70 mL with distilled water. 20 mL of 1.5 M KSCN was added, and after one minute, the sample was measured against the blank solution at 480 nm using a UV-visible spectrophotometer. The phytic acid concentration in the sample was determined using the slope of the phytic acid standard curve. The concentration of phytic acid (mg/100 g sample) was determined using the following formula:

Phytate P mg/100 g sample = $\frac{\mu g\ Fe \times 15}{Weight\ of\ sample\ (g)}$

3.4.3.2 Estimation of Total oxalate (mg 100 g^{-1})

The oxalate estimation was performed utilising the titrimetric method outlined by Adeniyi *et al*. (2009). The titration process is outlined as follows: A 2 g dry sample was measured and digested with 10 mL of 6M HCl for one hour, followed by a cooling period. The solution was prepared by transferring it to a 250 mL volumetric flask and subsequently filtered. 125 mL of the filtrate was transferred into the beakers, followed by the addition of 3 to 4 drops of methyl red. A concentrated NH4OH solution was gradually added to the test solution until the colour changed from salmon pink to pale yellow. The pH of the resultant solution was subsequently measured. The temperature of each component was elevated to 90°C, followed by cooling and filtration to remove the precipitate. The filtrate was subsequently heated to a temperature of 90°C. Subsequently, 10 mL of a 5% $CaCl_2$ solution was added while maintaining constant stirring. The solution underwent decantation, and the residue was completely dissolved in a 10 mL solution of 20% (v/v) H_2SO_4. The filtrate was adjusted to a volume of 300 mL, and a 125 mL aliquot was heated to near boiling temperature. The heated filtrate underwent titration with a standardised solution of 0.05M potassium tetraoxomanganate (VII). The titration produced a stable pink colour at the endpoint, which persisted for 30 seconds.

3.4.3.3 Estimation of Tannins (mg 100 g^{-1})

The tannin content was assessed utilising the Folin-Denis procedure (Schanderl 1970). A sample of 0.5 g was placed in a conical flask, and 75 mL of water was subsequently added. The sample flask was boiled for 30 minutes and subsequently centrifuged at 2000 rpm for 20 minutes. The supernatant was transferred to a volumetric flask and diluted with distilled water to a final volume of 100 ml. 1 mL of sample extract was added to a volumetric flask containing 75 mL of water. Following the addition of 5 mL of Folin-Denis reagent and 10 mL of sodium carbonate solution, the mixture was diluted to a final volume of 100 mL with water. After a 30-minute incubation period, the contents were analysed using a UV-visible spectrophotometer at a wavelength of 700 nm, utilising the blank solution as the reference.

3.4.4 Estimation of anticancer and antioxidant properties

3.4.4.1 Estimation of anticancer properties

3.4.4.1.1 Cell lines and culture

Type II human lung adenocarcinoma cell line (A549 cells) was procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were maintained in MEM supplemented with 10% FBS and 1% L-Glutamine in a humidified incubator with 5% CO_2 at 37^o C (Eppendorf, Hamburg, Germany).

3.4.4.1.2 Cytotoxicity assay (MTT assay)

The cytotoxicity of various fruit extracts was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) reduction test described by Mossman (1983). 100 µl of MEM with 1×10^4 cells were placed into 96-well plates. The cells were exposed to various doses of different fruit extracts (ranging from 5 to 500 µg/ml) for 24 hours, after a 24-hour adhesion period at 37°C and 5% CO2, alongside a control sample. Medium-containing extracts were removed, and cells were washed with medium without FBS after the treatments. Next, cells were exposed to 10 µl of MTT (5 mg/ml) and incubated for 2 hours at 37 °C in a CO2 incubator. Subsequently, 100 µl of DMSO was used to dissolve the insoluble purple formazan crystals. Following a 30-minute incubation period, the solution's absorbance was

assessed at 560 nm using a microplate reader (Spectramax m2e, Molecular Devices). Three separate trials with three replicates were performed for each treatment. Cytotoxicity was quantified as the percentage of inhibition using the following formula:

% inhibition = Control-Treatment/Control X 100.

3.4.4.1.3 Cell morphology analysis by fluorescent staining (Apoptotic assay)

The apoptosis-inducing potential of fruit extracts was evaluated using Acridine orange/Ethidium bromide (AO/EtBr) staining. 1.5×105 A549 cells were sown in six-well plates with 5 mL of medium. The cells adhered overnight and were treated with various doses of fruit extracts (10-50 µg/ml) and 5FU as a positive control (100 µg/ml) for 24 hours. Unprocessed control samples were likewise kept in the culture medium without further treatment. After treatments, cells were rinsed with sterile 1X PBS and removed with 1X trypsin EDTA. The cells in pellet form were mixed with 100 µl of medium without foetal bovine serum (FBS). Acridine orange and ethidium bromide, at a 100 g/mL concentration, were combined in a 1:1 ratio and put into a 25 µl cell solution. The mixture was then stained for 2 minutes. The structure of cells undergoing apoptosis was examined on a slide using a fluorescent microscope from Thermo Fisher Scientific, model EVOSR fluorescent Imaging, AMEP-4615. Analysed the apoptotic index of a minimum of 300 cells in the following manner:

Apoptotic index (%) = Number of apoptotic cells scored × 100/Total number of cells counted.

3.4.4.1.4 Antioxidant assays

2×10^6 cells were placed in a T-25 flask with 5 mL of media to evaluate antioxidant enzyme activity and lipid peroxidation levels. After 24 hours of treatment with various fruit extracts at a concentration of 50 µg/ml, the medium containing the medication was eliminated, The cells were collected after being rinsed with sterile 1XPBS. The cells were concentrated and disrupted using sound waves by a sonicator (PCI Analytics Pvt. Ltd., Mumbai, India), and a 5% mixture was made in cold, sterile PBS (pH-7.4) for biochemical tests. The total protein content was measured using the standard methodology outlined by Lowry *et al*. (1951), with bovine serum albumin as the reference standard. Glutathione levels were quantified by reacting it with DTNB

in Ellman's reaction, resulting in a molecule that absorbs light at 412 nm (Moron *et al*., 1979). An 80 µL cell homogenate was combined with 900 µL of 0.02M sodium phosphate buffer and 20 µL of 10 mM DTNB, then incubated for 2 minutes at room temperature. The sample's absorbance was measured at 412 nm using a UV-visible spectrophotometer (SW 3.5.1.0. Biospectrometer, Eppendorf, Chennai) by comparing it to a blank. The GSH concentration was determined from the standard graph and reported in µmol/mg protein.

3.4.4.1.4.1 *Glutathione (GSH)*

Glutathione (GSH) levels were quantified through its reaction with DTNB in Ellman's reaction, resulting in a compound that exhibits absorbance at 412 nm (Moron *et al*., 1979). In summary, 80 µL of the cell homogenate was combined with 900 µL of 0.02M sodium phosphate buffer and 20 µL of 10 mM DTNB, followed by a 2-minute incubation at room temperature. The blank comprised distilled water rather than cell homogenate. The sample's absorbance was measured against a blank at 412 nm using a UV-visible spectrophotometer (SW 3.5.1.0. Biospectrometer, Eppendorf India Ltd., Chennai). The GSH concentration was determined using the standard graph and expressed in µmol/mg protein.

3.4.4.1.4.2 *Glutathione-s-transferase (GST)*

Glutathione-s-transferase (GST) levels were evaluated according to the protocol established by Beutler (1984). Fifty microlitres of 20 mM CDNB were mixed with 850 microlitres of 0.1 M phosphate buffer at pH 6.5 and incubated for 10 minutes at 37 °C. Subsequently, 50 µL of 20 mM GSH and 50 µL of cell homogenate were incorporated into the mixture. Distilled water was used as a substitute for cell homogenate in the blank. Absorbance of the sample was recorded at one-minute intervals over a duration of 5 minutes, specifically at a wavelength of 340 nm. To determine GST activity, apply the formula: GST activity = (OD of test – OD of blank / 9.6 × volume of test sample) × 1000, with 9.6 denoting the molar extinction coefficient for GST.

3.4.4.1.4.3 *Superoxide dismutase (SOD)*

The activity of superoxide dismutase (SOD) was evaluated through the nitroblue tetrazolium (NBT) reduction method as outlined by Fried in 1975. One hundred microlitres of cell homogenate and 186 micromolar PMS were mixed with 300 microlitres of 3 mM NBT and 200 microlitres of 780 micromolar NADH. The mixture underwent incubation for 90 seconds, followed by the addition of 1 mL of acetic acid and 4 mL of n-butanol to terminate the process. The blank contained all reagents excluding the cell homogenate. Absorbance measurements of the test and blank samples were conducted at 560 nm to assess enzyme activity, quantified in units defined as 50% inhibition of NBT reduction per mg of protein.

3.4.4.1.4.4 Lipid peroxidation (LPO)

Lipid peroxidation (LPO) was assessed using the Beuege and Aust (1978) technique. Malondialdehyde (MDA) is a harmful byproduct produced when fatty acids, including polyunsaturated fatty acids (PUFA) and phospholipids, undergo oxidation. It is used as a useful indicator to measure the level of lipid peroxidation. Malondialdehyde (MDA) produced by lipid peroxidation combines with thiobarbituric acid (TBA) to form a red fluorescent compound that absorbs light at 535 nm. The cell homogenate was added to a mixture containing 10% TCA, 0.8% TBA, and 0.02 N HCl in a 1:2 ratio. The mixture was heated for 10 minutes, then promptly cooled to room temperature and centrifuged at 1000 revolutions per minute for 10 minutes. The supernatant was collected, and its absorbance was measured at 535 nm compared to a blank sample. The container held all the reagents except for the cell homogenate, which was replaced with distilled water. The MDA concentration of the sample was determined using the extinction coefficient of $1.56 \times 10^6\ M^{-1} \cdot cm^{-1}$.

3.4.4.1.5 qRT-PCR analysis of pro-apoptotic and anti-apoptotic gene expression

Briefly, 5×10^6 A549 cells were seeded in a T-25 flask with 5 mL media. After an overnight adherence, cells were treated with 50 µg/mL of fruit extracts or 5FU for 24 h along with a control sample. Cells were washed and harvested following treatments. Total RNA was extracted from the pelleted cells using Tri reagent (BR

Biochem, Life Science Pvt. Ltd, R1022). Extracted RNA was quantified using a Nanodrop Spectrophotometer (Eppendorf Biophotometer Plus, Hamburg, Germany) and an RQ1 DNase kit (Promega, M198A, Madison, WI, USA) was used to remove the genomic contamination. 3 µg of total RNA was used to synthesize cDNA with a first-strand cDNA synthesis kit (Thermo Scientific, K1621; Lithuania, Europe). Gene-specific primers were designed using Primer 3, Boston, MA, USA. The primer sequences used in qRT-PCR analysis are given in Table 3.2.

qPCR was performed using Quant-Studio 5 (ThermoFisher Scientific, Foster City, CA, USA). 1 µl of cDNA, 1 µl of gene-specific forward and reverse primers, 1 µl of nuclease-free water (ThermoFisher Scientific, A19938, Bangalore, India), and 3 µl of PowerUpTM SYBR^{TM} Green Master Mix (Thermo Fisher Scientific, A25742, Lithuania, Europe) constitute a 7 µl PCR reaction volume for each gene. The cycling condition of qPCR was 1 cycle at 95 °C (20 s), 35 cycles at 95 °C (01 s), 60 °C (20 s), and 95 °C (01 s), additional melt curve plot step included 1 cycle of 60 °C (20 s) and 1 cycle of 95 °C (01 s). Melting curves were subsequently generated to confirm a single uniform peak. The GAPDH gene was selected as a reference gene to determine the relative expression levels of specific target genes. Each sample was run in duplicate along with non-template and negative RT controls. The relative expression of genes was determined using the ΔΔCt method (Livak and Schmittgen, 2001).

Table 3.2 qRT-PCR primer sequence.

Genes	**Forward primer (5'→3')**	**Reverse primer (5'→3')**
Bax	TCCCCCCGAGAGGTCTTTT	CGGCCCCAGTTGAAGTTG
Bid	CCTTGCTCCGTGATGTCTTTC	GTAGGTGCGTAGGTTCTGGT
Bcl-X_L	GGCCACTTACCTGAATGACC	AAGAGTGAGCCCAGCAGAAC
Bcl-2	GGATGCCTTTGTGGAACTGT	AGCCTGCAGCTTTGTTTCAT

3.4.4.1.6 Caspase-3 and caspase-6 activities assay

A quantitative enzymatic activity test for caspase 3 and caspase 6 was conducted using the manufacturer's methods from BioVision Incorporated, USA. 2.5 × 10^6 A549 cells were exposed to 50 µg/mL of fruit extract or 5FU for 24 hours in a T-25 flask containing 5 mL of media, alongside an untreated control. After treatment, cells were rinsed and broken down in 50 µl of cold lysis solution, then left to sit on ice for 10 minutes. The cell lysates were centrifuged at 15,000 × g for 1 minute at 4 °C, and the supernatant was collected. The total protein content was determined using the Bradford test developed by Bradford in 1976. The test was conducted in a 100 µl total volume on 96-well plates. Each sample was tested for caspase-3/6 activity using 150 µg of protein and their particular colourimetric substrates: DEVD-pNA for caspase-3 and VEID-pNA for caspase-6. The solution was allowed to react for a further 2 hours at 37°C, and the amount of p-nitroanilide (pNA) released by activated caspase-3 and caspase-6 cleaving their substrates was quantified at 405 nm using a microplate reader.

3.4.4.2 Evaluation of antioxidant activity (AOA)

3.4.4.2.1 Estimation of antioxidant properties using DPPH free radical-scavenging assay.

The scavenging activity of methanolic extracts from fruits for DPPH radicals was assessed using the method outlined by Xiao *et al*. (2020), with minor modifications applied. In summary, 0.5 mL of different fruit extracts (20–1600 µg/ml) was combined with 1 mL of a 0.1 M DPPH methanol solution and incubated in the dark for 30 minutes. The solution's absorbance at 523 nm was compared to that of the control. The scavenging activity of the plant extract against DPPH was quantified as IC50, defined as the concentration (µg/mL) of extract required to inhibit 50% of DPPH radicals. Ascorbic acid (ASA) served as the standard. The test was conducted in triplicate for all concentrations of each sample. The scavenging activity was calculated by determining the percentage of DPPH radicals that were scavenged, utilising the following formula:

$$\% \text{ DPPH radical scavenging g activity} = \frac{Abs.\ of\ blank - Abs.\ of\ sample}{Abs.\ of\ blank} \times 100$$

Where Abs. of the blank is the absorbance of the control (solution containing all the reagents except the plant extracts) and Abs. of the sample is the absorbance of the solution containing the plant extract.

3.4.4.2.2 Estimation of antioxidant properties using ferric-reducing antioxidant power (FRAP).

The Ferric Reducing Antioxidant Potential (FRAP) method involves the reduction of a ferric Tripyridal Triazine (TPTZ) complex to its ferrous, coloured form in the presence of antioxidants (Xiao *et al*., 2020). The FRAP assay quantitatively assesses antioxidants that possess a reduction potential lower than that of the Fe3+/Fe2+ couple. The FRAP assay was conducted according to the established protocol. A 100 µl sample was combined with 3 mL of working FRAP reagent, and absorbance was measured at 593 nm immediately after vortexing. Subsequently, samples are incubated at 37°C in a water bath, and absorption is measured again after 4 minutes. The assay was standardised by creating a standard curve with Trolox solutions ranging from 0.2 to 1.2 µg/mL in water. The resulting values were reported as µmol/g Trolox equivalent.

3.4.4.2.3 Estimation of antioxidant properties using Superoxide anion scavenging activity.

The superoxide scavenging activity was assessed using the nitroblue tetrazolium (NBT) reduction method, incorporating minor modifications (Lalhminghlui and Jagetia, 2018). The reaction mixture comprised 0.2 mL of NBT (1 mg/mL in DMSO) and 0.6 mL of plant extract (20-1600 µg/mL). The volume of the mixture was adjusted to 2.8 mL by adding 2 mL of alkaline DMSO (1 mL DMSO in 5 mM NaOH). The absorbance of the mixture was measured at 560 nm, with pure DMSO serving as the blank. Ascorbic acid (ASA) was utilised as the standard, and the capacity of fruit extracts to scavenge the superoxide radical was assessed.

$$\% \text{ scavenging} = (Ae - Ao/ Ae) \times 100$$

where Ao is absorbance without plant extract, and Ae is absorbance with the plant extract.

3.4.4.2.4 Estimation of antioxidant properties using ABTS scavenging assay

The scavenging activity of fruit extracts against ABTS was assessed using the method outlined by Xiao *et al*. (2020). Five mL of 7 mM ABTS were combined with 5 mL of 2.45 mM potassium persulfate to prepare a stock solution. A stock solution was incubated at room temperature in the dark for 12 hours to produce a dark-coloured solution containing ABTS˙+ radicals. A freshly prepared working solution is derived from a stock solution diluted with 50% methanol, exhibiting an initial absorbance of 0.70 (±0.02) at 745 nm. The scavenging activity of ABTS˙+ radicals was evaluated by combining 150 µL of various fruit extract fractions (20-1600 µg/mL) with 1.5 mL of the ABTS working solution. The absorbance reduction was assessed promptly at 745 nm. The test was conducted in triplicate for all concentrations of each sample. Ascorbic acid (ASA) functioned as the standard. The scavenging activity of the plant extract was subsequently determined using the formula:

$$\text{Scavenging (\%)} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

Where, A_{blank} is the absorbance of the control (solution containing all the reagents except the plant extracts) and A_{sample} is the absorbance of the solution containing the plant extract.

3.5 GCMS analysis

The GC-MS instrument was employed to analyze the chemical constituents present in the fruits. It consisted of a Clarus 690 Perkin Elmer Gas Chromatograph, which was coupled with a mass detector Turbomass gold 5.1 spectrometer, and an Elite 1 (100% Dimethyl poly siloxane) capillary column measuring 123.5 M x 678 M. The instrument was initially set to a temperature of 40°C and then ramped up to 150°C at 10°C/min and held for 5 minutes. After that, the oven temperature was increased to 250°C at a rate of 40°C/min and maintained for eight minutes. The injection port temperature was set at 250°C, while the helium flow rate was maintained at 1.5 ml/min. The ionization voltage used was 70 eV, and the samples were injected in a 10:1 split mode. The mass spectral scanning range was set to 500-800 (m/z), and the

ion source temperature and interface temperature were kept at 230°C and 240°C, respectively. The MS start time was three minutes, and the end time was 31 minutes, with a solvent cut time of three minutes. The spectra of volatile compounds detected through GC-MS were compared and matched with NIST17 online library Ver. 2.339.

3.6 LC-HRMS analysis

Samples were measured in triplicate in a randomized order. After fifteen sample injections (three triplicates), a blank was injected, followed by a pooled sample for quality control. The concentrated samples (140 µL, i.e. 10.5 mL of the original sample) were injected into an LC system consisting of a PAL RTC autosampler (CTC Analytics, Switzerland), a reversed-phase C18 column (Atlantis T3, 3 µm, 3 ×150 mm; Waters, Ireland), and a Dionex UltiMate 30 0 0 RS pump (Thermo Fisher Scientific RS). The gradient elution started with 100% water (containing 0.1% formic acid) to achieve an optimal retention of polar compounds. Then, methanol (containing 0.1% formic acid) was added and increased to 95% from 1.5 to 18.5 min and finally kept constant for 10 min. The flow rate was 0.3 mL min $^{-1}$. Analytes were ionized in electrospray (3.5/ −2.5 kV) and detected on an Orbitrap mass spectrometer (Fusion Lumos, Thermo Fisher Scientific, U.S.) with a resolution R of 240,0 0 0 (at *m/z* 200, full width at half maximum (FWHM)) in MS1 full-scan mode (*m/z* 100–1000), followed by three to four data-dependant MS/MS full- scans (high-resolution product scans; R 30,0 0 0 FWHM at *m/z* 200; cycle time 1s; isolation window of precursor 1 *m/z*). Internal calibration (EASY-IC TM) ensured a mass accuracy of < ±2 ppm in MS1 scans for 99.8% of detected target compound peaks and internal standard peaks (< ±1 ppm for 98.4% of peaks).

3.6.1 Method of Peak Processing and Identification

The obtained raw LC-MS data files were processed using the MZmine 2.5 software (Pluskal *et al*., 2010). First, raw data (.d) files were converted to .mzmL using Proteowizard's MSConverter. Continuous data acquired in positive mode was processed. Data processing (feature extraction) with MZmine comprised the following

steps: import data, MS peak detection, chromatogram building, chromatogram deconvolution, isotope grouping, peak alignment, MS row filtering, and gap filling. The module “online database search” was used from MZmine features, and the search was conducted against KEGG (Kyoto Encyclopedia of Genes and Genomes) and Pubchem database. Accurate masses of the fragments obtained in the same ionization mode were matched with the database by setting a tolerance of 5 ppm. The identified metabolites were then exported as an Excel spreadsheet containing the following information for each compound, arranged in columns: ID, m/z, chemical name, and retention time, Peak area, peak height. The database was saved in .csv format. After database matching (Table 2), an adduct search and a complex search were performed. Finally, the peak list was directly exported from MZmine in .csv (comma-separated values) format

3.7 In silico works

3.7.1 Protein structure preparation and Molecular docking

High resolution X-ray crystallographic structure of AKT1 protein from the Protein Data Bank (PDB ID 20FO), was downloaded (https://www.rcsb.org/). The molecular compounds of selected underutilised fruits (*Elaeocarpus lanceifolius, Embelia subcoriacea, Terminalia chebula, Garcinia kydia and Emblica officinalis)* were docked on potential target receptor proteins using AutoDock Vina software. The SDF files of the compounds were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and then converted into PDBQT file format using Openbabel software. Protein-ligand Docking was performed using Autodock Vina 1.1.2 version on the Terminal of the Linux platform. Docking has been performed on the particular site of the target protein, the coordinates of the active site are x = 3.009, y = 4.859, z = 3.661, and the coordinates grid dimensions of x, y, and z are 40 x 40 40 was calculated based on the presence of their known inhibitor at active site region. Vina calculates the docking score and shows the promising docking conformations of ligands bonded to the receptor protein and the docking scores are arranged in a hierarchy according to their binding affinities (in kcal/mol). To confirm the connections between ligand and protein, a graphical analysis was performed on the

resulting structures and binding docking postures using the LigPlus (Laskowski R A et.al. 2011) and Pymol tools.

3.7.2 In silico ADME and drug-likeness screening

To undertake in silico ADME screening and drug-likeness assessment, the online tool SwissADME (https://www.swissadme.ch) was used to calculate the physicochemical, absorption, distribution, metabolism, and excretion (ADME), and drug-likeness parameters (Daina et al. 2017). This particular screening phase was limited to compounds with high binding energy values. Furthermore, the drug-likeness candidature was carried out following Lipinski's 2001 rule of five, which computed physicochemical parameters like molecular weight (MW), molecular refractivity (MR), atom counts, and polar surface area (PSA) etc. (Kumari et al. 2023).

3.7.3 Molecular dynamics simulation

To investigate the compound's stability the top three molecules with the highest binding affinities and the best docking score with AKT1 were then carried on to the MD analysis using the GROMACS 23.2 program. The SWISSPARAM online web service was used to build the ligand topology file (Zoete V et al., 2011), and the GROMACS tutorial was followed to create the protein topology file and other necessary files. (http://www.mdtutorials.com/gmx/complex/02_topology.html). The protein topology was generated under the CHARMM-36 all-atom force field following the protein-ligand tutorial (Orr AA et al. 2022). Additionally, the systems were developed using TIP3 water model and triclinic periodic boundary conditions (PBC). where four Na+ were added to a water solution to neutralize the systems. The bond lengths were constrained using the linear constraint solver (LINCS) methodology, while the long-range electrostatic interactions in the TIP3 water model were computed using the particle mesh Ewald (PME) method. NVT and NPT ensemble was run for 100 ps, where a fixed number of particles, volume, and temperature were used for the 300K in NVT equilibration (Berendsen et al. 1995). Similarly, 300K and 1 bar of

pressure were used for NPT equilibration (Berendsen, et al., 1984). Finally, the final production run of all protein-ligand complexes was performed for the duration of 100 ns and the coordinates of each simulation trajectory for each 2 fs were stored at every 20 ps. The results of generated trajectories were analyzed in terms of root mean square deviation (RMSD), root mean square fluctuation (RMSF), the radius of gyration (Rg), solvent-accessible surface area (SASA), and hydrogen bond (H-bond) using Xmgrace Linux based tool (Kumar et al 2022).

3.8 Correlation studies

Pearson's correlation coefficient (r) of quantitative characters of nutrients, bioactive, and anti-nutritional components of the twenty-one (21) underutilised fruits was calculated using IBM SPSS 25 software and the significance of the correlation was determined from the correlation table at 5% level of significance with N-2 degrees of freedom.

3.9 Principal component analysis

Principal component analysis was obtained from the twenty-one (21) underutilized fruits among their nutrient, bioactive and antinutritional components where the different components of the studied underutilised fruits were grouped into components and summarised the large datasets into a smaller set of uncorrelated variables known as principal components which capture as much information from the original dataset as possible. This analysis is carried out with IBM SPSS 25 software.

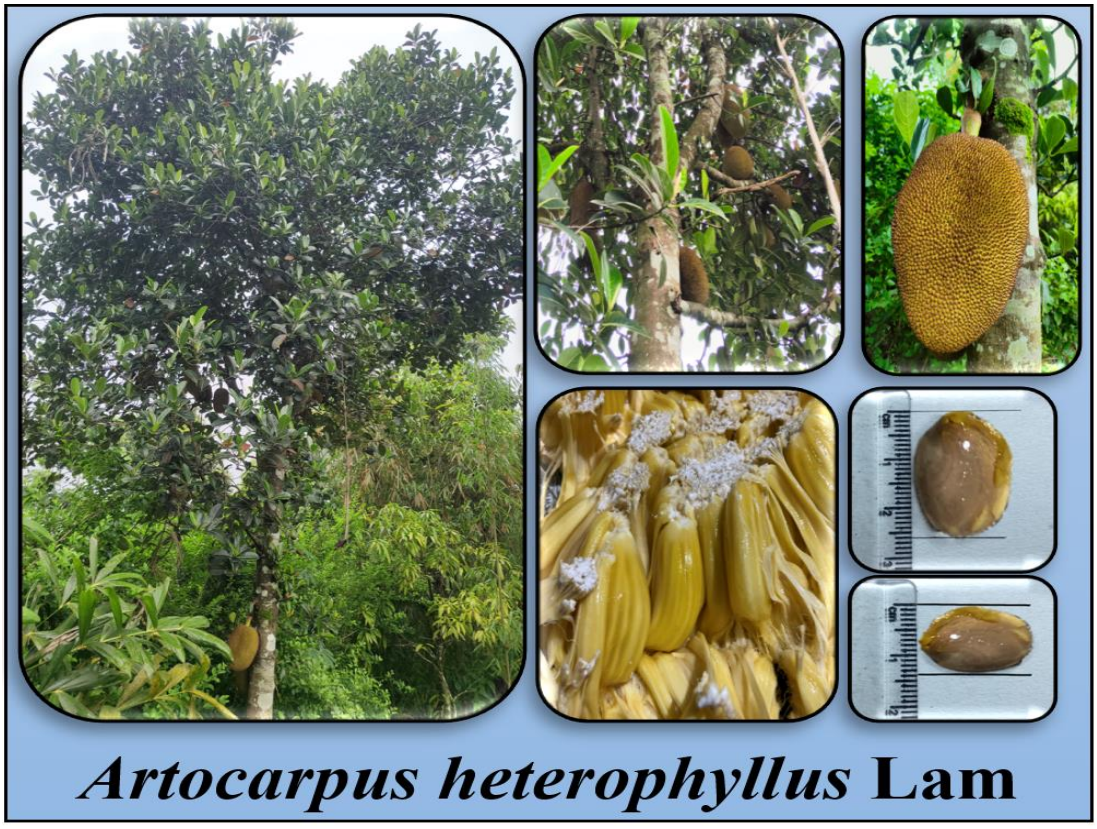
3.10 Cluster analysis

The nutritional, bioactive and antinutritional components characters of the twenty-one (21) underutilised fruits were scored and expressed in frequency percentage. The scores were subjected to cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with IBM SPSS 25 software.

3.11 Statistical analysis

All data were expressed as mean ± standard error of the mean. One-way ANOVA followed by Tukey’s test and Duncan's test was performed to test significant variations between control and treatment groups. SPSS ver. 20.0 software (SPSS Inc, Chicago, Illinois, USA) and Graph Pad Prism ver. 8.0 was used for statistical and graphical analyses. A p-value of less than 0.05 was considered statistically significant.

Photo Plate 3.1



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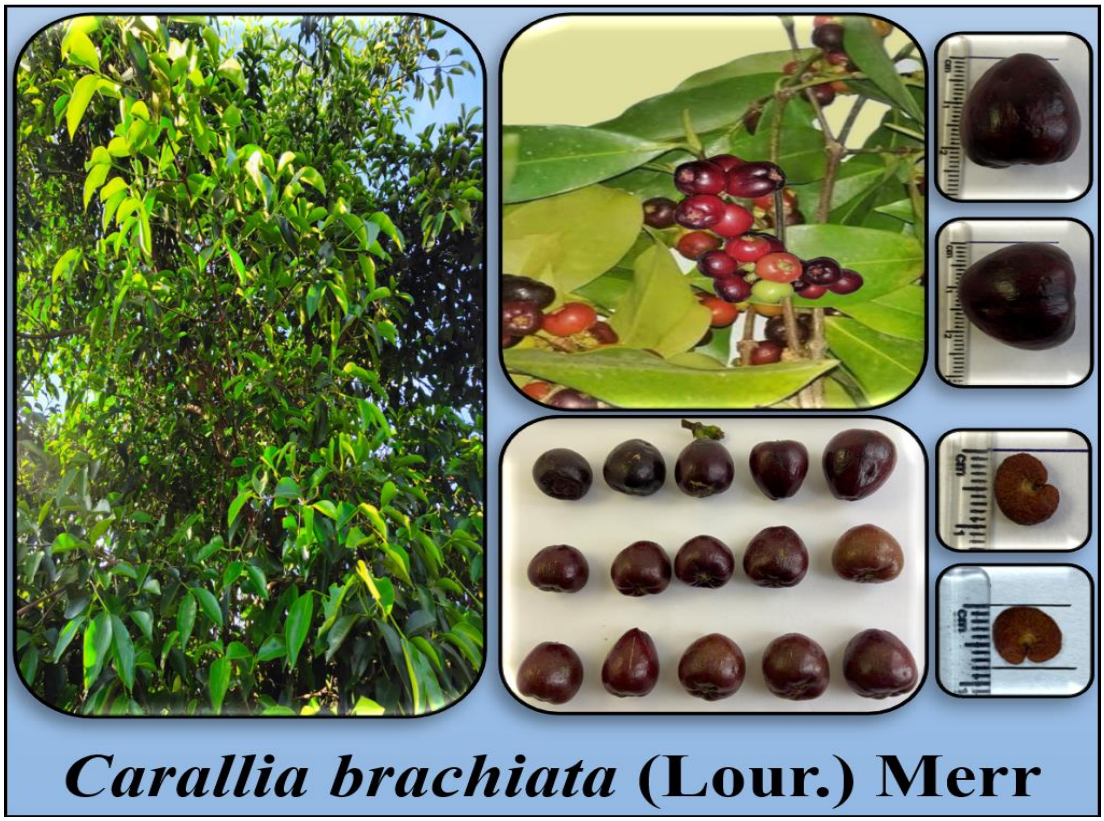

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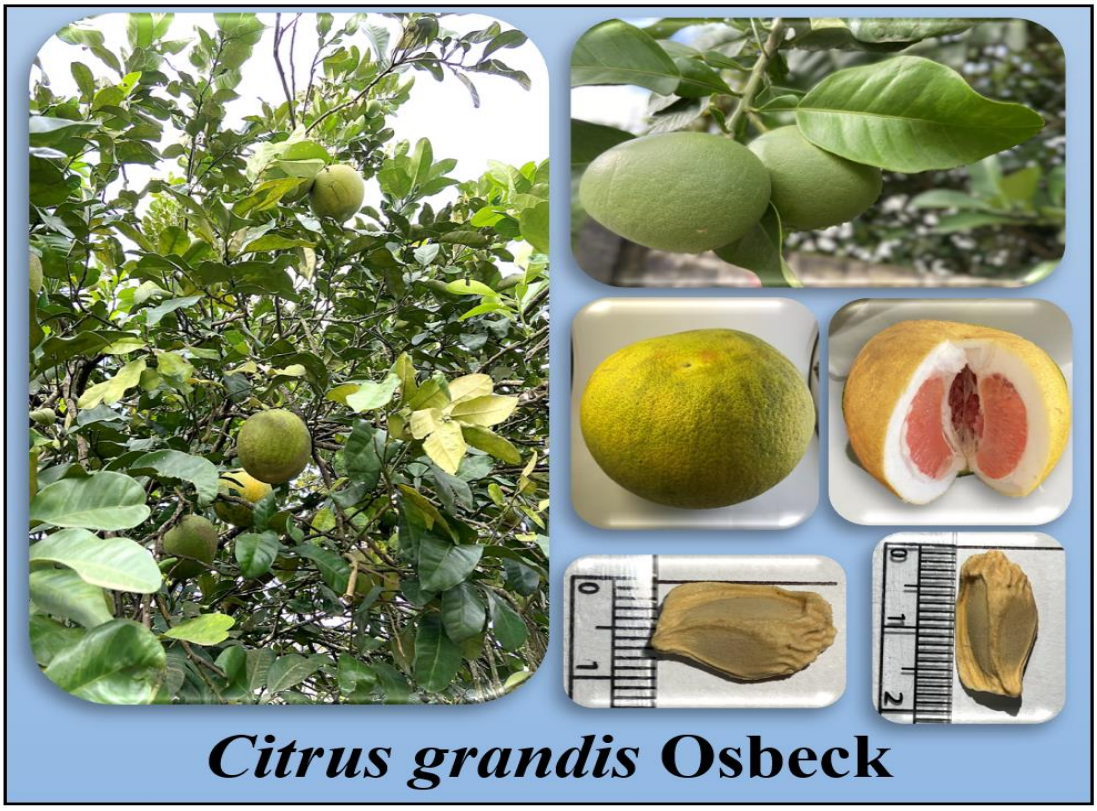
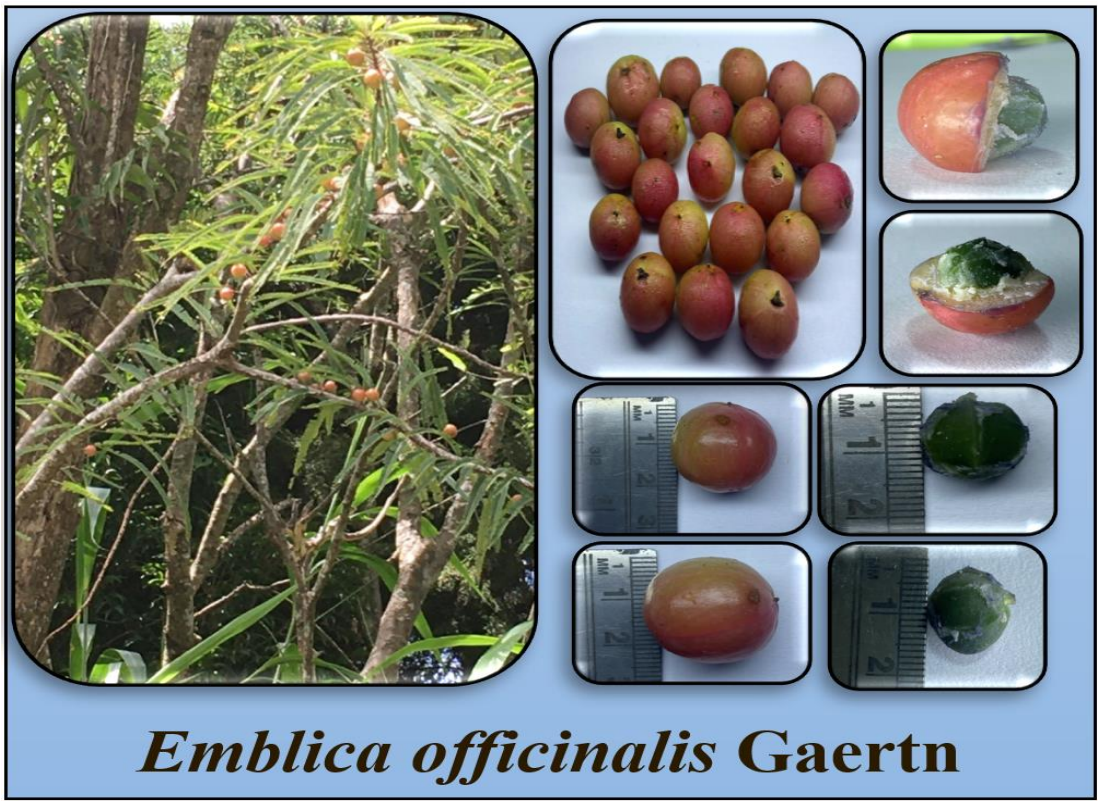


Photo Plate 3.4

Photo Plate 3.5



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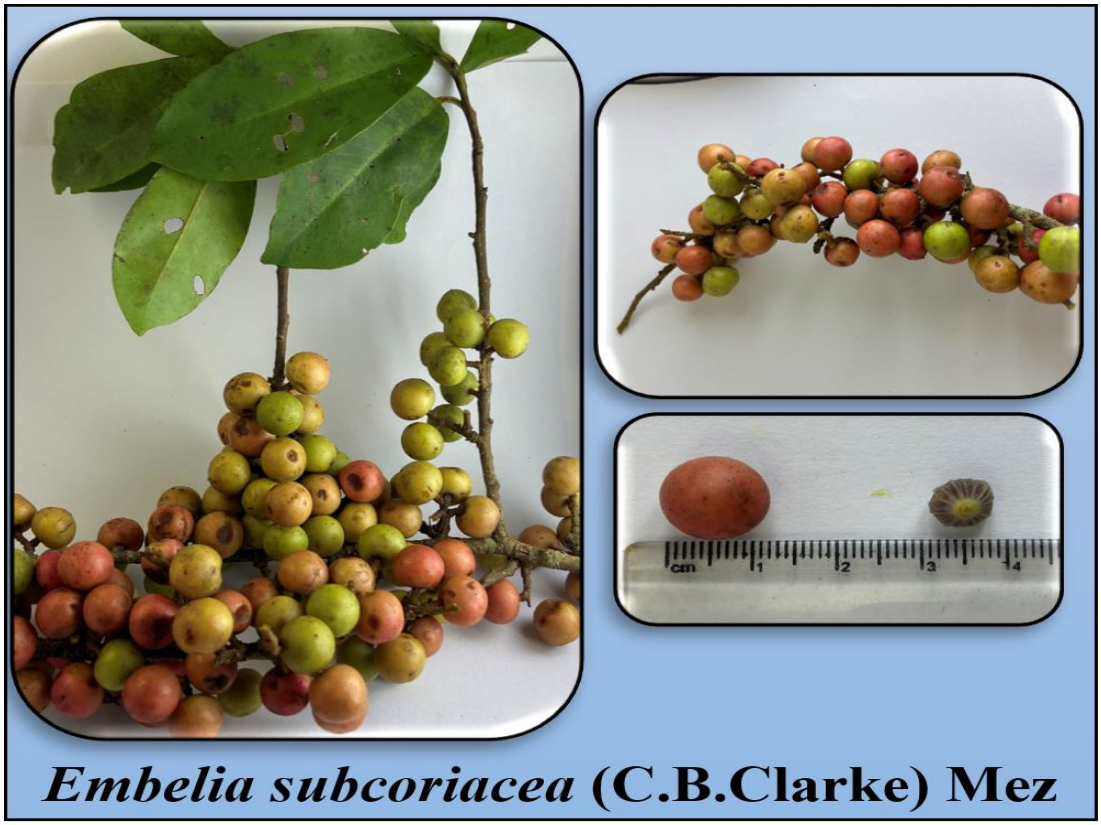

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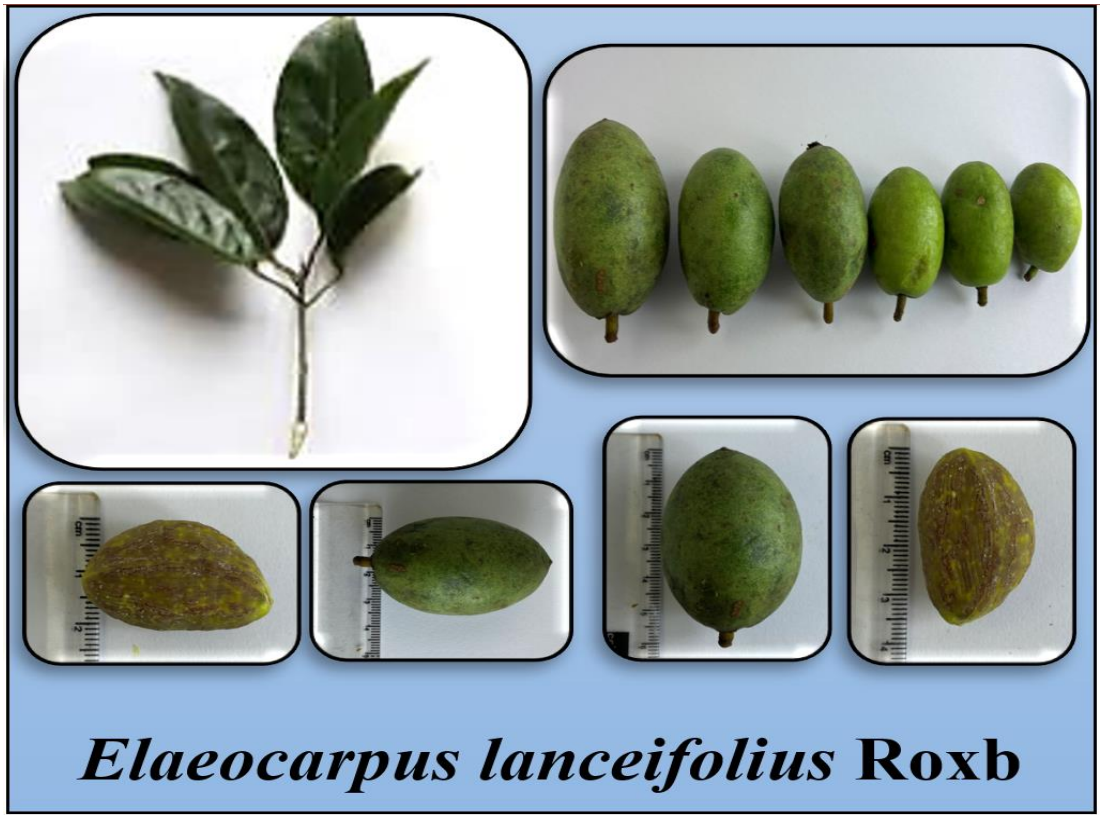

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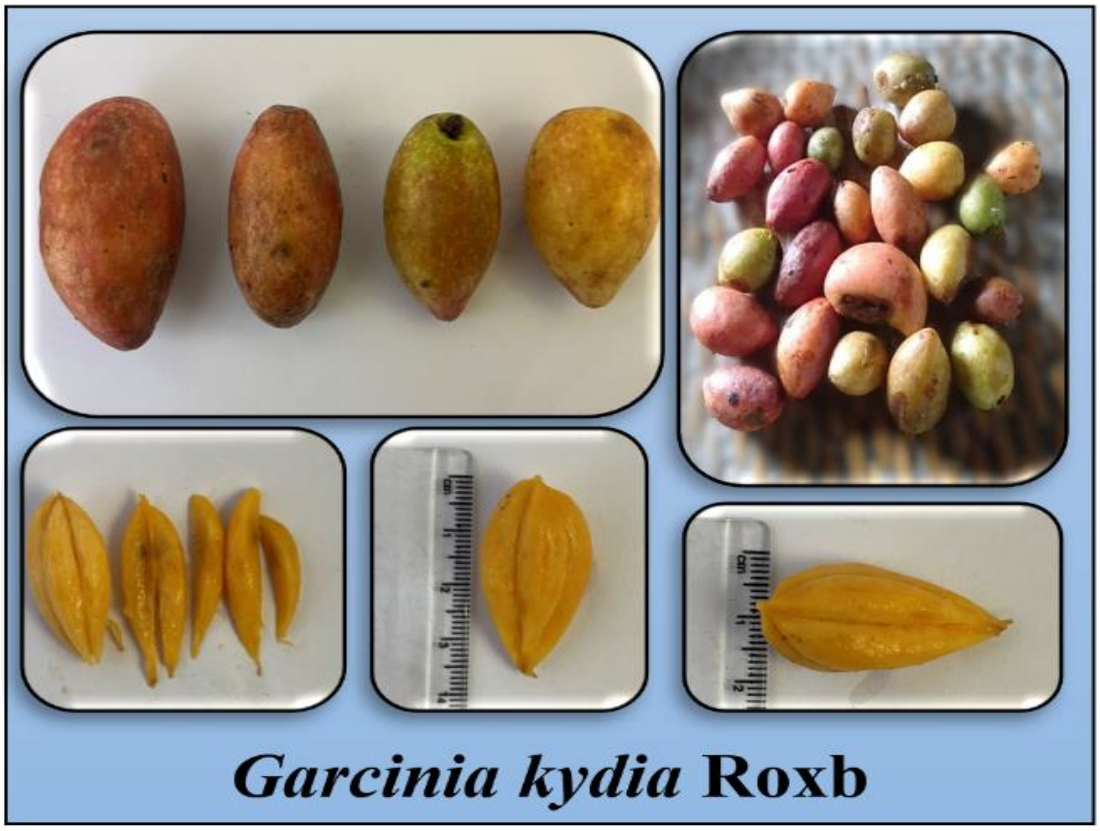


Photo Plate 3.9

Photo Plate 3.10

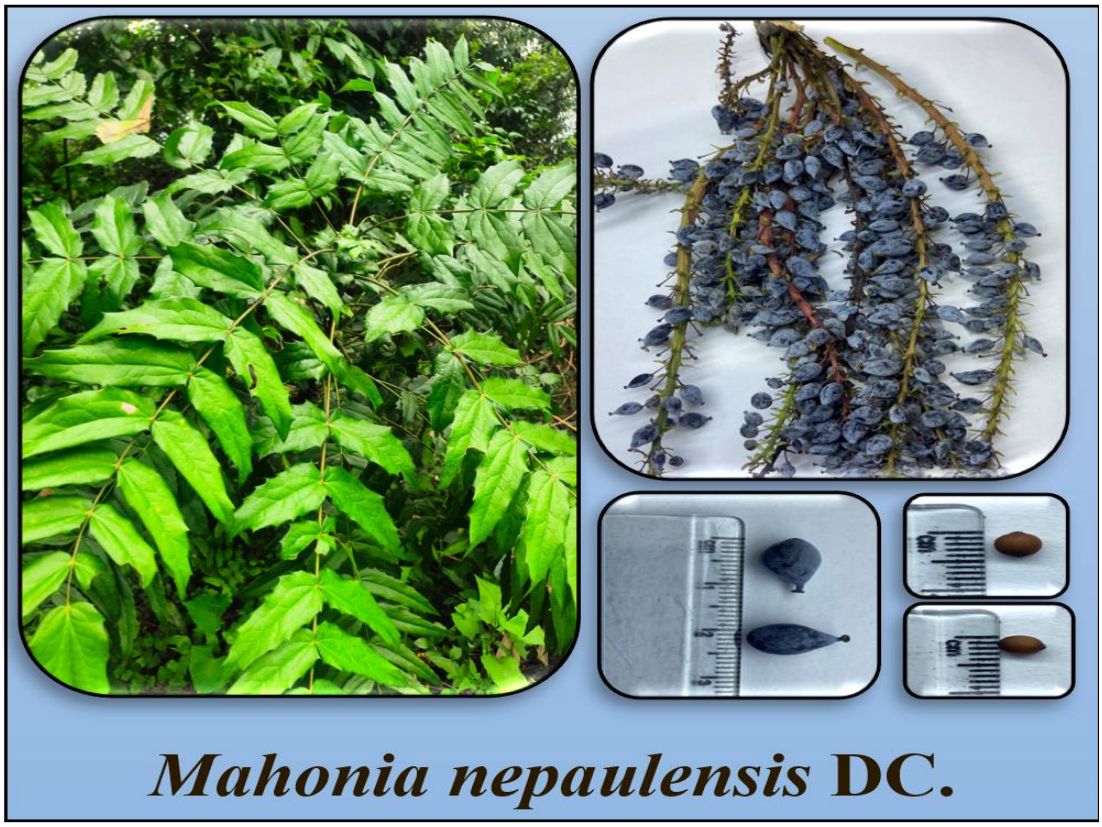


Photo Plate 3.11

Photo Plate 3.12

Photo Plate 3.13

Photo Plate 3.14

Photo Plate 3.15

Photo Plate 3.16

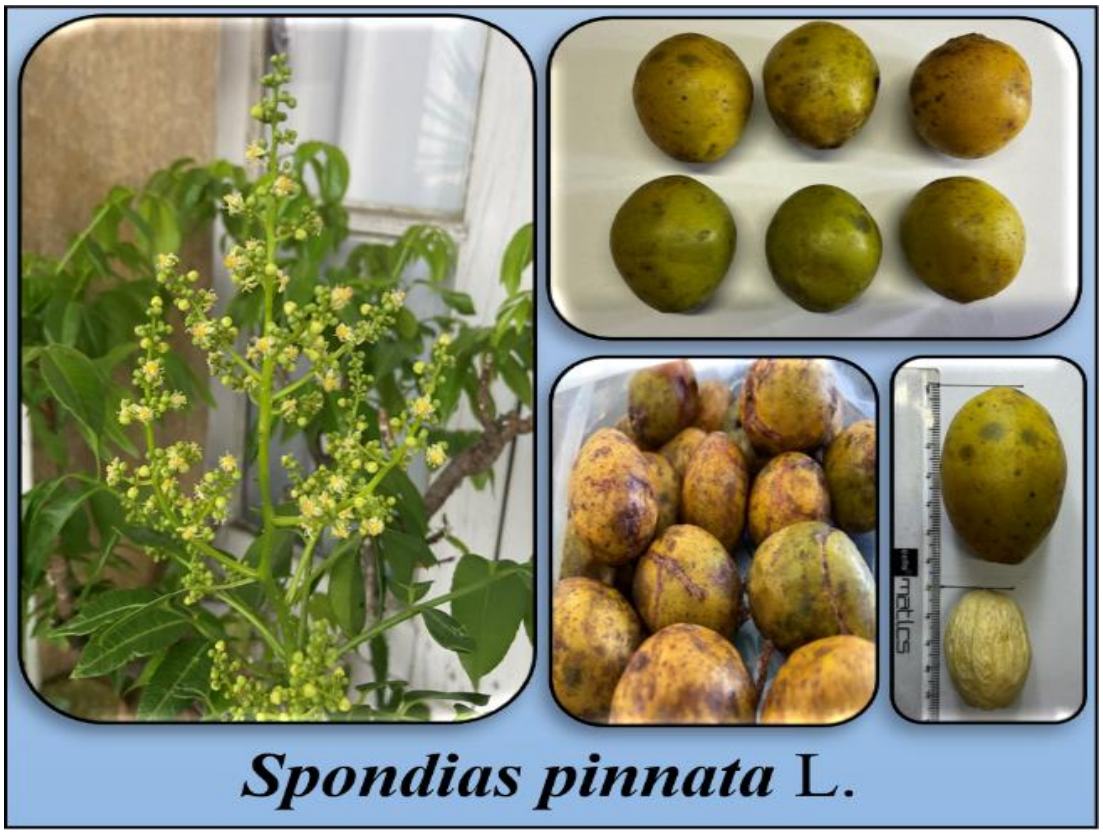


Photo Plate 3.17

Photo Plate 3.18

Photo Plate 3.19

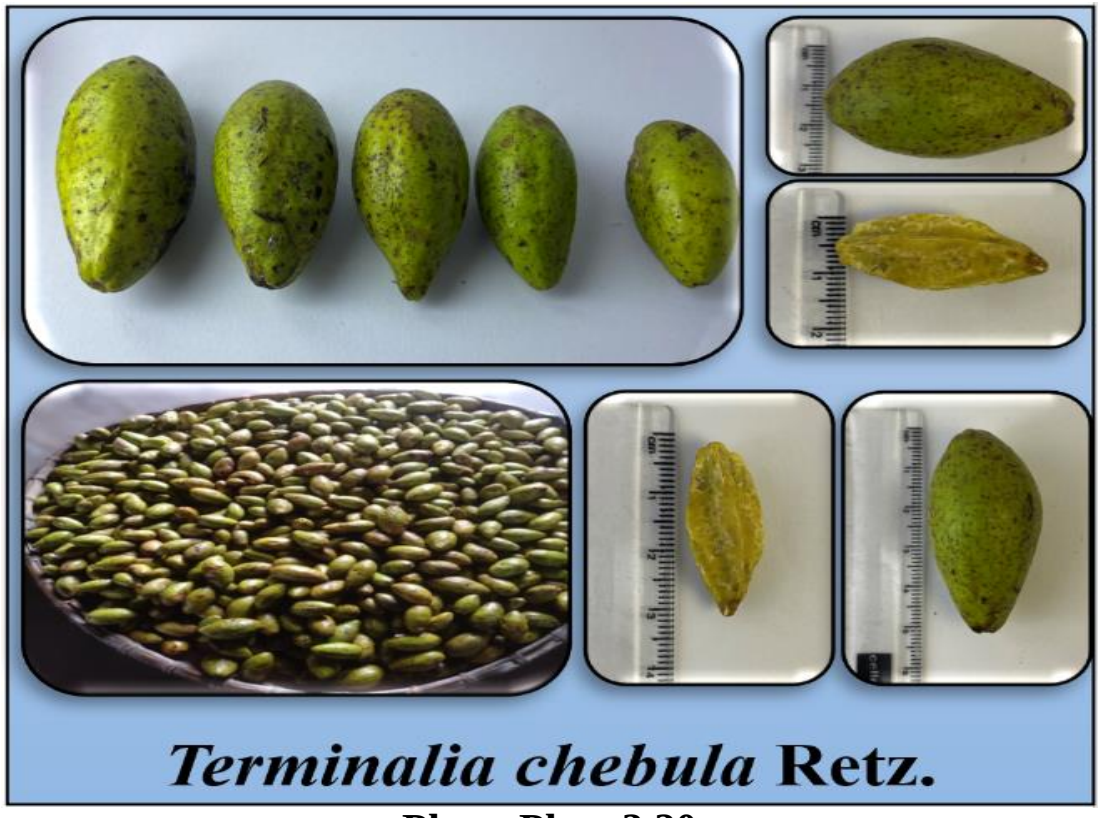


Photo Plate 3.20

Photo Plate 3.21

Chapter IV

RESULTS AND DISCUSSION

Results and Discussion

The present investigation entitled "Nutrient profiling, functional food compounds and antioxidant properties of some underutilised fruits of Mizoram" was conducted at Mizoram University Aizawl, Mizoram to establish a nutritional database of underutilised edible fruits of Mizoram, northeast India, to explore the untapped fruits of the state. The findings revealed that these fruits' nutritional and anti-nutritional components exhibited significant variations.

4.1 Fruit Nutrient Analysis

4.1.1 Proximate composition

4.1.1.1 Moisture (%)

The moisture content profoundly influences the quality, shelf life, and nutritional value of fruits. Elevated moisture levels provide juicier and more palatable fruits, but diminished levels may result in dryness and lower appeal, adversely impacting the concentration of nutrients in fruits.

The research data represented in Table 4.1a revealed that *Citrus jambhiri* had the highest moisture content (95.43 ± 1.68 %), followed by *Myrica esculenta* (89.00 ± 1.00 %), which is statistically *at par* with *Citrus grandis* (88.64 ± 6.88 %) and *Prunus jenkinsii* (88.00 ± 1.00 %). In contrast, *Tamarindus indica* had the lowest moisture content (59.40 ± 1.12 %), while *Mahonia nepaulensis* (65.17 ± 0.76%) and *Emblica officinalis* (62.16 ± 0.78 %) had moisture content that was statistically akin to each other but slightly higher than *Tamarindus indica.*

The moisture content observed in *Citrus jambhiri* was found to be within the range (87.1 ± 0.2 %), as documented by Rehman *et al*. (2019). Similarly, the moisture content in *Myrica esculenta* and *Prunus jenkinsii* aligned with the values (86.75 ± 1.33 %) and (87.00 %), as reported by Rymbai *et al*. (2023) and Borgohain *et al*. (2022) respectively. The heightened moisture content present in *Citrus* fruits can be ascribed to the substantial proportion, surpassing three-quarters (3/4), of moisture within these fruits, rendering them an attractive option for direct consumption or juice extraction (Shah *et al*., 2020). The observed variations

underscored the significance of accounting for moisture levels when evaluating fruit quality, shelf life, nutritional value, and economic considerations. The elevated moisture levels observed in *Citrus jambhiri* and *Myrica esculenta* (Figure 4.1) indicate a potentially reduced shelf life, attributable to heightened microbial proliferation and an associated risk of spoilage. Consequently, the implementation of appropriate storage conditions, including refrigeration and regulated humidity levels, is crucial for prolonging their shelf life. In contrast, the reduced moisture content observed in *Tamarindus indica* suggests an extended shelf life, thereby enhancing its suitability for storage and transportation (Ibrahim *et al*., 2021).

4.1.1.2 Dry Matter (%)

The dry matter content (DMC) of fruits serves as a pivotal indicator of quality attributes, bearing considerable implications for both consumers and producers. This method is frequently employed to assess the maturity of fruits. A higher dry matter content typically indicates superior quality, as it reflects the concentration of sugars, starches, proteins, and various other essential nutrients. The significance of this is especially pronounced for fruits like apples, pears, and avocados, in which DMC serves as a dependable measure of ripeness and the optimal period for harvesting (Goke *et al.*, 2018).

The dry matter exhibited an inverse relationship with the moisture content as observed from the data in Table 4.1a, with the greatest values observed in *Tamarindus indica* (40.60 ± 1.12 %) and *Emblica officinalis* (37.84 ± 0.78 %), which were statistically indistinguishable from one other, followed by *Mahonia nepaulensis* (34.83 ± 0.76 %). Conversely, *Citrus jambhiri* displayed the lowest dry matter content (4.57 ± 1.68 %). At the same time, being statistically similar, *Myrica esculenta* (11.00 ± 1.00 %) and *Prunus jenkinsii* (12.00 ± 1.00 %) reported slightly higher dry matter content than *Citrus jambhiri* which reported the lowest dry matter content.

Dry matter constitutes the nutrient-rich component of the fruit, omitting the water content and it increases as the fruits reach maturity which results to the variation in the studied underutilised fruits. The results suggest that *Tamarindus indica* and *Emblica officinalis* (Figure 4.2) are likely to exhibit elevated

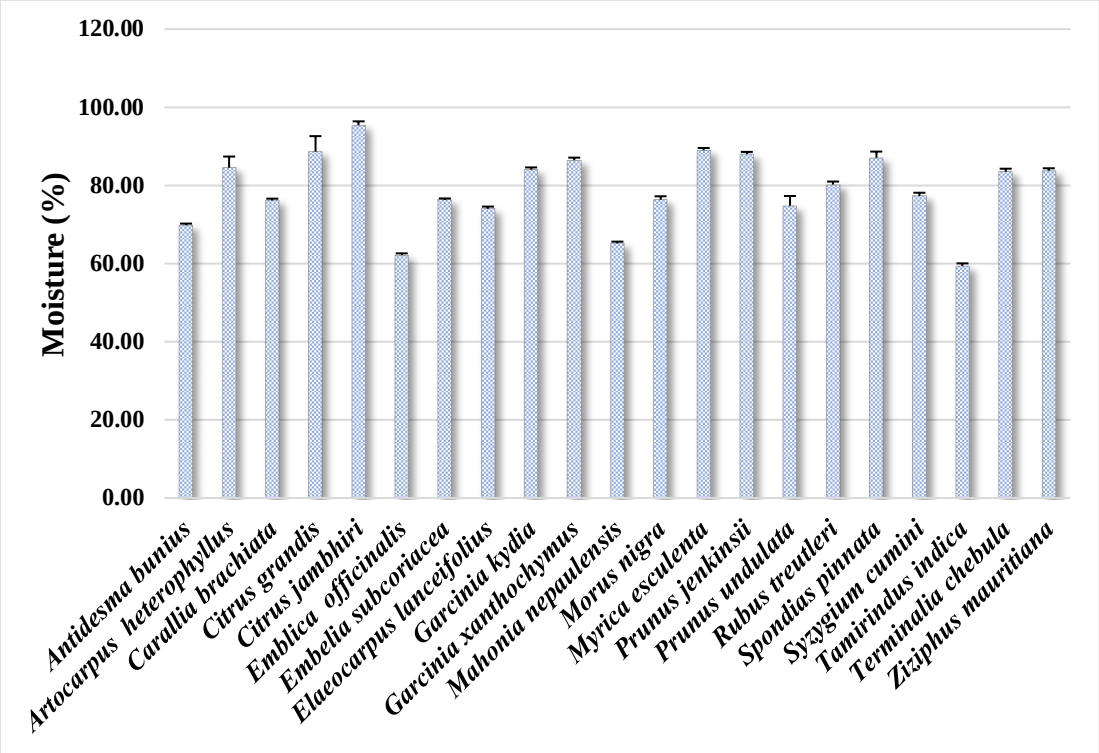


Fig. 4.1 Moisture content in the studied underutilised fruits

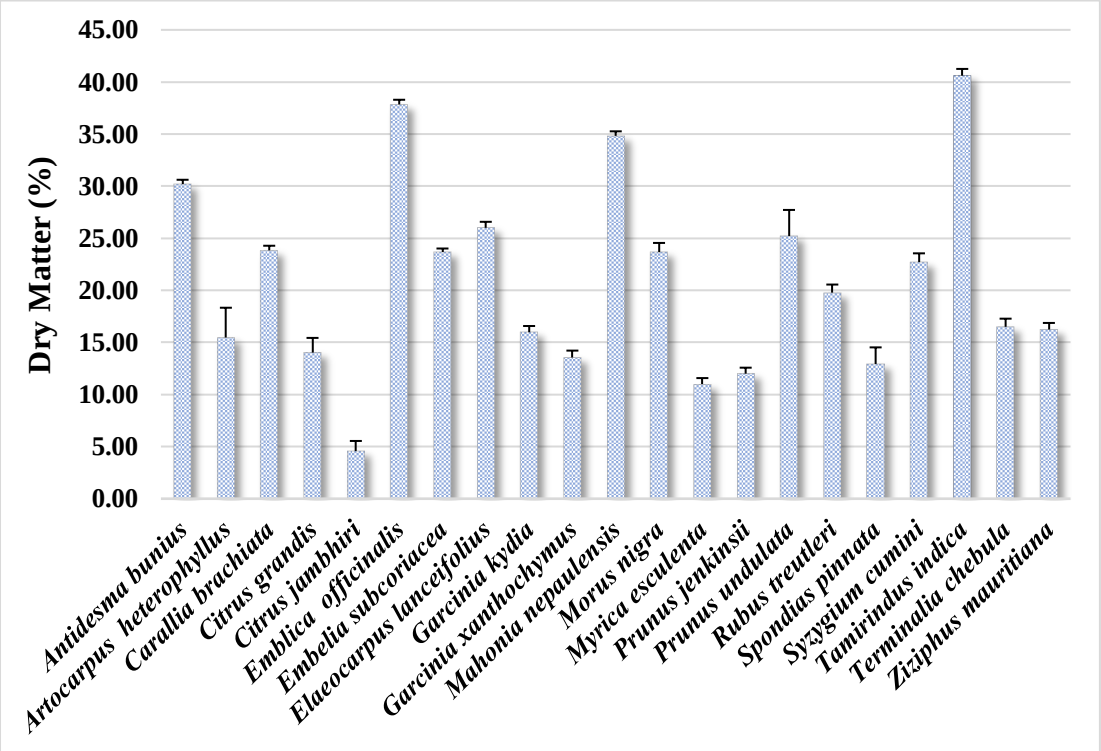


Fig 4.2 Dry Matter content of the studied underutilised fruits

concentrations of nutrients, sugars, and beneficial compounds, thereby enhancing their nutrient density and contributing to an increased dry matter content. (Achaglinkame *et al*., 2019). The values documented for *Prunus jenkinsii* exceed the 0.5% reported by Borgohain *et al*. (2022). Furthermore, the elevated dry matter content observed in these fruits indicates an extended shelf life attributable to lower water content, thereby mitigating the potential for microbial proliferation and subsequent spoilage. Comprehending the dry matter content in fruits is essential for making informed decisions regarding their nutritional value, shelf life, and attractiveness to consumers (Homma *et al*., 2022).

4.1.1.3 Total Ash (%)

The ash content of fruits serves as a significant indicator of their overall mineral composition and plays a crucial role in nutritional labelling and dietary evaluations. Typically, a higher ash content correlates with an elevated mineral content, which in turn enhances the nutritional value and flavour profile of the fruit (Puri *et al*., 2024). Comprehending the ash content of these underutilised fruits can inform dietary selections and nutritional advisories. The ash content of food can significantly affect various physicochemical and nutritional characteristics.

The proximate analysis is utilised to determine the ash content present in the fruits. The study revealed from the data in Table 4.1a showed that *Citrus grandis* exhibited the greatest ash concentration (9.21 ± 0.06 %), followed by *Prunus undulata* (6.87 ± 0.07 %) and *Spondias pinnata* (5.44 ± 0.08 %). *Emblica officinalis* which reported the lowest ash content (1.16 ± 0.12 %). However, *Embelia subcoriacea* (1.47 ± 0.12 %) and *Syzygium cumini* (1.82 ± 0.07 %) exhibited lower levels of ash content.

The results indicate that *Citrus grandis* fruit possesses a significant ash content (Figure 4.3), suggesting a higher concentration of dietary minerals in comparison to other underutilised fruits examined. The significant concentration of calcium, magnesium, sodium, potassium, manganese, zinc, and iron may result in an increased occurrence of ash (Ismail, 2024). The documented findings aligned with the values reported (0.99 – 4.95%) for underutilised fruits in the eastern Himalayas as noted by Rymbai *et al*. (2023).

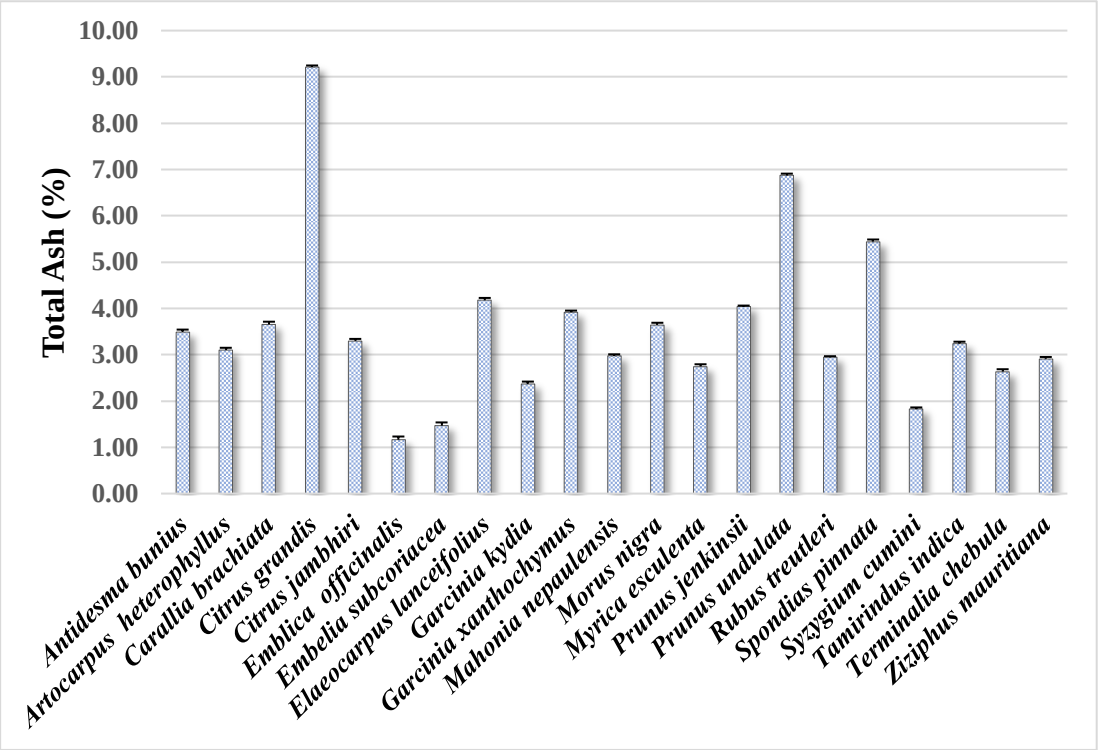


Fig. 4.3 Total ash of the studied underutilised fruits

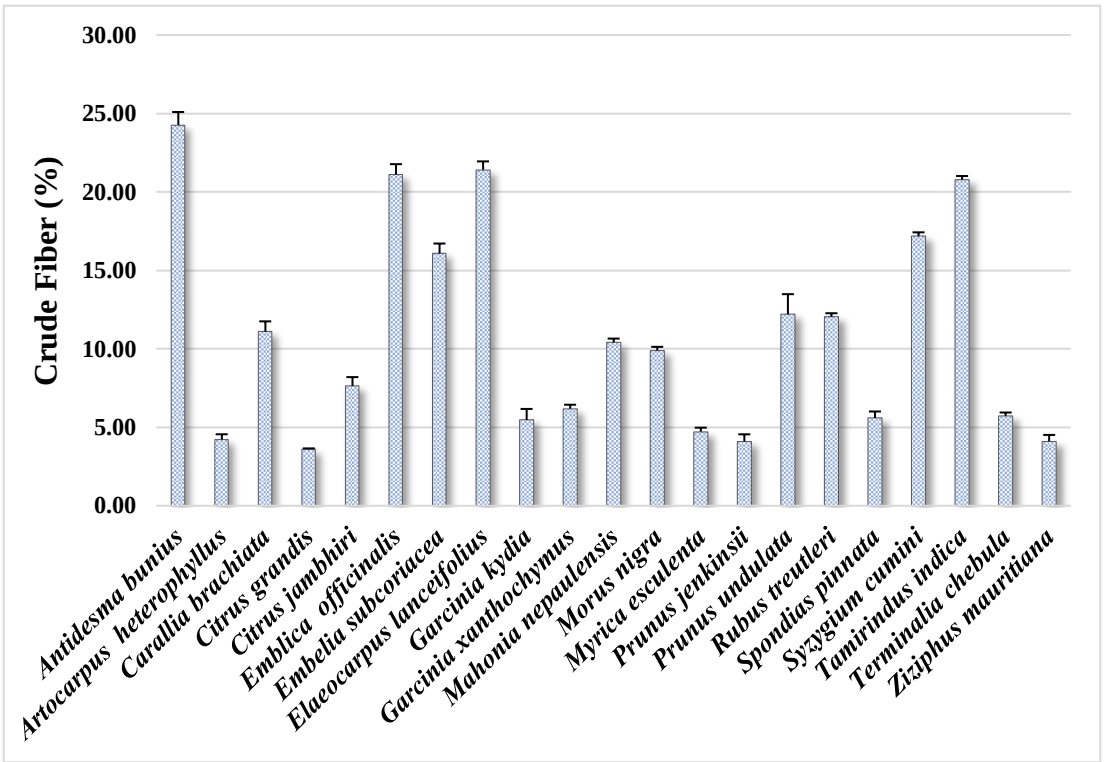


Fig 4.4 Crude fibre content of the studied underutilised fruits

Table 4.1(a) Nutrient composition of the studied underutilised fruits

Botanical Name	Moisture (%)	Dry matter (%)	Total Ash (%)	Crude Fibre (%)	Carbohydrate (%)	Protein (%)	Fat (%)	Energy (kcal)
Antidesma bunius	69.81 ± 0.73^{c}	30.19 ± 0.73^{g}	3.49 ± 0.09^{i}	24.27 ± 1.43^{i}	5.91 ± 0.04^{a}	6.98 ± 0.06de	0.82 ± 0.03^{ef}	59.00 ± 0.58^{a}
Artocarpus heterophyllus	84.53 ± 4.96^{fghi}	15.47 ± 4.96^{cd}	3.10 ± 0.09^{g}	3.89 ± 1.04^{ab}	53.29 ± 0.98^{i}	1.88 ± 0.04^{a}	0.71 ± 0.01^{de}	227.08 ± 4.08^{g}
Carallia brachiata	76.16 ± 0.77^{de}	23.84 ± 0.77^{f}	3.66 ± 0.10^{j}	11.09 ± 1.14^{ef}	14.68 ± 0.22^{c}	32.23 ± 0.26^{k}	2.30 ± 0.26^{k}	175.05 ± 38.59^{f}
Citrus grandis	88.64 ± 6.88^{ij}	14.02 ± 2.44^{de}	9.21 ± 0.06^{o}	3.61 ± 0.08^{ab}	34.32 ± 0.90^{f}	8.28 ± 0.13^{e}	0.92 ± 0.02^{fg}	178.64 ± 4.09^{f}
Citrus jambhiri	95.43 ± 1.68^{k}	4.57 ± 1.68^{a}	3.30 ± 0.08^{h}	7.64 ± 0.97^{d}	26.33 ± 0.87^{e}	4.17 ± 0.31^{b}	1.04 ± 0.05^{g}	131.37 ± 4.64^{e}
Emblica officinalis	62.16 ± 0.78^{ab}	37.84 ± 0.78^{hi}	1.16 ± 0.12^{a}	21.10 ± 1.16^{h}	12.18 ± 0.04^{b}	114.30 ± 0.94^{n}	2.08 ± 0.08^{j}	524.67 ± 3.07^{n}
Embelia subcoriacea	76.32 ± 0.59^{de}	23.68 ± 0.59^{f}	1.47 ± 0.12^{b}	16.07 ± 1.11^{g}	11.96 ± 0.04^{b}	44.47 ± 0.33l	2.50 ± 0.10^{l}	248.25 ± 1.94^{h}
Elaeocarpus lanceifolius	74.00 ± 1.00^{d}	26.00 ± 1.00^{f}	4.18 ± 0.09^{l}	21.41 ± 0.92^{h}	11.95 ± 0.68^{b}	28.37 ± 0.71^{j}	0.35 ± 0.05^{bc}	164.43 ± 4.38^{f}
Garcinia kydia	84.02 ± 1.02^{fgh}	15.98 ± 1.03^{d}	2.37 ± 0.09^{d}	5.47 ± 1.22^{bc}	43.04 ± 0.48^{g}	7.21 ± 0.98^{de}	6.23 ± 0.06^{n}	257.09 ± 5.69^{h}
Garcinia xanthochymus	86.45 ± 1.15^{ghij}	13.55 ± 1.15^{bcd}	3.92 ± 0.06^{k}	6.19 ± 0.44^{cd}	10.94 ± 0.45^{b}	5.19 ± 0.29^{bc}	1.88 ± 0.08^{i}	81.48 ± 0.47^{b}
Mahonia nepaulensis	65.17 ± 0.76^{b}	34.83 ± 0.76^{h}	2.97 ± 0.06^{fg}	10.41 ± 0.43^{e}	71.68 ± 0.95^{m}	24.95 ± 0.29^{i}	4.43 ± 0.06^{m}	426.42 ± 2.24^{l}
Morus nigra	76.33 ± 1.53^{de}	23.67 ± 1.53^{f}	3.64 ± 0.08^{j}	9.89 ± 0.41^{e}	52.32 ± 0.71^{i}	11.83 ± 0.13^{g}	4.35 ± 0.05^{m}	295.73 ± 2.42^{k}
Myrica esculenta	89.00 ± 1.00^{j}	11.00 ± 1.00^{b}	2.75 ± 0.08^{e}	4.72 ± 0.44^{abc}	79.84 ± 3.74^{n}	25.02 ± 0.43^{i}	1.64 ± 0.03^{h}	434.17 ± 13.36^{l}
Prunus jenkinsii	88.00 ± 1.00^{hij}	12.00 ± 1.00^{bc}	4.04 ± 0.03^{k}	3.43 ± 0.46^{a}	15.84 ± 0.04^{c}	6.29 ± 0.14^{cd}	0.62 ± 0.03^{d}	94.08 ± 0.60^{c}
Prunus undulata	74.78 ± 4.33^{d}	25.22 ± 4.33^{f}	6.87 ± 0.07^{n}	12.20 ± 2.24^{f}	20.67 ± 0.06^{d}	16.97 ± 0.69^{h}	2.32 ± 0.03^{k}	171.39 ± 2.74^{f}
Rubus treutleri	80.22 ± 1.34^{ef}	19.78 ± 1.34^{e}	2.94 ± 0.04^{f}	12.03 ± 0.41^{f}	46.37 ± 0.29^{h}	10.17 ± 0.03^{f}	4.35 ± 0.05^{m}	265.30 ± 1.31^{ij}
Spondias pinnata	87.07 ± 2.76^{ghij}	12.93 ± 2.76^{bcd}	5.44 ± 0.08^{m}	5.60 ± 0.71^{bc}	53.05 ± 0.90^{i}	12.30 ± 0.13^{g}	1.65 ± 0.05^{h}	276.24 ± 3.29^{i}
Syzygium cumini	77.29 ± 1.45^{de}	22.72 ± 1.45^{ef}	1.82 ± 0.07^{c}	17.18 ± 0.42^{g}	59.13 ± 1.31^{j}	12.40 ± 0.17^{g}	2.15 ± 0.05^{j}	305.50 ± 4.37^{j}
Tamirindus indica	59.40 ± 1.12^{a}	40.60 ± 1.12^{i}	3.24 ± 0.07^{h}	20.78 ± 0.40^{h}	61.85 ± 3.46^{k}	59.68 ± 1.35^{m}	0.45 ± 0.05^{c}	490.17 ± 11.65^{m}
Terminalia chebula	83.50 ± 1.35^{fg}	16.50 ± 1.35^{d}	2.64 ± 0.09^{e}	5.73 ± 0.37^{bc}	65.02 ± 1.71^{l}	129.49 ± 3.41^{o}	0.25 ± 0.05^{ab}	780.31 ± 8.01^{o}
Ziziphus mauritiana	83.76 ± 1.09^{fgh}	16.24 ± 1.09^{d}	2.91 ± 0.07^{f}	3.78 ± 0.40^{ab}	14.38 ± 0.04^{c}	17.01 ± 0.76^{h}	0.15 ± 0.05^{a}	126.89 ± 3.22^{d}

Values with different lower-case superscript ($^{a-d}$) letters in a column are significantly different among the fruits at $p < 0.05$ (DMRT test performed for separation of mean).
Values are expressed as mean ± SD with three replications (n = 3) for each experiment.

4.1.1.4 Crude Fibre (%):

A diet abundant in fibre has been linked to a reduced risk for gastrointestinal disorders, including diverticulitis, irritable bowel syndrome (IBS), and colorectal cancer. Fibre contributes to the maintenance of the digestive tract's efficiency (Mathers, 2023), thereby promoting digestive health, enhancing the process of digestion, and facilitating regular bowel movements (Madhu *et al*., 2017).

From the data recorded in Table 4.1a, the greatest crude fibre levels were recorded in *Antidesma bunius* (24.27 ± 1.43 %) followed by *Elaeocarpus lanceifolius* (21.41 ± 0.92 %) which is statistically *at par* with *Emblica officinalis* (21.10 ± 1.16 %) and *Tamarindus indica* (20.78 ± 0.40 %). The lowest level was reported in *Prunus jenkinsii* (3.43 ± 0.46%) which was statistically *at par* with *Citrus grandis* (3.61 ± 0.08 %), *Ziziphus mauritiana* (3.78 ± 0.40%) and *Artocarpus heterophyllus* (3.89 ± 1.04 %).

The documented crude fibre content in *Emblica officinalis* is in the range (8.60 ± 0.50 % to 23.4 ± 0.20 %), reported by Bulo *et al.* (2024). The elevated crude fibre content in *Antidesma bunius* (Figure 4.4) suggests its significance as a noteworthy dietary source of fibre, recognised for its role in enhancing digestive health through the improvement of digestion and the facilitation of regular bowel movements. In contrast, *Prunus jenkinsii* (Figure 4.4), characterised by its reduced fibre content, may be advantageous for enhancing infant nutrition and developing food formulations for children (Jiru *et al*., 2023). The fibre content of the examined underutilised fruits may vary based on their maturity and ripeness stages, as well as the decomposition of cellulose and lignin (Cvrk *et al*., 2022; Yimer *et al*., 2023).

4.1.1.5 Total carbohydrates (%)

Carbohydrates, proteins, and lipids represent the primary macronutrients in food, essential nutrients that must be consumed in significant quantities on a daily basis (Hans and Jana, 2018). Carbohydrates constitute a fundamental component in a diverse array of fruits, including those frequently disregarded, and play a crucial role in supplying energy within human nutrition. These substances can be classified into

two primary categories: simple sugars, which include monosaccharides and disaccharides, and complex carbohydrates, comprising starch and fibre.

The study revealed from the data presented in Table 4.1a, *Myrica esculenta* exhibited the maximum carbohydrate content (79.84 ± 3.74 %), followed by *Mahonia nepaulensis* (71.68 ± 0.95 %) and *Terminalia chebula* (65.02 ± 1.71 %). *Antidesma bunius* exhibited the lowest values (5.91 ± 0.04 %) for carbohydrates and *Garcinia xanthochymus* (10.94 ± 0.45 %) which is statistically *at par* with *Elaeocarpus heterophyllus* (11.95 ± 0.68 %), *Embelia subcoriacea* (11.46 ± 0.04 %) recorded lower carbohydrate contents.

Among the fruits examined, *Myrica esculenta* demonstrates the highest carbohydrate levels, comparable to the values (78.03 %) reported by Bachheti *et al*. (2023). This finding underscores its considerable potential as an energy source and provides important insights into its nutritional profile, as noted by Murthy *et al*. (2020). The differences in carbohydrate composition across various fruits can be attributed to the distinct genetic makeups of their respective genera and species (Hazarika and Lalnunsangi, 2019). Throughout the ripening process, there is a gradual depletion of starch, which is paralleled by an increase in the levels of glucose, fructose, and sucrose (Miller, 1958). Fruits that exhibit elevated carbohydrate content, including *Myrica esculenta, Mahonia nepaulensis,* and *Terminalia chebula* (Figure 4.5), may be prioritised for the development of energy-dense food products (Shah *et al*., 2020).

4.1.1.6 Total Protein (%)

Proteins serve as vital macronutrients, playing a crucial role in numerous physiological processes such as tissue repair, enzyme synthesis, and the immune response. These fruits play a vital role in the processes of muscle repair and growth, rendering them advantageous for individuals engaged in physical activity as well as those in the recovery phase following injuries. Their inadequacy may result in various health complications, especially within the younger demographic, including oedema, hepatic steatosis, dermal degeneration, increased vulnerability to infections,

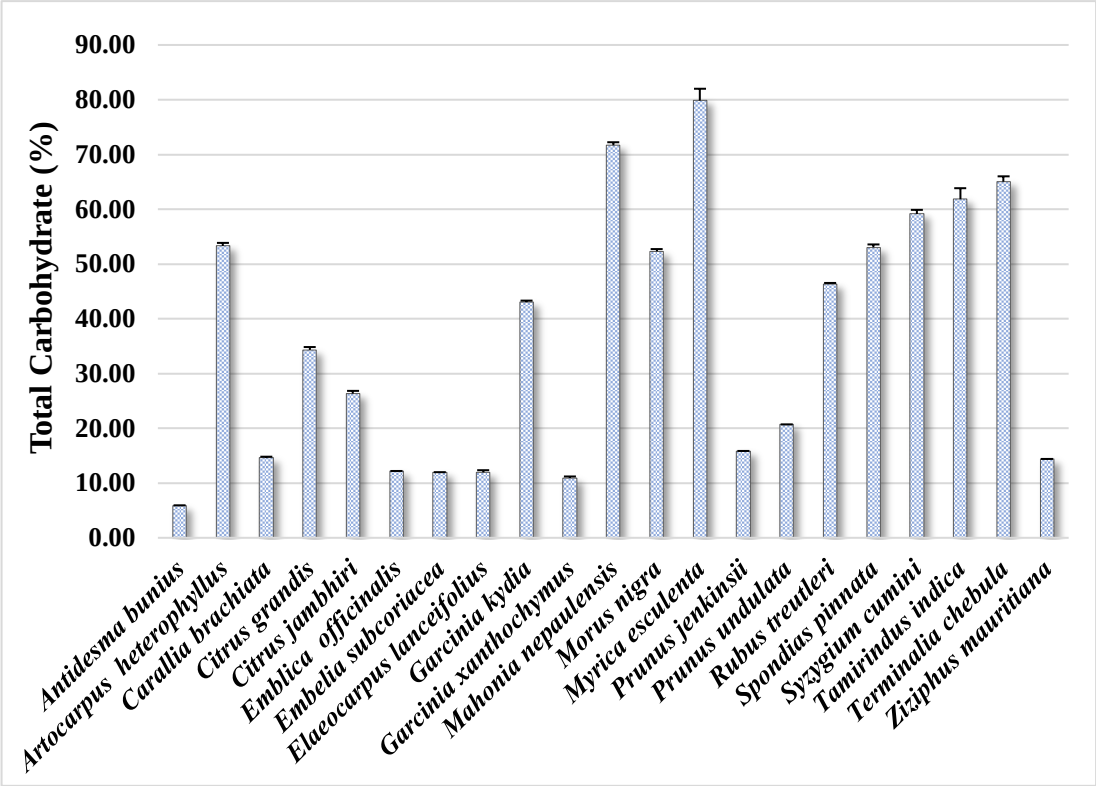


Fig. 4.5 Total Carbohydrate content of the studied underutilised fruits

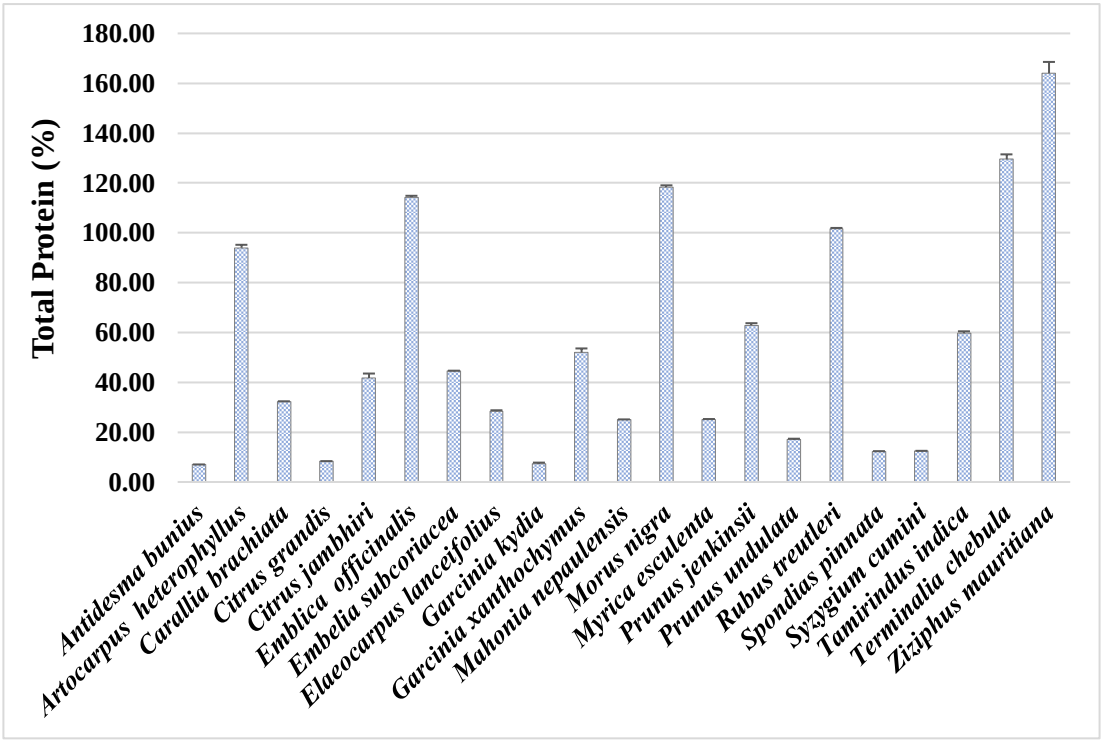


Fig. 4.6 Total Protein content of the studied underutilised fruits

and hindered physical development (Biswas *et al*., 2022a). Although fruits are generally not recognised for their high protein content in comparison to other food categories, certain underutilised fruits possess significant levels of protein (Murthy and Bapat, 2020).

The recorded data from the study in Table 4.1a revealed that *Terminalia chebula* exhibited the maximum protein (89.16 ± 3.41 %) content, followed by *Emblica officinalis* (74.30 ± 0.94 %) and *Tamarindus indica* (59.68 ± 1.35 %). *Artocarpus heterophyllus* exhibited the lowest amount (1.88 ± 0.04 %) while *Citrus jambhiri* (4.17 ± 0.31 %) reported lower protein content while being statistically similar to *Garcinia xanthochymus* (5.19 ± 0.29 %).

The protein content observed in *Garcinia xanthochymus* and *Artocarpus heterophyllus* aligns with the values of 4.01 % and 1.4% to 2.3 % as documented by Parthsarathy *et al*. (2014) and Sreeja *et al*. (2021), respectively. The notable protein content present in *Terminalia chebula* and *Emblica officinalis* (Figure 4.6) in comparison to other enumerated fruits is particularly compelling, especially considering its classification as a relatively obscure tropical species (Moutaly *et al*., 2022). The differences in protein content can be attributed to environmental conditions and physiological factors that alter the expression of quality attributes; however, the genetic background of the fruit species serves as the primary determinant, exerting a direct influence on the quality traits of fruits (Huyskens-Keil and Schreiner, 2004).

4.1.1.7 Total fats (%)

Fats play a vital role in the absorption of fat-soluble vitamins, namely A, D, E, and K, thereby enhancing the overall uptake of nutrients. They additionally function as a concentrated source of energy, crucial for numerous physiological processes (Murthy and Bapat, 2020).

From the observation in Table 4.1a, *Garcinia kydia* reported the highest fat percentage (6.23 ± 0.06 %) followed by *Mahonia nepaulensis* (4.43 ± 0.06 %) which was statistically *at par* with *Morus nigra* (4.35 ± 0.05 %) and *Rubus treutleri* (4.35 ± 0.0 5%) species. Conversely, the lowest fat percentage was observed in *Ziziphus*

mauritiana (0.15 ± 0.05 %), which was statistically *at par* with *Terminalia chebula* (0.25 ± 0.05 %).

The observed fat percentage in *Ziziphus mauritiana* was found to be lower than the range documented by Butt *et al.* (2021). *Garcinia kydia* and *Mahonia nepaulensis* (Figure 4.7) exhibit the highest fat percentages among the enumerated fruits, indicating a correspondingly elevated caloric content. The elevated fat percentages suggest that these fruits may serve as significant sources of healthy fats and oil-soluble vitamins, essential for their antioxidant properties. The observed variation among the genus could be linked to environmental and physiological factors, as well as the ripeness of these underutilised fruits. (Memete *et al*., 2022).

4.1.1.8 Energy (kcal 100g^{-1})

The energy content of fruits plays a pivotal role in assessing their nutritional value, especially in the context of underutilised fruits. The energy is predominantly sourced from the carbohydrate, protein, and fat composition of the fruits. The diverse elements of the human body, including muscles, the brain, the heart, and the liver, necessitate energy to perform their distinct physiological functions. (Achaglinkame *et al*., 2019).

As depicted in Table 4.1a from the present investigation, the *Terminalia chebula* had the maximum energy content (780.31 ± 8.01 kcal 100 g^{-1}), followed by *Emblica officinalis* (524.67 ± 3.07 kcal 100 g^{-1}) and *Tamarindus indica* (490.7 ± 11.65 kcal 100 g^{-1}). Conversely, the lowest energy level was recorded in *Antidesma bunius* (59.00 ± 0.58 kcal 100 g^{-1}) at the same time, *Garcinia xanthochymus* (81.84 ± 0.47 kcal 100 g^{-1}) and *Prunus jenkinsii* (94.08 ± 0.60 kcal 100 g^{-1}) reported lower energy level.

The observed energy level values in *Terminalia chebula, Garcinia xanthochymus*, and *Antidesma bunius* exceeded those documented by Angami *et al*. (2024), a discrepancy that may be ascribed to variations in environmental conditions and climatic influences. The elevated energy content of the fruit can be attributed to its increased lipid composition, given that lipids possess the highest caloric density per unit mass, attaining values of up to 9 kcal per g (Insel *et al*., 2012). The elevated energy content of *Terminalia chebula* and *Emblica officinalis* (Figure 4.8) indicates

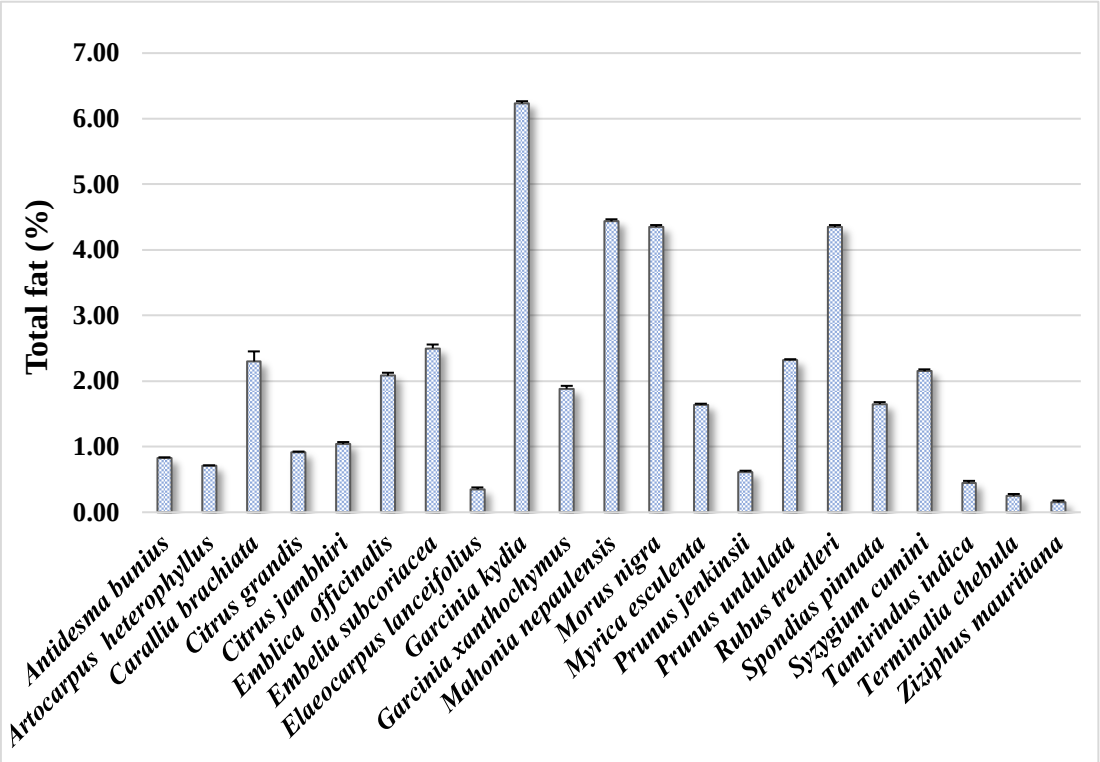


Fig. 4.7 Total fat content of the studied underutilised fruits

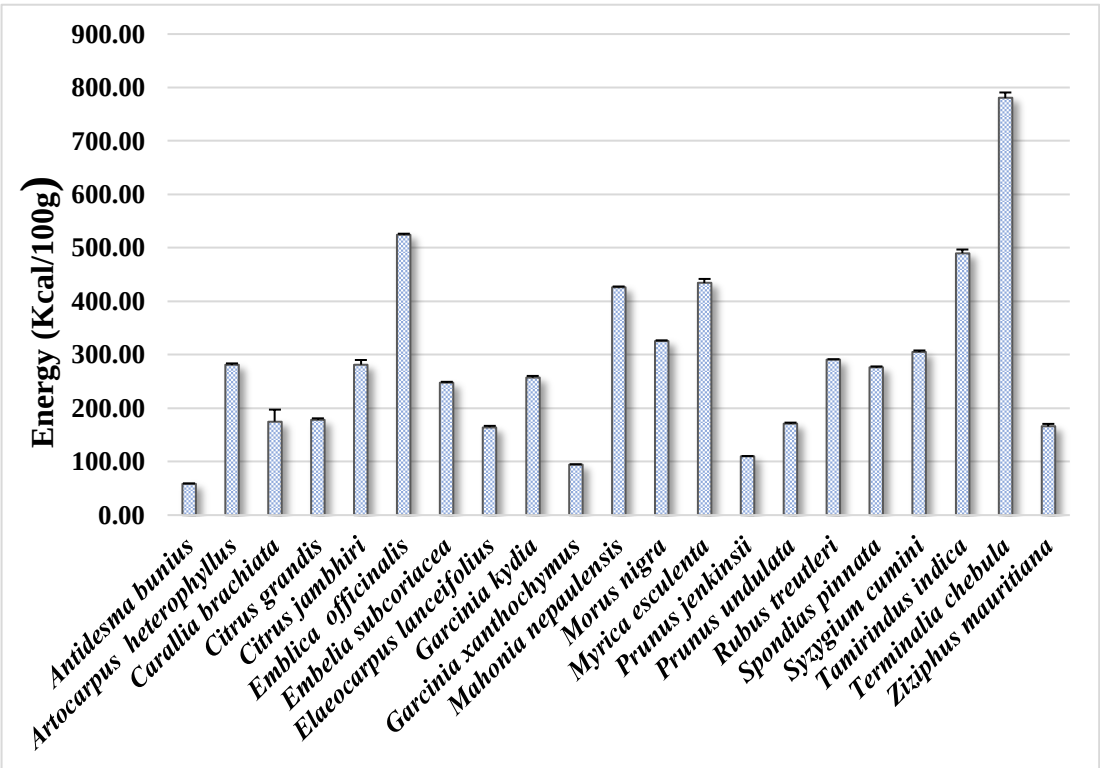


Fig 4.8 Energy content of the studied underutilised fruits

their potential as a significant source of calories, thereby rendering them especially advantageous for dietary regimens necessitating increased energy consumption. They could potentially serve as a highly effective energy source in urban environments where commercial fruits are limited (Murthy and Bapat, 2020).

4.1.1.9 Starch content (mg100 g^{-1})

Starch constitutes an essential element in a variety of fruits, including those that are frequently disregarded. It serves as a fundamental carbohydrate reserve and is essential in human nutrition for the provision of metabolic energy. The concentration of starch can vary considerably across different fruits, thereby influencing their texture, flavour, and nutritional advantages (Ho and Wang, 2020).

From the observed data in Table 4.1b, *Artocarpus heterophyllus* had the highest concentration of starch (222.67 ± 6.83 mg 100 g^{-1}), followed by *Ziziphus mauritiana* (147.05 ± 0.48 mg 100 g^{-1}), *Rubus treutleri* (89.84 ± 0.96 mg100 g^{-1}) which was statistically a par with *Prunus jenkinsii* (88.23 ± 0.16 mg 100 g^{-1}). In contrast, *Antidesma bunius* displayed the lowest quantity of starch (3.12 ± 0.004 mg 100 g^{-1}), which was statistically *at par* with *Embelia subcoriacea* (3.75 ± 0.26 mg 100 g^{-1}) and *Terminalia chebula* (4.12 ± 0.14 mg100 g^{-1}).

The hydrolysis of starch into sugars typically occurs during the maturation and ripening processes of fruits (Castillo *et al.*, 1997). Consequently, the variation in starch content observed among the underutilised fruits examined may be ascribed to the differing genetic compositions present within the species (Hazarika and Lalnunsangi, 2019). In the current investigation, of all the examined fruits, *Artocarpus heterophyllus* (Figure 4.9) exhibits the highest concentrations of starch, underscoring its considerable energy providing capacity and offering valuable insights into its nutritional characteristics. This suggests its applicability in the production of starch based bioplastics or its role as a thickening agent in food processing.

4.1.1.10 Total sugars (%)

The composition of sugars in fruits, encompassing glucose, fructose, and sucrose, plays a pivotal role in determining their sweetness and flavour profile. Fruits characterised by elevated sugar content are typically regarded as possessing greater

sweetness and are thus more appealing to consumers. Moreover, the sugars present in fruits serve as an immediate source of energy, as they can be rapidly metabolised into glucose, which is essential for cognitive processes and physical exertion (Yan *et al*., 2019). The total sugar composition in fruits encompasses both reducing and non-reducing sugars.

In the present investigation, as depicted from the data presented in Table 4.1b among all the studied fruits the maximum total sugar level was detected in *Emblica officinalis* (28.13 ± 1.33 %), which was statistically *at par* with *Terminalia chebula* (26.75 ± 1.79 %), *Garcinia xanthochymus* (20.93 ± 4.08 %) and *Mahonia nepaulensis* (20.56 ± 4.19 %). Conversely, *Antidesma bunius* exhibited the lowest total sugars (2.24 ± 0.25 %) which was statistically *at par* with *Citrus jambhiri* (2.28 ± 0.16 %), *Carallia brachiata* (2.60 ± 0.25 %) and *Citrus grandis* (2.72 ± 0.02 %) which all reported lower levels total sugar.

The sugar content observed in various species of underutilised fruits can be ascribed to the process of starch hydrolysis, resulting in the production of sucrose as the fruits mature and ripen (Angami *et al*., 2018). This phenomenon renders *Emblica officinalis, Terminalia chebula, Garcinia xanthochymus,* and *Mahonia nepaulensis* (Figure 4.10) significant sources of sugar, which ultimately contribute to energy and nutritional value. Assessing sugar levels is instrumental in ascertaining the ideal timing for harvest, thereby guaranteeing superior flavour and nutritional quality (Nair *et al*., 2012).

4.1.1.11 Reducing sugars (%)

The role of reducing sugars in the transfer of electrons to other molecules significantly influences the colour and flavour profiles of food products. The consumption of this substance offers critical nutrients such as fibre, protein, a low glycaemic index, and carbohydrates, all of which are indispensable for the sustenance of our bodies (Biswas *et al*., 2022). The sugars fulfil a range of biological roles, encompassing the provision of essential caloric intake and functioning as energy reserves within an organism.

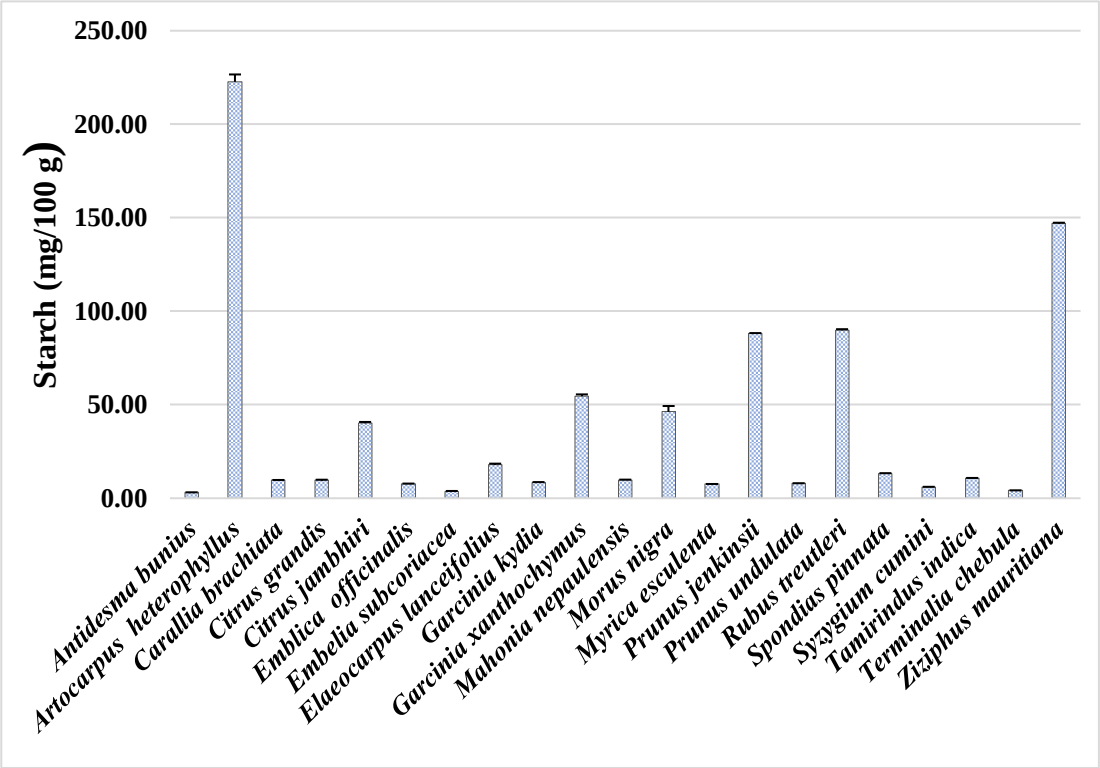


Fig. 4.9 Starch content of the selected underutilised fruits

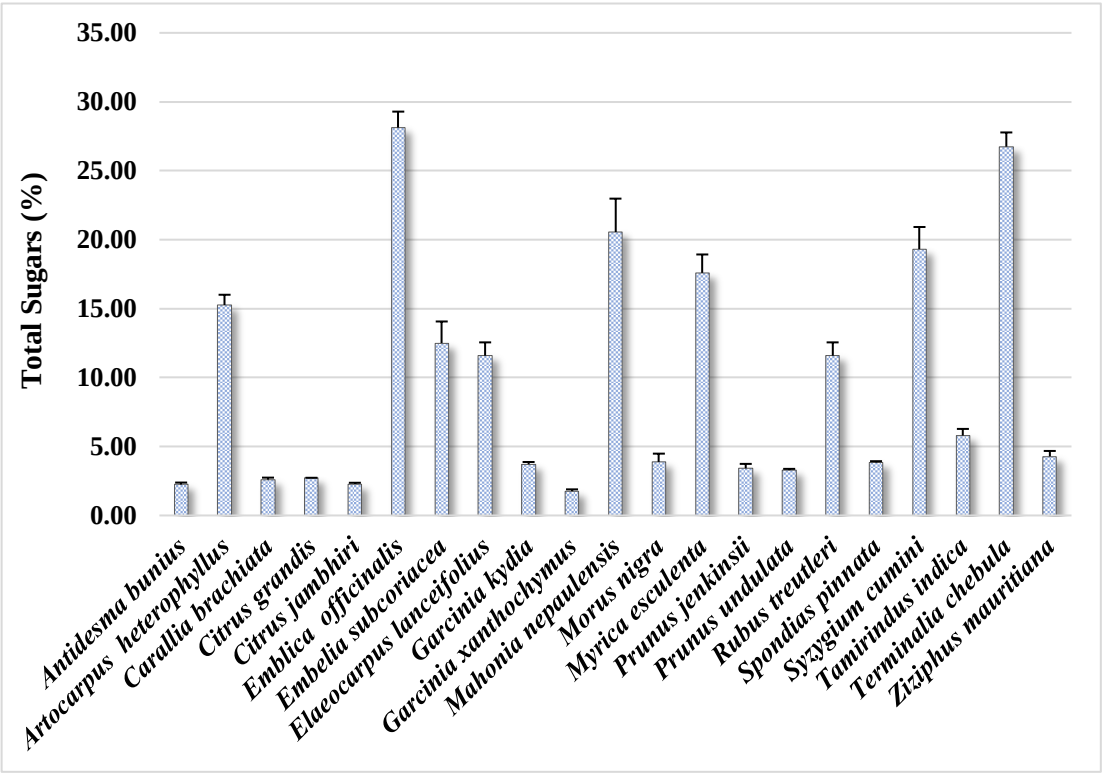


Fig. 4.10 Total sugars of the studied underutilised fruits

The recorded findings in Table 4.1b revealed that *Garcinia xanthochymus* exhibit the highest reducing sugars (14.61 ± 1.76 %), which was statistically *at par* with *Emblica officinalis* (14.44 ± 5.09 %), *Terminalia chebula* (12.19 ± 1.11 %) and *Syzygium cumini* (12.02 ± 2.15 %). Conversely, *Citrus jambhiri* displays the lowest reducing sugars (1.35 ± 0.42 %), which was statistically *at par* with *Antidesma bunius* (1.43 ± 0.10 %), *Morus nigra* (1.72 ± 0.72 %) and *Prunus jenkinsii* (1.90 ± 0.42 %).

The present values of *Terminalia chebula* and *Antidesma bunius* align closely with the results reported by Angami *et al*. (2024), which are 8.38 ± 0.07% and 0.37 ± 0.04%, respectively. The propensity to exhibit elevated or diminished levels of reducing sugar is an inheritable characteristic (Cunningham *et al*., 1963). Moreover, it is important to highlight that the presence of amylase, a potent enzyme found in plants, facilitates the breakdown of carbohydrates, which leads to the formation of polysaccharides and hydrolysis reactions throughout the ripening process, ultimately resulting in variations in reducing sugars (Al-Qarni, 2020). Elevated concentrations of reducing sugar in *Emblica officinalis* and *Terminalia chebula* (Figure 4.11) may contribute to the prevention and management of a range of health conditions, including obesity, type 2 diabetes, cardiovascular diseases, and the reducing sugar content in these fruits may play a significant role in promoting a healthier dietary regimen (Mahato *et al*., 2024).

4.1.1.12 Non-reducing sugars (%)

Non-reducing sugars include disaccharides such as sucrose and tetrasaccharides. These compounds are carbohydrates that do not possess a free aldehyde or ketone group, thereby preventing them from acting as reducing agents. This attribute holds significant implications for the development of flavour in cooked foods (Sakthivel *et al*., 2019).

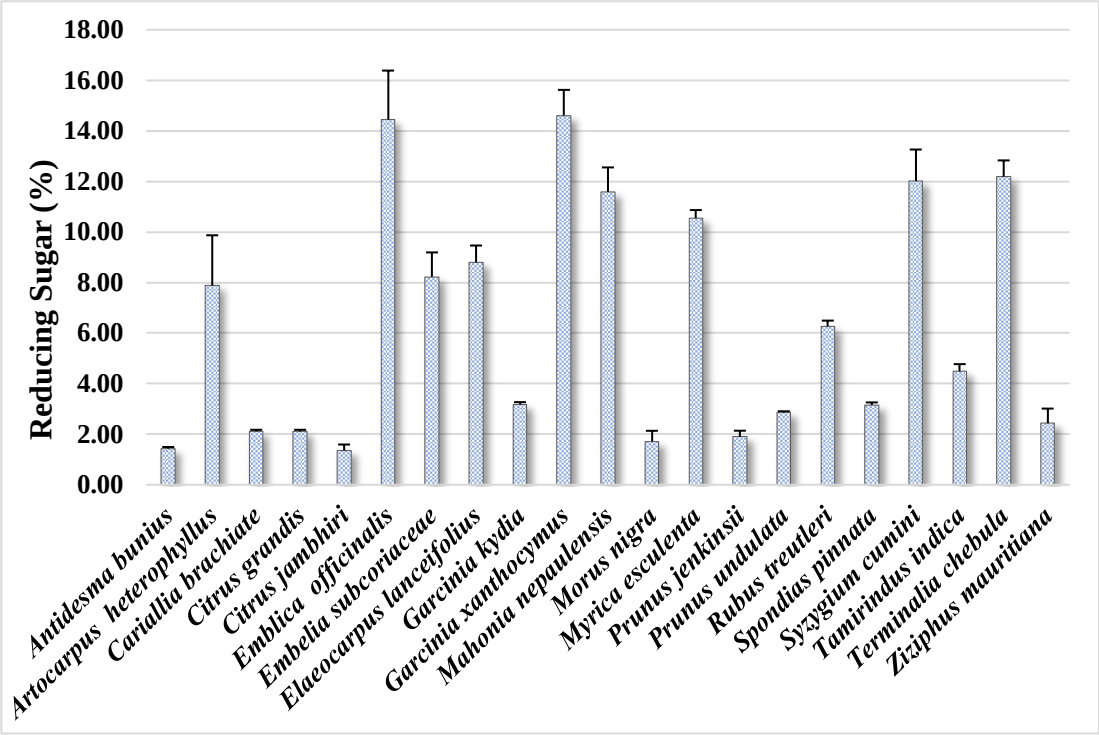


Fig. 4.11 Reducing sugar content of studied underutilised fruits

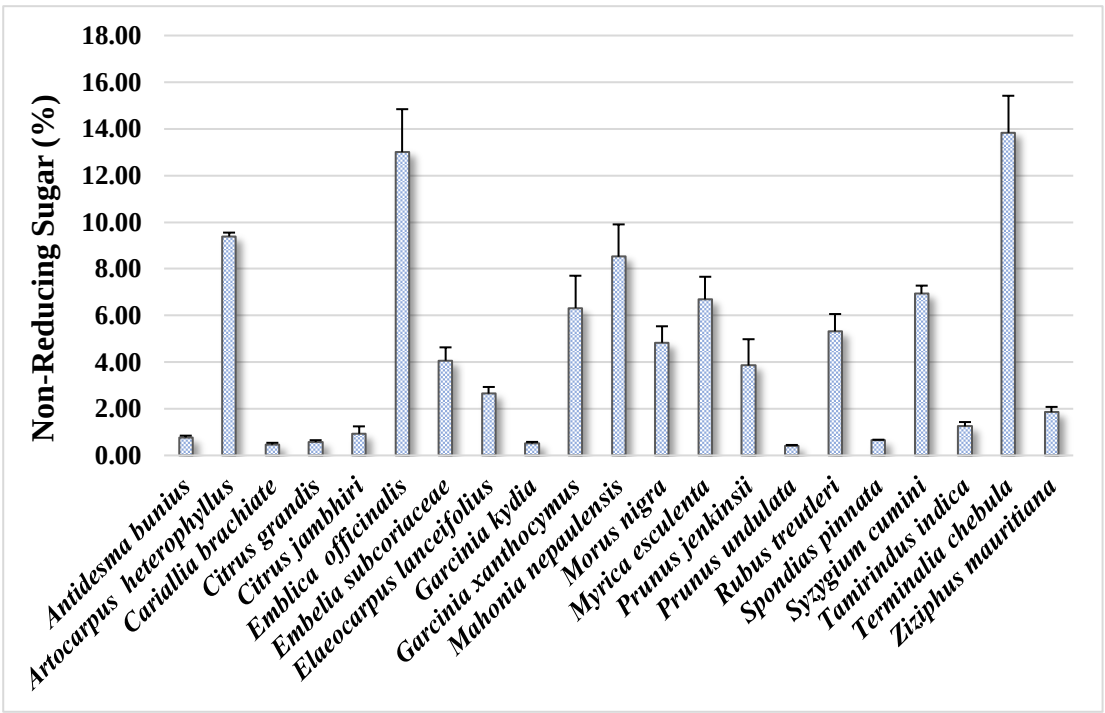


Fig. 4.12 Non- reducing sugar content of selected underutilised fruits

Table 4.1b displays the data for non-reducing sugars and was observed that *Terminalia chebula* had the highest concentration of non-reducing sugars (13.83 ± 2.75 %), followed by *Emblica officinalis* (13.00 ± 1.58 %) and *Artocarpus heterophyllus* (9.39 ± 0.28 %). In contrast, *Prunus undulata* exhibited the lowest non-reducing sugar (0.40 ± 0.08 %), which was statistically *at par* with *Carallia brachiata* (0.46 ± 0.13 %), *Garcinia kydia* (0.51 ± 0.12 %) *and Citrus grandis* (0.58 ± 0.12 %).

The variations in non-reducing sugar content among these fruits may be due to the decline in non-reducing sugars observed with the progression of maturity and during storage in underutilised fruits, potentially attributable to the hydrolysis or inversion of non-reducing sugars into reducing sugars (Biswas *et al*., 2022). The notable concentrations of non-reducing sugars identified in *Terminalia chebula* and *Emblica officinalis* (Figure 4.12) suggest their potential applicability in contexts where non-reducing sugars confer benefits in particular food preservation methodologies and formulation (Chaurasia *et al*., 2023).

4.1.1.13 Titratable acidity (%)

Microorganisms encounter significant challenges regarding their survival and growth when subjected to highly acidic foods that are characterised by low pH levels. The intrinsic acidity of a product fulfils a dual role, functioning both as a preservation technique and as a safeguard for food safety in consumption. Acidic fruits enhance the activity of digestive enzymes, facilitating the process of food breakdown (Hossain *et al*., 2021). The acidity of fleshy fruits, ascertainable through titratable acidity or pH measurements play a crucial role in influencing the sensory quality of the fruit (Bugaud *et al*., 2011).

The acidity levels observed in Table 4.1b revealed the highest acidity in *Citrus jambhiri* (6.19 ± 0.37 %), followed by *Citrus grandis* (5.64 ± 0.45 %) and *Tamarindus indica* (5.54 ± 0.98 %). In comparison, *Syzygium cumini* exhibited the lowest acidity levels (0.74 ± 0.25 %), which was statistically *at par* with *Artocarpus heterophyllus* (0.88 ± 0.09 %), *Mahonia nepaulensis* (0.88 ± 0.21 %) and *Spondias pinata* (0.88 ± 0.21 %).

The elevated acidity levels observed in these species can be attributed to the presence of primary organic acids, namely malic acid and citric acid, found in ripe fruits. These acids accumulate within the mesocarp cells throughout the fruit development process, which is influenced by both genetic and environmental factors. The reduced acidity observed in *Syzygium cumini*, *Artocarpus heterophyllus*, *Mahonia nepaulensis*, and *Spondias pinata* (Figure 4.13) demonstrates an inverse correlation with their elevated total soluble solids (TSS), rendering them more appealing to a broader demographic and establishing them as a vital nutritional resource (Julhia *et al*., 2019).

4.1.1.14 TSS (°Brix)

The Total Soluble Solids (TSS), conventionally measured in degrees Brix, represent the comprehensive concentration of all solubilised constituents within the fruit, including sugars, acids, proteins, and minerals. Total soluble solids (TSS) serve as a pivotal metric in the evaluation of fruit quality, significantly impacting flavour, texture, and overall consumer satisfaction (Hossain *et al*., 2021).

The investigation data depicted in Table 4.1b revealed that *Terminalia chebula* reported the highest TSS content (20.77 ± 0.32 ˚B), followed by *Artocarpus heterophyllus* (19.10 ± 2.31 ˚B) and *Mahonia nepaulensis* (16.07 ± 0.32 ˚B). On the other hand, *Antidesma bunius* had the lowest TSS content (3.27 ± 0.25 ˚B), while *Citrus grandis* reported lower TSS content (7.43 ± 0.40 ˚B), which was statistically *at par* with *Citrus jambhiri* (7.93 ± 0.35 ˚B).

The total soluble solids (TSS) level in *Terminalia chebula* aligns closely with the findings of Angami *et al*. (2024), recorded at 19.33 ± 0.24 ˚B, and *Antidesma bunius* exhibits a lower TSS level of 10.33 ± 0.24 ˚B. This discrepancy may be attributed to environmental stressors, particularly the limited availability of water in natural habitats, as such stress conditions typically result in an increased concentration of soluble solids, reflecting the plant's allocation of resources towards dry matter production (Rymbai *et al*., 2023). The heightened TSS levels in *Terminalia chebula, Elaeocarpus lancefolius*, and *Spondias pinnata* (Figure 4.14)

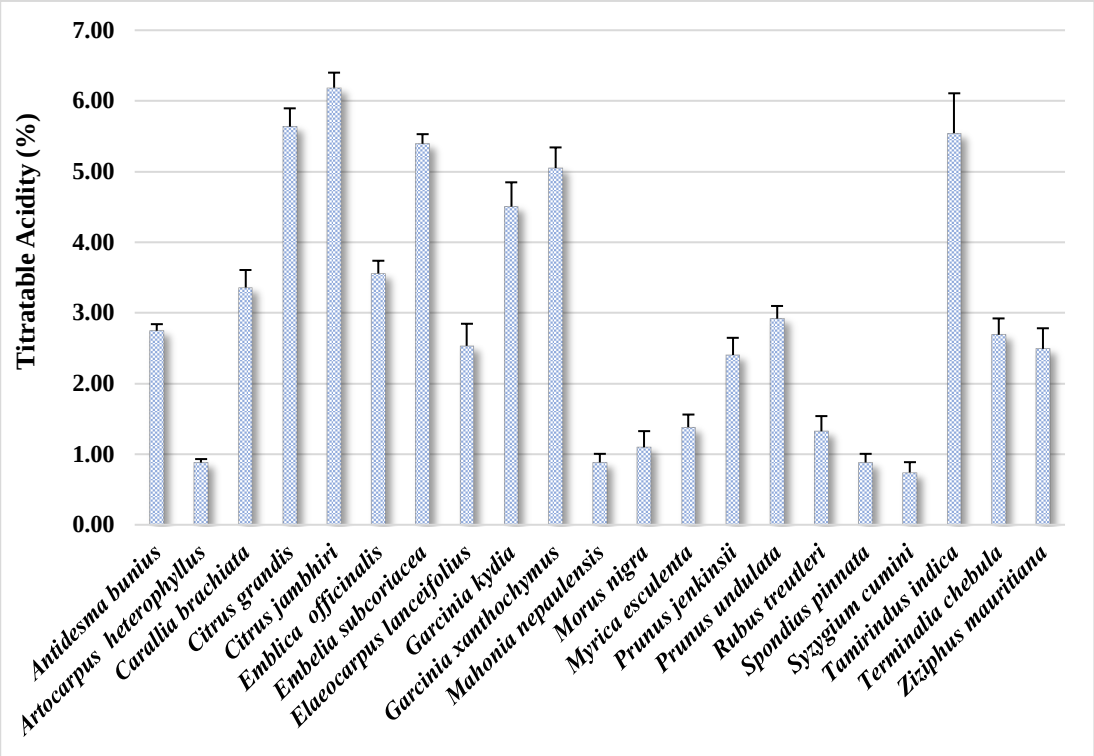


Fig 4.13 Titratable acidity of the selected underutilised fruits

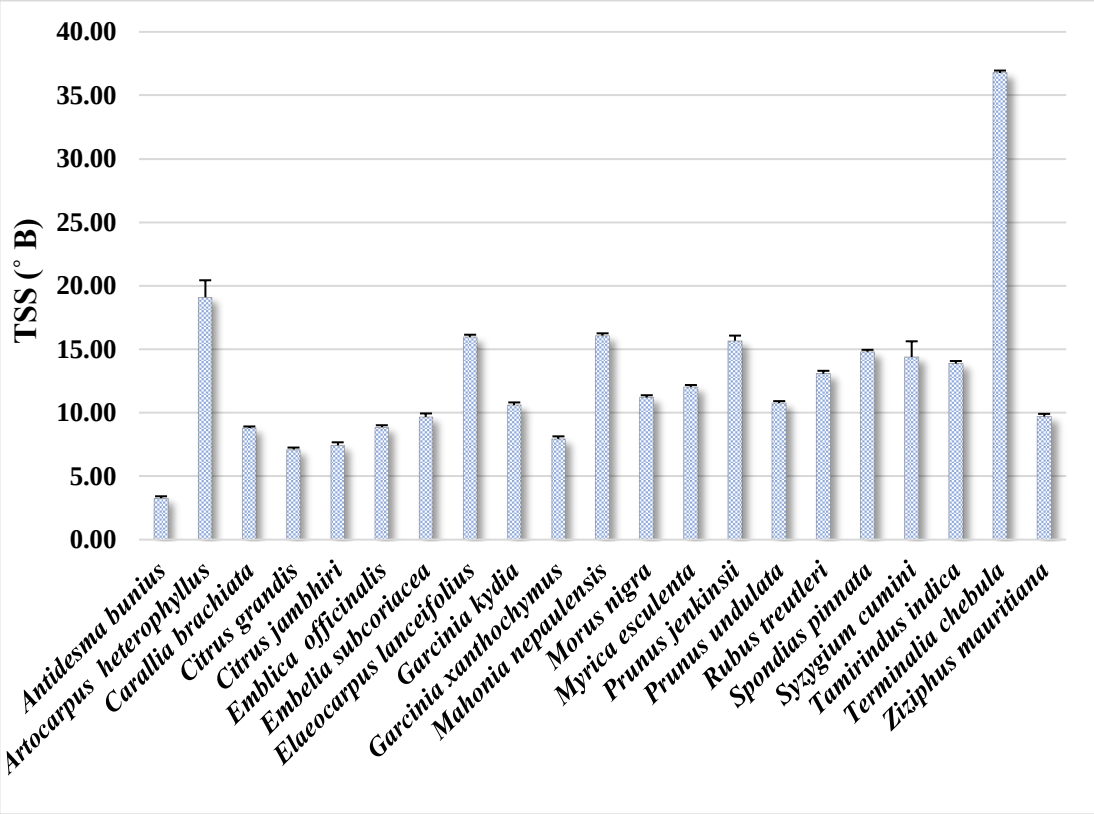


Fig 4.14 TSS content of the selected underutilised fruits

can be regarded as a significant criterion for assessing their quality traits, reflecting the maturity of the fruit and may indicate a more comprehensive nutritional profile (Sakthivel *et al*., 2019). *Terminalia chebula*, characterised by its bitter and astringent properties, demonstrated a significant level of total soluble solids (TSS). The underlying reason for this phenomenon may be attributed to the presence of various components, including organic acids, amino acids, and soluble materials, in addition to sugar, which collectively contributes to the total soluble solids (TSS) of this fruit (Crowther *et al*., 2005; Chope *et al*., 2006; Angami *et al*., 2024).

4.1.1.15 Lignin (%)

Lignin constitutes a complex organic polymer that imparts rigidity to plant cell walls; its presence may affect the texture and perceived freshness of fruit. This factor is essential for assessing the marketability of fruit and its acceptance by consumers (Zhu *et al*., 2018). Understanding the role of lignin in the lignification of fruit is essential for improving postharvest storage methods.

The most significant levels of lignin as observed in Table 4.1b were seen in *Tamarindus indica* (13.69 ± 0.01 %), followed by *Syzygium cumini* (12.66 ± 0.01 %) and *Embelia subcoriacea* (10.64 ± 0.01 %). The lowest levels of lignin were identified in *Terminalia chebula* (2.06 ± 0.01 %), which was statistically *at par* with *Artocarpus heterophyllus* (2.09 ± 0.58 %), *Prunus jenkinsii* (2.19 ± 0.665 %) and *Morus nigra* (2.31 ± 0.57 %).

The concentration of lignin in fruits generally rises with maturation, leading to variations in lignin content among reported underutilised fruits. The process of lignification involves the deposition of lignin, which can influence the ripening and softening of fruit. The elevated lignin concentrations in *Tamarindus indica* and *Syzygium cumini* (Figure 4.15) offer significant implications for their effective application in dietary planning and food processing, (Vinardell and Mitjans 2017). Effective management of lignin levels can mitigate quality deterioration and extend the shelf life of fruits. The activity of enzymes involved in lignin synthesis diminishes during the ripening process, leading to the development of softer and more palatable fruits (Khan *et al*., 2023).

4.1.1.16 Cellulose (mg 100 g^{-1})

Cellulose and hemicellulose are essential for imparting rigidity and strength to cell walls, thus preserving the structural integrity of fruits. The preservation of firmness and texture in fruits is crucial, as it directly influences their marketability and consumer appeal. Understanding the cellulose content in fruits is crucial for their processing and preservation, as it affects the texture and stability of processed fruit products such as jams, jellies, and dried fruits (Xu *et al*., 2023).

The present research data depicted in Table 4.1b revealed that *Embelia subcoriacea* had the highest cellulose content (75.43 ± 2.61 mg 100 g^{-1}), followed by *Myrica esculenta* (67.39 ± 0.65 mg 100 g^{-1}), which was shown to be statistically *at par* with *Carallia brachiata* (66.85 ± 1.63 mg 100 g^{-1}) and *Emblica officinalis* (65.87 ± 0.001 mg 100 g^{-1}), while *Mahonia nepaulensis* displayed the lowest cellulose (37.72 ± 0.54 mg 100 g^{-1}) content which was statistically *at par* with *Elaeocarpus lanceifolius* (39.02 ± 0.54 mg 100 g^{-1}) at the same time *Garcinia xanthochymus* (41.30 ± 0.22 mg 100 g^{-1}) recorded lower cellulose content and was found to be statistically *at par* with *Spondias pinnata* (41.85 ± 0.33 mg 100 g^{-1}).

The results demonstrate that *Embelia subcoriacea, Myrica esculenta, Carallia brachiata*, and *Emblica officinalis* (Figure 4.16) exhibit significant cellulose content, essential for preserving the structural integrity of fruits and enhancing resistance to environmental stressors. Additionally, cellulose functions as dietary fibre, fostering a healthy gut microbiome through its prebiotic effects. The elevated cellulose content in these fruits may be associated with their maturity level, exhibiting higher concentrations in the immature stage (de Souza and Kawaguti, 2021).

4.1.1.17 Hemicellulose (%):

Hemicellulose, cellulose, and lignin play essential roles in conferring structural integrity to plant cell walls. Hemicellulose facilitates the adhesion of cellulose fibres, thus improving the overall strength and rigidity of the cell wall. In contrast to cellulose, hemicellulose exhibits greater flexibility and is more susceptible to hydrolysis. The flexibility of cell walls is crucial for facilitating

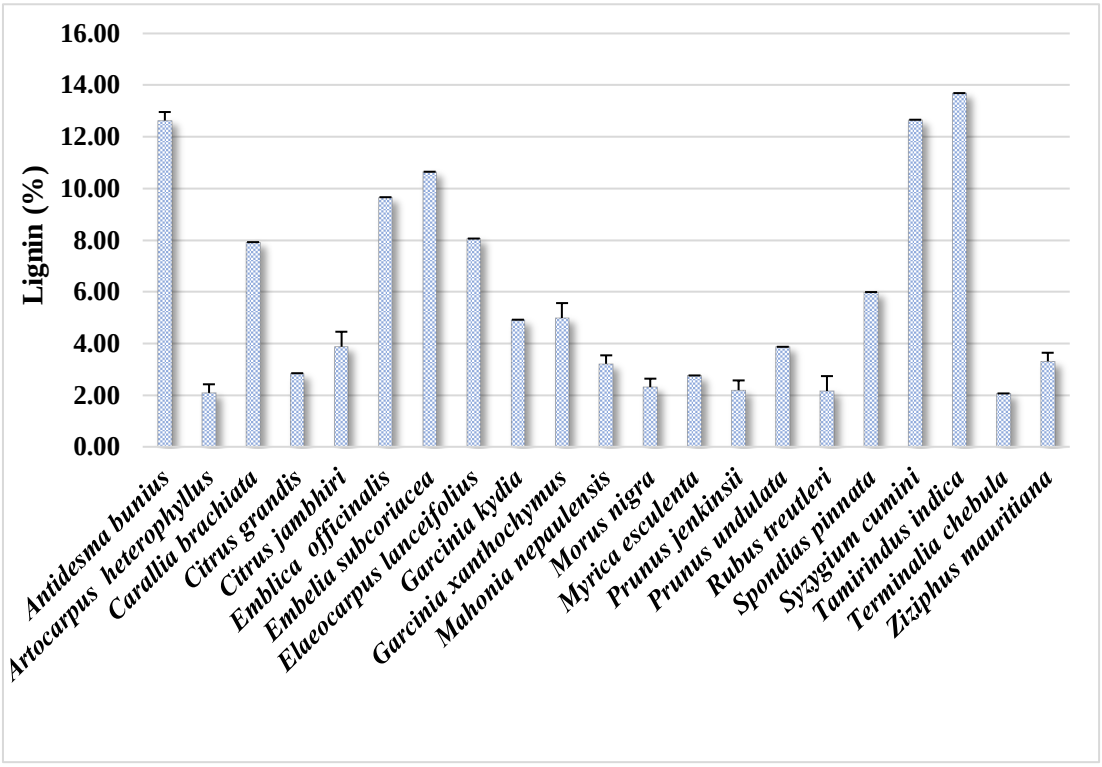


Fig 4.15 Lignin content of the selected underutilised fruits

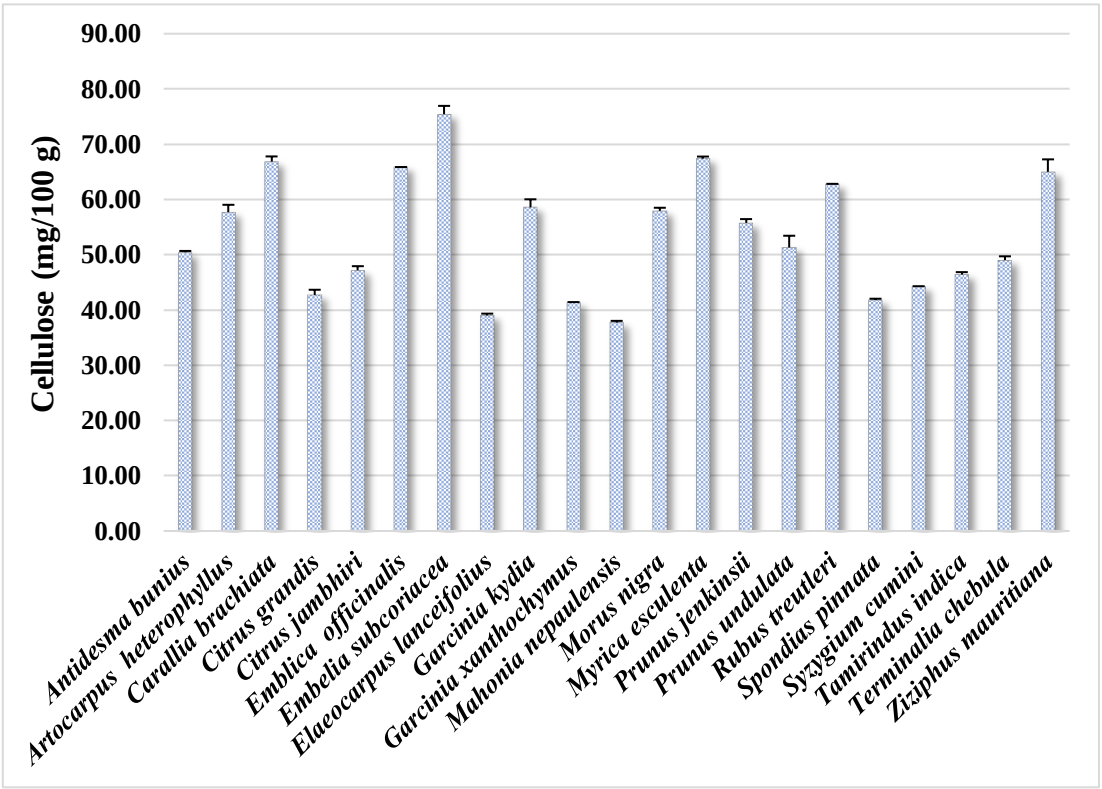


Fig 4.16 Cellulose content of the selected underutilised fruits

expansion and growth, especially during the processes of fruit development and ripening (de Souza and Kawaguti, 2021).

In the present investigation among all the studied fruits as presented in Table 4.1b the hemicellulose content was the highest in *Tamarindus indica* (12.09 ± 0.01%), followed by *Antidesma bunius* (6.84 ± 0.01 %) which was found to be statistically *at par* with *Elaeocarpus lanceifolius* (6.36 ± 0.01 %), *Mahonia nepaulensis* (6.40 ± 0.01 %) and *Spondias pinnata* (5.97 ± 0.01 %). Conversely, the lowest hemicellulose content was seen in *Carallia brachiata* (1.87 ± 0.01 %) and was observed to be statistically *at par* with *Citrus grandis* (1.98 ± 0.01 %), *Citrus jambhiri* (2.05 ± 0.99 %) and *Artocarpus heterophyllus* (2.33 ± 0.58 %).

The substantial hemicellulose content in *Tamarindus indica* and *Spondias pinnata* (Figure 4.17) may serve as a significant source of dietary fibre that resists human enzymatic digestion. This fibre undergoes fermentation by gut bacteria, resulting in the production of short-chain fatty acids that confer various health benefits, including the enhancement of gut health and a reduction in the risk of specific diseases (Szymańska-Chargot *et al*., 2017).

Comprehending the variations in nutritional values of the examined underutilised fruits is essential for their application in relation to plant biomass, as these variations can markedly influence the quality and efficiency of the resultant products. The variability in the collected data regarding the examined underutilised fruits is contingent upon the maturity stage and the analytical methodology utilised (Holtzapple 2003).

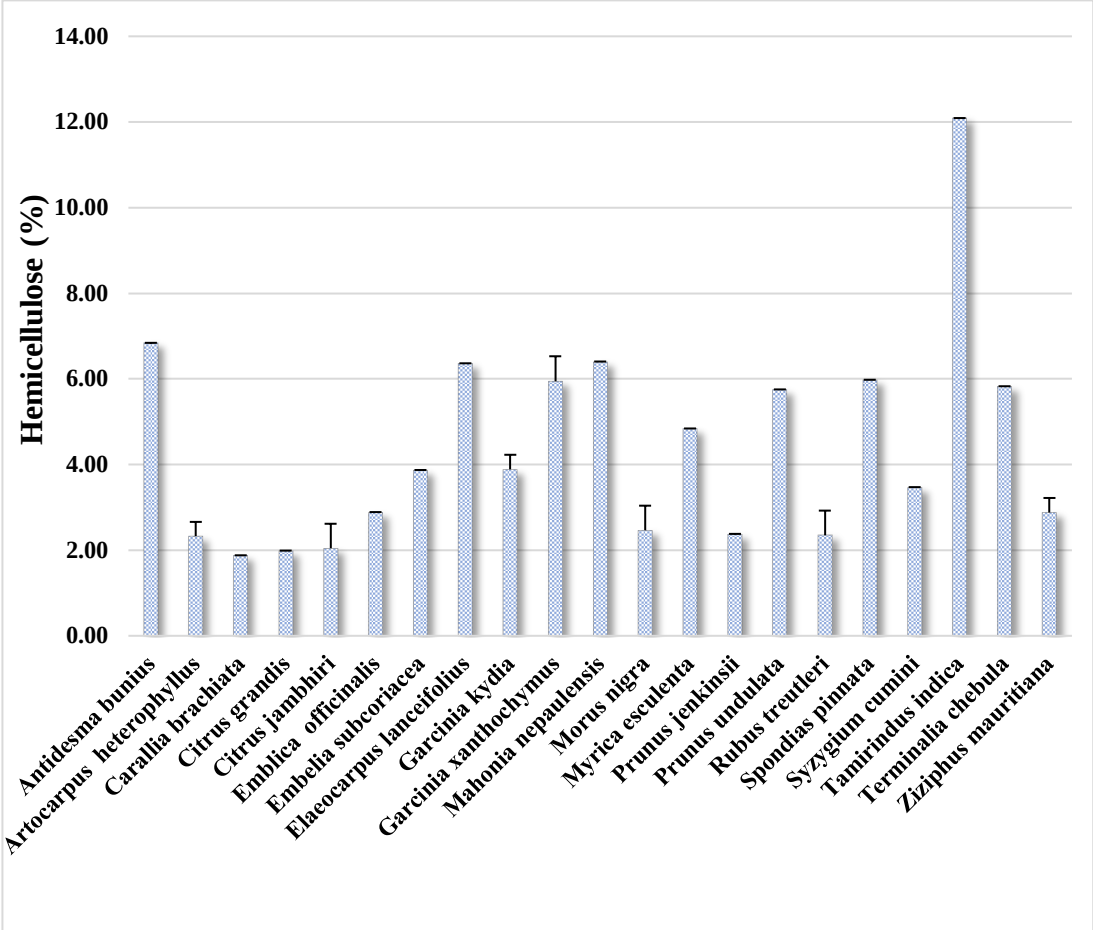


Fig 4.17 Hemicellulose content of the selected underutilised fruits

Table 4.1(b) Nutrient composition of underutilised fruits

Botanical Name	Starch (%)	Total Sugar (%)	Reducing Sugar %	Non-Reducing Sugar %	Titratable Acidity (%)	TSS (° B)	Lignin (%)	Cellulose (mg100 g^{-1})	Hemicellulose (%)
Antidesma bunius	3.13 ± 0.01^{a}	2.24 ± 0.25^{a}	1.43 ± 0.10^{a}	0.77 ± 0.14^{ab}	2.75 ± 0.16^{bc}	3.27 ± 0.25^{a}	12.63 ± 0.57^{i}	50.44 ± 0.43^{f}	6.84 ± 0.01^{g}
Artocarpus heterophyllus	222.67 ± 6.83^{k}	17.27 ± 3.29^{de}	7.88 ± 3.45^{cd}	9.39 ± 0.28^{de}	0.88 ± 0.09^{a}	19.10 ± 2.31^{m}	2.09 ± 0.58^{a}	57.72 ± 2.28^{g}	2.33 ± 0.58^{ab}
Carallia brachiata	9.76 ± 0.01^{bcd}	2.60 ± 0.25^{a}	2.11 ± 0.11^{a}	0.46 ± 0.13^{a}	3.36 ± 0.43^{cd}	8.80 ± 0.20cd	7.92 ± 0.01^{f}	66.85 ± 1.63^{i}	1.87 ± 0.01^{a}
Citrus grandis	9.62 ± 0.51^{bcd}	2.72 ± 0.02^{a}	2.11 ± 0.11^{a}	0.58 ± 0.12^{a}	5.64 ± 0.45^{fg}	7.10 ± 0.26^{b}	2.84 ± 0.01^{ab}	42.72 ± 1.63^{c}	1.98 ± 0.01^{ab}
Citrus jambhiri	40.10 ± 1.21^{f}	2.28 ± 0.16^{a}	1.35 ± 0.42^{a}	0.93 ± 0.54^{ab}	6.19 ± 0.37^{g}	7.43 ± 0.40^{b}	3.87 ± 1.00^{c}	47.17 ± 1.30^{e}	2.05 ± 0.99^{ab}
Emblica officinalis	7.84 ± 0.02^{bc}	28.13 ± 1.33^{g}	14.44 ± 5.09^{gh}	13.00 ± 1.58^{e}	3.56 ± 0.31^{d}	8.87 ± 0.25^{cd}	9.66 ± 0.01^{g}	65.87 ± 0.001^{i}	2.88 ± 0.01^{bc}
Embelia subcoriacea	3.75 ± 0.26^{a}	12.50 ± 2.71^{cde}	8.22 ± 1.68^{cd}	4.07 ± 0.98^{abcd}	5.39 ± 0.24^{f}	9.67 ± 0.47^{de}	10.64 ± 0.01^{h}	75.43 ± 2.61^{j}	3.87 ± 0.01^{d}
Elaeocarpus lanceifolius	18.11 ± 0.68^{e}	11.59 ± 1.67^{bcd}	8.80 ± 1.16^{cde}	2.65 ± 0.49^{abc}	2.53 ± 0.54^{b}	15.97 ± 0.31^{kl}	8.06 ± 0.01^{f}	39.02 ± 0.54^{ab}	6.36 ± 0.01^{fg}
Garcinia kydia	8.29 ± 0.59^{bc}	3.72 ± 0.28^{ab}	3.18 ± 0.15^{a}	0.51 ± 0.12^{a}	4.50 ± 0.60^{e}	10.60 ± 0.36^{ef}	4.91 ± 0.01^{d}	58.59 ± 2.50^{g}	3.89 ± 0.59^{d}
Garcinia xanthochymus	54.56 ± 1.69^{h}	20.93 ± 4.08^{efg}	14.61 ± 1.76^{h}	6.32 ± 2.40^{abcd}	5.05 ± 0.50^{ef}	7.93 ± 0.35^{bc}	4.98 ± 1.00^{d}	41.30 ± 0.22^{bc}	5.94 ± 1.01^{fg}
Mahonia nepaulensis	9.88 ± 0.14^{bcd}	20.56 ± 4.19^{efg}	11.59 ± 1.67^{efg}	8.52 ± 2.40^{cde}	0.88 ± 0.21^{a}	16.07 ± 0.32^{l}	3.21 ± 0.57^{bc}	37.72 ± 0.54^{a}	6.40 ± 0.01^{fg}
Morus nigra	46.21 ± 5.38^{g}	6.55 ± 1.48^{ab}	1.72 ± 0.72^{a}	4.83 ± 1.22^{abcd}	1.10 ± 0.40^{a}	11.20 ± 0.30^{fg}	2.31 ± 0.57^{a}	57.94 ± 0.98^{g}	2.47 ± 0.99^{ab}
Myrica esculenta	7.62 ± 0.02^{bc}	17.59 ± 2.31^{de}	10.55 ± 0.56^{def}	6.69 ± 1.67^{abcd}	1.38 ± 0.31^{a}	12.03 ± 0.25^{gh}	2.76 ± 0.01^{ab}	67.39 ± 0.65^{i}	4.84 ± 0.01^{e}
Prunus jenkinsii	88.23 ± 0.16^{i}	5.75 ± 1.54^{ab}	1.90 ± 0.42^{a}	3.86 ± 1.95^{abcd}	2.41 ± 0.42^{b}	15.67 ± 0.70^{kl}	2.19 ± 0.65^{a}	55.76 ± 1.20^{g}	2.38 ± 0.01^{ab}
Prunus undulata	8.00 ± 0.02^{bc}	3.28 ± 0.16^{ab}	2.86 ± 0.08^{a}	0.40 ± 0.08^{a}	2.92 ± 0.31^{bcd}	10.77 ± 0.25^{efg}	3.86 ± 0.01^{c}	51.30 ± 3.70^{f}	5.75 ± 0.01^{f}
Rubus treutleri	89.84 ± 0.96^{i}	11.59 ± 1.67^{bcd}	6.27 ± 0.39^{bc}	5.32 ± 1.28^{abcd}	1.32 ± 0.38^{a}	13.07 ± 0.40^{hi}	2.16 ± 1.00^{a}	62.83 ± 0.00^{h}	2.35 ± 1.00^{ab}
Spondias pinnata	13.16 ± 0.43d	3.85 ± 0.15^{ab}	3.16 ± 0.17a	0.66 ± 0.03^{ab}	0.88 ± 0.21^{a}	14.80 ± 0.26^{jkl}	5.98 ± 0.01^{e}	41.85 ± 0.33bc	5.97 ± 0.01^{fg}
Syzygium cumini	6.12 ± 0.001^{ab}	19.31 ± 2.79^{def}	12.02 ± 2.15^{fgh}	6.93 ± 0.60^{bcd}	0.74 ± 0.25^{a}	14.38 ± 2.15^{ijk}	12.66 ± 0.01^{i}	44.24 ± 0.11^{cd}	3.47 ± 0.01^{cd}
Tamirindus indica	10.68 ± 0.30^{cd}	5.79 ± 0.84^{abc}	4.48 ± 0.50^{ab}	1.25 ± 0.32^{ab}	5.54 ± 0.98^{fg}	13.87 ± 0.35^{ij}	13.69 ± 0.01^{j}	46.41 ± 0.76^{de}	12.09 ± 0.01^{h}
Terminalia chebula	4.12 ± 0.14a	26.75 ± 1.79^{fg}	12.19 ± 1.11^{fgh}	13.83 ± 2.75^{e}	2.69 ± 0.40^{bc}	20.77 ± 0.32^{n}	2.06 ± 0.01^{a}	49.02 ± 1.20^{ef}	5.82 ± 0.02^{f}
Ziziphus mauritiana	147.05 ± 0.48^{j}	4.28 ± 0.68^{abc}	2.43 ± 1.01^{a}	1.85 ± 0.39^{ab}	2.49 ± 0.50^{b}	9.70 ± 0.36^{de}	3.31 ± 0.58^{bc}	$65.\ 00 \pm 3.91^{hi}$	2.88 ± 0.59^{bc}

Values with different lower-case superscript ($^{a–d}$) letters in a column are significantly different among the fruits at $p < 0.05$ (DMRT test performed for separation of mean).
Values are expressed as mean ± SD with three replications (n = 3) for each experiment.

4.1.1.18 Correlation between proximate nutrient composition:

A linear regression analysis was conducted to ascertain the link among the proximate nutrient compositions in the fruits under investigation. A correlation approach is deemed effective in establishing the interrelationship between diverse variables that quantify factors associated with fruit quality. The correlation coefficients among the nutrients found in the analysis are presented in Table 4.2.

There was a strong negative association between moisture content with dry matter (r = -0.99), as well as fibre (r = -0.77), protein (r = -0.48), energy (r = -0.32), total sugar (r = -0.28), lignin (r = -0.60) and hemicellulose (r = -0.51). A positive correlation between moisture content and starch (r = 0.31) along with total ash (r = 0.27).

Similarly, dry matter and moisture had a strong negative correlation (r = -0.99) with starch (r = -0.32), and a strong positive correlation was exhibited for dry matter with fibre (r = 0.77), protein (r = 0.48), energy (r = 0.31), total sugar (r = 0.28), lignin (r = 0.61) and hemicellulose (r = 0.50).

The correlation coefficient (r = 0.27) indicates a positive relationship between total ash and moisture. Conversely, a negative correlation was observed between ash and fibre (r = -0.29), protein (r = -0.36), energy (r = -0.37), total sugar (r = -0.49), reducing sugar (r = -0.44), non-reducing sugar (r = -0.42), lignin (r = -0.30), cellulose (r = -0.44).

A negative correlation coefficient was exhibited between fibre and moisture (r = -0.77), ash (r = -0.30), carbohydrate (r = -0.28) and starch (r = -0.41), while a positive correlation was observed between fibre with dry matter (r = 0.77), protein (r = 0.34), lignin (r = 0.82) and hemicellulose (r = 0.43).

A significant positive correlation was exhibited between carbohydrates with energy (r = 0.64), total sugar (r = 0.38), non-reducing sugar (r= 0.29), TSS (r = 0.52) and conversely a significant negative association with fibre (r = -0.28) and acidity (r = -0.43).

Protein exhibit a negative correlation with moisture (r = -0.48), ash (r = -0.36) and starch (r = -0.38) while a high positive correlation with energy (r = 0.81), dry matter (r = 0.48), fibre (r = 0.34), total sugar (r = 0.59), reducing sugar (r = 0.44), non reducing sugar (r = 0.44), TSS (r = 0.50), lignin (r = 0.28) and hemicellulose (r = 0.35).

A strong positive correlation was observed for energy with protein (r = 0.81), total sugars (r = 0.70), TSS (r = 0.68), carbohydrate (r = 0.64) along with dry matter (r = 0.31), reducing sugar (r = 0.48), non-reducing sugars (r = 0.57) and hemicellulose (r = 0.28) conversely a negative correlation was observed between energy and moisture (r = -0.32), ash (r = -0.37) and starch (r = -0.27).

Starch recorded a positive correlation with moisture (r = 0.31) and a negative correlation with dry matter (r = -0.32), fibre (r = -0.41), protein (r = -0.38), energy (r = -0.27), acidity (r = -0.28), lignin (r = -0.44) and hemicellulose (r = -0.37).

A negative correlation was observed for total sugar with moisture (r = -0.28), ash (r = -0.49), and acidity (r = -0.34) while a strong positive correlation with non-reducing sugar (r = 0.89), reducing sugar (r = 0.76), energy (r = 0.70), dry matter (r = 0.28), carbohydrate (r = 0.38), protein (r = 0.59), and TSS (r = 0.52).

Reducing sugar was negatively correlated with dry matter (r = -0.44) with a positive correlation with protein (r = 0.44), energy (r = 0.48), total sugar (r = 0.78), non-reducing sugar (r = 0.28) and TSS (r = 0.28).

Non-reducing sugar exhibited a negative correlation between ash (r = -0.42) and acidity (r = -0.29) and a positive correlation with carbohydrate (r = 0.29), protein (r = 0.44), energy (r = 0.57), total sugar (r = 0.89), reducing sugar (r = 0.76) and TSS (r = 0.50).

Titratable acidity was negatively correlated with carbohydrate (r = -0.43), starch (r = -0.28), total sugar (r = -0.34), non-reducing sugar (r = -0.29) and TSS (r = -0.35)

TSS exhibited a negative correlation with acidity (r = -0.35) while reporting a positive correlation with carbohydrate (r = 0.52), protein (r = 0.50), energy (r = 0.68), total sugar (r = 0.52), reducing sugar (r = 0.35), non-reducing sugar (r = 0.50) and lignin (r = 0.29).

Lignin showed a high positive correlation with fibre (r = 0.817), dry matter (r = 0.61), protein (r = 0.28) and hemicellulose (r = 0.47). In contrast, lignin was negatively correlated with moisture (r = -0.60), total ash (r = -0.30), starch (r = -0.44) and TSS (r = -0.29).

Cellulose reported a negative correlation with total ash (r = -0.44) and hemicellulose (r = -0.44).

Hemicellulose exhibited negative correlation with moisture (r = -0.51), dry matter (r = -0.50), starch (r = -0.37) and cellulose (r = -0.44) while a positive correlation with fibre (r = 0.43), protein (r = 0.35), energy (r = 0.28) and lignin (r = 0.47).

Table 4.2 Correlation among the nutrient contents of the underutilised fruits

	Moisture	*Dry matter*	*Ash*	*Fibre*	*Carbohydrate*	*Protein*	*Fat*	*Energy*	*Starch*	*Total sugar*	*Reducing sugar*	*Non reducing sugar*	*Acidity*	*TSS*	*Lignin*	*Cellulose*	*Hemicellulose*
Moisture	1																
Dry matter	**-0.99****	1															
Ash	**0.27***	-0.23	1														
Fibre	**-0.77****	**0.77****	**-0.30***	1													
Carbohydrate	0.02	-0.03	-0.11	**-0.28***	1												
Protein	**-0.48****	**0.48****	**-0.36****	**0.34****	0.13	1											
Fat	-0.13	0.12	-0.20	-0.05	0.23	-0.21	1										
Energy	**-0.32***	**0.31***	**-0.37****	0.05	**0.64****	**0.81****	0.06	1									
Starch	**0.31***	**-0.30***	-0.08	**-0.41****	-0.04	**-0.38****	-0.21	**-0.27***	1								
Total sugar	**-0.28***	**0.28***	**-0.49****	0.17	**0.38****	**0.59****	0.00	**0.70****	-0.08	1							
Reducing sugar	-0.21	0.20	**-0.44****	0.15	0.22	**0.44****	0.01	**0.48****	-0.10	**0.78****	1						
Non reducing sugar	-0.13	0.12	**-0.42****	-0.01	**0.29***	**0.44****	-0.02	**0.57****	0.12	**0.89****	**0.76****	1					
Acidity	0.06	-0.04	0.15	0.08	**-0.43****	0.16	-0.15	-0.18	**-0.26***	**-0.34****	-0.17	**-0.29***	1				
TSS	0.05	-0.06	-0.17	-0.23	**0.52****	**0.50****	-0.18	**0.68****	0.10	**0.52****	**0.35****	**0.50****	**-0.35****	1			
Lignin	**-0.60****	**0.61****	**-0.30***	**0.82****	-0.24	**0.28***	-0.15	-0.02	**-0.44****	0.04	0.10	-0.14	0.24	**-0.29***	1		
Cellulose	0.02	-0.03	**-0.44****	-0.04	-0.18	0.17	0.18	0.06	0.20	0.10	-0.08	0.08	0.01	-0.18	-0.03	1	
Hemicellulose	**-0.51****	**0.50****	-0.01	**0.43****	0.23	**0.35****	-0.19	**0.28***	**-0.37****	0.01	0.16	-0.06	0.12	0.17	**0.47****	**-0.44****	1

**. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*

4.1.2 Mineral content of the fruits

Minerals constitute essential components of our diet. These elements fulfil various functions, such as offering structural support for bones, influencing muscle and nerve operations, and regulating the body's water balance (Weyh *et al*., 2022). Furthermore, they function as integral components of hormones, enzymes, and various physiologically active compounds. Specific minerals are essential for the optimal functioning of the immune system. This relates to both the innate defence system and the adaptive immune response. The presence of minerals can influence susceptibility to infections as well as the progression of chronic diseases (Gharibzahedi and Jafari, 2017). Mineral elements are distinct from other essential nutrients, such as proteins, fats, carbohydrates, and vitamins, and are crucial for a balanced diet. Micronutrient deficiencies represent a considerable public health challenge in numerous developing nations, particularly affecting infants and pregnant women (Batra and Seth, 2002).

The human body necessitates approximately twenty unique minerals for the maintenance of optimal physiological function. Minerals are typically classified into two main categories, micro-minerals and macro-minerals, according to their recommended daily intake rather than their relative importance or physiological functions (Morris and Mohiuddin, 2023). Macroelements, including calcium, phosphorus, magnesium, sulphur, sodium, and potassium, are typically necessitated in amounts exceeding 100 mg per day. Conversely, microminerals are required in amounts less than 100 mg per day and include vital elements such as Fe, Zn, I, Se, Mn, Cr, Cu, Mo, F, B, Co, Si, Al, Ar, Sn, Li, and Ni ((Morris and Mohiuddin, 2023). Minerals, essential for promoting optimal biological functions within the human body, constitute a significant advantage of fruit consumption. The Atomic Absorption Spectrometer (AAS) is recognised as a reliable tool for the quantitative analysis of mineral compositions in fruit. This investigation involved a comprehensive analysis of eleven elements: nitrogen, phosphorus, potassium, calcium, cobalt, copper, iron, manganese, magnesium, sodium, and zinc. The findings of this analysis are delineated in Table 4.3

4.1.2.1 Macroelements

The examination of underutilised fruits involves the analysis of macro-elements, including nitrogen, phosphorus, potassium, calcium, magnesium, and sodium. These elements are crucial in considerable amounts for sustaining a balanced diet, rendering them a significant factor in evaluating the nutritional value of fruits. Nitrogen and phosphorus are integral to various biological processes and are an essential component as fundamental units of DNA and RNA, playing a critical role in the storage and transmission of genetic information. Furthermore, phosphorus exists as phospholipids, which play a crucial role in maintaining the structural integrity of cell membranes. Moreover, nitrogen and phosphorus play a crucial role in the synthesis of adenosine triphosphate (ATP), which serves as the principal energy currency within cells. The phosphorylation of various proteins and carbohydrates is essential (Sun *et al*., 2020).

4.1.2.1.1 Nitrogen (N)

Nitrogen (N) is a critical component of amino acids, which are the fundamental building blocks of proteins. Proteins are essential for the development, restoration, and maintenance of bodily tissues. Nitrogen serves as a fundamental element in nucleotides, which are the foundational units of DNA and RNA. These molecules play a vital role in the storage and transmission of genetic information (Brender, 2020).

The results of the present study depicted in Table 4.3 revealed that *Citrus grandis* exhibited the highest N content (12.14 ± 0.49 %), followed by *Myrica esculenta* (6.85 ± 0.19 %) which is statistically *at par* with *Citrus jambhiri* (6.05 ± 0.13 %). *Embelia subcoriacea* recorded the least N content (0.41 ± 0.03%) while *Morus nigra* (2.76 ± *0.01 %*), *Terminalia chebula* (2.88 ± *0.019 %*) and *Mahonia nepaulensis* (2.76 ± 0.01 %) had a comparatively lower quantity of N and are statistically *at par* with each other,

The differences in N composition among various fruits can be ascribed to the nitrogen levels present in the soil environment. The nitrogen content observed in *Morus nigra* aligns with the range reported by Mehta and Kumar, (2021). The findings

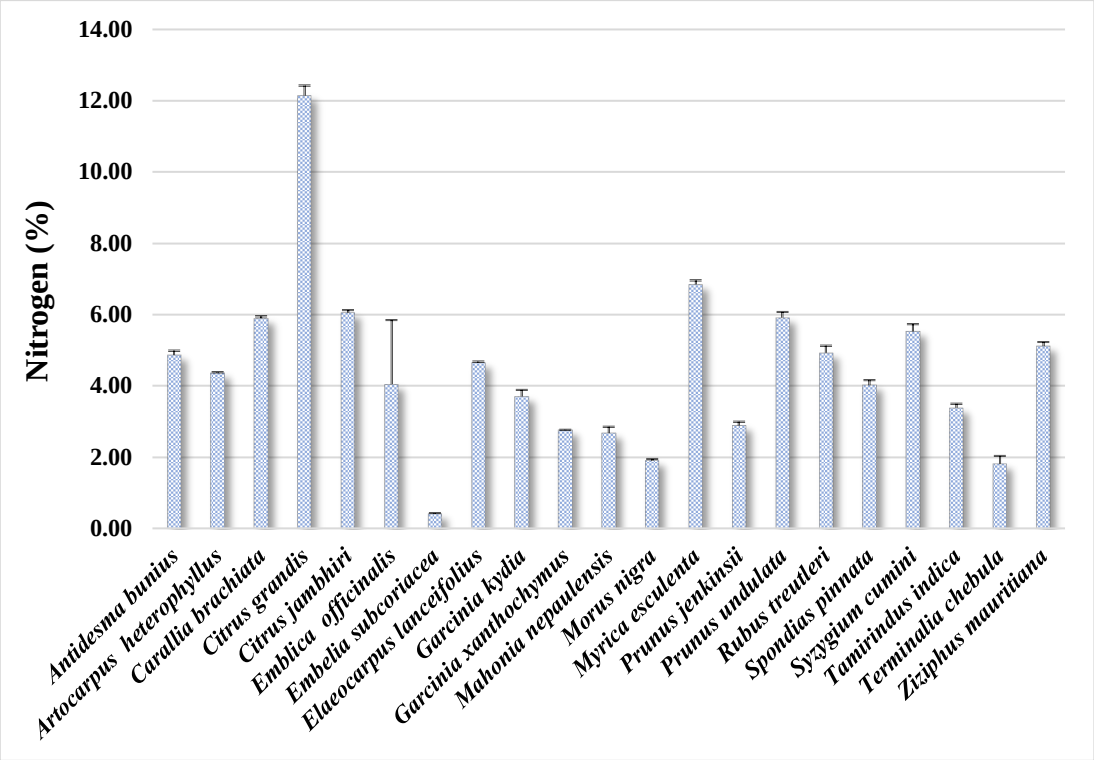


Fig 4.18 Nitrogen content of the selected underutilised fruits

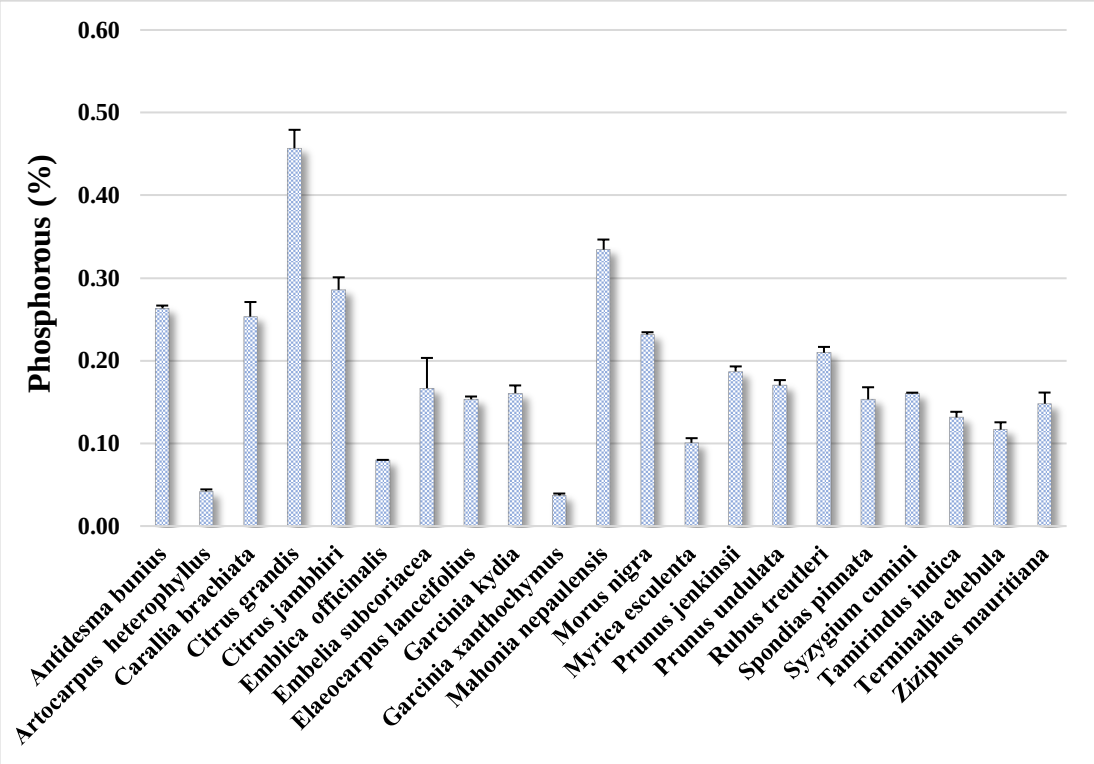


Fig 4.19 Phosphorus content of the selected underutilised fruits

underscore the significance of *Citrus grandis* and other species with elevated nitrogen levels, indicating their strong potential for protein synthesis. This is advantageous for human consumption, contributes to dietary nitrogen intake, and may enhance soil fertility through the decomposition of organic matter. Conversely, *Embelia subcoriacea* (Figure 4.18) exhibits the lowest nitrogen content, potentially indicative of varying soil conditions or genetic influences.

4.1.2.1.2 Phosphorus (P)

Phosphorus (P) constitutes a vital element of adenosine triphosphate (ATP), the molecule that facilitates the storage and transfer of energy within cellular structures. This is essential for energy production and metabolic processes. Phosphorus, in conjunction with calcium, plays a critical role in the development and maintenance of robust bones and teeth. Approximately 85% of the body's phosphorus is found within the skeletal system, specifically in bones and teeth (Bird *et al*., 2021).

The results of our present investigation recorded in Table 4.3 revealed that *Citrus grandis* had the highest P content (0.46 ± 0.04 %), followed by *Mahonia nepaulensis* (0.33 ± 0.02 %*)* and *Citrus jambhiri* (0.29 ± 0.03 %). Conversely, *Garcinia xanthochymus* and *Artocarpus heterophyllus* displayed the lowest P levels (0.04 ± 0.004 %) and (0.04 ± 0.005 %) respectively which are statistically *at par* while *Emblica officinalis* (0.08 ± 0.001 %) and *Myrica esculenta* (0.10 ± 0.01 %) showed very less P content.

The elevated phosphorus levels in *Citrus grandis*, *Mahonia nepaulensis*, and *Citrus jambhiri* (Figure 4.19) suggest their possible nutritional advantages, especially regarding bone health and energy metabolism. The phosphorus content in *Artocarpus heterophyllus* aligns with the values documented by Kamdem *et al*. (2023). The elevated phosphorus content in edible plants may be attributed to both environmental factors and the genetic composition of the plant (Onwordi *et al*., 2009). The phosphorus content in *Emblica officinalis* exceeded the values documented by Bulo *et al*. (2024).

4.1.2.1.3 Potassium (K)

Potassium (K) plays a crucial role in counteracting the effects of sodium and alleviating tension in blood vessel walls, thereby contributing to the reduction of

blood pressure. The consumption of potassium is associated with a reduced risk of stroke and cardiovascular diseases. Furthermore, potassium is crucial for the proper functioning of muscles, encompassing the contraction of both skeletal and smooth muscle types (Cruz *et al*., 2017). This is essential for sustaining consistent digestive and muscular function.

The results of our present investigation depicted in Table 4.3 indicate that *Terminalia chebula* had the greatest K content (4.37 ± 0.10 %), followed by *Elaeocarpus lanceifolius* (4.00 ± 0.03 %*)* and Prunus undulata (3.93 ± 0.05 %). Additionally, *Garcinia xanthochymus* demonstrated the lowest K content (0.03 ± 0.004 %) among the tested samples and is significantly *at par* with *Artocarpus heterophyllus* (0.41 ± 0.03 %) and *Citrus jambhiri* (0.22 ± 0.001 %) which showed relatively lower levels of K.

The elevated potassium concentration in *Terminalia chebula* (Figure 4.20) underscores its importance in the regulation of various metabolic processes, including glucose and protein metabolism, as well as the maintenance of fluid balance within the body. This is essential for cellular function, overall homeostasis, and various physiological activities, including the regulation of blood pressure, muscle function, and nerve signalling (D'Elia, 2024).

4.1.2.1.4 Calcium (Ca)

Calcium (Ca) is a vital macroelement integral to neuromuscular function and the mineralisation of the skeletal system (Lombardi *et al*., 2020). Approximately 99% of the body's calcium is sequestered within the skeletal system, specifically in bones and teeth, which are essential for providing structural support and strength. Calcium ions play a crucial role in the transmission of nerve impulses. They promote the release of neurotransmitters, which are chemical agents essential for the transmission of impulses between neuronal cells (Li *et al*., 2018).

The Ca concentration observed in Table 4.3 was found to be highest in *Morus nigra* (474.42 ± 14.56 mg 100 g^{-1}), followed by *Tamarindus indica* (397.7 ± 2.47 mg 100 g^{-1}) and *Antidesma bunius* (376.10 ± 4.88 mg 100 g^{-1}) and are statistically *at par* with each other. Conversely, *Terminalia chebula* had the lowest Ca level (7.40 ± 5.93 mg 100 g^{-1}) which was statistically *at par* with *Embelia subcoriacea*

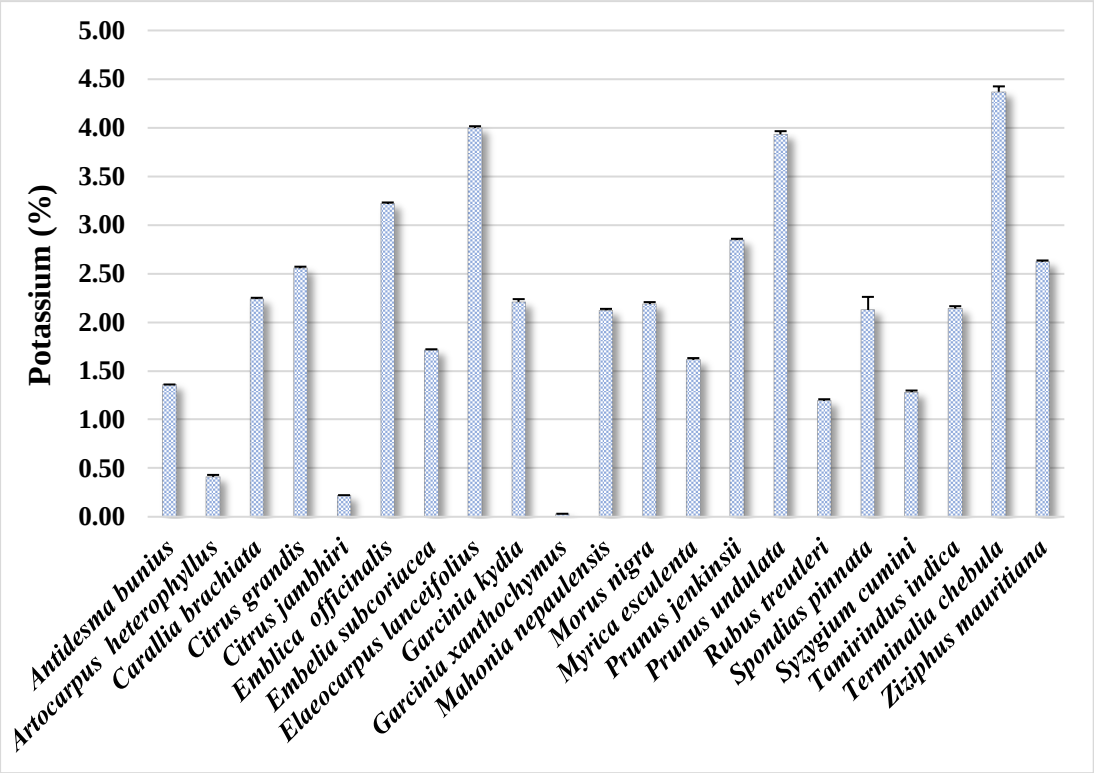


Fig 4.20 Potassium content of the selected underutilised fruits

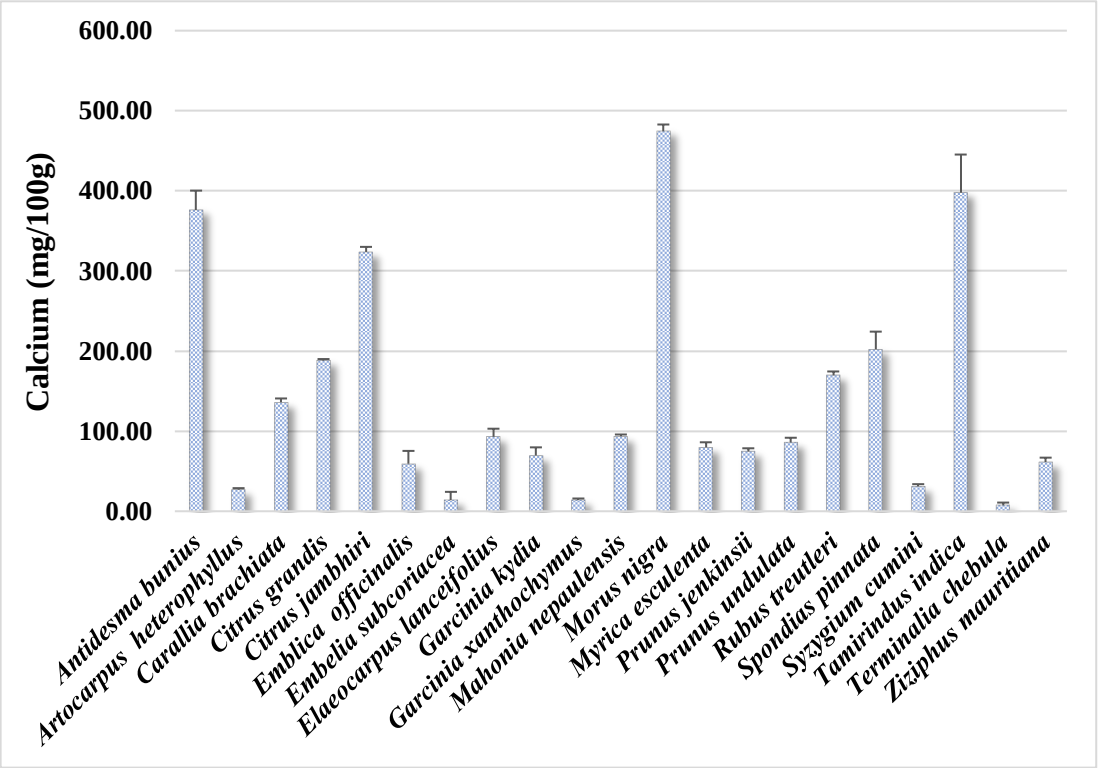


Fig 4.21 Calcium content of the selected underutilised fruits

(13.95 ± 1.75 mg 100 g^{-1}) and *Garcinia xanthochymus* (14.03 ± 3.28 mg 100 g^{-1}).

The current study reveals that *Morus nigra* exhibits the highest calcium content among the examined fruits, which is advantageous for bone health and neuromuscular function. The findings of this study align with the calcium content (94.37 mg 100 g^{-1}–217.77 mg 100 g^{-1}) documented for underutilised plant species in Ethiopia (Mokria *et al*., 2022). The calcium content in *Garcinia xanthochymus* aligns with the findings of Parthsarathy and Nandakishore, (2014), which reported a level of 13.7 mg/100g.

4.1.1.1.5 Magnesium (Mg)

Magnesium (Mg) serves as a co-factor for over 300 enzymatic activities, playing a crucial role in energy generation, DNA synthesis, and protein synthesis. It is essential in processes such as adenosine triphosphate (ATP) synthesis, oxidative phosphorylation, and glycolysis (Grober *et al*., 2015). Magnesium plays a crucial role in facilitating the active transport of calcium and potassium ions across cellular membranes. This process is critical for various physiological functions, including nerve impulse transmission, muscular contraction, maintenance of vasomotor tone, and regulation of heart rhythm (Firouzi *et al*., 2015; Goff 2018).

In our present investigation represented in Table 4.3, among all the studied fruits, *Prunus undulata* exhibited the maximum values for Mg (377.33 ± 3.45 mg 100 g^{-1}) which was statistically *at par* with *Carallia brachiata* (373.67 ± 4.78 mg 100 g^{-1}) and *Mahonia nepaulensis* (353.33 ± 3.40 mg 100 g^{-1}). On the other hand, *Artocarpus heterophyllus* exhibited the lowest value (28.33 ± 15.04 mg 100 g^{-1}) which was statistically *at par* with *Garcinia xanthochymus* (31.62 ± 1.20 mg 100 g^{-1}) while *Terminalia chebula* (98.17±5.22 mg 100 g^{-1}) and *Citrus grandis* (108.17±3.27 mg 100 g^{-1}) reported lower content and are statistically at par with each other.

The results suggest that *Prunus undulata* (Figure 4.22) could serve as a valuable source of magnesium, essential for various physiological functions including enzyme activity, muscle and neuronal function, bone health, regulation of blood glucose, cardiovascular health, and mental health (Tian *et al*., 2021). The values obtained for *Artocarpus heterophyllus* and *Garcinia xanthochymus* align with

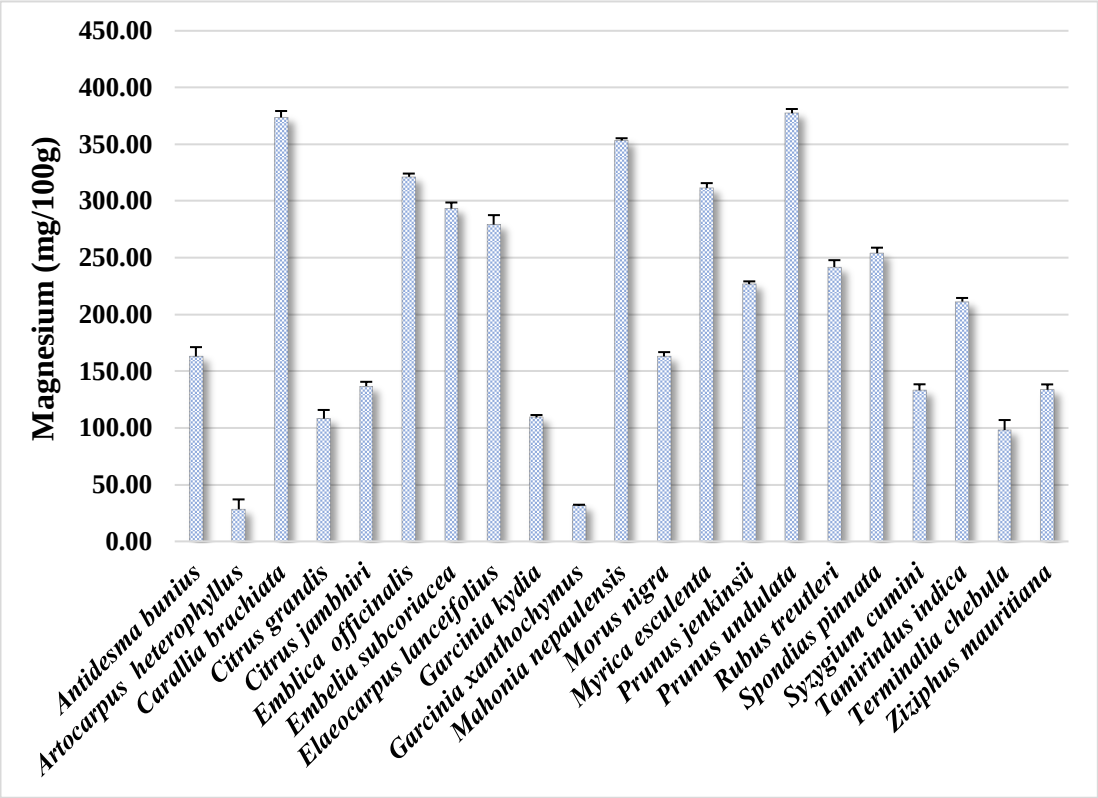


Fig 4.22 Magnesium content of the selected underutilised fruits

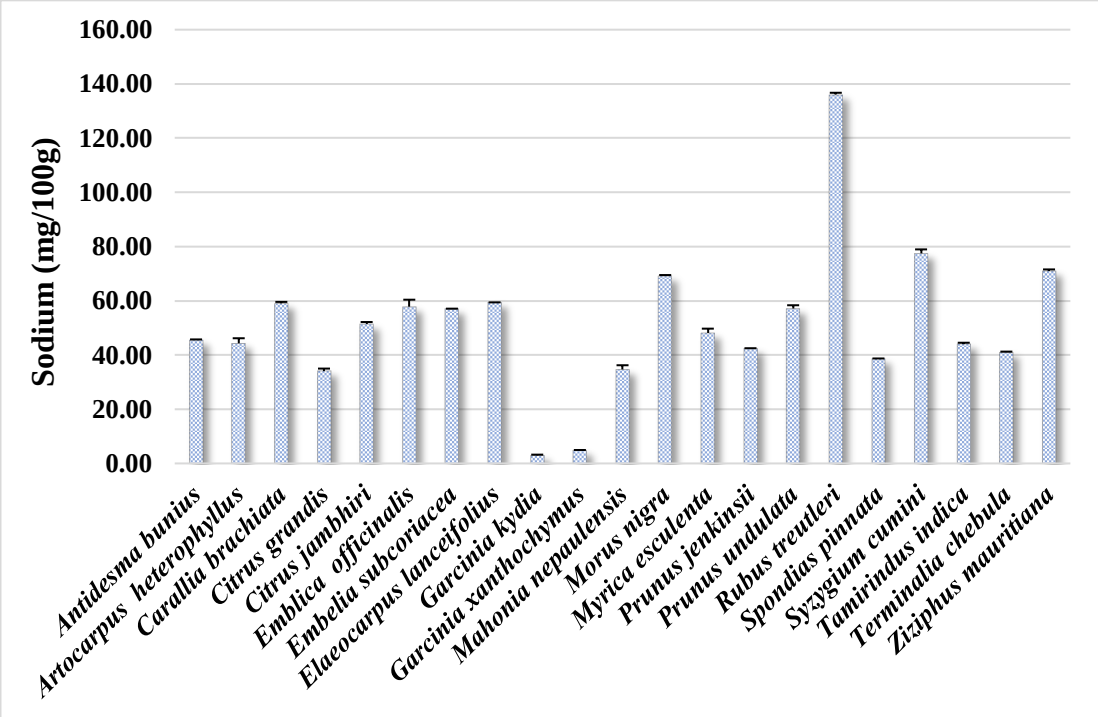


Fig 4.23 Sodium content of the selected underutilised fruits

Table 4.3 The mineral content of the selected underutilised fruits

Botanical Name	*N (%)*	*P (%)*	*K (%)*	*Ca (mg100 g^{-1})*	*Mg (mg100 g^{-1})*	*Na (mg100 g^{-1})*	*Co (mg100 g^{-1})*	*Cu (mg100 g^{-1})*	*Fe (mg100 g^{-1})*	*Mn (mg100 g^{-1})*	*Zn (mg100 g^{-1})*
Antidesma bunius	4.87 ± 0.21^{fghij}	0.26 ± 0.01^{ij}	1.36 ± 0.01^{b}	376.10 ± 4.88^{h}	163.00 ± 4.85^{e}	45.59 ± 0.26^{fg}	0.10 ± 0.03^{bcdef}	2.72 ± 0.18^{abcd}	8.17 ± 0.02k	13.11 ± 0.07i	3.50 ± 0.04
Artocarpus heterophyllus	4.36 ± 0.03^{efgh}	0.04 ± 0.004^{a}	0.41 ± 0.03^{a}	27.07 ± 3.07^{ab}	28.33 ± 5.04^{a}	44.33 ± 3.21^{ef}	0.29 ± 0.03^{i}	1.45 ± 0.58^{abcd}	1.13 ± 0.11^{a}	11.41 ± 1.04^{h}	5.41 ± 1.06
Carallia brachiata	5.90 ± 0.09^{ijk}	0.25 ± 0.03^{ij}	2.24 ± 0.02^{cde}	136.00 ± 4.32^{e}	373.67 ± 4.78^{l}	59.05 ± 0.93^{i}	0.11 ± 0.02^{cdefg}	2.09 ± 0.01^{abcd}	7.56 ± 0.05^{jk}	15.89 ± 0.02^{j}	4.05 ± 0.03
Citrus grandis	12.14 ± 0.49^{l}	0.46 ± 0.04^{l}	2.56 ± 0.03^{de}	188.05 ± 3.39^{f}	108.17 ± 3.27^{bc}	34.06 ± 1.62^{b}	0.03 ± 0.02^{ab}	1.42 ± 0.26^{abcd}	1.46 ± 0.32^{ab}	0.50 ± 0.04^{a}	2.95 ± 0.25^{l}
Citrus jambhiri	6.05 ± 0.13^{jk}	0.29 ± 0.03^{j}	0.22 ± 0.001^{a}	323.72 ± 1.97^{g}	136.83 ± 3.60^{de}	51.47 ± 1.21^{h}	0.11 ± 0.05^{bcdefg}	1.01 ± 0.002^{ab}	2.11 ± 0.02^{abc}	0.61 ± 0.01^{a}	2.57 ± 0.00
Emblica officinalis	4.05 ± 3.11^{defg}	0.08 ± 0.001^{b}	3.22 ± 0.02^{f}	59.30 ± 2.80^{bcd}	321.33 ± 4.91^{k}	57.82 ± 4.48^{i}	0.05 ± 0.04^{abcd}	1.82 ± 0.01^{abcd}	5.90 ± 0.05^{ghi}	4.24 ± 0.02^{f}	4.34 ± 0.01
Embelia subcoriacea	0.41 ± 0.03^{a}	0.17 ± 0.06^{ef}	1.71 ± 0.02^{bc}	13.95 ± 1.75^{a}	293.33 ± 3.13^{ij}	56.98 ± 0.19^{i}	0.18 ± 0.05^{gh}	2.35 ± 0.01^{abcd}	5.01 ± 0.02^{fgh}	1.92 ± 0.01^{cd}	3.32 ± 0.01
Elaeocarpus lanceifolius	4.65 ± 0.05^{efghi}	0.15 ± 0.01^{def}	4.00 ± 0.03^{g}	92.95 ± 1.47^{d}	279.17 ± 4.51^{i}	59.37 ± 0.08^{i}	0.02 ± 0.01a	1.11 ± 0.44^{ab}	5.64 ± 2.84^{ghi}	9.89 ± 0.08^{g}	4.54 ± 0.03
Garcinia kydia	3.70 ± 0.32^{cdef}	0.16 ± 0.02^{ef}	2.21 ± 0.05^{cd}	69.65 ± 1.53^{bcd}	109.33 ± 3.51^{bc}	30.06 ± 0.33^{a}	3.95 ± 0.10^{k}	5.78 ± 0.75^{e}	43.09 ± 1.56^{n}	11.30 ± 0.12^{h}	6.85 ± 0.25
Garcinia xanthochymus	2.76 ± 0.01^{bcd}	0.04 ± 0.004^{a}	0.03 ± 0.004^{a}	14.03 ± 3.28^{a}	31.62 ± 1.20^{a}	4.94 ± 0.02^{a}	0.32 ± 0.02^{i}	3.49 ± 1.41^{bcd}	10.89 ± 1.03^{l}	2.08 ± 0.002^{d}	2.92 ± 0.03
Mahonia nepaulensis	2.68 ± 0.31^{bc}	0.33 ± 0.02^{k}	2.12 ± 0.02^{cd}	93.65 ± 3.77^{d}	353.33 ± 3.40^{l}	34.69 ± 2.6b^{e}	0.16 ± 0.02^{fgh}	3.85 ± 0.25^{de}	13.67 ± 0.83^{m}	9.48 ± 0.20^{g}	6.32 ± 0.15
Morus nigra	1.89 ± 0.09^{b}	0.23 ± 0.005^{hi}	2.19 ± 0.03^{a}	474.42 ± 4.56^{i}	163.00 ± 3.61^{e}	69.18 ± 0.53^{j}	0.07 ± 0.02^{abcde}	0.45 ± 0.04^{a}	4.00 ± 0.90^{def}	4.27 ± 0.04^{f}	6.42 ± 0.33
Myrica esculenta	6.85 ± 0.19^{k}	0.10 ± 0.01^{bc}	1.61 ± 0.03^{cd}	79.93 ± 1.67^{d}	311.55 ± 4.27^{jk}	48.13 ± 2.75^{g}	0.17 ± 0.02^{fgh}	1.09 ± 0.01^{ab}	13.23 ± 0.29^{m}	27.66 ± 1.04^{m}	3.46 ± 0.07
Prunus jenkinsii	2.88 ± 0.19^{bcd}	0.19 ± 0.01^{fg}	2.85 ± 0.02^{cd}	74.63 ± 3.86^{cd}	226.67 ± 4.31^{fg}	42.36 ± 0.23^{de}	0.05 ± 0.03^{abc}	2.95 ± 0.16^{abcd}	3.07 ± 0.01^{cde}	21.51 ± 0.06^{l}	5.35 ± 0.05
Prunus undulata	5.91 ± 0.28^{ijk}	0.17 ± 0.01^{ef}	3.93 ± 0.05^{bc}	85.98 ± 1.00^{d}	377.33 ± 3.45^{l}	57.15 ± 2.08^{i}	0.04 ± 0.02^{abc}	3.22 ± 0.18^{bcd}	7.13 ± 1.50^{ijk}	3.30 ± 0.15^{e}	5.36 ± 0.10
Rubus treutleri	4.93 ± 0.34^{fghij}	0.21 ± 0.01^{gh}	1.19 ± 0.03^{ef}	169.67 ± 4.41^{ef}	241.83 ± 1.42^{gh}	136.05 ± 1.18^{l}	0.96 ± 0.02^{j}	3.70 ± 0.01^{cde}	11.20 ± 0.05^{l}	20.68 ± 0.03^{k}	4.48 ± 0.02
Spondias pinnata	4.02 ± 0.25^{defg}	0.15 ± 0.03^{def}	2.13 ± 1.58^{g}	202.15 ± 3.27^{f}	254.00 ± 2.54^{h}	38.58 ± 0.23^{c}	0.04 ± 0.02^{abc}	1.02 ± 0.46^{ab}	2.85 ± 0.04^{bcd}	3.29 ± 0.01e	1.97 ± 0.01
Syzygium cumini	5.52 ± 0.36^{hij}	0.16 ± 0.001^{ef}	1.28 ± 0.03^{b}	31.05 ± 4.76^{abc}	133.17 ± 3.17^{cd}	77.48 ± 2.55^{k}	0.06 ± 0.03^{abcde}	1.20 ± 0.04^{ab}	3.75 ± 0.47^{def}	1.39 ± 0.08^{bc}	3.33 ± 0.09
Tamirindus indica	3.38 ± 0.20^{cde}	0.13 ± 0.01d^{cde}	2.14 ± 0.04^{cd}	397.70 ± 2.47^{h}	211.33 ± 5.53^{f}	44.01 ± 0.88^{def}	0.13 ± 0.10^{efg}	32.94 ± 1.62^{f}	4.68 ± 0.56^{fg}	1.38 ± 0.02^{bc}	11.43 ± 0.7
Terminalia chebula	1.81 ± 0.39^{b}	0.12 ± 0.02^{bcd}	4.37 ± 0.10^{g}	7.40 ± 5.93^{a}	98.17 ± 5.22^{b}	41.09 ± 0.27^{cd}	0.21 ± 0.02^{h}	1.91 ± 0.02^{abcd}	4.53 ± 0.02^{efg}	0.91 ± 0.01^{ab}	3.42 ± 0.06
Ziziphus mauritiana	5.11 ± 0.20^{ghij}	0.15 ± 0.02^{def}	2.62 ± 0.02^{def}	61.40 ± 5.56^{bcd}	133.67 ± 4.13^{cd}	71.00 ± 0.98^{j}	0.13 ± 0.03^{defg}	3.25 ± 0.01^{bcd}	6.50 ± 0.03^{hij}	2.25 ± 0.01^{d}	3.53 ± 0.03

***Values with different lower-case superscript (a–d) letters in a column are significantly different among the fruits at p < 0.05 (DMRT test performed for separation of me**
Values are expressed as mean ± SD with three replications (n = 3) for each experiment.

the earlier research conducted by Baliga *et al*. (2011) and Parthsarathy *et al*. (2014), reported values of 27 mg 100 g^{-1} and 30.62 mg 100 g^{-1}, respectively.

4.1.2.1.6 Sodium (Na)

Sodium (Na) is integral to the regulation of fluid balance within the body, as it governs the movement of water across cellular membranes, thereby ensuring adequate hydration and optimal cellular function. Additionally, it is vital for the absorption of several macronutrients, including amino acids, water, and carbohydrates (Goff, 2018). Sodium is widely acknowledged as the principal cation in blood. Sodium is essential for the transmission of nerve impulses, facilitating the generation of electrical signals within the nervous system, which are crucial for muscle contractions and intercellular communication among nerve cells (Wai *et al*., 2024).

As observed from the data presented in Table 4.3 *Rubus treutleri* had the highest concentration of Na (136.05 ± 1.18 mg 100 g^{-1}), followed by *Syzygium cumini* (77.48 ± 2.55 mg 100 g^{-1}) and *Ziziphus mauritiana* (71.00 ± 0.98 mg 100 g^{-1}) which is statistically *at par* with *Morus nigra* (69.18 ± 0.53 mg 100 g^{-1}). Conversely, the lowest Na content was found in *Garcinia xanthochymus* (4.94 ± 0.02 mg 100 g^{-1}) while *Garcinia kydia* (30.06 ± 0.33mg 100 g^{-1}) showed a minor level of Na content.

The higher sodium content in *Rubus treutleri*, *Syzygium cumini*, and *Ziziphus mauritiana* (Figure 4.23) indicates that these fruits may represent significant dietary sources of sodium, underscoring their importance in fluid balance, nerve function, and muscle contraction. The findings for *Morus nigra* and *Garcinia xanthochymus* exhibited values that were comparably aligned with those reported by Mehta and Kumar (2021) and Parthsarathy and Nandakishore (2014), specifically ranging from 51 to 65 mg 100 g^{-1} and 2.06 mg 100 g^{-1}, respectively. The observed variations can be ascribed to differences in moisture levels and the particular environmental conditions of its habitat (Cook *et al*., 2020). Maintaining a balanced sodium intake is crucial, as excessive consumption may result in hypertension and cardiovascular diseases (Amarra *et al*., 2021).

The elevated concentrations of the aforementioned macronutrients in fruits that include *Citrus jambhiri*, *Prunus jenkinsii*, *Morus nigra*, and *Rubus treutleri* distinctly underscore their essential contribution to a balanced diet, especially for individuals with particular mineral needs.

4.1.2.2 Microelements

The microelements examined in this study comprise cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). The body requires these elements in lesser amounts. Nevertheless, they are crucial for sustaining optimal physiological health. Microelements, or trace elements, are essential for numerous physiological functions within the human body, even though they are required in very small amounts. The microelements examined include cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Microelements such as Zn, Cu, and Mn serve as co-factors for various enzymes, thereby enabling critical biochemical reactions necessary for metabolism and cellular function. Iron and zinc are essential for brain development and cognitive function. Iron is crucial for the transport of oxygen to the brain, whereas zinc plays a significant role in neurotransmitter function (Nieder *et al*., 2018).

4.1.2.2.1 Cobalt (Co)

The primary function of cobalt in the human body pertains to its involvement in cobalamin (vitamin B-12), which is crucial for metabolism, nerve function, and the production of red blood cells. Cobalt, an essential trace element frequently neglected and overshadowed by its more prominent elemental counterparts, subtly integrates itself into various domains including science, industry, health, and the natural environment (Yamada 2013).

Among the studied underutilised fruits as represented in Table 4.3 the species *Garcinia kydia* had the highest concentrations of cobalt (3.95 ± 0.10 mg 100 g^{-1}) followed by *Rubus treutleri* (0.96 ± 0.02 mg 100 g^{-1}) and *Garcinia xanthochymus* (0.32 ± 0.02 mg 100 g^{-1}) which was statistically *at par* with *Artocarpus heterophyllus* (0.29 ± 0.03 mg 100 g^{-1}), whereas *Elaeocarpus lanceifolius* displayed

the lowest Co content (0.02 ± 0.01 mg 100 g^{-1}) which was statistically *at par* with *Citrus grandis* (0.03 ± 0.02 mg 100 g^{-1}) and *Spondias pinnata* (0.04 ± 0.02 mg 100 g^{-1}).

In the current study, *Garcinia kydia* emerged as the species of underutilised fruits exhibiting the highest concentration of Co. Research indicates that cobalt (Co) effectively activates antioxidant mechanisms (Srivastava and Shukla 2016). Furthermore, cobalt has been identified as essential for the biosynthesis of vitamin B-12 in humans (Banerjee and Bhattacharya, 2021). This finding indicates that this relatively underutilised fruit may serve as an unrecognised source of nutrition, offering cobalt to its consumers (Yamada 2013).

4.1.2.2.2 Copper (Cu)

Copper (Cu) is an essential micronutrient for humans, playing a critical role that is often underappreciated. It constitutes a fundamental element of proteins and metalloenzymes. Copper is essential for the structural and catalytic functions of cuproenzymes. These enzymes are integral to numerous physiological processes (Li *et al*., 2023).

From the present investigation as depicted in Table 4.3 Cu was found to be maximum in *Tamarindus indica* (32.94 ± 1.62 mg 100 g^{-1}) followed by *Garcinia Kydia* (5.78 ± 0.75mg 100 g^{-1}) which was statistically *at par* with *Mahonia nepaulensis* (3.85 ± 0.25 mg 100 g^{-1}). Conversely, it was found to be the lowest in *Morus nigra* (0.45 ± 0.04 mg 100 g^{-1}) which is statistically *at par* with *Citrus jambhiri* (1.01 ± 0.002 mg 100 g^{-1}), *Spondias pinnata* (1.02 ± 0.46 mg 100 g^{-1}), *Myrica esculenta* (1.09 ± 0.01 mg 100 g^{-1}) and *Syzygium cumini* (1.20 ± 0.04 mg 100 g^{-1}).

Tamarindus indica (Figure 4.25) exhibits the highest copper content, underscoring its significance as a vital source of this trace mineral, which plays a crucial role in numerous physiological processes, such as iron metabolism and antioxidant defence. Therefore, it is crucial to uphold a varied diet to fulfil comprehensive nutritional needs. Copper deficiency may result in anaemia, neutropenia, and skeletal abnormalities (Li *et al*., 2023).

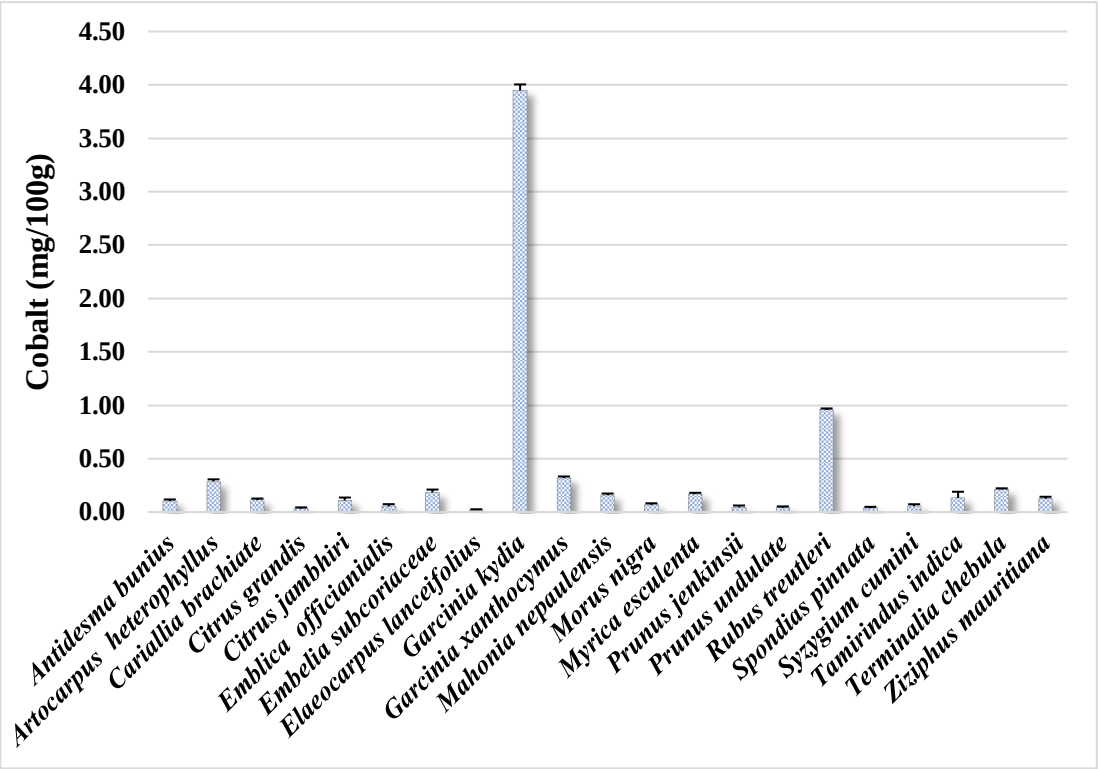


Fig 4.24 Cobalt content of the selected underutilised fruits

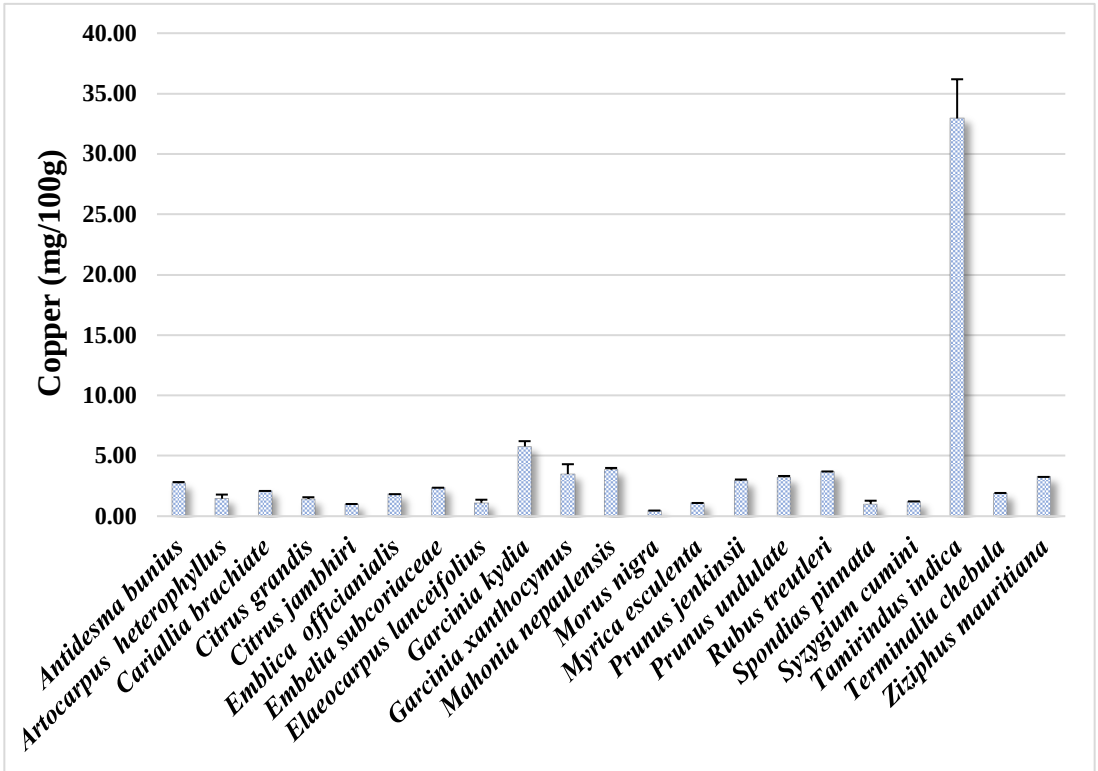


Fig 4.25 Copper content of the selected underutilised fruits

4.1.2.2.3 Iron (Fe)

Iron (Fe) serves as a crucial element of haemoglobin, a protein found in erythrocytes (red blood cells) responsible for the transport of oxygen from the lungs to bodily tissues. Iron, as a constituent of myoglobin, a protein responsible for oxygen transport, plays a crucial role in muscle metabolism and the maintenance of healthy connective tissue. Iron is essential for physical growth, neurological development, cellular function, and the synthesis of certain hormones (Charlebois and Pantopoules, 2023).

In our present investigation as represented in Table 4.3 among the studied fruits, *Garcinia kydia* had the highest concentrations of Fe (43.09 ± 1.56 mg 100 g^{-1}), followed by *Mahonia nepaulensis* (13.67 ± 0.89 mg 100 g^{-1}) which is statistically *at par* with *Myrica esculenta* (13.23 ± 0.29 mg 100 g^{-1}) whereas *Artocarpus heterophyllus* showed the lowest amount of iron (1.13 ± 0.11 mg 100 g^{-1}) being statistically *at par* with *Citrus grandis* (1.46 ± 0.32 mg 100 g^{-1}) and *Citrus jambhiri* (2.11 ± 0.02 mg 100 g^{-1}).

The concentration of Fe in *Garcinia xanthochymus* and *Artocarpus heterophyllus* aligns closely with the values reported by Parthasarathy *et al*. (2014) at 10.82 mg 100 g^{-1} and by Goswami *et al*. (2016) ranging from 0.5 to 1.1 mg 100 g^{-1}. *Garcinia kydia, Mahonia nepaulensis,* and *Myrica esculenta* (Figure 4.26) exhibited elevated iron content, underscoring their significance as nutritional supplements for iron and their potential role in addressing iron deficiency management. (Briguglio *et al*., 2020). The mean daily iron intake from dietary sources is reported to be 11.5–13.7 mg/day for children aged 2–11 years, 15.1 mg/day for adolescents aged 12–19 years, 16.3–18.2 mg/day for men, and 12.6–13.5 mg/day for women over the age of 19 (Charlebois and Pantopoules, 2023).

4.1.2.2.4 Manganese (Mn)

Manganese (Mn) is a trace mineral that is vital for human health in minimal quantities. Manganese plays a critical role in numerous vital processes. It serves as a cofactor for enzymes essential in the metabolism of carbohydrates and cholesterol.

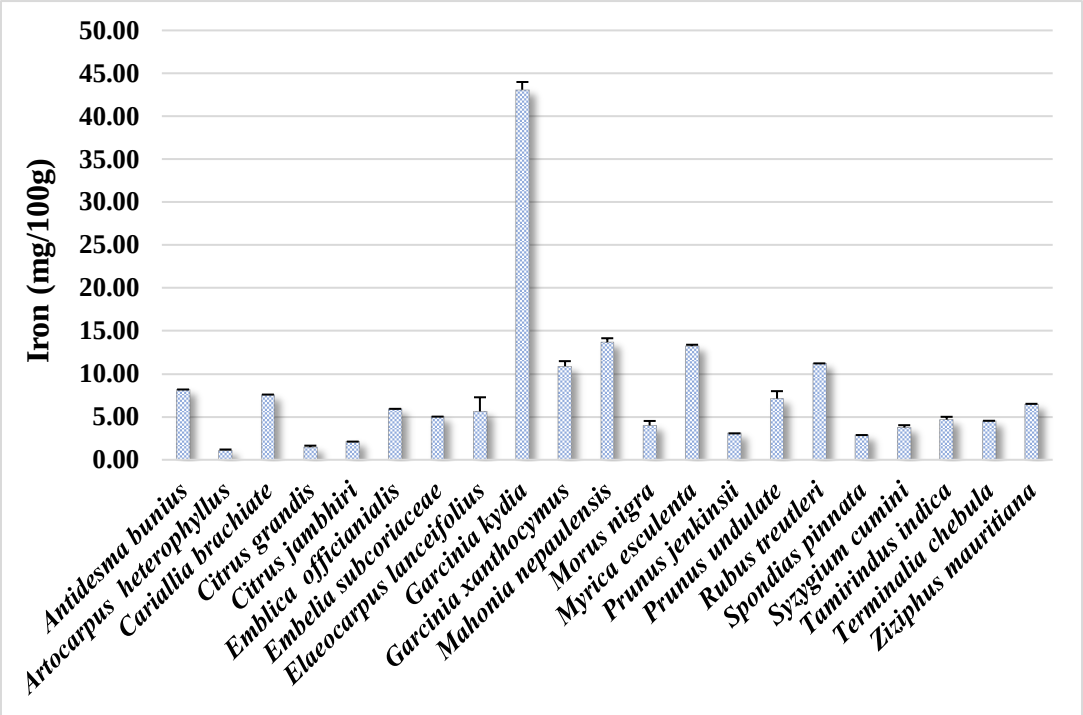


Fig 4.26 Iron content of the selected underutilised fruits

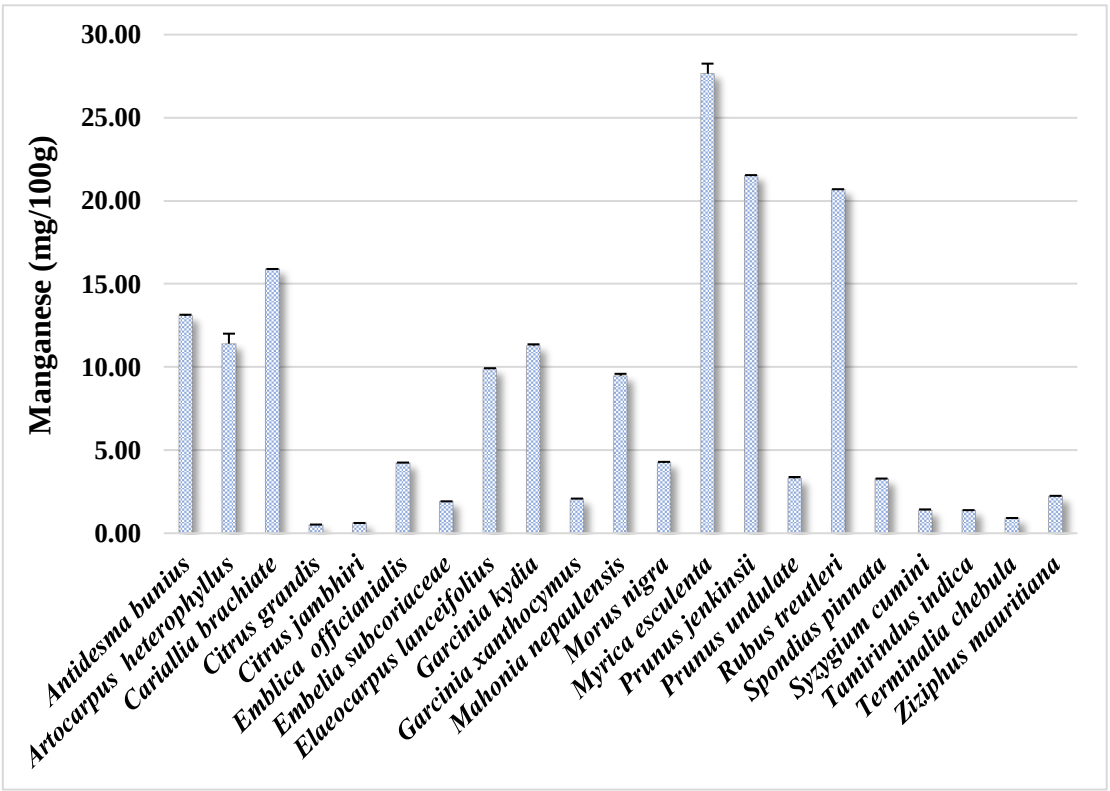


Fig 4.27 Manganese content of the selected underutilised fruits

Manganese is essential for bone formation and is involved in urea synthesis (Yang *et al*., 2024).

As depicted in Table 4.3 from our present investigation Mn was recorded to be highest in *Myrica esculenta* (27.66 ± 1.04 mg 100 g^{-1}) followed by *Prunus jenkinsii* (21.51 ± 0.06 mg 100 g^{-1}) and *Rubus treutleri* (20.68 ± 0.03 mg 100 g^{-1}), conversely, it was reported to be lower in *Citrus grandis* (0.50 ± 0.04 mg 100 g^{-1}) which is statistically *at par* with *Citrus jambhiri* (0.61 ± 0.01 mg 100 g^{-1}).

The recommended dietary allowance (RDA) of manganese (Mn) for adults aged 19 years and older is 2.3 mg per day for males and 1.8 mg per day for females. The Recommended Dietary Allowance (RDA) for pregnant and lactating women is 2.0 mg and 2.6 mg, respectively (Rondanelli *et al*., 2021). The current study indicates that *Myrica esculenta*, *Prunus jenkinsii, and Rubus treutleri* (Figure 4.27) serve as significant sources of manganese, which is crucial for multiple nutritional applications.

4.1.2.2.5 Zinc (Zn)

Zinc (Zn) interacts with more than 300 enzymes and over 2000 transcription factors. The significance of its role in biochemical pathways and cellular functions is substantial. This element participates in various biological functions, serving as a versatile trace element that contributes to protein synthesis, collagen formation, wound healing, and the maintenance of healthy skin. Zinc influences the composition of gut microbial communities (Ceballos-Rasgado *et al*., 2024).

From the data represented in Table 4.3 the species *Tamarindus indica* had the highest concentration of Zn (11.43 ± 0.71 mg 100 g^{-1}), followed by *Garcinia kydia* (6.88 ± 0.25 mg 100 g^{-1}), *Morus nigra* (6.42 ± 0.33 mg 100 g^{-1}) and *Myrica esculenta* (6.32 ± 0.15 mg 100 g^{-1}) which was statistically *at par*. In contrast, *Spondias pinnata* reported the lowest Zn content (1.97 ± 0.01 mg 100 g^{-1}) while being statistically *at par Citrus jambhiri* (2.57 ± 0.004 mg 100 g^{-1}) and *Citrus grandis* (2.95 ± 0.25 mg 100 g^{-1}) reported lower quantity of Zinc.

The findings of this study align with the reported Fe (11.70–37.13 mg 100 g^{-1}) and Zn (0.26–1.53 mg 100 g^{-1}) concentrations in underutilised plant species from

Ethiopia (Mokria *et al*., 2022). The elevated zinc concentration in *Tamarindus indica, Garcinia kydia*, and *Morus nigra* signifies their critical role as a source of zinc supplements across different age groups. Zinc deficiency continues to pose a significant global issue, impacting immune function, growth, and developmental processes. Promoting dietary diversity, particularly the inclusion of zinc-rich fruits, may mitigate deficiencies (Li *et al*., 2022).

The current investigation suggests that variations in macro and micro components can be ascribed to differences in mineral levels, potentially influenced by soil conditions, light exposure, and moisture availability in the respective region. Incorporating a diverse array of fruits into the diet facilitates a more comprehensive acquisition of vital nutrients. The findings may guide dietary recommendations and encourage the intake of indigenous fruits that are abundant in these essential elements.

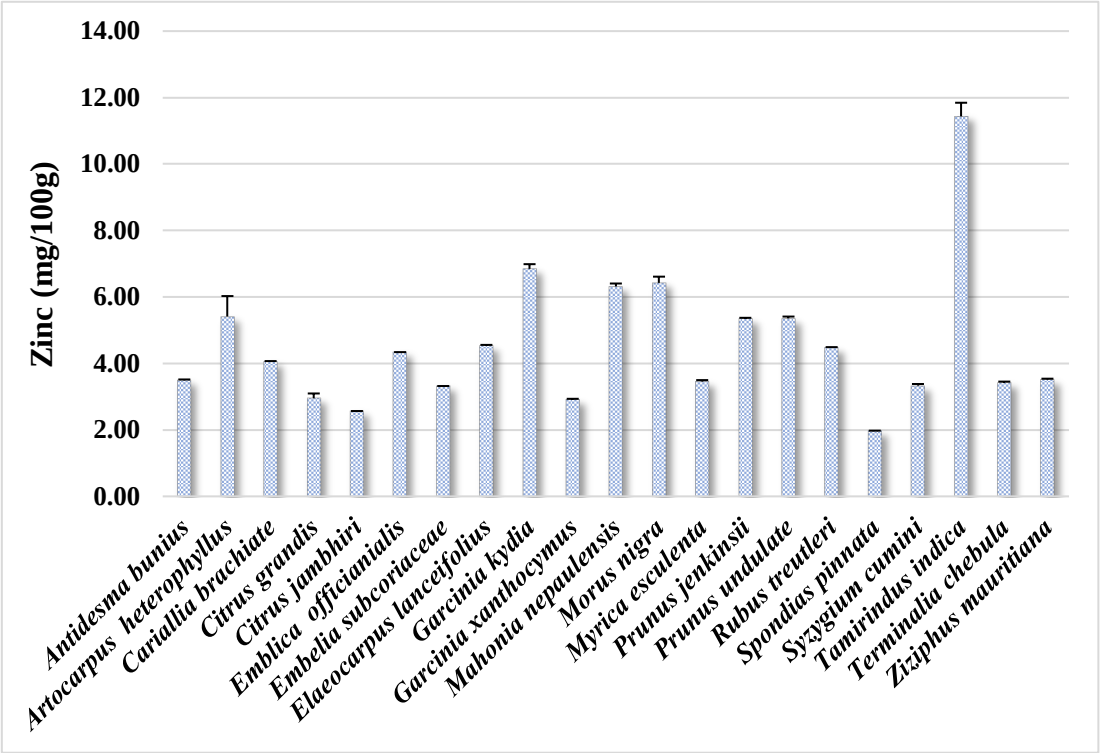


Fig 4.28 Zinc content of the selected underutilised fruits

4.1.2.3 Correlation between mineral composition of underutilised fruits

The correlation coefficient analysis quantifies the nature and magnitude of relationships among various plant traits, thereby identifying the component traits suitable for selection purposes. The understanding of the interplay between genetic and phenotypic data is crucial and beneficial when evaluating traits for selection purposes. Amulya *et al*. (2022). Comprehending these correlations enables breeders and cultivators to make informed decisions regarding the enhancement of fruit quality (Debbarma and Hazarika, 2024).

The establishment of correlations among factors identified in the investigation was accomplished by examining linear regression correlations. The corresponding values of correlation coefficients are shown in Table 4.4 From our investigation a significant positive association was exhibited between N with P (r = 0.48) and Zn (r = 0.26). The variable P has a positive correlation with N (r = 0.48) and Ca (r = 0.40). The element K was positively associated with Co (r = 0.39). The element Ca was positively correlated with the variables P, Mn and Zn as indicated by a correlation coefficient of 0.40, 0.37 and 0.33 respectively. Likewise, significant positive correlations were observed between Co and K (r = 0.39), Cu (r = 0.28), and Na (r = 0.31). The elements Cu exhibited significant positive correlations with Co (r = 0.28) conversely showing a negative correlation with Fe (r = -0.28), and Mg (r = -0.34). The element Fe had a negative correlation with Cu (r = -0.28) and Mg (r = -0.34). Mn positively correlated with Ca (r = 0.37) and Zn (r = 0.78). The element Mg had significant positive correlations with Na (r = 0.28), demonstrating a notably strong association with Fe (r = 0.92) and a negative correlation with Cu (r = -0.34). The element Na exhibited positive correlations with Co (r = 0.31) and Mg (r = 0.28). The element Zn exhibited positive correlations with Ca (r = 0.47) and Mn (r = 0.48) and a negative correlation with N (r = -0.26).

Table 4.4 Correlation among the mineral composition of underutilised fruits

	N	*P*	*K*	*Ca*	*Co*	*Cu*	*Fe*	*Mn*	*Mg*	*Na*	*Zn*
N	1.00										
P	**0.48****	1.00									
K	-0.03	0.05	1.00								
Ca	0.08	**0.40****	-0.17	1.00							
Co	-0.03	0.14	**0.39****	-0.02	1.00						
Cu	0.07	0.08	0.02	0.13	**0.28***	1.00					
Fe	-0.10	-0.08	-0.07	-0.14	-0.23	**-0.28***	1.00				
Mn	-0.14	-0.12	0.00	**0.37****	0.01	-0.10	0.07	1.00			
Mg	-0.11	-0.07	-0.02	-0.17	-0.02	**-0.34****	**0.92****	0.06	1.00		
Na	0.07	-0.06	-0.09	-0.09	**0.31***	0.21	0.17	-0.15	**0.28***	1.00	
Zn	**-0.26***	-0.09	0.14	**0.33****	0.10	-0.07	0.25	**0.78****	0.24	0.05	1.00

***. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*

4.2 Bioactive components of the fruits

4.2.1 Total phenolics (mg 100 g^{-1})

Phenolic compounds, such as phenols, flavonoids, and tannins, represent a class of bioactive molecules present in a diverse array of plant-based foods. Recent studies suggest that the intake of polyphenols may significantly influence health through the regulation of metabolism, body weight, chronic diseases, and cellular proliferation (Kumar *et al*., 2023). Phenolic compounds, integral to the overall phytochemical composition of plants, are recognised for their significant free radical scavenging capacity, as well as their potential in disease prevention, including antimicrobial, antiviral, and anti-inflammatory properties (Deng *et al*., 2012; Boakye *et al*., 2015).

As depicted in Table 4.5, in the present investigation the total phenol content was found to be highest in *Terminalia chebula* (792.28 ± 0.32 mg 100 g^{-1}), followed by *Emblica officinalis* (782.92 ± 3.98 mg 100 g^{-1}) and *Mahonia nepaulensis* (463.78 ± 5.81 mg 100 g^{-1}), while the lowest amount was seen in *Ziziphus mauritiana* (38.95 ± 0.97 mg 100 g^{-1}) and a lower amount was observed in *Citrus grandis* (94.32±0.43 mg 100 g^{-1}) and *Rubus treutleri* (69.91±2.05 mg 100 g^{-1}).

The elevated phenol content in *Terminalia chebula* aligns with the values documented by Angami *et al*. (2024). The differences in phenol content among various fruit species can be ascribed to the inherent genetic factors of the species, as well as varietal distinctions arising from diverse climatic and pre-harvest conditions, as indicated by Rawat *et al*. (2011). Deng *et al*. (2012) reported that the phenolic constituents of plant foods can be either hydrophilic or lipophilic, indicating that their analysis is invariably influenced by the solvent used, the extraction methods, and the duration of the extraction process. The examination of phenolic content in fruits such as *Terminalia chebula, Emblica officinalis*, and *Mahonia nepaulensis* (Figure 4.29) reveals a significant concentration of phenols.

4.2.2 Total flavonoids (mg 100 g^{-1})

Flavonoids, classified as polyphenolic compounds, can be divided into six distinct categories: anthocyanidin, flavone, flavanone, flavan-3-ol, flavanol, and isoflavone. A multitude of studies has established the therapeutic efficacy of flavonoids and their subclasses in addressing inflammation, oxidative stress, and bacterial infections (Rathod *et al*., 2023). Flavonoids have been shown to possess antioxidant properties by inhibiting reactive oxygen species and free radicals within the organism, thus alleviating oxidative stress. These compounds exhibit a range of beneficial biochemical and antioxidant properties linked to various diseases, including cancer, Alzheimer's disease (AD), and atherosclerosis (Boakye *et al*., 2015). Flavonoids are linked to a wide range of health benefits and serve as essential elements in numerous nutraceutical, pharmaceutical, medicinal, and cosmetic applications (Kumar *et al*., 2023).

In our present investigation among all the studied fruits as depicted in Table 4.5, the total flavonoid content was found to be highest in *Carallia brachiata* (632.62 ± 0.65 mg 100 g^{-1}), followed by *Emblica officinalis* (516.31 ± 0.65 mg 100 g^{-1}) and *Terminalia chebula* (494.04 ± 5.81 mg 100 g^{-1}), while the lowest amount was recorded in *Tamarindus indica* (22.41 ± 0.24 mg 100 g^{-1}) followed by *Syzygium cumini* (23.69 ± 0.65 mg 100 g^{-1}), *Citrus jambhiri* (30.78 ± 0.88) which is statistically *at par* with *Garcinia xanthochymus* (31.06 ± 0.85 mg 100 g^{-1}) and *Ziziphus mauritiana* (38.95 ± 0.97 mg 100 g^{-1}).

The values recorded for *Terminalia chebula* and *Ziziphus mauritiana* align closely with those reported by Angami *et al*. (2024), (445.0 ± 1.18 mg 100 g^{-1} and 49.73 ± 1.24 mg 100 g^{-1}, respectively). The differences in flavonoid content observed in the examined fruits can be ascribed to genetic factors and hormonal influences, in addition to external variables such as temperature, light conditions, nutritional status, and biotic stresses, which significantly contribute to flavonoid accumulation in fruits (Azuma *et al*., 2012; Goławska *et al*., 2023). *Carallia brachiata* flourishes in particular regions, which correlates with its elevated

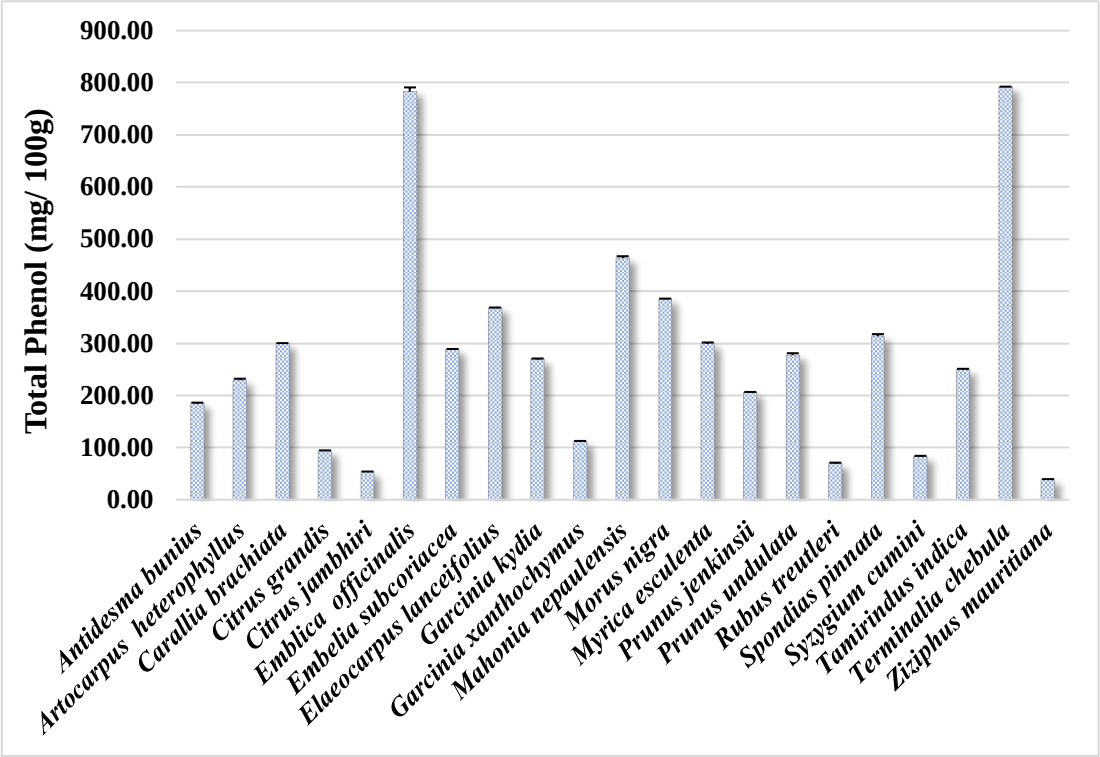


Fig 4.29 Total Phenol content of the selected underutilised fruits

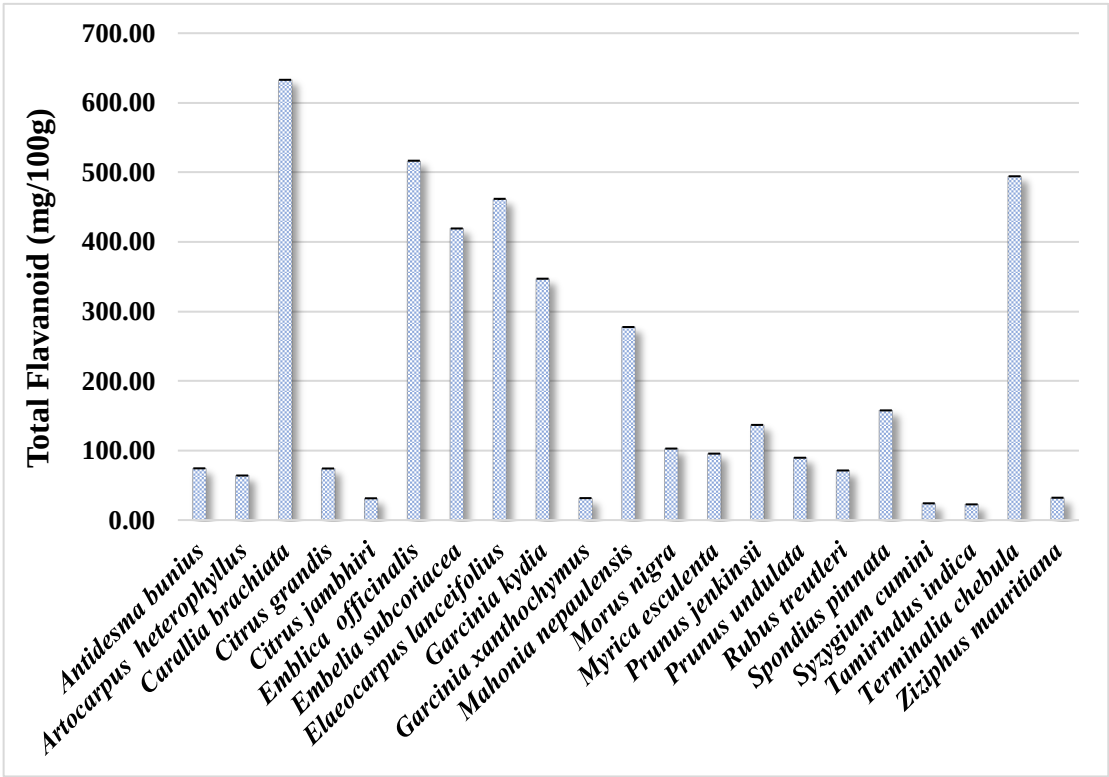


Fig 4.30 Total Flavonoid content of the selected underutilised fruits

flavonoid concentrations, indicating its promise as a functional food. *Tamarindus indica*, although it has relatively low flavonoid content, continues to be favoured by local populations due to its distinctive tangy flavour and various nutritional attributes (Silva *et al*., 2020). Analysing the flavonoid content in these underutilised fruits (Figure 4.30) enhances our comprehension of their nutritional diversity.

4.2.3 Total Carotenoids (mg 100 g^{-1})

Carotenoids play a crucial role in human health due to their efficacy as powerful antioxidants, protecting cells from damage caused by free radicals (Crupi *et al*., 2023). This contributes to reducing the risk of chronic conditions, including cancer and cardiovascular disease. Certain carotenoids, such as beta-carotene, are capable of being metabolised into vitamin A within the human organism. Vitamin A is essential for the optimal functioning of vision, development, immune response, and reproductive processes (Bas, 2024).

The total carotenoid pigment as represented in Table 4.5 the observed data was found to be highest in *Prunus jenkinsii* (1.97 ± 0.21 mg 100 g^{-1}), followed by *Terminalia chebula* (1.75 ± 0.005 mg 100 g^{-1}) and *Artocarpus heterophyllus* (1.70 ± 0.020), while the lowest amount was seen in *Syzygium cumini* (0.03 ± 0.005 mg 100 g^{-1}) and a lower amount was observed in *Citrus jambhiri* (0.70 ± 0.001 mg 100 g^{-1}) and *Tamarindus indica* (0.11 ± 0.005 mg 100 g^{-1}).

Fruits such as *Prunus jenkinsii, Terminalia chebula,* and *Artocarpus heterophyllus* (Figure 4.31) exhibit elevated carotene concentrations, reinforcing their status as significant sources of vitamin A and antioxidants (Jafari *et al*., 2023). The coordinated transcriptional activation of carotenoid biosynthetic genes significantly regulates the accumulation and composition of carotenoids in fruit throughout maturation. The relative expression profile between upstream and downstream genes within the pathway is a critical determinant of the variability observed in fruit colour and carotenoid content (Alquezar *et al*., 2008). The notable concentration of carotene in *Terminalia chebula* (Figure 4.31) aligns with its extensively documented pharmacological properties, including antioxidant and

anti-inflammatory effects (Upadhyay *et al*., 2014). Carotenoids have the potential to improve ocular health, augment immune function, and reduce the risk of chronic diseases.

4.2.4 Total Chlorophyll (mg 100 g^{-1})

Chlorophyll, the green pigment present in plants, exerts considerable influence on human nutrition through its various health benefits, including antioxidant properties, detoxification capabilities, facilitation of wound healing, enhancement of blood health, and improvement of digestive health. Chlorophyll's significance in human nutrition is well established, with research highlighting its benefits in improving blood health, aiding detoxification, and promoting overall well-being (Martins *et al*., 2023).

As depicted from the data presented in Table 4.5, it is revealed that the total chlorophyll pigment content was found to be highest in *Artocarpus heterophyllus* (0.507 ± 0.053 mg 100 g^{-1}), followed by *Carallia brachiata* (0.378 ± 0.003 mg 100 g^{-1}) and *Elaeocarpus lanceifolius* (0.297 ± 0.001), while the lowest amount was seen in *Citrus grandis* (0.004 ± 0.001 mg 100 g^{-1}) which was statistically *at par* with *Prunus undulata* (0.006 ± 0.001 mg 100 g^{-1}), *Citrus jambhiri* (0.009 ± 0.001 mg 100 g^{-1}), *Tamarindus indica* (0.015 ± 0.001 mg 100 g^{-1}), *Ziziphus mauritiana* (0.017 ± 0.0012mg 100 g^{-1}) *and Syzygium cumini* (0.021 ± 0.001 mg 100 g^{-1}).

Artocarpus heterophyllus, Carallia brachiata, and *Elaeocarpus lanceifolius* (Figure 4.32) exhibit elevated chlorophyll concentrations, suggesting their potential as advantageous dietary sources of chlorophyll. Chlorophyll is acknowledged for its antioxidant, detoxifying, and wound-healing attributes (Alsuhaibani *et al*., 2017). This metric is instrumental in evaluating fruit quality and maturity, as variations in chlorophyll content can be linked to both the maturity stage and the genetic characteristics of the fruit species. The maintenance of the green colouration in the fruit during the mature stage is primarily attributed to a restricted accumulation of pigments aside from chlorophylls (Rodrigo *et al*., 2012).

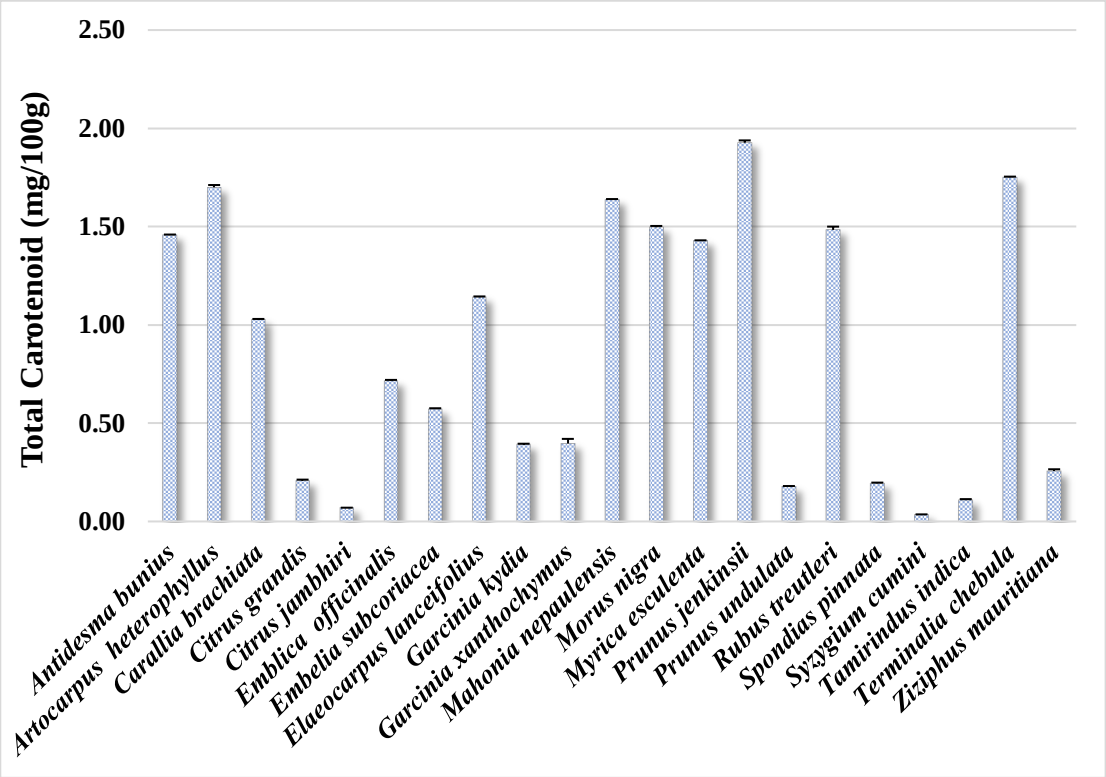


Fig 4.31 Total Carotenoid content of the selected underutilised fruits

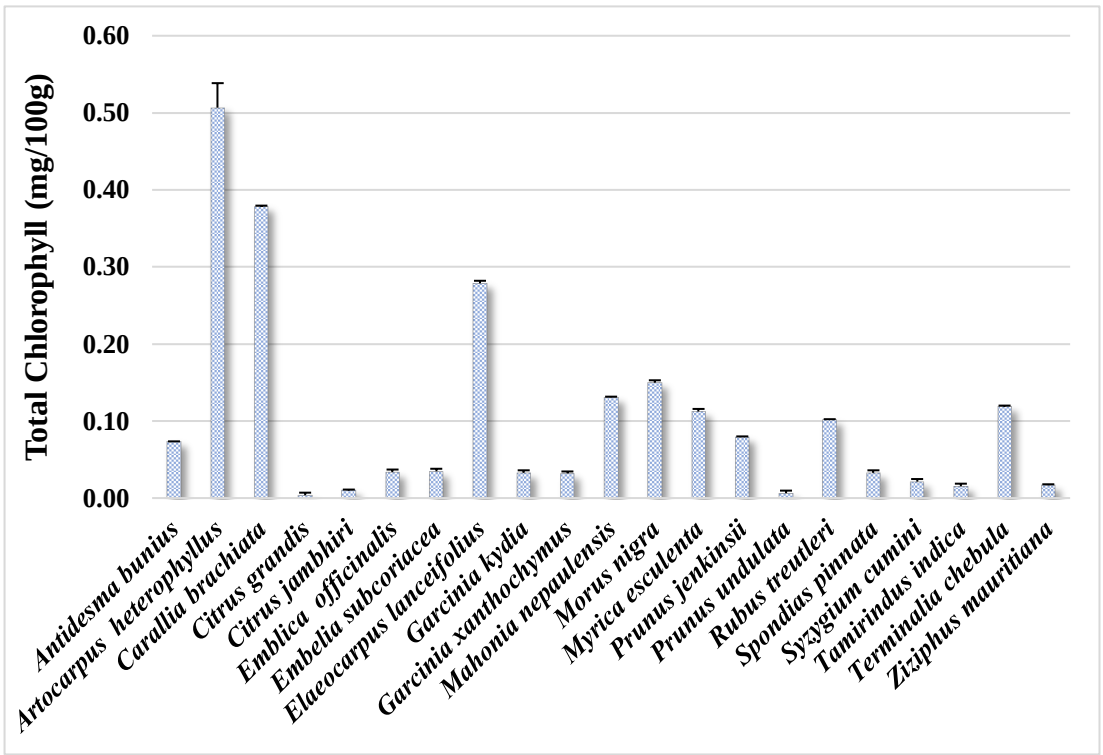


Fig 4.32 Total Chlorophyll content of the selected underutilised fruits

Table 4.5 Bioactive composition of underutilised fruits

Botanical Name	*Phenol (mg100 g^{-1})*	*Flavonoid (mg100 g^{-1})*	*Total Carotenoid (mg100 g^{-1})*	*Total Chlorophyll (mg100 g^{-1})*	*Anthocyanin (mg100 g^{-1})*	*Vitamin C (mg100 g^{-1})*	*Vitamin E (mg100 g–1)*	*Alkaloid (mg100 g^{-1} AE)*	*Saponin (mg100 g^{-1} DE)*
Antidesma bunius	184.86 ± 2.37^{g}	73.76 ± 1.07^{f}	1.458 ± 0.004^{m}	0.073 ± 0.001^{d}	336.67 ± 7.64^{g}	126.00 ± 4.00^{def}	36.69 ± 0.35^{g}	188.01 ± 3.34^{j}	9.78 ± 0.15^{hi}
Artocarpus heterophyllus	229.81 ± 4.09^{i}	63.69 ± 0.65^{d}	1.700 ± 0.020^{p}	0.507 ± 0.053^{j}	14.21 ± 1.05^{a}	38.33 ± 3.51^{a}	27.98 ± 0.80^{ef}	110.78 ± 3.78^{c}	10.38 ± 0.66^{j}
Carallia brachiata	299.59 ± 1.40^{n}	632.62 ± 0.65^{r}	1.028 ± 0.005^{j}	0.378 ± 0.003^{i}	816.67 ± 5.28^{m}	130.00 ± 14.00^{def}	23.24 ± 0.62^{d}	147.44 ± 4.57^{gh}	13.70 ± 0.50^{k}
Citrus grandis	94.32 ± 0.43^{e}	73.76 ± 0.65^{f}	0.21 ± 0.007^{e}	0.004 ± 0.001^{a}	226.67 ± 7.64^{b}	82.20 ± 5.71^{abcd}	21.40 ± 0.56^{b}	122.11 ± 2.39^{d}	6.92 ± 0.96^{ef}
Citrus jambhiri	53.89 ± 0.22^{b}	30.78 ± 0.88^{c}	0.070 ± 0.000^{b}	0.009 ± 0.001^{ab}	ND	202.67 ± 9.87^{g}	19.55 ± 0.49^{a}	142.89 ± 6.61^{fg}	1.13 ± 1.16^{a}
Emblica officinalis	782.92 ± 3.98^{s}	516.31 ± 0.65^{q}	0.717 ± 0.006^{i}	0.034 ± 0.001^{c}	281.67 ± 5.77^{d}	756.00 ± 4.00^{i}	28.02 ± 0.93^{ef}	89.93 ± 4.58^{b}	4.71 ± 0.49^{cd}
Embelia subcoriacea	288.41 ± 1.40^{m}	418.86 ± 0.65^{n}	0.573 ± 0.005^{h}	0.035 ± 0.001^{c}	231.67 ± 2.89^{b}	84.00 ± 28.00^{bcd}	27.86 ± 0.59^{ef}	122.92 ± 4.08^{d}	3.45 ± 0.73^{bc}
Elaeocarpus lanceifolius	368.62 ± 0.11^{p}	461.13 ± 1.30^{o}	1.141 ± 0.006^{k}	0.279 ± 0.001^{h}	313.33 ± 5.41^{ef}	42.00 ± 14.00^{ab}	22.88 ± 0.65^{cd}	164.50 ± 5.01^{i}	4.73 ± 0.52^{cd}
Garcinia kydia	269.38 ± 2.58^{k}	346.52 ± 0.89^{m}	0.39 ± 0.006^{g}	0.033 ± 0.001b^{c}	218.33 ± 7.64^{b}	60.87 ± 4.43^{abc}	23.17 ± 0.69^{d}	143.26 ± 3.75^{fg}	8.24 ± 1.53^{fg}
Garcinia xanthochymus	111.42 ± 2.26^{f}	31.06 ± 0.85^{c}	0.397 ± 0.040^{q}	0.032 ± 0.005^{bc}	14.56 ± 1.31^{a}	80.00 ± 3.61^{abc}	28.83 ± 0.51^{f}	41.67 ± 2.17^{a}	ND
Mahonia nepaulensis	463.78 ± 5.81^{r}	277.16 ± 0.65^{l}	1.64 ± 0.005^{o}	0.131 ± 0.001^{fg}	678.33 ± 27.54^{l}	100.93 ± 6.97^{cde}	22.03 ± 0.58^{bc}	150.81 ± 1.19^{h}	10.23 ± 1.16^{i}
Morus nigra	384.97 ± 1.40^{q}	102.41 ± 0.65^{i}	1.497 ± 0.012^{n}	0.150 ± 0.006^{g}	436.12 ± 5.93^{h}	137.00 ± 3.61^{ef}	23.20 ± 0.33^{d}	133.94 ± 5.56^{e}	5.42 ± 0.41^{d}
Myrica esculenta	299.48 ± 3.87^{n}	95.18 ± 0.65^{h}	1.43 ± 0.005^{l}	0.112 ± 0.001^{ef}	310.00 ± 8.66^{e}	60.68 ± 4.65^{abc}	23.27 ± 0.61^{d}	153.47 ± 3.53^{h}	6.94 ± 0.48^{ef}
Prunus jenkinsii	206.47 ± 0.54^{h}	136.45 ± 0.65^{j}	1.927 ± 0.021^{r}	0.079 ± 0.001^{d}	626.78 ± 0.51^{k}	136.00 ± 4.00^{ef}	19.13 ± 0.55^{a}	151.79 ± 2.88^{h}	8.72 ± 1.13^{gh}
Prunus undulata	277.87 ± 5.48^{l}	89.36 ± 0.43^{g}	0.18 ± 0.006^{d}	0.006 ± 0.001^{a}	328.33 ± 7.64^{fg}	126.26 ± 41.70^{def}	21.74 ± 0.61^{b}	123.94 ± 5.57^{d}	5.58 ± 0.65^{de}
Rubus treutleri	69.91 ± 2.05^{c}	71.06 ± 0.43^{e}	1.483 ± 0.029^{n}	0.102 ± 0.001^{e}	258.83 ± 0.77^{c}	50.67 ± 4.73^{ab}	23.12 ± 0.56^{d}	120.24 ± 1.76^{d}	2.58 ± 0.58^{b}
Spondias pinnata	313.68 ± 6.88^{o}	157.45 ± 0.43^{k}	0.195 ± 0.005^{de}	0.033 ± 0.001^{bc}	484.17 ± 0.76^{i}	644.00 ± 8.00^{h}	27.15 ± 0.28^{e}	95.36 ± 3.36^{b}	8.29 ± 0.55^{fg}
Syzygium cumini	83.25 ± 1.62^{d}	23.69 ± 0.65^{b}	0.03 ± 0.005^{a}	0.021 ± 0.001^{abc}	313.33 ± 5.77^{ef}	104.21 ± 6.37^{cde}	23.17 ± 0.4^{d}	136.89 ± 2.39^{ef}	10.24 ± 0.55^{i}
Tamirindus indica	250.34 ± 1.40^{j}	22.41 ± 0.24^{a}	0.11 ± 0.005^{c}	0.015 ± 0.001^{abc}	290.00 ± 0.001^{d}	65.13 ± 7.78^{abc}	21.97 ± 0.67^{bc}	124.03 ± 5.47^{d}	11.93 ± 1.08^{j}
Terminalia chebula	792.28 ± 0.32^{t}	494.04 ± 0.43^{p}	1.752 ± 0.005^{q}	0.119 ± 0.000^{ef}	521.67 ± 7.64^{j}	154.00 ± 14.00^{f}	27.01 ± 0.23^{e}	192.84 ± 4.84^{j}	11.93 ± 1.08^{j}
Ziziphus mauritiana	38.95 ± 0.97^{n}	31.91 ± 0.43^{c}	0.257 ± 0.015^{f}	0.017 ± 0.002^{abc}	15.27 ± 5.10^{a}	67.33 ± 2.52^{abc}	19.93 ± 0.60^{a}	108.42 ± 3.58^{c}	0.98 ± 0.38^{a}

**Values with different lower-case superscript ($^{a–d}$) letters in a column are significantly different among the fruits at $p < 0.05$ (DMRT test performed for separation of mean).*
Values are expressed as mean ± SD with three replications (n = 3) for each experiment.

4.2.5 Total anthocyanin (mg 100 g^{-1})

Anthocyanins are water-soluble pigments that impart red, purple, and blue hues to various fruits and vegetables. This class of flavonoids is responsible for the red, purple, and blue pigmentation observed in various fruits and vegetables. Their significant influence on human nutrition and health is attributed to beneficial characteristics such as the promotion of cardiovascular health, enhancement of cognitive function, and anticancer properties (Mesiano *et al*., 2024).

In the present investigation, as depicted in Table 4.5 It was observed that *Carallia brachiata* had the highest concentration of anthocyanin pigment (816.67 ± 5.28 mg 100 g^{-1}), followed by *Mahonia nepaulensis* (678.33 ± 5.93 mg 100 g^{-1}) and *Prunus jenkinsii* (626.78 ± 0.51 mg 100 g^{-1}), which are likely to have a deep colouration and potentially offer greater health benefits associated with anthocyanins. while *Artocarpus heterophyllus* displayed the lowest concentration (14.21 ± 1.05 mg 100 g^{-1}) which is statistically *at par* with *Garcinia xanthochymus* (14.56 ± 1.31mg 100 g^{-1}), *Ziziphus mauritiana* (15.27 ± 5.10 mg 100 g^{-1}), and no anthocyanin content was observed on *Citrus jambhiri*.

The anthocyanin content in *Prunus jenkinsii* was found to be higher than the values (313.03 ± 11.79 mg cyd-3-glu eq./100 g fw) reported by Rymbai et al., 2024. The variation in anthocyanin concentration among these underutilised fruits can be attributed to a range of external and internal factors, such as genetic and agronomic characteristics, light intensity and quality, temperature, and other pertinent variables (Angami *et al*., 2024), as the distinctive dark blue hue is linked to anthocyanins. The concentration of anthocyanins escalates with the darkening of the fruit. The deep purple and blue colouration of *Prunus jenkinsii* and *Morus nigra* fruits may be attributed to their high anthocyanin concentrations (Figure 4.33), thereby enhancing their antioxidant properties (Rymbai *et al*., 2023).

4.2.6 Ascorbic acid (mg 100 g^{-1})

Ascorbic acid, commonly known as vitamin C, is crucial for enhancing the immune system. It enhances the production and efficacy of leukocytes, which are essential for the defence against infections. Furthermore, it improves the

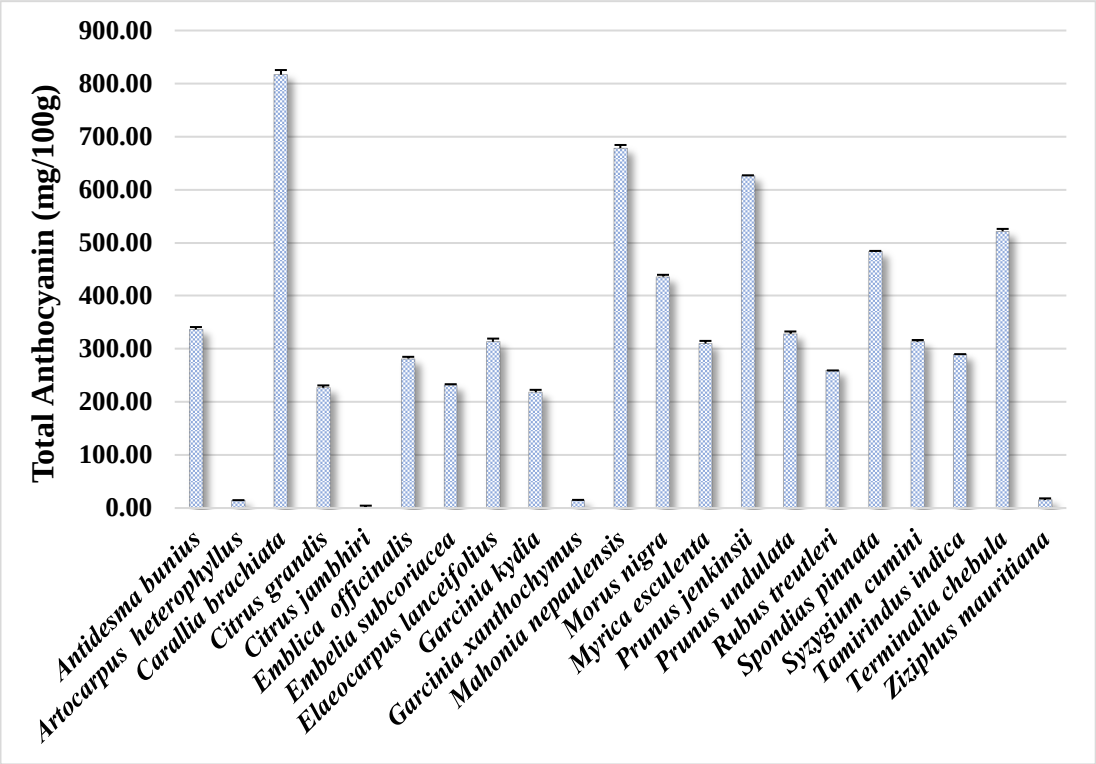


Fig 4.33 Total Anthocyanin content of the selected underutilised fruits

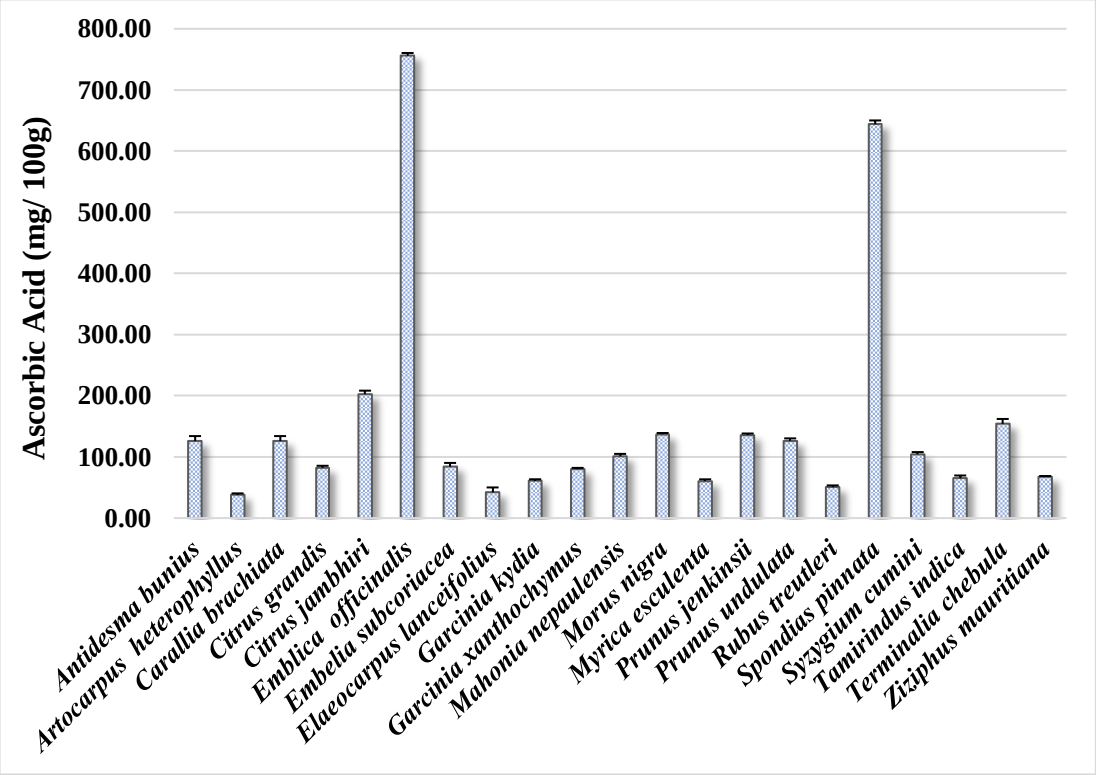


Fig 4.34 Ascorbic acid content of the selected underutilised fruits

Vitamin C plays a vital role in the synthesis of collagen, L-carnitine, and various neurotransmitters, among other physiological functions. This compound facilitates protein metabolism and exhibits antioxidant properties that may reduce the risk of specific cancers (Carr and Maggini 2017).

Among our studied fruits as represented in Table 4.5, *Emblica officinalis* had the greatest concentration of vitamin C (756.00 ± 4.00 mg 100 g^{-1}), followed by *Spondias pinnata* (644.00 ± 8.00 mg 100 g^{-1}) and *Citrus jambhiri* (202.67 ± 9.87 mg 100 g^{-1}). *Artocarpus heterophyllus* had the lowest reported concentration of vitamin C (38.33 ± 3.51 mg 100g^{-1}) which is statistically *at par* with *Elaeocarpus lanceifolius* (42.00 ± 14.00 mg 100 g^{-1}), *Rubus treutleri* (50.67 ± 4.73 mg 100 g^{-1}).

The observed vitamin C content in *Emblica officinalis* exceeds the range documented by Bulo *et al*. (2024). The concentration of ascorbic acid in fruits results from the hydrolysis of starch into glucose, which subsequently participates in the biosynthetic pathway of ascorbic acid (Khomdram and Devi, 2010). Moreover, the concentration of ascorbate is influenced by abiotic factors, including light and temperature (Gautier *et al*., 2008). *Emblica officinalis* is characterised by its remarkable concentration of vitamin C, as illustrated in Figure 4.34. This is consistent with its reputation for high ascorbic acid content, underscoring its role as a potent antioxidant that protects cells from oxidative damage and reduces the risk of chronic diseases such as heart disease and cancer (Feszterová *et al*., 2023).

4.2.7 Vitamin E (mg 100 g^{-1})

Vitamin E exhibits neuroprotective properties that may aid in the prevention of neurodegenerative disorders, such as Alzheimer's disease. It additionally maintains the structural integrity of cell membranes and enhances cognitive health (Börmel *et al*., 2024).

It is evident from the data presented in Table 4.5, that among the studied fruits the maximum concentration of vitamin E was observed in *Antidesma bunius* (36.69 ± 0.80 mg 100 g^{-1}) followed by *Garcinia xanthochymus* (28.83 ± 0.51 mg 100 g^{-1}) and *Emblica officinalis* (23.20 ± 0.51 mg 100 g^{-1}). Contrastingly the least quantity was discovered in *Prunus jenkinsii* (19.13 ± 0.55 mg 100 g^{-1}) which was

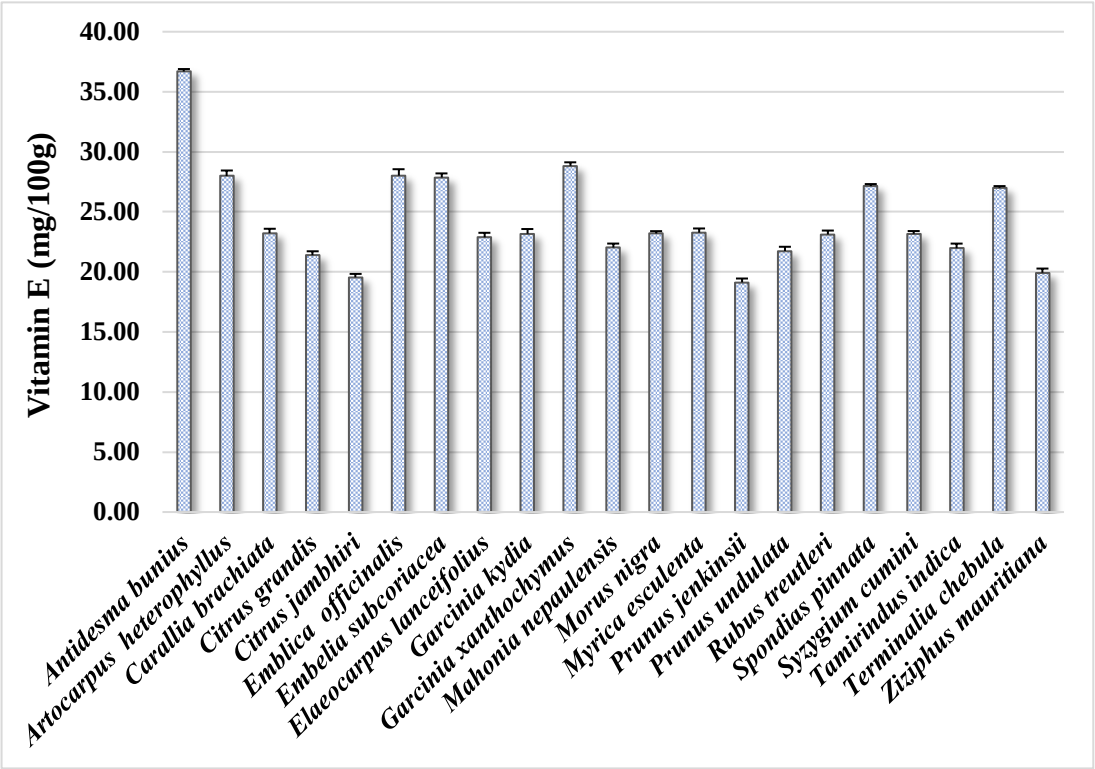


Fig 4.35 Vitamin E content of the selected underutilised fruits

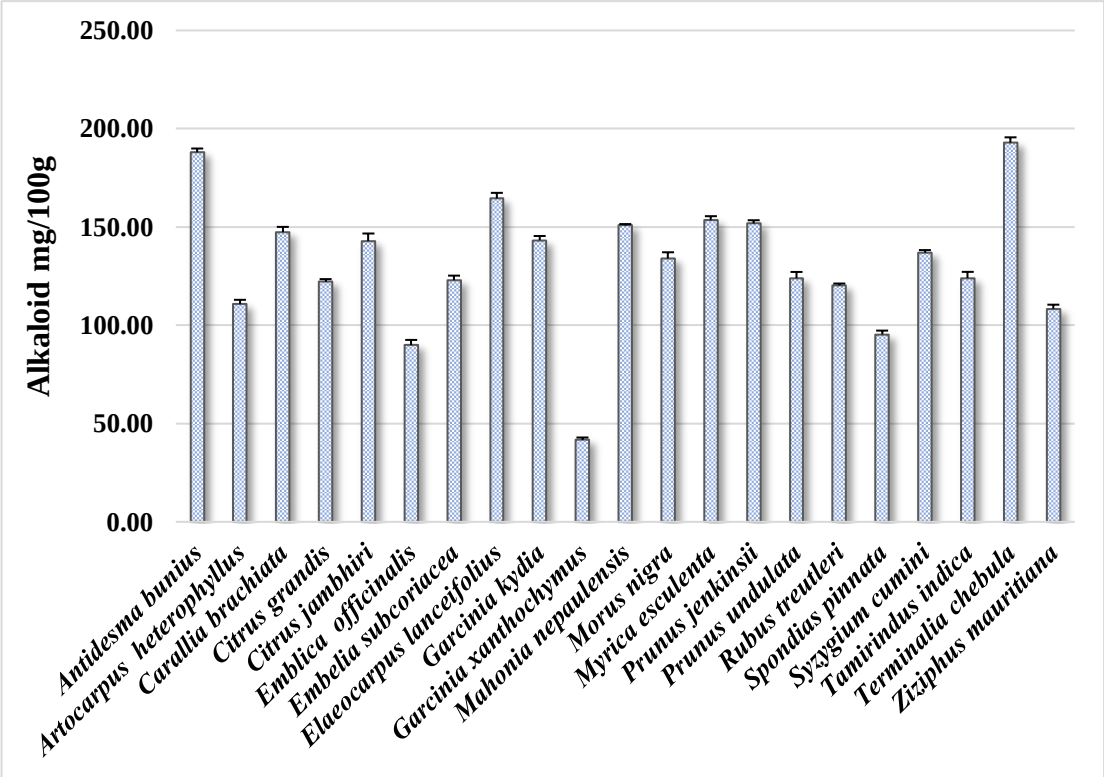


Fig 4.36 Alkaloid content of the selected underutilised fruits

found to be statistically *at par* with *Citrus jambhiri* (19.55 ± 0.49 mg 100 g^{-1}) and *Ziziphus mauritiana* (19.93 ± 0.60 mg 100 g^{-1}).

The concentration of vitamin E in *Garcinia xanthochymus* and *Ziziphus mauritiana* was slightly higher compared to the results reported by Angami *et al*. (2024), who examined these properties in wild plants located in the Eastern Himalayas. The differences in vitamin content among fruits can be attributed to factors including genetic characteristics, environmental conditions, agricultural practices, and the stage of maturity, among others (Vicente *et al*., 2022). *Garcinia xanthochymus* and *Emblica officinalis* exhibited the highest levels of Vitamin E (Figure 4.35), suggesting their potential as fruits rich in antioxidants (Méne-Saffrané 2018).

4.2.8 Alkaloids (mg 100 g^{-1})

Alkaloids represent a heterogeneous class of naturally occurring compounds characterised by the presence of nitrogen atoms. These compounds are predominantly located in plants and play crucial roles in human health owing to their diverse biological activities. Morphine and codeine an alkaloid are recognised for their analgesic properties and are utilised in medical contexts for the management of severe pain (Gutiérrez-Grijalva *et al*., 2020).

In the present investigation as observed from Table 4.5, the maximum concentration of alkaloid was found in *Terminalia chebula* (192.84 ± 4.84 mg 100 g^{-1}) which was statistically *at par* with *Antidesma bunius* (188.01 ± 3.34 mg 100 g^{-1}) followed by *Elaeocarpus lanceifolius* (164.50 ± 5.01 mg 100 g^{-1}) and the least quantity was discovered in *Garcinia xanthochymus* (41.67 ± 2.17 mg 100 g^{-1}). At the same time, *Emblica officinalis* (89.93 ± 4.58 mg 100 g^{-1}) and *Spondias pinnata* (95.36 ± 3.36 mg 100 g^{-1}) are found to be statistically *at par* with each other and exhibited lower levels of alkaloids.

The substantial concentrations of alkaloids in *Terminalia chebula* (Figure 4.36) suggest its potential as a significant source of these bioactive compounds. The quantities and varieties of phytochemicals, such as alkaloids, present in plants are influenced by several factors, including genotypes, ontogeny, and environmental conditions (Zhao *et al*., 2006). The concentration of alkaloids in fruit is primarily

influenced by the biosynthetic pathways governed by various enzymes throughout the growth and development of the plant (Tiwari and Cummins, 2013). The examination of alkaloid concentrations in diverse underutilised fruits demonstrated notable variations.

4.2.9 Saponins (mg 100 g^{-1})

Saponins exhibit the capacity to bind with cholesterol within the digestive tract, thereby impeding its absorption and facilitating its excretion. This mechanism contributes to the reduction of blood cholesterol levels and the mitigation of cardiovascular disease risk (Timilsena *et al*., 2023). Saponins exhibit cytotoxic properties towards cancer cells through the induction of apoptosis and the inhibition of tumour proliferation. Evidence suggests efficacy in the treatment of multiple cancer types (Shen *et al*., 2023).

It is revealed from the data presented in Table 4.5 that among the studied fruits, the highest saponin content was found in *Carallia brachiata* (13.70 ± 0.50 mg 100 g^{-1}), followed by *Terminalia chebula* (12.98 ± 1.09 mg 100 g^{-1}) which is statistically *at par* with *Tamarindus indica* (11.93 ± 1.08 mg 100 g^{-1}). Conversely, the lowest saponin content was reported in *Ziziphus mauritiana* (0.98 ± 0.38 mg 100 g^{-1}) which was found statistically *at par* with *Citrus jambhiri* (1.13 ± 1.16 mg 100 g^{-1}) while no saponin content was found in *Garcinia xanthochymus*.

Plants accumulate saponins as a protective strategy throughout their growth and development, which accounts for the observed variations in the examined underutilised fruits. These variations are influenced by the specific plant species, the particular plant part, and various environmental factors. The analysis of saponins in *Carallia brachiata, Terminalia chebula,* and *Tamarindus indica* (Figure 4.37) indicates their considerable potential to enhance human health and development. Their properties, which include cholesterol-lowering, anticancer, immune modulation, antioxidant activity, anti-inflammatory effects, antimicrobial action, and blood glucose regulation, render them significant bioactive compounds.

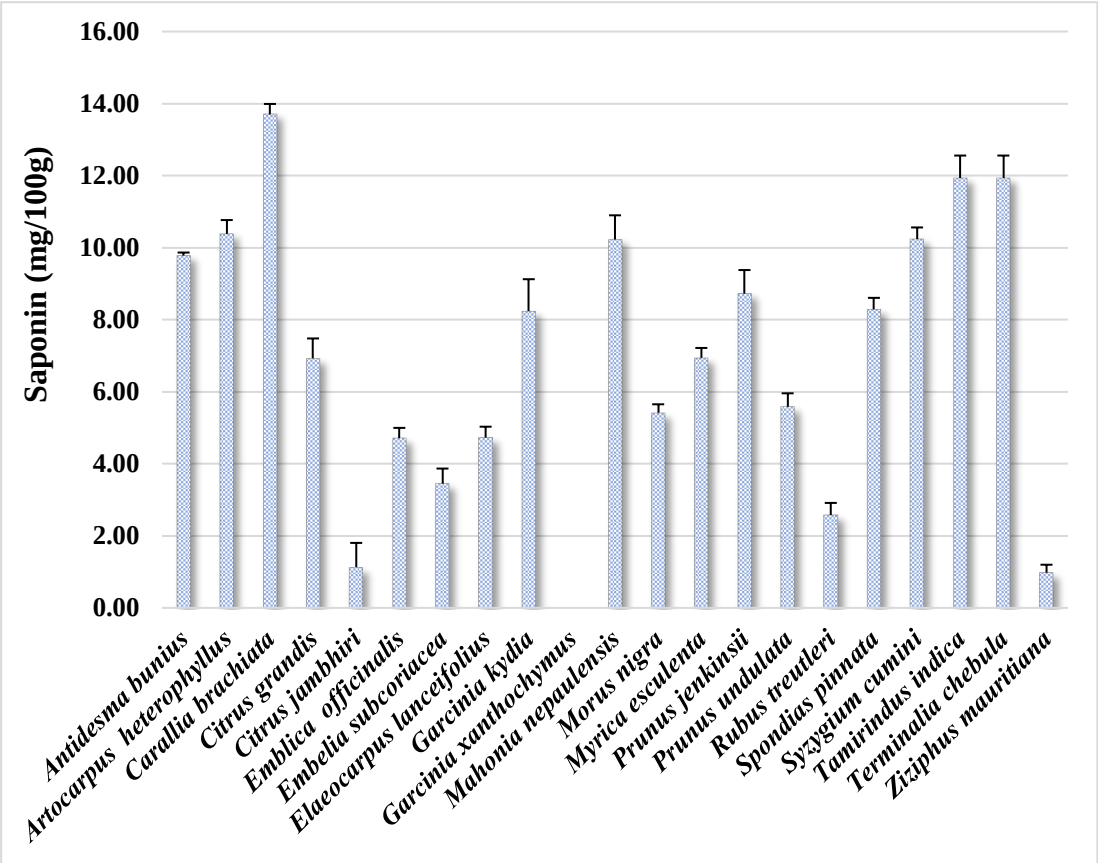


Fig 4.37 Saponin content of the selected underutilised fruits

4.2.10 Correlation between the bioactive composition of underutilised fruits:

The investigation established correlations among the factors identified by examining linear regression correlations. The corresponding correlation coefficients are shown in Table 4.6. Phenol exhibited a high positive correlation with flavonoid (r = 0.70), carotenoid (r = 0.37), anthocyanin (r = 0.44), vitamin C (r = 0.49) and saponin (r = 0.33). Flavonoid was found to be strongly correlated with phenol (r = 0.70), total chlorophyll (r = 0.32), anthocyanin (r = 0.51), vitamin C (r = 0.27), alkaloid (r = 0.27) and saponin (r = 0.28). A strong positive correlation was observed between total carotenoid with total chlorophyll (r = 0.56), anthocyanin (r = 0.44), phenol (r = 0.37), alkaloid (r = 0.27), and saponin (r = 0.31). The study found a positive correlation between total chlorophyll with flavonoid (r = 0.32), total carotenoid (r = 0.56) and saponin (r = 0.38). Anthocyanin was observed to be positively correlated with phenol (r = 0.44), flavonoid (r = 0.51), total carotenoid (r = 0.44), alkaloid (r = 0.46) and saponin (r = 0.65). The study found a negative correlation between vitamin C and alkaloid (r = -0.31), while vitamin C exhibited a positive correlation with phenol (r = 0.49), flavonoid (r = 0.27) and vitamin E (r = 0.25). There exists a positive correlation between vitamin E and vitamin C (r = 0.25). Alkaloids exhibited a negative correlation with vitamin C (r = -0.31) and a positive correlation with flavonoid (r = 0.27), total carotenoid (r = 0.47), anthocyanin (r = 0.46), and saponin (r = 0.51). Saponin was observed to be positively correlated with alkaloid (r = 0.51), phenol (r = 0.33), flavonoid (r = 0.28), total carotenoid (r = 0.30), total chlorophyll (r = 0.38) and anthocyanin (r = 0.65).

Table 4.6 Correlation among the bioactive compounds of underutilized fruits

	Phenol	*Flavonoid*	*Total Carotenoid*	*Total Chlorophyll*	*Anthocyanin*	*Vitamin C*	*Vitamin E*	*Alkaloid*	*Saponin*
Phenol	1.00								
Flavanoid	**0.70****	1.00							
Total Carotenoid	**0.37****	0.23	1.00						
Total Chlorophyll	0.14	**0.32***	**0.56****	1.00					
Anthocyanin	**0.44****	**0.51****	**0.44****	0.20	1.00				
Vitamin C	**0.49****	**0.27***	-0.19	-0.23	0.14	1.00			
Vitamin E	0.25	0.13	0.20	0.12	-0.07	**0.25***	1.00		
Alkaloid	0.23	**0.27***	**0.47****	0.18	**0.46****	**-0.31***	-0.02	1.00	
Saponin	**0.33****	**0.28***	**0.301***	**0.38****	**0.65****	-0.04	0.11	**0.51****	1.00

***. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*

4.3 Antinutrients

4.3.1 Phytic acid (mg 100 g^{-1})

Phytic acid, existing as phytate, is a naturally occurring compound frequently found in fruits. This compound is frequently classified as an antinutrient due to its ability to chelate essential minerals such as iron, zinc, calcium, and magnesium, consequently diminishing their bioavailability and absorption within the human body (Hossain *et al*., 2021). It has the potential to inhibit digestive enzymes, thereby impacting the breakdown of proteins and starches, as well as overall digestion and nutrient absorption.

It is observed from the data presented in Table 4.7 that among all the studied fruits the presence of phytic acid, namely in the form of phytate, was detected highly in *Embelia subcoriacea* (7.60 ± 0.16mg 100 g^{-1}) followed by *Citrus jambhiri* (6.02 ± 0.07 mg 100 g^{-1}) which is statistically *at par* with *Rubus treutleri* (6.05 ± 0.06 mg 100 g^{-1}). The species *Syzygium cumini* had the lowest observed phytate level (3.26 ± 0.12 mg 100 g^{-1}) and was found to be statistically *at par* with *Prunus undulata* (3.33 ± 0.12 mg 100 g^{-1}) and *Elaeocarpus lanceifolius* (3.41±0.02 mg 100 g^{-1}).

The synthesis of phytic acid commences post-flowering in plants and accumulates within the seeds throughout their development (Bohn *et al*., 2008; Iwai *et al*., 2012). The accumulation site of phytic acid differs among species, which may account for the variation in phytate content observed in the underutilised fruits examined (Raes *et al*., 2014). Fruits with elevated phytic acid content, including *Embelia subcoriacea*, *Citrus jambhiri*, and *Rubus treutleri* (Figure 4.38), may necessitate processing techniques such as soaking, sprouting, or fermentation to diminish phytic acid concentrations and improve mineral bioavailability (Murthy and Bapat, 2020).

4.3.2 Total oxalate (mg 100 g^{-1})

Oxalates, also known as oxalic acid, occur naturally in a variety of plant-based foods, particularly fruits. These compounds are classified as antinutrients due to their ability to bind with minerals such as calcium, resulting in the formation of

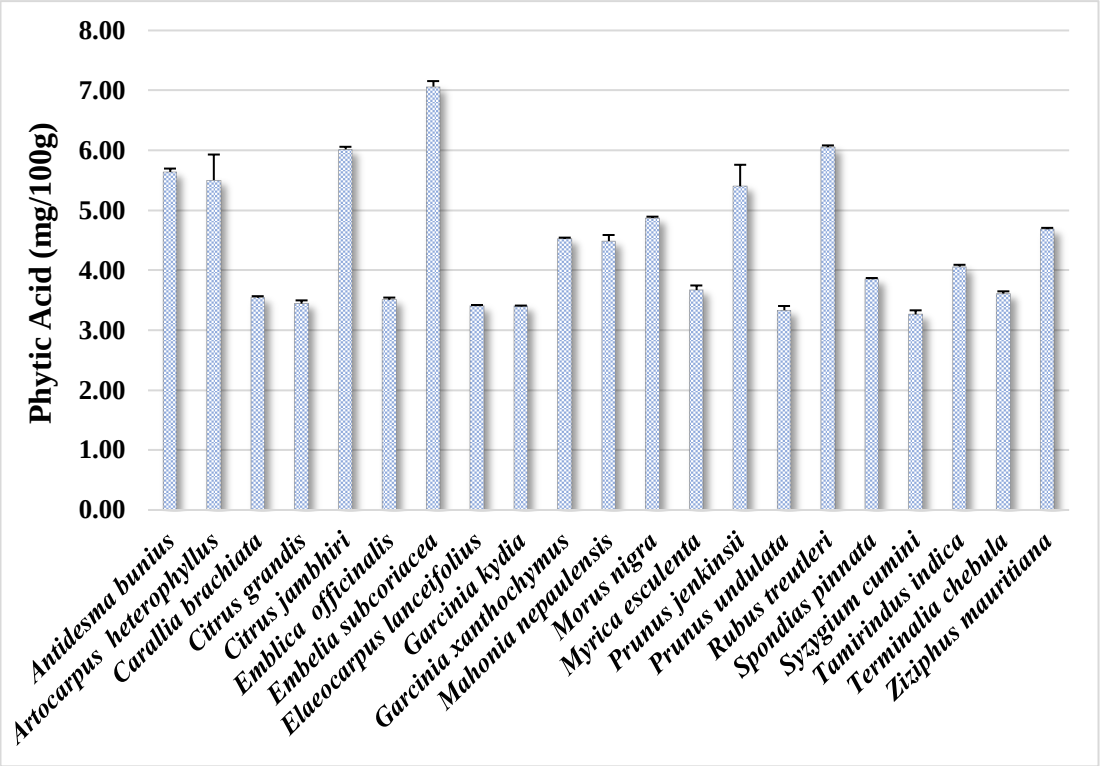


Fig 4.38 Phytic acid content of the selected underutilised fruits

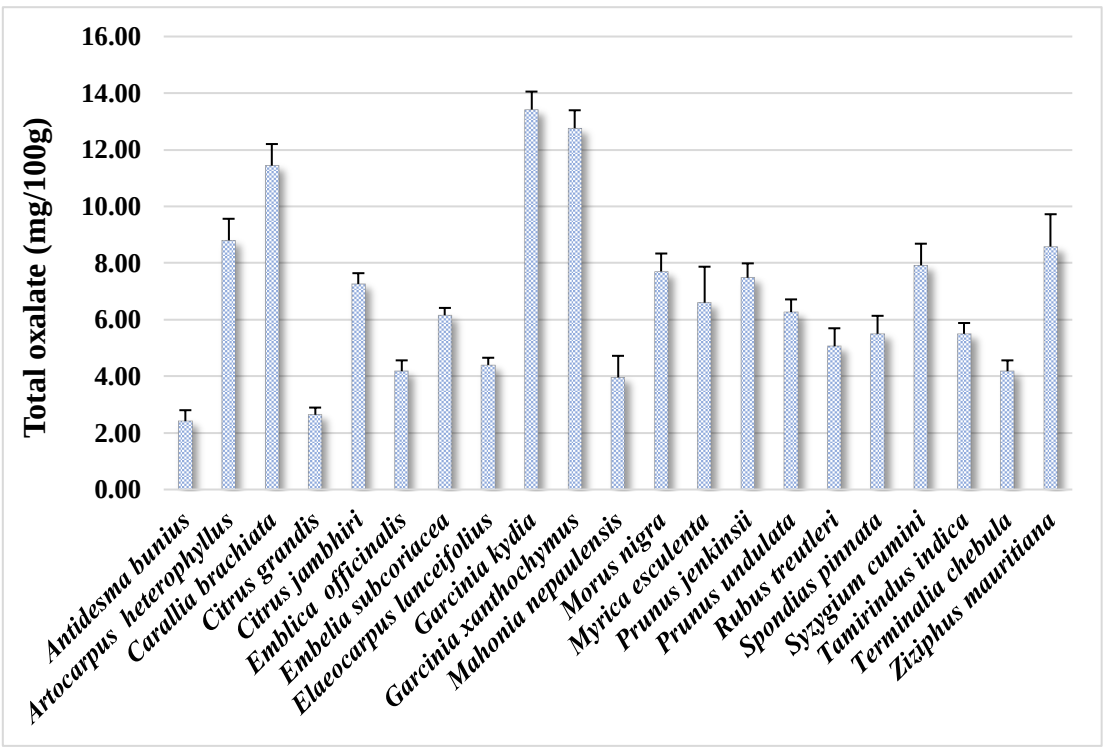


Fig 4.39 Total oxalate content of the selected underutilised fruits

Table 4.7. Antinutritional compounds of underutilised fruits

Botanical Name	*Phytic acid (mg100 g^{-1})*	*Total oxalate (mg100 g^{-1} FW)*	*Tannin (mg100 g^{-1})*
Antidesma bunius	5.64 ± 0.10^{e}	2.42 ± 0.66^{a}	48.22 ± 1.54^{a}
Artocarpus heterophyllus	5.50 ± 0.74^{e}	8.80 ± 1.32^{h}	51.11 ± 1.68^{a}
Carallia brachiata	3.54 ± 0.04^{ab}	11.44 ± 1.32^{i}	114.00 ± 2.00^{f}
Citrus grandis	3.44 ± 0.09^{ab}	2.64 ± 0.44^{a}	86.44 ± 2.34^{cd}
Citrus jambhiri	6.02 ± 0.07^{f}	7.26 ± 0.66^{efgh}	52.66 ± 1.15^{a}
Emblica officinalis	3.51 ± 0.06^{ab}	4.18 ± 0.66^{abc}	317.55 ± 4.91^{i}
Embelia subcoriacea	7.06 ± 0.16^{g}	6.16 ± 0.44^{cdef}	89.78 ± 1.02^{de}
Elaeocarpus lanceifolius	3.41 ± 0.02^{a}	4.40 ± 0.44^{abc}	97.56 ± 0.77^{e}
Garcinia kydia	3.40 ± 0.02^{a}	13.42 ± 1.10^{j}	46.44 ± 0.39^{a}
Garcinia xanthochymus	4.52 ± 0.04^{d}	12.76 ± 1.10^{ij}	85.56 ± 3.79^{f}
Mahonia nepaulensis	4.49 ± 0.17^{d}	3.96 ± 1.32^{ab}	114.44 ± 3.42^{f}
Morus nigra	4.87 ± 0.04^{d}	7.70 ± 1.10^{fgh}	64.22 ± 0.77^{b}
Myrica esculenta	3.67 ± 0.13^{abc}	6.60 ± 2.20^{defg}	138.44 ± 1.02^{g}
Prunus jenkinsii	5.40 ± 0.62^{e}	7.48 ± 0.88^{efgh}	109.56 ± 1.39^{e}
Prunus undulata	3.33 ± 0.12^{a}	6.27 ± 0.77^{cdef}	78.00 ± 0.67^{c}
Rubus treutleri	6.05 ± 0.06^{f}	5.06 ± 1.10^{bcd}	82.67 ± 0.67^{cd}
Spondias pinnata	3.85 ± 0.03^{bc}	5.50 ± 1.10^{bcde}	211.55 ± 1.91^{h}
Syzygium cumini	3.26 ± 0.12^{a}	7.92 ± 1.32^{fgh}	111.56 ± 5.43^{f}
Tamirindus indica	4.06 ± 0.05^{c}	5.50 ± 0.66^{bcde}	88.44 ± 1.39^{d}
Terminalia chebula	3.61 ± 0.07^{ab}	4.18 ± 0.66^{abc}	483.78 ± 7.49^{i}
Ziziphus mauritiana	4.68 ± 0.04^{d}	8.58 ± 1.98^{gh}	61.11 ± 1.02^{b}

**Values with different lower-case superscript ($^{a-d}$) letters in a column are significantly different among the fruits at $p < 0.05$ (DMRT test performed for separation of mean). Values are expressed as mean ± SD with three replications (n = 3) for each experiment.*

insoluble complexes that impede mineral absorption and may, in certain individuals, lead to the development of kidney stones (Salgado *et al*., 2023).

In our present investigation as depicted in Table 4.7 among all the studied fruits, the highest concentration of oxalate was seen in *Garcinia kydia* (13.42 ± 1.10 mg 100 g^{-1}) which was statistically *at par* with *Garcinia xanthochymus* (12.76 ± 1.10 mg 100 g^{-1}), followed by *Carallia brachiata* (11.44 ± 1.32 mg 100 g^{-1}). In contrast, *Antidesma bunius* had the lowest oxalate content (2.42 ± 0.66 mg 100 g^{-1}) and was found to be statistically *at par* with *Citrus grandis* (2.64 ± 0.44 mg 100 g^{-1}) (3.96 ± 1.32 mg 100 g^{-1}) and *Mahonia nepaulensis* (3.96 ± 1.32 mg 100 g^{-1})

Garcinia kydia (Figure 4.39) exhibits the highest oxalate content among the listed fruits, albeit in minimal quantities, indicating a potential anti-nutrient effect when ingested in substantial amounts. Oxalate is synthesised endogenously in plants via the degradation of ribulose-1,5-bisphosphate, a product of photosynthesis, to phosphoglycine. This process may be influenced by genetic factors, leading to variability in oxalate levels among underutilised fruits (Franceschi *et al*., 2005). The elevated oxalate concentration may interfere with mineral absorption and potentially present a risk for individuals predisposed to nephrolithiasis (Jiru *et al*., 2023).

4.3.3 Tannins (mg 100 g^{-1})

Tannins are natural polyphenolic compounds, commonly present in various plants, including fruits. These compounds are classified as antinutrients due to their ability to interact with proteins and other macromolecules, leading to decreased digestibility and hindered nutrient absorption (Hoque *et al*., 2024). It is widely acknowledged that foods rich in tannins have limited nutritional value due to their ability to cause protein precipitation, hinder digestive enzymes and iron absorption, and impact the absorption of vitamins and minerals from food (Yiblet, 2024).

The study data presented in Table 4.7 revealed that *Terminalia chebula* had the greatest concentration of tannin (483.78 ± 7.49 mg 100 g^{-1}) and was statistically *at par* with *Emblica officinalis* (317.55 ± 4.91 mg 100 g^{-1}) followed by *Spondias pinnata* (211.55 ± 1.91 mg 100 g^{-1}). The lowest value was observed in *Garcinia*

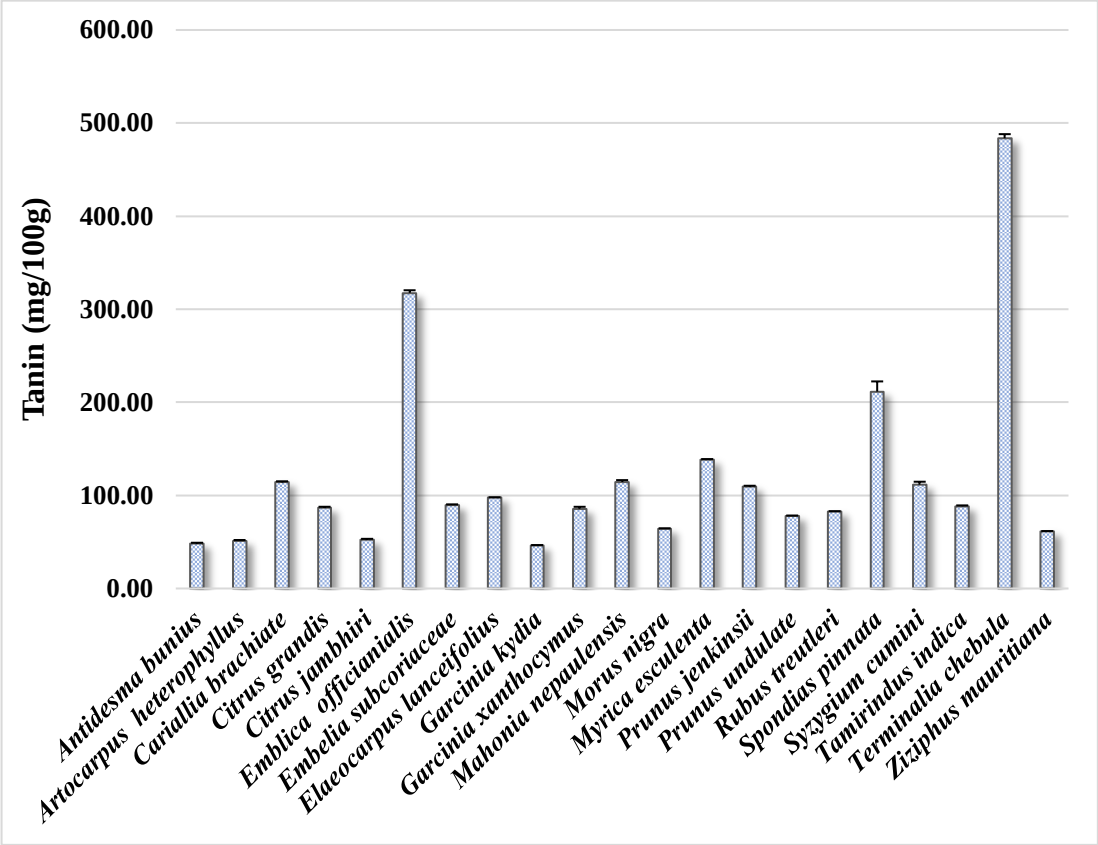


Fig 4.40 Tannin content of the selected underutilised fruits

kydia (46.44 ± 0.39 mg 100 g^{-1}) and was statistically *at par* with *Antidesma bunius* (48.22 ± 1.68mg 100 g^{-1}) and *Artocarpus heterophyllus* (51.11 ± 1.68 mg 100 g^{-1}).

The findings indicate that *Terminalia chebula* exhibits the highest tannin content (Figure 4.40) among the studied fruits, potentially influencing its protein digestibility and overall nutrient absorption. The regulation of tannin accumulation is influenced by temperature, light, moisture, and various environmental factors, which may account for the observed variation in tannin content among the examined fruits (He *et al.*, 2015). The perception of astringency can be ascribed to tannins, whose regulation is influenced by various structural and regulatory genes (Tsurunaga *et al.*, 2024). It is important to recognise that tannin compounds are non-toxic when consumed in amounts less than 560 mg per 100 g. The specified level is regarded as an acceptable daily intake of tannic acid for individuals (Fekadu *et al.*, 2013).

4.3.4 Correlation between antinutrient composition of underutilised fruits

The correlation analysis of the anti-nutritional factors in Table 4.8 revealed several significant relationships. Phytic acid was observed to be negatively correlated with tannin (r = -0.34). A negative correlation was recorded for total oxalate with tannin (r = -0.30) and phytic acid (r = -0.68). Lastly, tannin was negatively correlated with both phytic acid (r = -0.34) and oxalate (r = -0.30).

Table 4.8 Correlation among the Antinutrient compounds of underutilised fruits

	Phytic acid	**Total oxalate**	**Tanin**
Phytic acid	1		
Total oxalate	-0.07	1	
Tanin	**-0.34****	**-0.30***	1

***. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*

4.4 Evaluation of anti-cancerous and anti-oxidant properties

4.4.1 Evaluation of anti-cancerous activity

4.4.1.1 Cytotoxic effects of methanolic extracts of different fruits extracts in A549 cells

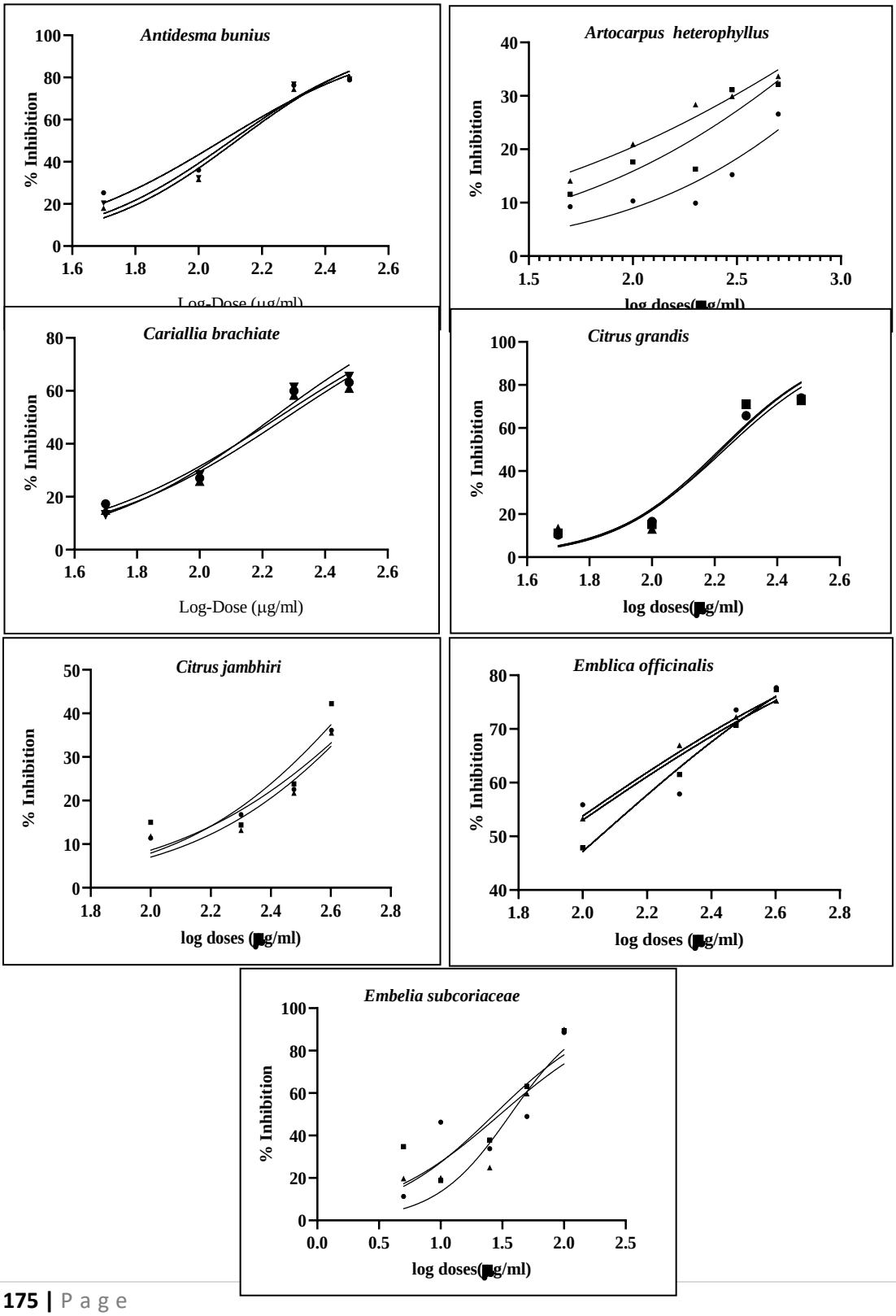
The inhibitory and cytotoxic effects of the fruits on human lung adenocarcinoma A549 cells were examined. The aim of targeting cell proliferation in cancer is to induce cell death or arrest the cell cycle through the application of cytotoxic agents. The MTT assay is a rapid and well-established method employed to evaluate the cytotoxicity of pharmaceuticals in various cultured cell types, wherein the reduction of MTT occurs exclusively in metabolically active cells. A549 cells underwent treatment with a methanolic extract of the specified fruit, resulting in a dose-dependent increase in cytotoxicity.

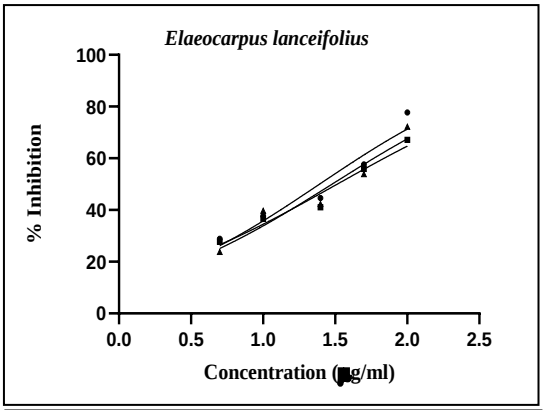
To evaluate the cytotoxic effects of methanolic extracts derived from various fruits, A549 cells were exposed to different concentrations for a duration of 24 hours. The percentage inhibition of A549 cells by methanolic extracts from various fruits was plotted against logarithmic doses to determine the IC_{50} value (Figure 4.41). Among the various fruit extracts, *Elaeocarpus lanceifolius* exhibited the highest cytotoxicity, with an IC_{50} of 29.5 ± 3.2 µg/ml, comparable to that of *Embelia subcoriacea* (31.54 ± 2.83 µg/ml), *Terminalia chebula* (IC50 = 39.6 ± 3.2 µg/ml) followed by *Garcinia kydia* (64.73 ± 7.07 µg/ml), and *Emblica officinalis* (94.62 ± 4.89 µg/ml). The five extracts exhibiting higher cytotoxic activity, as indicated by their lower IC_{50} values, were employed in the subsequent experiments. The extract of *Artocarpus heterophyllus* (2074.33 ± 47.85 µg/ml) demonstrated the lowest cytotoxic activity against A549 cancer cells, as indicated in Table 4.9.

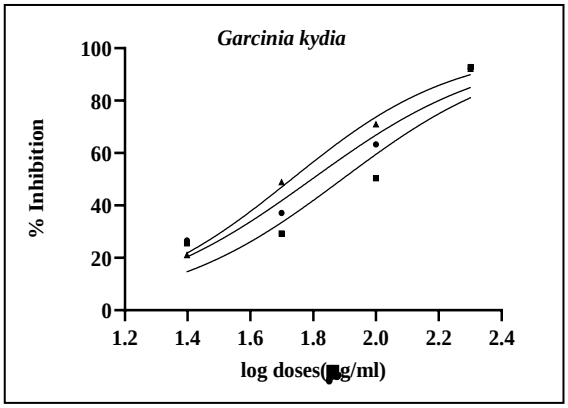
The administration of varying concentrations of different fruit extracts to A549 cells elicited a dose-dependent enhancement in cytotoxicity. The extracts of the fruits examined include *Elaeocarpus lanceifolius, Embelia subcoriacea*. *Terminalia chebula, Garcinia kydia, and Emblica officinalis* exhibiting the most significant cytotoxic effects following a 24-hour treatment period (Table 4.9). The studies underscore the significance of investigating underutilised fruits for their prospective health advantages. The cytotoxic effects on cancer cells may be attributed to the presence of bioactive compounds in these fruits.

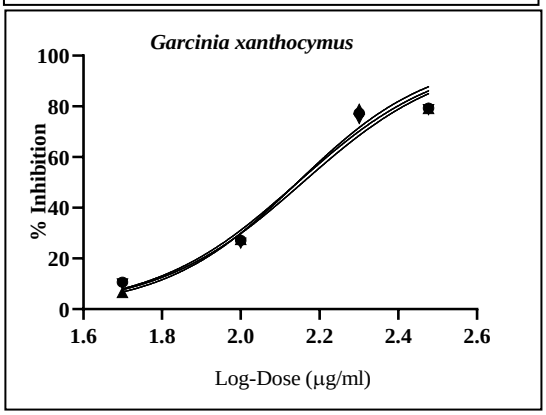
Recent studies have identified the cytotoxic effects on A549 cells of various fruits, including *Dimocarpus longan* Lour. seeds (Wang *et al*., 2014), *Annona muricata* leaves (Moghadamtousi *et al*., 2014), *Syzygium samarangense* (Thampi *et al*., 2015), *Citrus* extracts (Abosharaf *et al*., 2020), *Syzygium cumini* (Gibbert *et al*., 2021), *Actinidia deliciosa* (Manivasagan *et al*., 2021), *Rubus chingii* leaf (Khafagy *et al*., 2022), *Ziziphus mauritiana* (Prakash *et al*., 2022), and *Aegle marmelos* fruit juice (Devendrapandi *et al*., 2023).

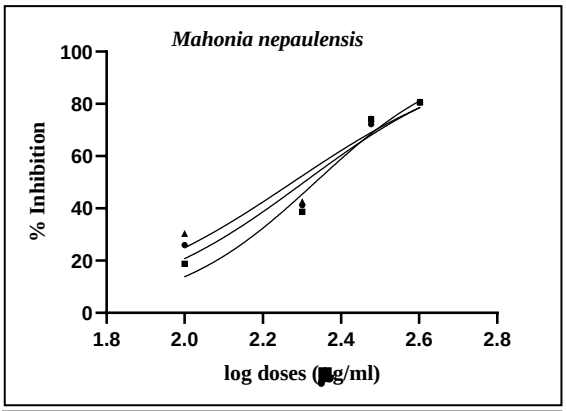
Fig. 4.41 Plots of log-doses of methanolic extracts of different underutilized fruits against inhibition (%) of A549 cells after 24 hr treatment for the calculation of IC_{50}

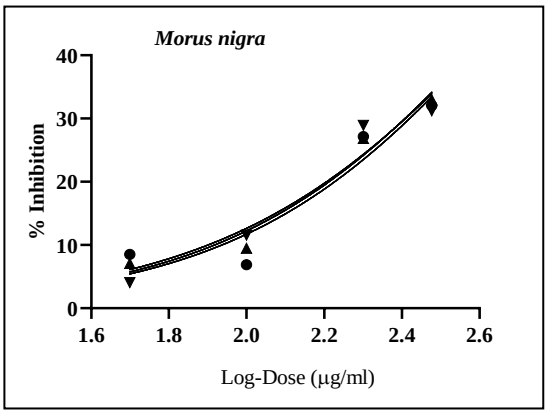


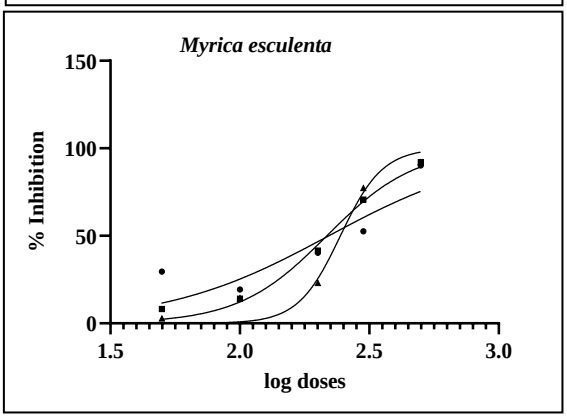

Elaeocarpus lanceifolius
% Inhibition
Concentration (µg/ml)

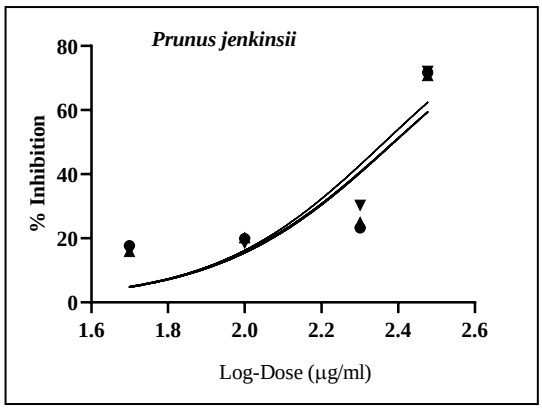

Garcinia kydia
% Inhibition
log doses(µg/ml)

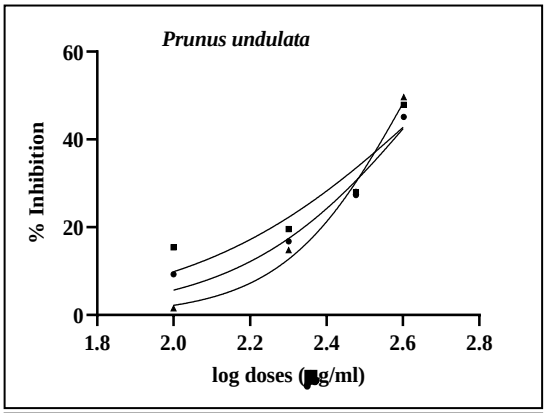

Garcinia xanthocymus
% Inhibition
Log-Dose (µg/ml)

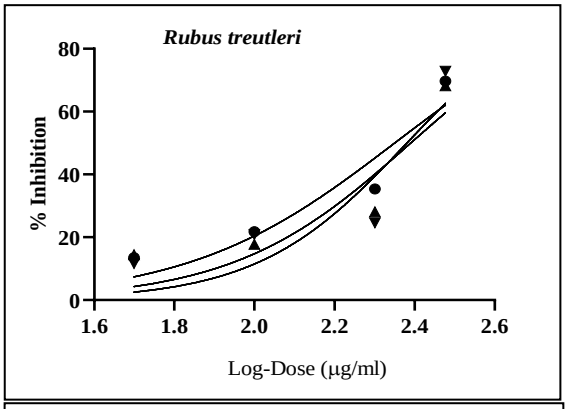

Mahonia nepaulensis
% Inhibition
log doses (µg/ml)

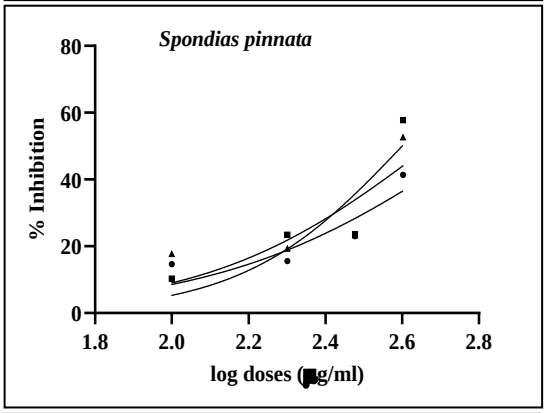

Morus nigra
% Inhibition
Log-Dose (µg/ml)

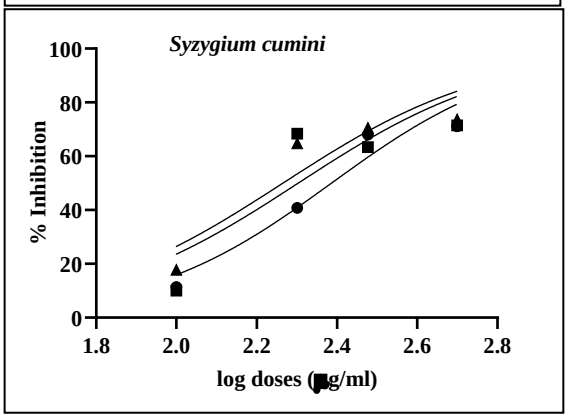

Myrica esculenta
% Inhibition
log doses

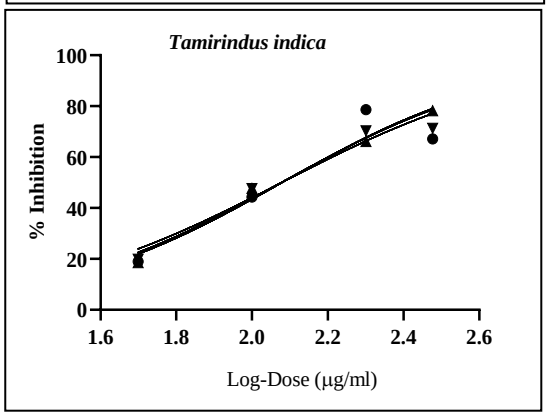

Prunus jenkinsii
% Inhibition
Log-Dose (µg/ml)

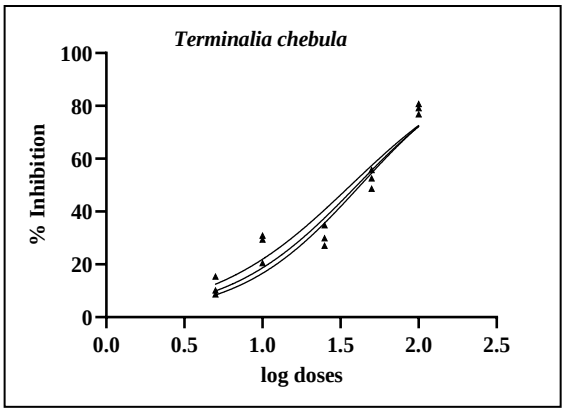

Prunus undulata
% Inhibition
log doses (µg/ml)


Rubus treutleri
% Inhibition
Log-Dose (µg/ml)


Spondias pinnata
% Inhibition
log doses (µg/ml)


Syzygium cumini
% Inhibition
log doses (µg/ml)


Tamirindus indica
% Inhibition
Log-Dose (µg/ml)


Terminalia chebula
% Inhibition
log doses

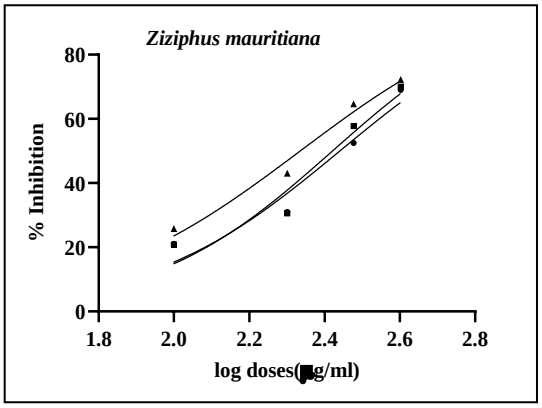

Ziziphus mauritiana
% Inhibition
log doses(µg/ml)

Table 4.9: Cytotoxic effects (IC_{50}) of methanolic extracts of different underutilised fruits on A549 cells after 24 hr treatment

Botanical Name	MTT Assay IC_{50} (µg/ml)
Antidesma bunius	125.63 ± 3.96^{abc}
Artocarpus heterophyllus	2074.33 ± 47.85^{f}
Carallia brachiata	179.93 ± 5.00^{abc}
Citrus grandis	176.63 ± 10.66^{abc}
Citrus jambhiri	659.47 ± 42.55^{e}
Emblica officinalis	92.82 ± 4.89^{abc}
Embelia subcoriacea	31.54 ± 2.83^{a}
Elaeocarpus lanceifolius	29.16 ± 2.38^{a}
Garcinia kydia	64.73 ± 7.07^{abc}
Garcinia xanthochymus	140.83 ± 1.43^{abc}
Mahonia nepaulensis	202.23 ± 7.80^{abc}
Morus nigra	527.87 ± 4.40^{de}
Myrica esculenta	227.30 ± 8.72^{abc}
Prunus jenkinsii	242.00 ± 5.39^{bc}
Prunus undulata	456.97 ± 24.90^{d}
Rubus treutleri	236.70 ± 6.49^{abc}
Spondias pinnata	486.13 ± 12.66^{de}
Syzygium cumini	209.53 ± 17.57^{abc}
Tamirindus indica	119.77 ± 0.20^{abc}
Terminalia chebula	39.93 ± 1.64^{ab}
Ziziphus mauritiana	251.90 ± 18.09^{c}

**Values with different lower-case superscript ($^{a-d}$) letters in a column are significantly different among the fruits at $p < 0.05$ (DMRT test performed for separation of mean). Values are expressed as mean ± SD with three replications (n = 3) for each experiment.*

4.4.1.2 Morphological evidence of apoptosis induced by *Elaeocarpus lanceifolius, Embelia subcoriacea, Terminalia chebula, Garcinia kydia and Emblica officinalis*

Comprehending the precise mechanisms through which anticancer agents operate is essential for evaluating and enhancing the development of anticancer therapeutics. Apoptosis represents a vital and meticulously regulated mechanism of cellular demise, facilitating the removal of dysfunctional cells while preserving the integrity of healthy ones. The dysregulation of apoptosis is associated with various diseases, including cancer. Any agent that induces apoptosis is considered a potential therapeutic option for cancer chemotherapy.

To investigate whether the selected fruit extracts induced inhibition of A549 cell growth via apoptosis, acridine orange/ethidium bromide (AO/EB) dual staining was used to identify and quantify the apoptotic morphology. Treatment of A549 cells with different concentrations (10–50 µg/ml) for *Elaeocarpus lanceifolius* (ELME), *Embelia subcoriacea* (ESME), *Terminalia chebula* (TCME) extracts and 25-100 µg/mL concentrations for *Garcinia kydia* (GKME) and *Emblica officinalis* (EOME) extracts for 24 hr resulted in a dose-dependent increase in the number of apoptotic cells (Figure 4.42). Fluorescence microscopic images of the treated cells (Figure 4.43) revealed morphological alterations such as membrane blebbing, nuclear condensation, and nuclear fragmentation which are the distinct characteristics of apoptotic cells. At 50 µg/ml, *E. lanceifolius* induced the highest apoptosis with an apoptotic index of 70.73 % when compared to *E. subcoriacea* (64.56 %), *T. chebula* (58.4 %), *G. kydia* (40.43 %) and *E. officinalis* (34.78 %) extracts. Furthermore, *E.*

lanceifolius and *E. subcoriacea* extracts were found to possess an apoptotic index which is significantly *at par* with the standard drug at 100 µg/mL (5FU-100).

A549 cells treated with 50 µg/mL of *E. lanceifolius, E. subcoriacea,* and *T. chebula*, as well as 100 µg/mL of *G. kydia* and *E. officinalis* fruit extracts, exhibited apoptotic characteristics, including distinctly orange-red condensed nuclei. In contrast, untreated control cells maintained round and intact green nuclei, signifying viable cells. Numerous plant-derived anti-cancer agents exhibit similar efficacy across various cancer types, including podophyllotoxin (Motati *et al*., 2019), vinblastine and vincristine (Katnoria *et al*., 2020), and curcumin (Yin *et al*., 2018).

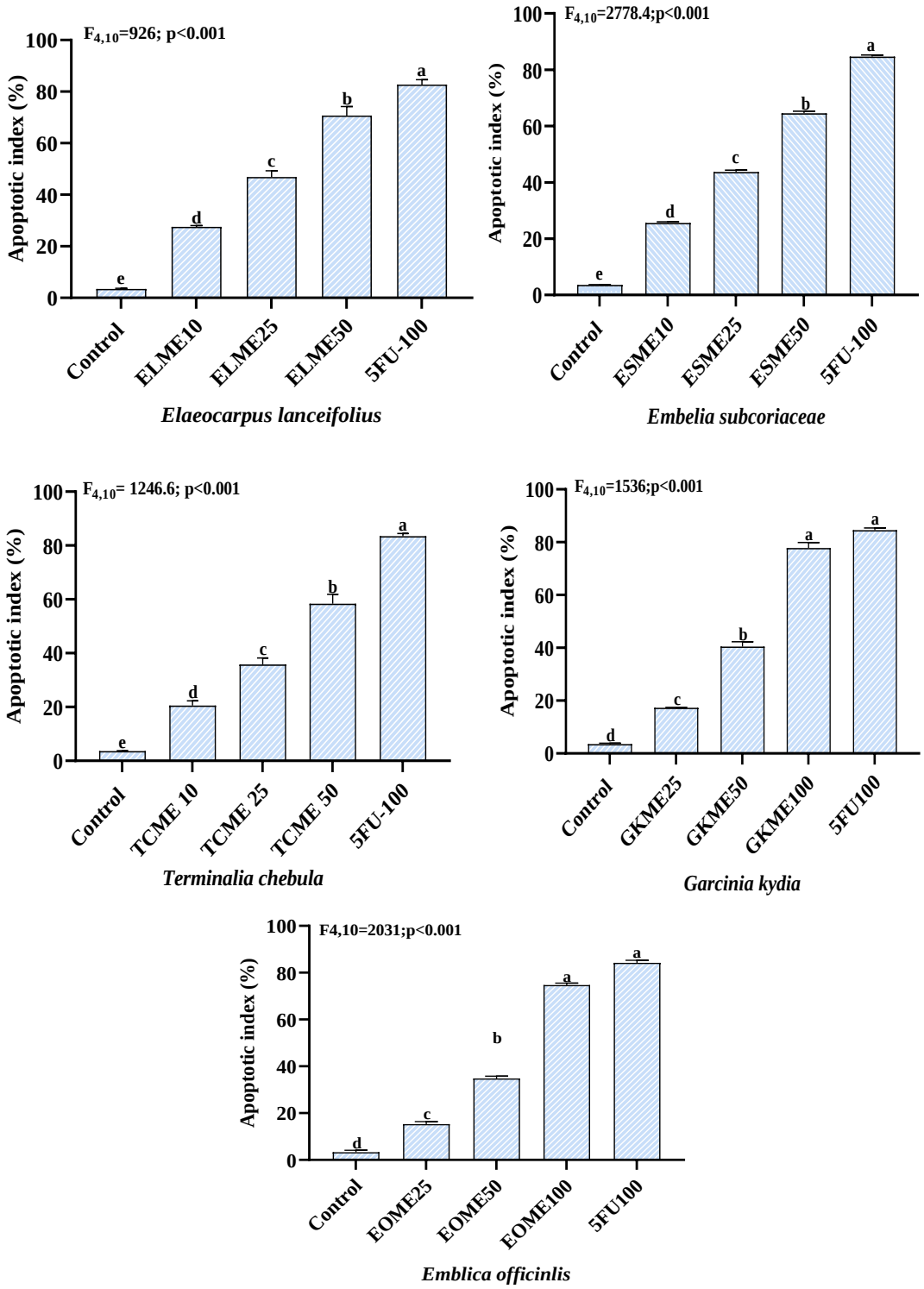


Fig. 4.42: Percentage of dead cells after treatment of A549 with different doses of methanolic extracts of the selected underutilized fruits. Control: A549 cells without treatment. ELME, ESME and TCME (10, 25, 50): A549 cells treated with 10, 25 and 50 µg/mL of the fruit methanolic extract respectively. GKME and EOME (25, 50, 100): A549 cells treated with 25, 50 and 100 µg/mL of the fruit methanolic extract respectively. 5FU-100: A549 cells treated with 100 µg/mL of 5FU (positive control). Values are expressed as Mean ± SEM. Different letters indicate significant variation

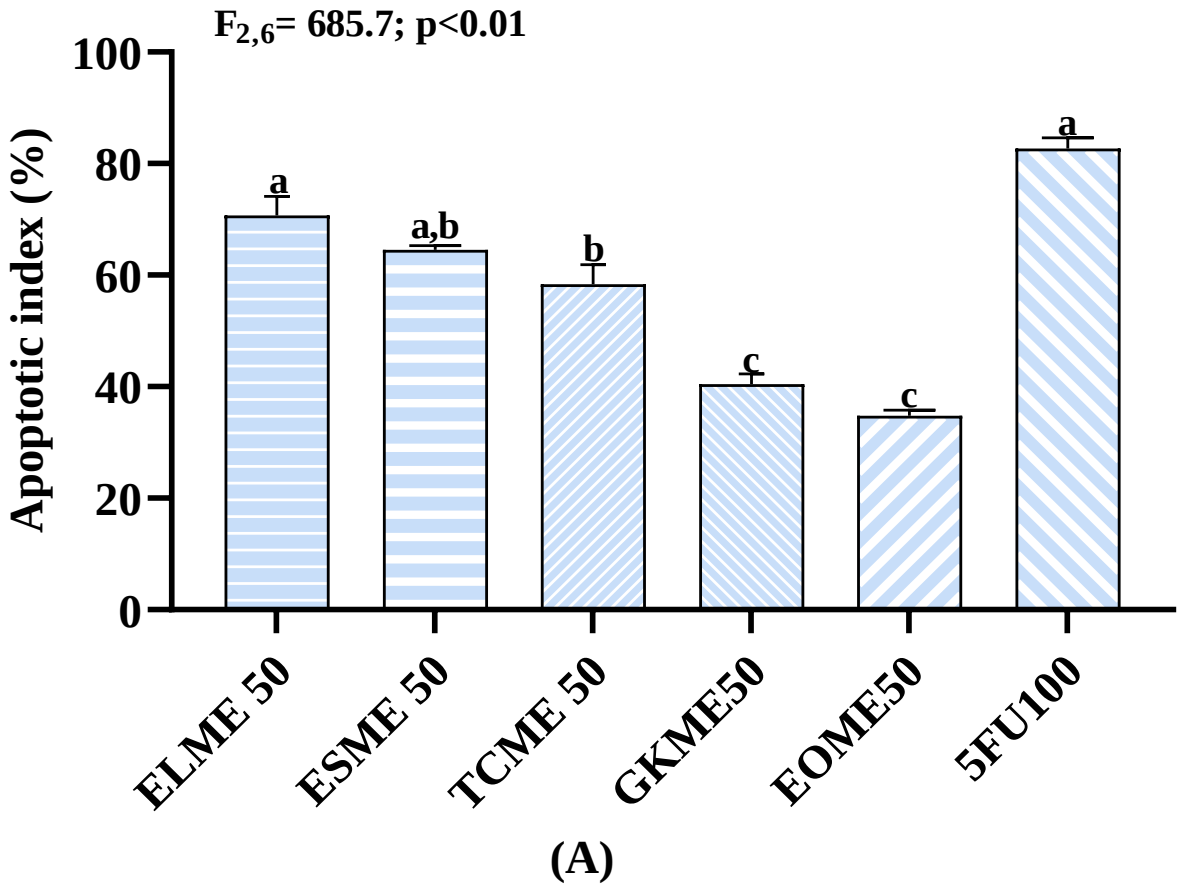


Fig. 4.42 (A) Comparison of Apoptotic index of the methanolic extracts at the dose of 50 µg/ml

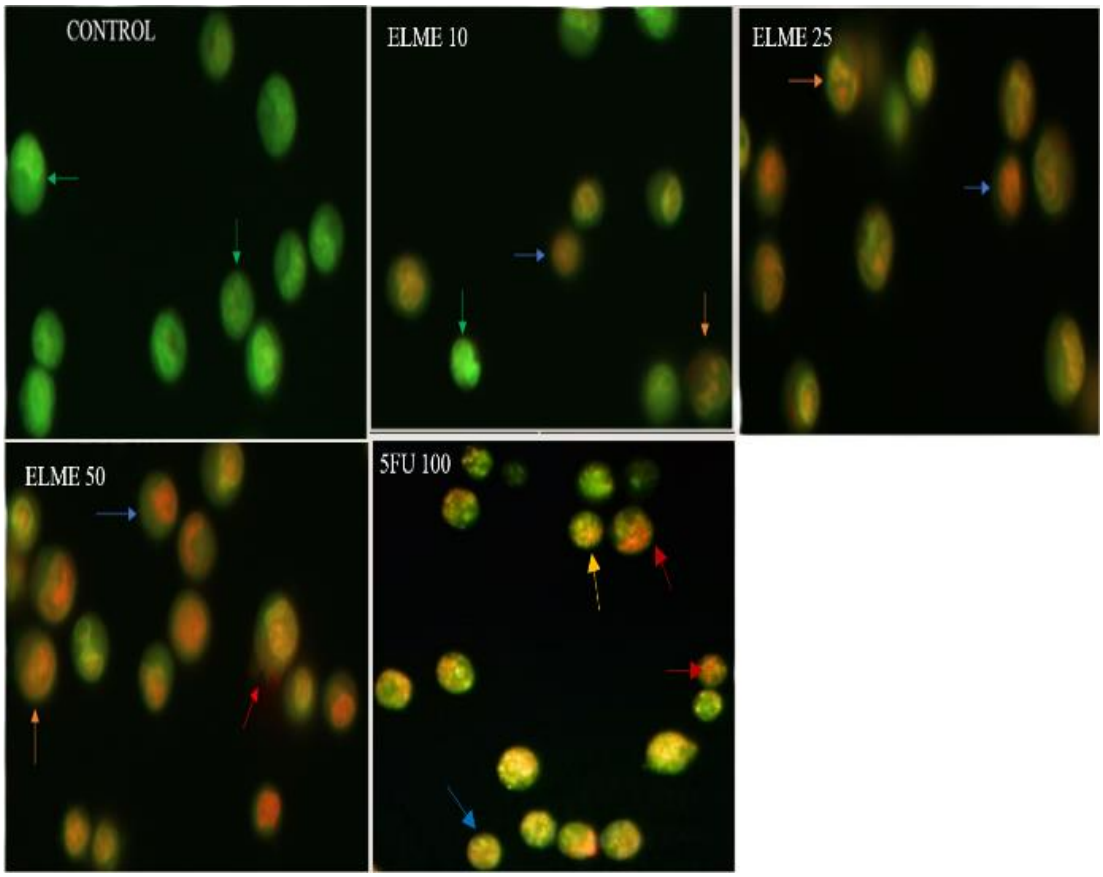


Photo plate 4.1

Fluorescence microscopic images of A549 treated cells with different doses of ELME- *Elaeocarpus lanceifolius* Methanolic extract reveal morphological alterations such as membrane blebbing, nuclear condensation, and nuclear fragmentation which are the distinct characteristics of apoptotic cells.

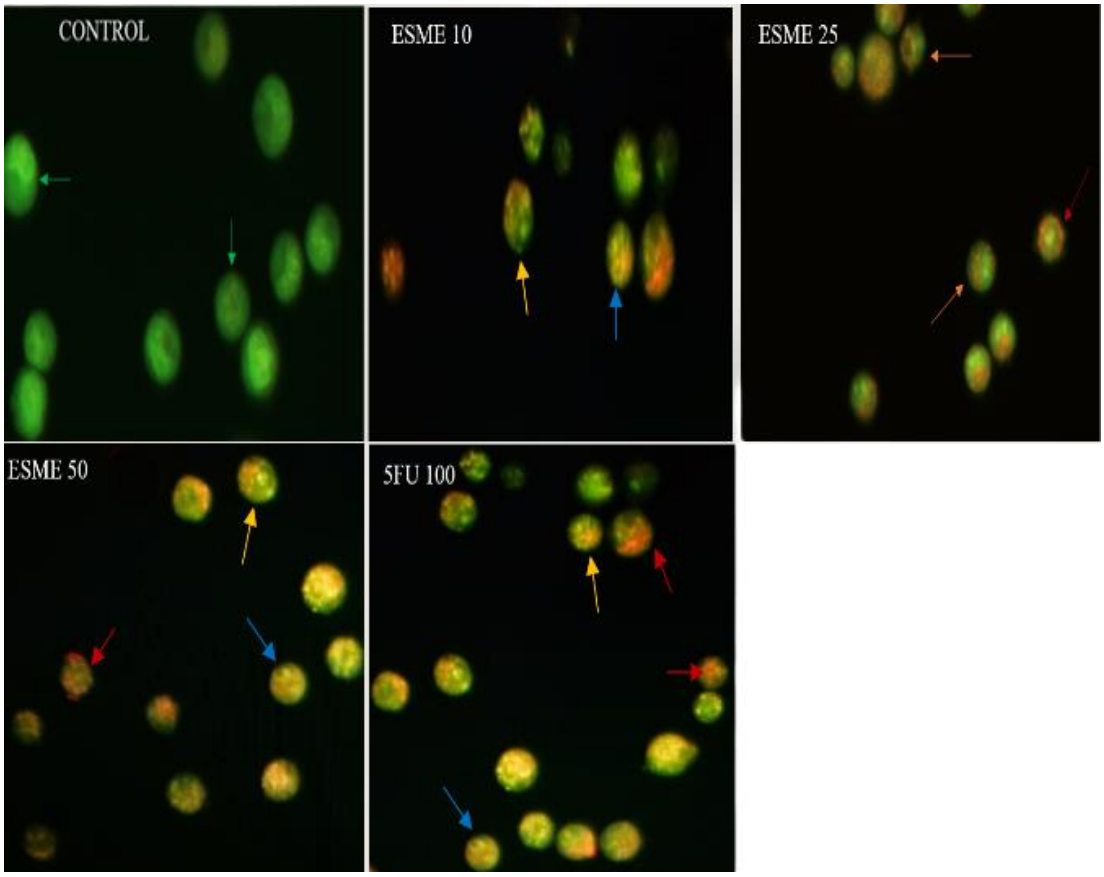


Photo plate 4.2

Fluorescence microscopic images of A549 treated cells with different doses of ESME- *Embelia subcoriacea* Methanolic extract reveal morphological alterations such as membrane blebbing, nuclear condensation, and nuclear fragmentation which are the distinct characteristics of apoptotic cells.

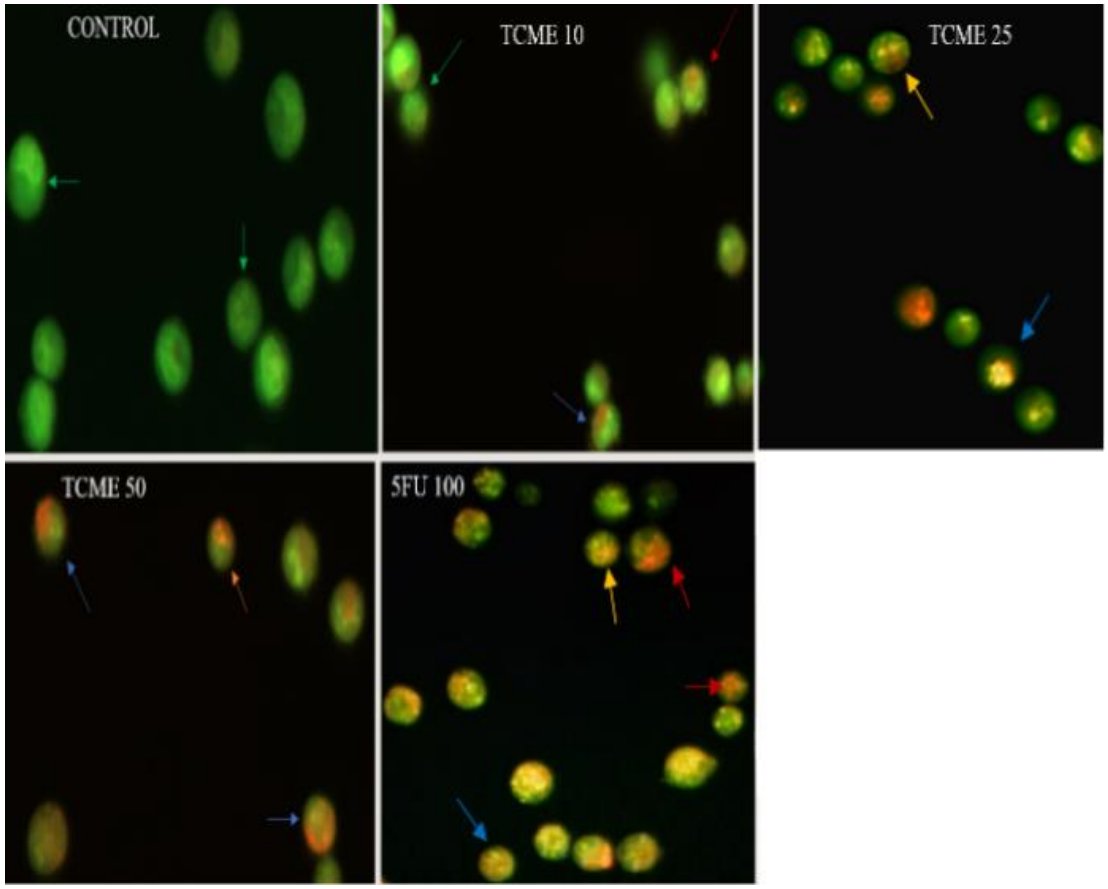


Photo plate 4.3

Fluorescence microscopic images of A549 treated cells with different doses of TCME *Terminalia chebula* Methanolic extract reveals morphological alterations such as membrane blebbing, nuclear condensation, and nuclear fragmentation which are the distinct characteristics of apoptotic cells.

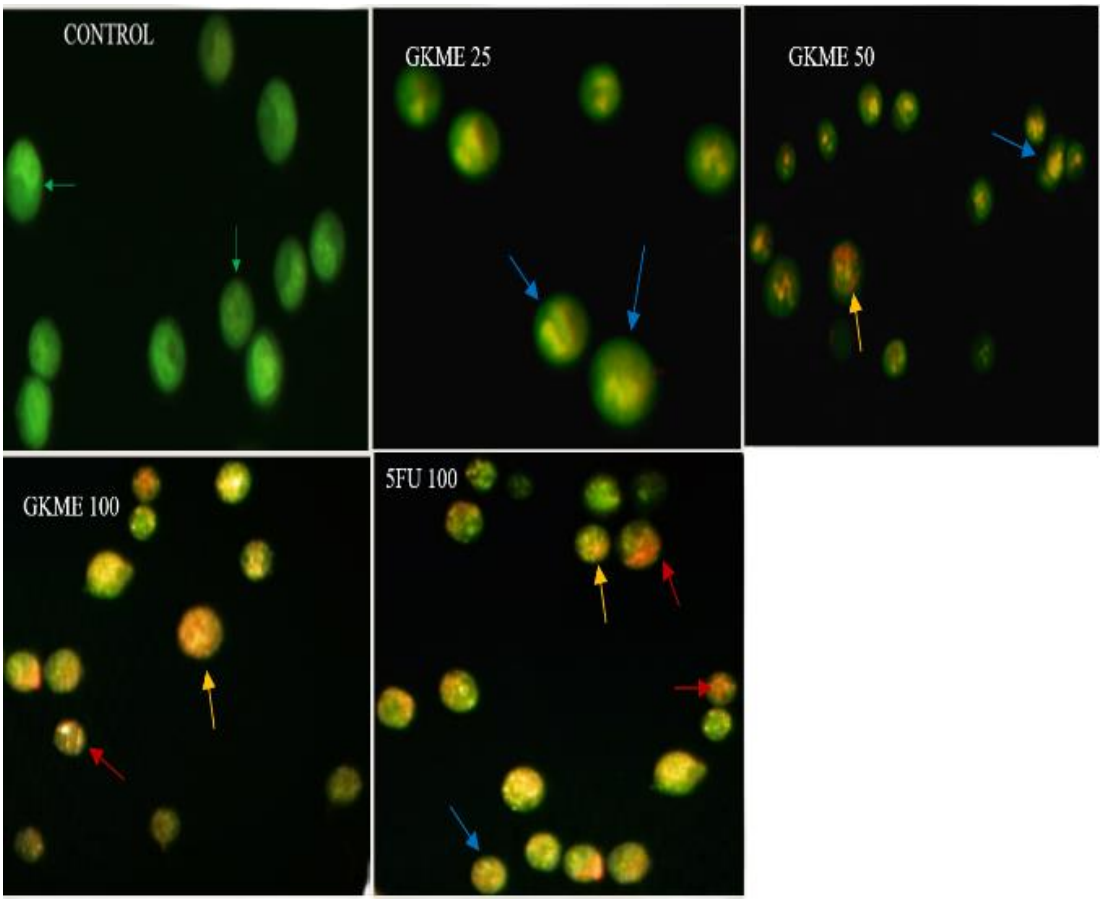


Photo plate 4.4

Fluorescence microscopic images of A549 treated cells with different doses of GKME- *Garcinia kydia* Methanolic extract reveal morphological alterations such as membrane blebbing, nuclear condensation, and nuclear fragmentation which are the distinct characteristics of apoptotic cells.

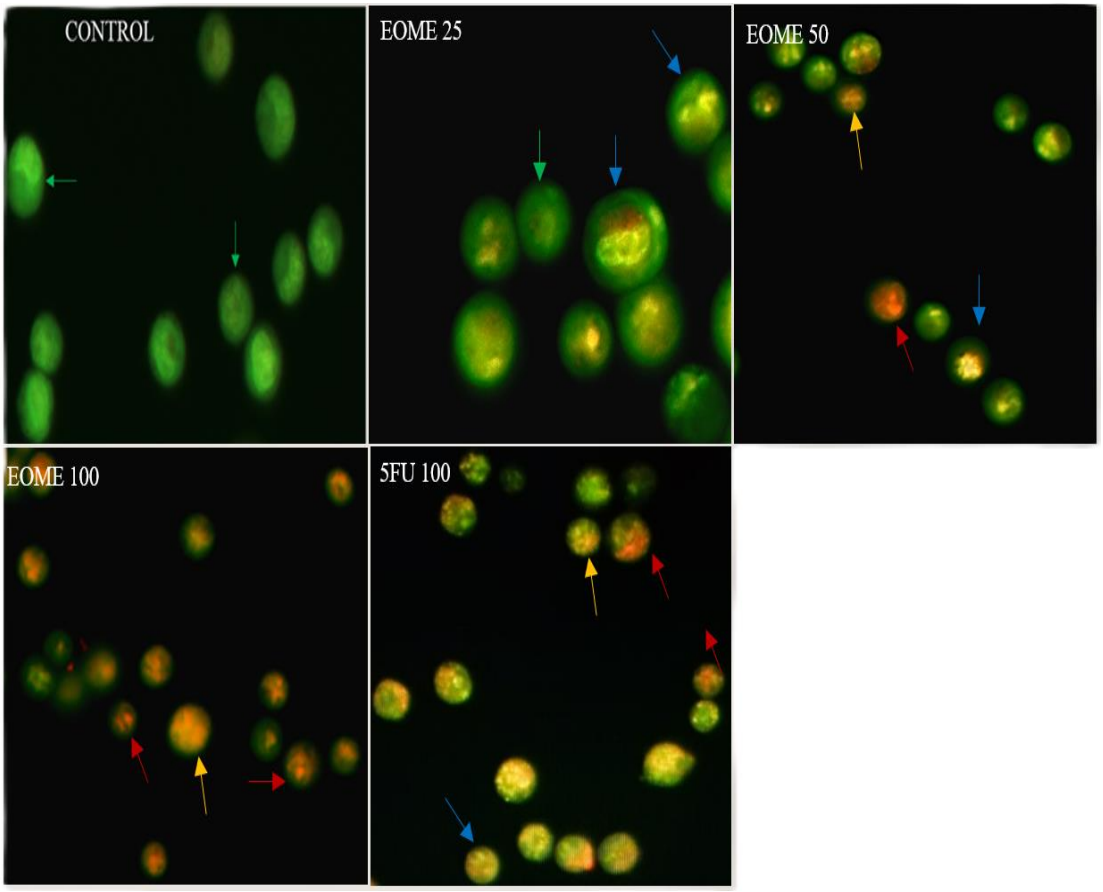


Photo plate 4.5

Fluorescence microscopic images of A549 treated cells with different doses of *Emblica officinalis* Methanolic extract reveal morphological alterations such as membrane blebbing, nuclear condensation, and nuclear fragmentation which are the distinct characteristics of apoptotic cells.

4.4.1.3 Antioxidants/Oxidant Status

Maintaining an equilibrium between the production and elimination of reactive oxygen species (ROS) is essential, as elevated ROS levels have been associated with the onset of various diseases, including cancer (Dickinson and Chang, 2011; Weidinger and Kozlov, 2015; Reczek and Chandel, 2017). The redox balance within a cell is upheld by a diverse array of the antioxidant system, encompassing both enzymatic components (including CAT, SOD, GPx, and GST) and non-enzymatic elements (such as GSH, ascorbic acid, and lipoic acid) (Hanschmann *et al*., 2013). Cancer cells frequently demonstrate higher levels of reactive oxygen species (ROS) in comparison to healthy cells, primarily due to enhanced metabolic activity and mitochondrial dysfunction. Elevated levels of reactive oxygen species (ROS) are intricately associated with multiple phases of cancer progression, including tumour initiation, angiogenesis, cellular invasion, metastasis, and the development of resistance to chemotherapy across various cancer models. Consequently, agents capable of significantly increasing reactive oxygen species (ROS) production or inhibiting the antioxidant defence mechanisms may present a viable strategy for the eradication of cancer cells. Consequently, the application of antioxidants or agents that enhance the antioxidant system may reduce intracellular ROS-mediated tumorigenesis and the progression of cancer (Lalremruati 2022).

The concentrations and activities of antioxidants and oxidants in A549 cells were assessed following treatment with the five fruit extracts. The administration of *Elaeocarpus lanceifolius* Methanolic extract (ELME), *Embelia*

subcoriacea Methanolic extract (ESME), *Terminalia chebula* Methanolic extract (TCME), *Garcinia kydia* Methanolic extract (GKME), and *Emblica officinalis* Methanolic extract (EOME) at a concentration of 50 µg/mL resulted in a significant reduction in glutathione (GSH) levels, as well as the activities of glutathione-s-transferase (GST) and superoxide dismutase (SOD), in comparison to the untreated control (Figure 4.44 A-C). The study aimed to determine the impact of fruit extract treatment on intracellular oxidant levels by evaluating lipid peroxidation (LPO) as a biomarker of oxidative stress. The findings indicate that treatments with ELME, ESME, TCME, GKME, and EOME significantly elevated MDA levels in A549 cells (Figure 4.44 D), suggesting that these treatments may contribute to an increase in intracellular ROS levels. The treatments ELME, ESME, and TCME demonstrated comparable efficacy to the standard pharmacological agent 5FU. The various fruit extracts administered at a concentration of 50 µg/mL resulted in a notable enhancement of antioxidant levels and activities in comparison to untreated cells (Fig. 4.44). The observed enhancement in antioxidant enzyme activities corresponded with a significant reduction in oxidative stress levels following various fruit extract treatments in A549 cells.

The elevation of reactive oxygen species (ROS) levels may consequently account for the cytotoxic effects of the fruit extracts on the A549 cells observed in this study. The findings of our study indicate that treatments with ELME, ESME, TCME, GKME, and EOME may elevate intracellular ROS levels by compromising the antioxidant system in A549 cells. Furthermore, this suggests that the cell death associated with these treatments may be related to the down-regulation of antioxidant

activities. Similarly, many anti-cancer agents, including doxorubicin, exert their cytotoxic effects primarily through the induction of reactive oxygen species (ROS) (Mai *et al*., 2016). While effective antioxidants are essential for sustaining redox homeostasis, elevated concentrations of these compounds have been implicated in the advancement of cancer and the development of chemoresistance. Elevated levels of glutathione have been documented in cancer cells (Godwin *et al*., 1992), resulting in enhanced neoplastic transformation, drug resistance, and subsequent failure of cancer treatments (Traverso *et al*., 2013; Ramsay and Dilda, 2014). Increased activities of GST and SOD have been documented in various human cancer cells, leading to resistance against therapeutic interventions (Jagetia and Venkatesha, 2012; Che *et al*., 2016; Tew, 2016; Allocati *et al*., 2018; Glorieux *et al*., 2018). Various plant species, such as *Cyathula prostrata*, *Hypericum hookerianum*, *Momordica charantia*, and *Cocculus hirsutus* (Thiagarajan *et al*., 2019), *Rhynchosia rufescens* (Khader *et al*., 2019), and *Malvapseudo lavatera* (Khoury *et al*., 2020), have demonstrated anti-cancer properties through the enhancement of the antioxidant defence system and the reduction of lipid peroxidation. Numerous plant-derived anti-cancer agents have demonstrated similar effectiveness across various cancer types. The current investigation demonstrated a notable lipid-damaging effect of fruit extracts on A549 cells. Lipid peroxidation represents a critical phenomenon linked to cellular mortality, having been documented to cause significant disruption in membrane functionality. This disruption manifests through heightened membrane permeability, oxidation of membrane proteins, and DNA damage, ultimately culminating in cell death (Chen and Niki, 2011).

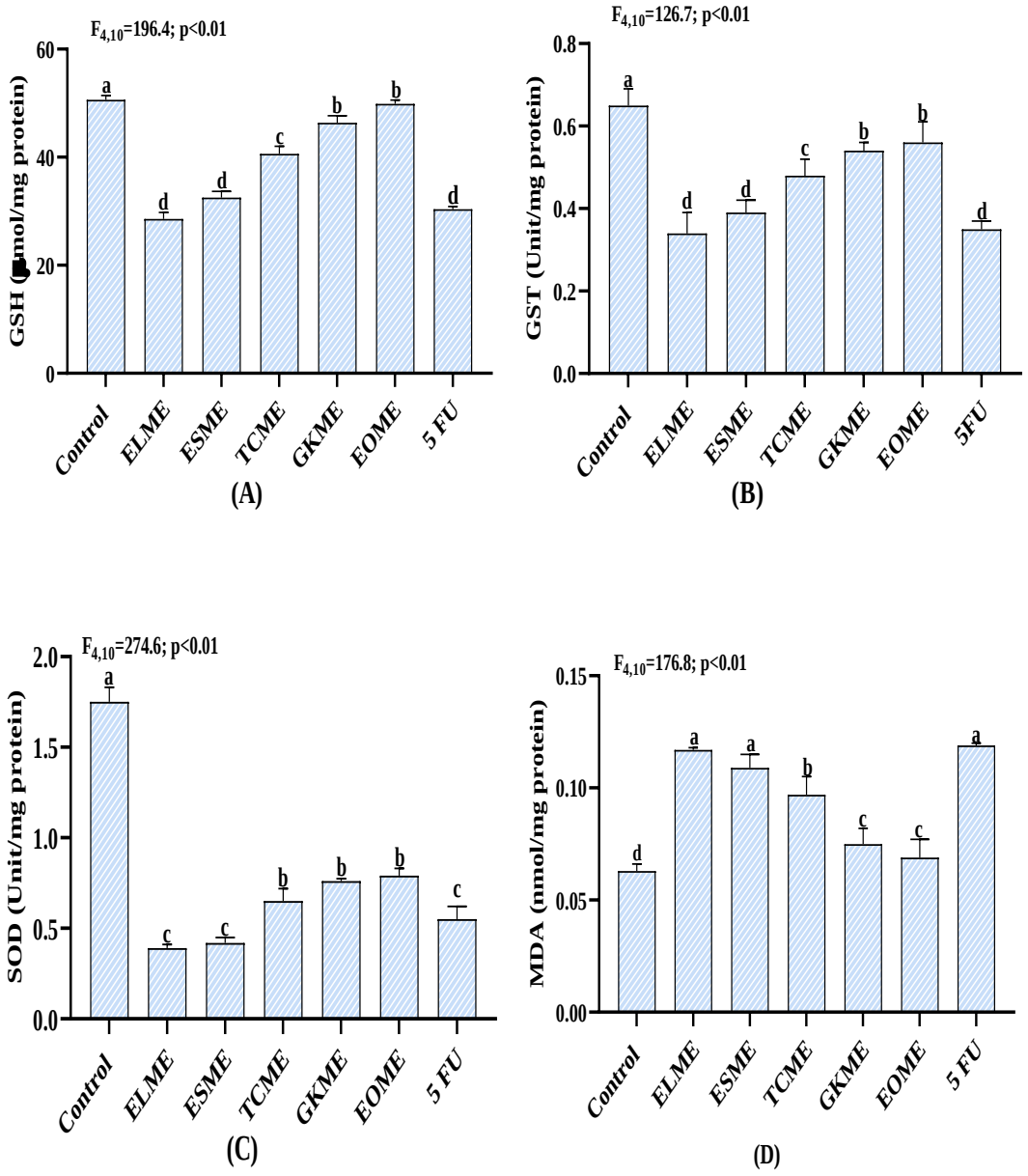


Figure 4.44: Effects of the methanolic extract of different underutilised fruits on (A) glutathione (GSH) level; (B) glutathione-s-transferase (GST) activity; (C) superoxide dismutase (SOD) activity; and (D) lipid peroxidation (LPO) expressed in malondialdehyde (nmol/mg protein) in A549 cells after 24 hr treatment. Control: A549 cells without treatment; ELME, ESME and TCME: A549 cells treated with 50 µg/mL of methanolic extract of *E. lanceifolius*, *E. subcoriacea* and *T. chebula* respectively; GKME and EOME: A549 cells treated with 100 µg/mL of methanolic extract of *G. kydia* and *E. officinalis* respectively; 5FU: A549 cells treated with 100 µg/mL of 5FU. Values are expressed as Mean ± SEM. Different letters indicate significant variation.

4.4.1.4 Effect of fruit extracts on the relative expression of pro-apoptotic and anti-apoptotic genes

To ascertain the involvement of Bcl-2 family proteins in apoptosis induced by fruit extracts, the relative mRNA expression levels of pro-apoptotic genes (Bax and Bid) and anti-apoptotic genes (Bcl-XL and Bcl-2) were evaluated utilising qRT-PCR methodologies. The treatment of A549 cells with 100 µg/mL of fruit extracts (*E. lanceifolius, E. subcoriacea, T. chebula, G. kydia*, and *E. officinalis*) for a duration of 24 hours resulted in the up-regulation of pro-apoptotic genes and the down-regulation of anti-apoptotic genes. Among the various fruit extracts examined, E. *lanceifolius* extract demonstrated the greatest efficacy, resulting in an up-regulation of pro-apoptotic genes, specifically Bax and Bid, by factors of 5.62 and 10.89, respectively. This was followed by *E. subcoriacea*, which induced an up-regulation of Bax and Bid by 4.62 and 8.89 folds, respectively, in comparison to the untreated control (Figure 4.45 A & B). The effects of *E. lanceifolius* and *E. subcoriacea* extracts on the relative expression of Bax and Bid in A549 cells surpassed those of the standard drug. No notable alterations were detected in the mRNA expression levels of Bax and Bid when comparing the standard drug (5 FU) with the groups treated with *G. kydia* and *E. officinalis* (Figure 4.45 A & B). Among the diverse fruit extracts, *E. lanceifolius* and *E. subcoriacea* extracts demonstrated a significant down-regulation of the anti-apoptotic genes Bcl-XL and Bcl-2 (Figure 4.45 C & D). The up-regulation of pro-apoptotic genes and the down-regulation of anti-apoptotic genes observed in this study indicate that apoptosis induced by fruit extracts in A549 cells may be regulated through the mitochondrial pathway.

The Bcl-2 (B-cell lymphoma/leukemia-2) family comprises pro- and anti-apoptotic proteins that, through their interactions, are pivotal in the intrinsic apoptosis pathway mediated by mitochondria, primarily by regulating mitochondrial membrane permeabilization (Xiong *et al.*, 2014). The sensitivity of cells to apoptotic stimuli is contingent upon the equilibrium between pro-apoptotic and anti-apoptotic Bcl-2 proteins. The presence of elevated pro-apoptotic Bcl-2 proteins on the mitochondrial surface leads to the formation A concentration-dependent increase in

the apoptotic index (%) was noted in the treated cells relative to the control group. Numerous studies indicate that a range of anticancer agents function by triggering apoptosis in malignant cells, with several compounds derived from medicinal plants demonstrating anticancer efficacy via the induction of apoptosis (Wang and Fang, 2009). The permeability transition (PT) pore facilitates a sequential release of pro-apoptotic proteins, including cytochrome c, Smac/Diablo, and apoptosis-inducing factor (AIF), which subsequently activates caspase-3 and caspase-6 (Arnoult *et al*., 2003). Consequently, changes in the expression of these genes play a significant role in the pathogenesis and progression of cancers, thereby offering potential targets for the discovery of anti-cancer drugs. The up-regulation of pro-apoptotic genes observed in this study indicates that the methanolic extracts of the fruit may induce apoptosis in A549 cells, potentially through modulation of the mitochondrial pathway. The modulation of anti-apoptotic genes, including Bcl-XL and Bcl-2, alongside pro-apoptotic genes such as Bax and Bid, by various plant extracts has been previously reported in A549 cells (Hansakul *et al*., 2014; Sharifi *et al*., 2018; Thiagarajan *et al*., 2019; Panicker *et al*., 2020).

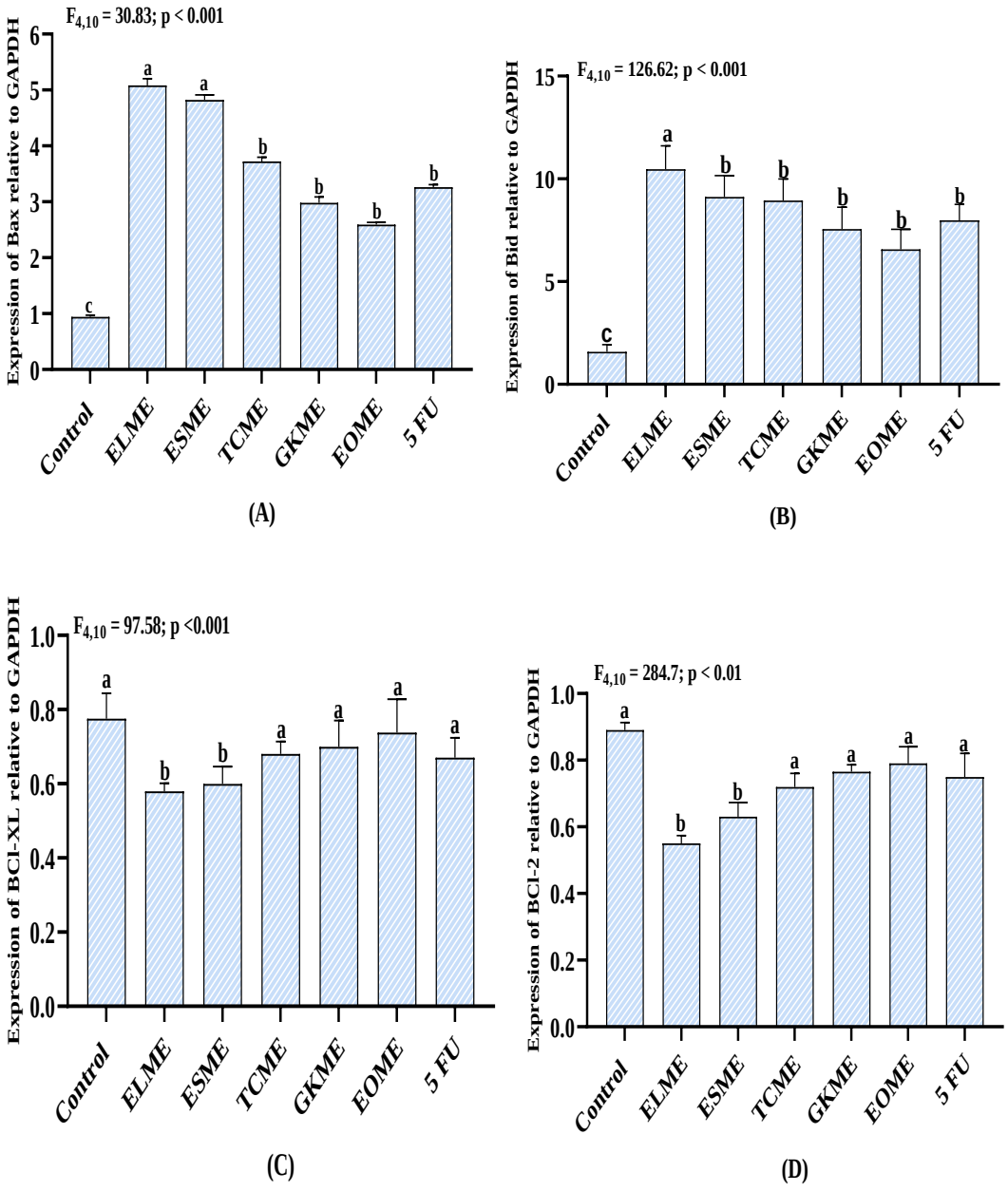
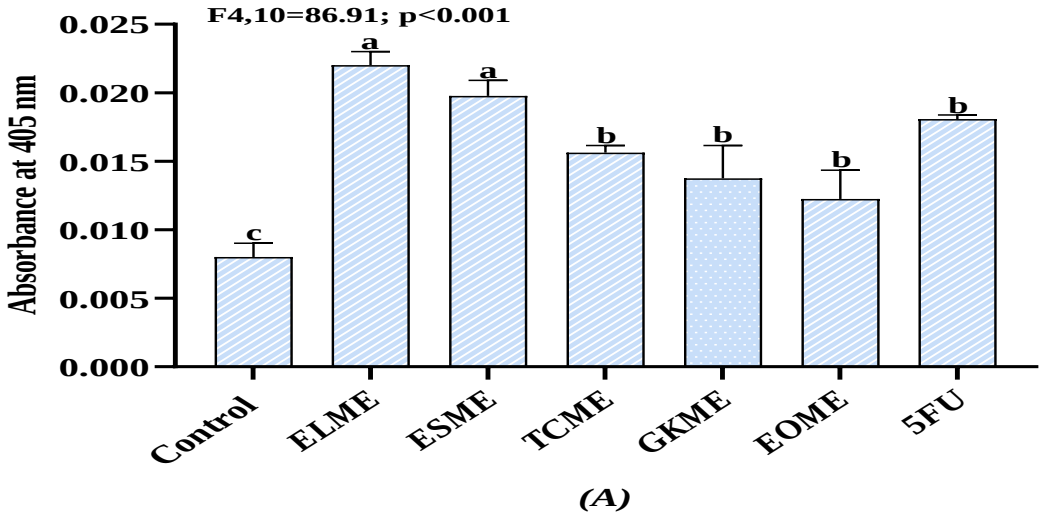


Fig. 4.45. Effects of the methanolic extract of different underutilised fruits on (A) Bax; (B) Bid; (C) BCl-X_L; and (D) BCl-2 in A549 cells after 24 h treatment. Control: A549 cells without treatment; ELME, ESME, TCME, GKME and EOME: A549 cells treated with 100 µg/mL of methanolic extract of *E. lanceifolius*, *E. subcoriacea*, *T. chebula*, *G. kydia* and *E. officinalis* respectively; 5FU: A549 cells treated with 100 µg/mL of 5FU. Values are expressed as Mean ± SEM. Different letters indicate significant variation.

4.4.1.5 Effects of fruit extracts on the activities of caspase-3 and caspase-6

The impact of drupe extracts on apoptosis in A549 cells was assessed through the evaluation of caspase-3 and caspase-6 activities. Treatment of A549 cells with 100 µg/mL of fruit extracts for 24 hours resulted in a notable enhancement in the activities of caspase-3 and caspase-6. Among various fruit extracts, *E. lanceifolius* extract demonstrated the greatest efficacy, resulting in a 3.92-fold and 6.6-fold increase in the activities of caspase-3 and caspase-6, respectively. This was followed by *E. subcoriacea*, which exhibited a 2.32-fold and 4.21-fold increase in the activities of caspase-3 and caspase-6, respectively, in comparison to the untreated control. The activities of caspase-3 and caspase-6 were significantly elevated in the groups treated with extracts of *E. lanceifolius* and *E. subcoriacea* compared to the standard drug 5FU (Figure 4.46). Nonetheless, no notable differences were detected in the activities of caspase-3 and caspase-6 across *G. Kydia* extract, *E. officinalis* extract, and 5 FU-treated A549 cells.

Caspase-3 and caspase-6 are responsible for the cleavage of several essential proteins involved in the intrinsic (mitochondrial-mediated) and extrinsic (death receptor-mediated) pathways of apoptosis. These proteins include the inhibitor of caspase-activated DNase (ICAD), poly (ADP ribose) polymerase (PARP), and various intra-nuclear proteins (Porter and Janicke, 1999). The cleavage facilitates the disassembly of the cell, resulting in apoptotic morphological alterations such as cell shrinkage, chromatin condensation, and nuclear fragmentation (Elmore, 2007). Consequently, the activation of caspase-3 and caspase-6 serves as a robust biomarker for cells in the process of apoptosis. The administration of fruit extracts to A549 cells resulted in elevated activities of caspase-3 and caspase-6, thereby strongly suggesting that the apoptosis induced by the fruit extracts occurs via a caspase-dependent mechanism. In summary, the anticancer mechanisms of the fruit extract are characterised by the induction of mitochondria-mediated apoptosis, which is facilitated by the enhanced activities of caspase-3 and caspase-6. Additionally, there is an upregulation of pro-apoptotic genes such as Bax and Bid, accompanied by a downregulation of anti-apoptotic genes including Bcl-2 and Bcl-XL. These findings indicate that fruit extracts may serve as a promising candidate for cancer therapy.



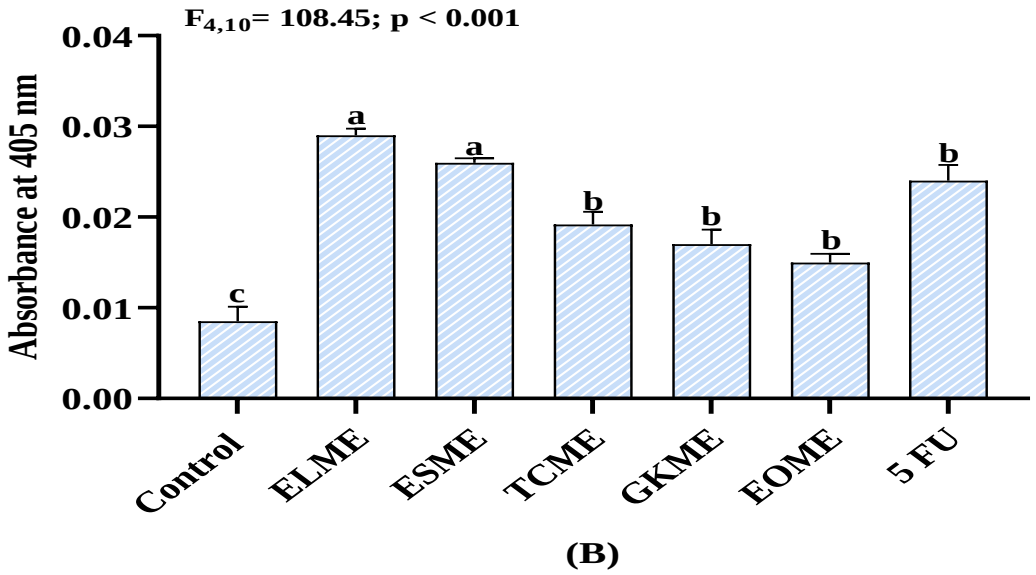


Fig. 4.46. Effects of the methanolic extract of different underutilised on **(A)** Caspase-3 and **(B)** Caspase-6 in A549 cells after 24 h treatment. Control: A549 cells without treatment; ELME, ESME, TCME, GKME and EOME: A549 cells treated with 100 µg/mL of methanolic extract of *E. lanceifolius*, *E. subcoriacea*, *T. chebula*, *G. kydia* and *E. officinalis* respectively; 5FU: A549 cells treated with 100 µg/mL of 5FU. Values are expressed as Mean ± SEM. Different letters indicate significant variation.

4.4.1.6 LC-HRMS of the five underutilised fruits having the highest cytotoxicity.

LC-HRMS was performed on the five underutilised samples having the highest cytotoxicity in MTT Assay and were further analysed to identify the compounds which might be responsible for the cytotoxicity effect of the fruit against A549 cells. These compounds would then be further analysed through molecular docking and simulation for their interaction and binding affinity for the lung cancer cells.

4.4.1.6.1 *Elaeocarpus lanceifolius*

From the LC-HRMS analysis, forty-one (41) compounds were isolated (Figure 4.10) and identified from *Elaeocarpus lanceifolius* out of which the compounds showing higher peaks were Tyr-Oet (R.T = 2.08), Isoflurophate (R.T = 4.09), Uh-301 (R.T = 5.36), L-Kynurenine (R.T = 24.13), Dodine (R.T = 25.27), Estrone (R.T = 26.21), Brn 0575813 (R.T = 26.85), Soyasapogenol B 3-O-D-Glucuronide (R.T = 27.40) (Figure 4.47 and 4.48).

4.4.1.6.2 *Embelia subcoriacea*

As depicted in Table 4.11, fifty-two (52) compounds were isolated and identified in our present investigation with LC-HRMS analysis in *Embelia subcoriacea* out of which the compounds having higher peaks were Bergaptol (R.T = 1.67), Resistomycin (R.T = 2.19), (R)-Mevalonate (R.T = 4.35), Chitobiose (R.T = 24.03), Prostaglandin B1 (R.T = 25.48), (15Z)-Tetracosenoic acid (R.T = 26.19), Acetylleucyl-leucyl-norleucinal (R.T = 27.61) (Figure 4.49 and 4.50).

4.4.1.6.3 *Terminalia chebula*

Among the 38 compounds isolated from the underutilised fruit *Terminalia chebula* in the LC-HRMS analysis, as represented in Table 4.12 the compound having higher peaks were Dihydro-heme d1(R.T = 1.67), Deoxyuridine (R.T = 1.67), trans-Cinnamate (R.T = 1.67), Terpendole C (R.T = 1.67) and Isopropamide (R.T = 1.67) (Figure 4.51 and 4.52).

4.4.1.6.4 *Garcinia Kydia*

In the present investigation of the underutilised fruit, *Garcinia kydia*, thirty-three (33) compounds were isolated and identified as depicted in Table 4.13. The compounds having higher peaks were Taurine (R.T = 1.67), trans-Cinnamate (R.T = 1.67) and (15Z)-Tetracosenoic acid (R.T = 1.67) (Figure 4.53 and 4.54).

4.4.1.6.5 *Emblica officinalis*

From the data depicted in Table 4.14, fifty-three (53) compounds were isolated and identified from *Emblica officinalis* in the present study, out of which Citrate (R.T = 2.06), Lancerin (R.T = 2.81), Hydroxytamoxifen (R.T = 23.62) and L-Octanoylcarnitine (R.T = 26.20) were the compounds having a higher peak (Figure 4.55 and 4.56).

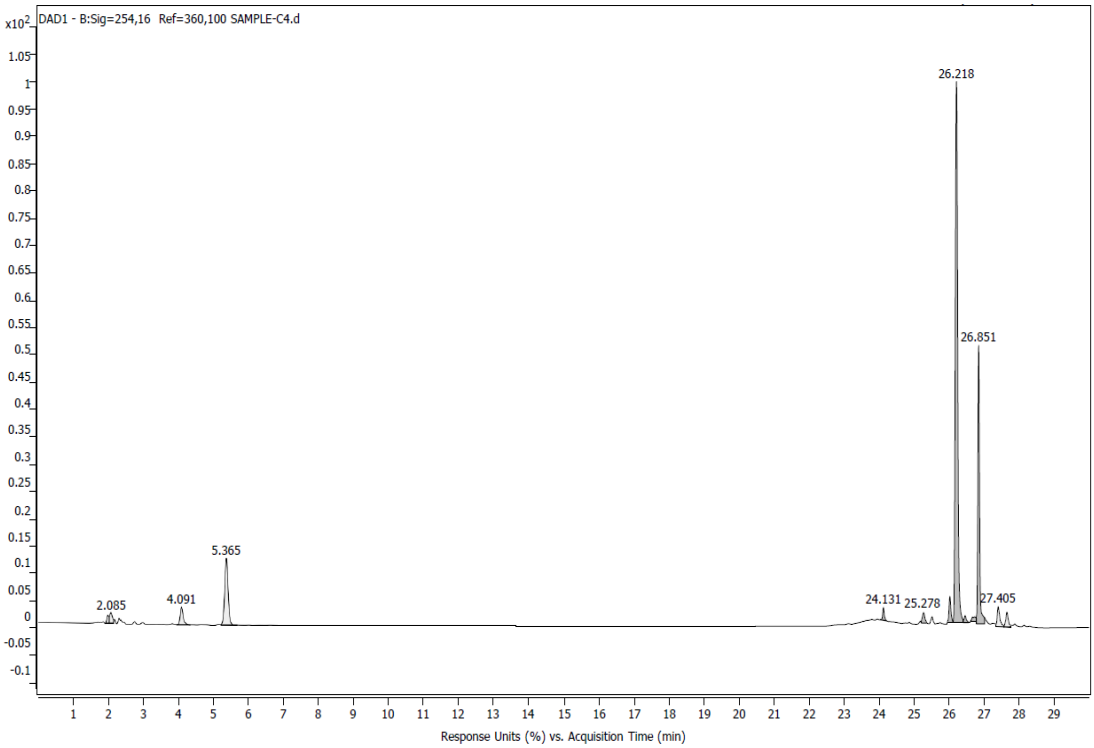


Fig. 4. 47 LC-HRMS UV Chromatogram for *Elaeocarpus lanceifolius*

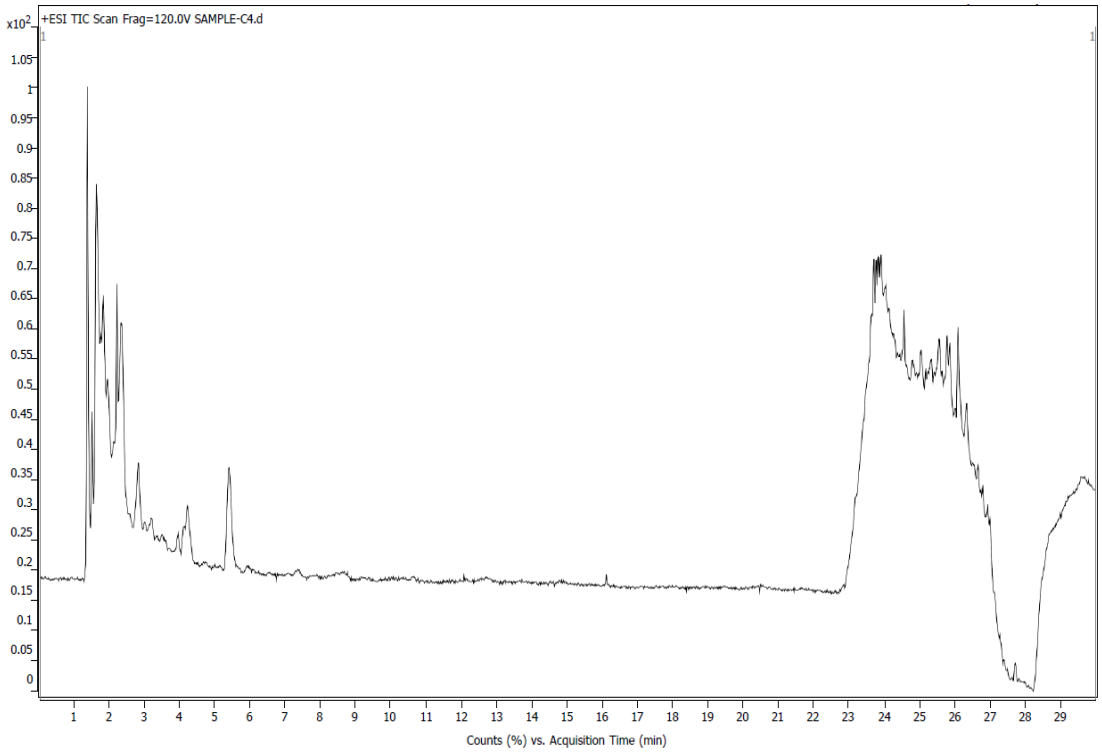


Fig. 4.48 LC-HRMS TIC Chromatogram for *Elaeocarpus lanceifolius*

Table 4.10 Compounds identified through LC-HRMS in *Elaeocarpus lanceifolius*

Sl.No	Retention Time (Minutes)	Compound Name	Molecular Weight (g/mol)	Chemical Formula
1.	1.72	L-Valine	117.15	$C_5H_{11}NO_2$
2.	1.77	Anthranilate	137.14	$C_7H_7NO_2$
3.	2.37	2-Deoxy-D-Ribose 1-Phosphate	214.11	$C_5H_{11}O_7P$
4.	1.69	N-Benzoyl-4-Hydroxyanthranilate	256.23	$C_{14}H_{10}NO_4^-$
5.	2.35	4-Nitrophenol	139.11	$C_6H_5NO_3$
6.	23.71	Stearoylglycerone Phosphate	436.5	$C_{21}H_{41}O_7P$
7.	26.21	Estrone	270.4	$C_{18}H_{22}O_2$
8.	26.09	Sterol	248.4	$C_{17}H_{28}O$
9.	24.13	L-Kynurenine	208.21	$C_{10}H_{12}N_2O_3$
10.	25.27	Dodine	287.44	$C_{15}H_{33}N_3O_2$
11.	1.64	Deoxyadenosine	251.24	$C_{10}H_{13}N_5O_3$
12.	2.08	Tyr-Oet	209.24	$C_{11}H_{15}NO_3$
13.	23.78	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$
14.	27.40	Soyasapogenol B 3-O-D-Glucuronide	634.8	$C_{36}H_{58}O_9$
15.	24.56	Curcumin Monoglucoside	530.5	$C_{27}H_{30}O_{11}$
16.	23.63	Betamethasone	392.5	$C_{22}H_{29}FO_5$
17.	26.85	Brn 0575813	634.94	$C_{12}H_8I_3N_5O_2$
18.	1.40	Aromatic Aldehyde	107.05	C_7H_6O
19.	2.85	Naringenin	272.25	$C_{15}H_{12}O_5$
20.	1.69	N-Acetylmuramate	293.27	$C_{11}H_{19}NO_8$
21.	25.86	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$
22.	1.40	(S)-2,3,4,5-Tetrahydropyridine-2-Carboxylate	127.14	$C_6H_9NO_2$
23.	23.66	Hydroxytamoxifen	387.5	$C_{26}H_{29}NO_2$
24.	4.09	Isoflurophate	184.15	$C_6H_{14}FO_3P$
25.	23.79	Netilmicin	475.6	$C_{21}H_{41}N_5O_7$
26.	1.90	Tolmetin Sodium	279.27	$C_{15}H_{14}NNaO_3$
27.	1.64	Thiamine	265.36	$C_{12}H_{17}N_4OS^+$
28.	2.35	Aciclovir	225.2	$C_8H_{11}N_5O_3$
29.	2.40	Lancerin	406.3	$C_{19}H_{18}O_{10}$
30.	1.69	N-Acetylmuramoyl-Ala	364.35	$C_{14}H_{24}N_2O_9$
31.	23.86	Protoporphyrinogen Ix	568.7	$C_{34}H_{40}N_4O_4$
32.	24.56	Flavonol 3-O-D-Xylosylglucoside	532.5	$C_{26}H_{28}O_{12}$
33.	1.67	Thymine	126.11	$C_5H_6N_2O_2$
34.	25.79	Fenpropimorph	303.5	$C_{20}H_{33}NO$
35.	26.34	Octadecanamide	283.5	$C_{18}H_{37}NO$
36.	1.65	D-Fructose 6-Phosphate	260.14	$C_6H_{13}O_9P$
37.	23.56	Veatchine	343.5	$C_{22}H_{33}NO_2$
38.	1.40	3-(3,4-Dihydroxyphenyl)Lactate	197.16	$C_9H_9O_5^-$
39.	2.37	Citrate	192.12	$C_6H_8O_7$
40.	5.36	Uh-301	265.37	$C_{16}H_{24}FNO$
41.	1.40	Adenine	135.13	$C_5H_5N_5$

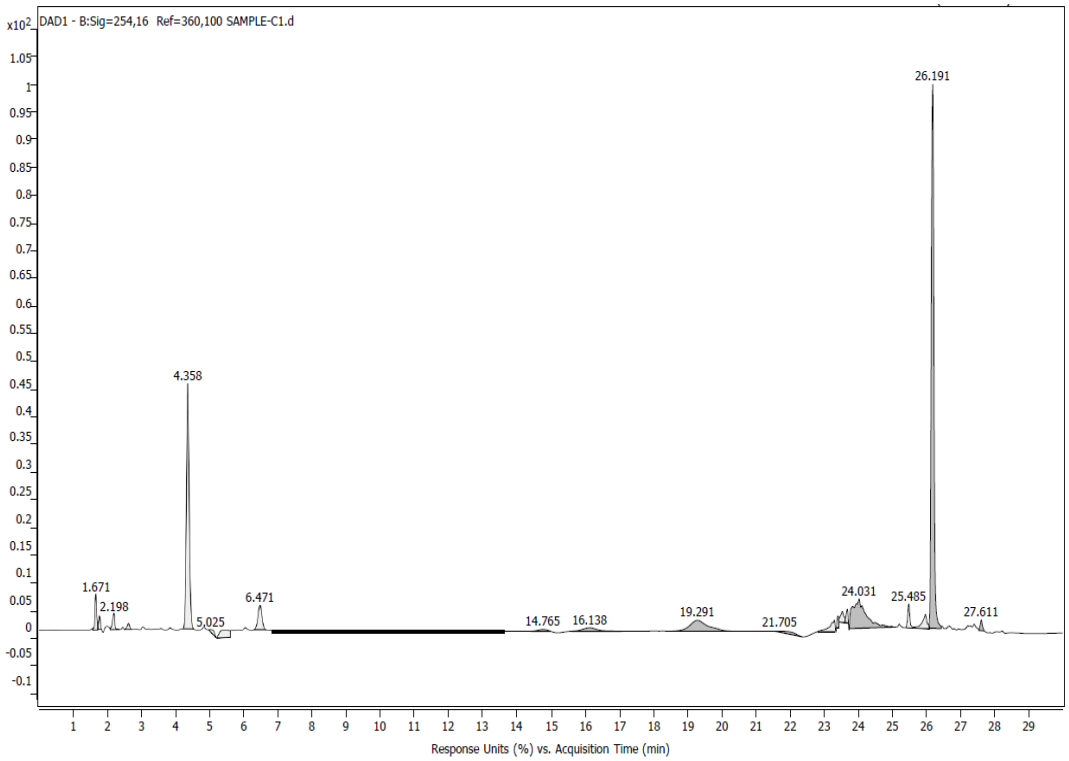


Fig. 4.49 LC-HRMS UV Chromatogram for *Embelia subcoriacea*

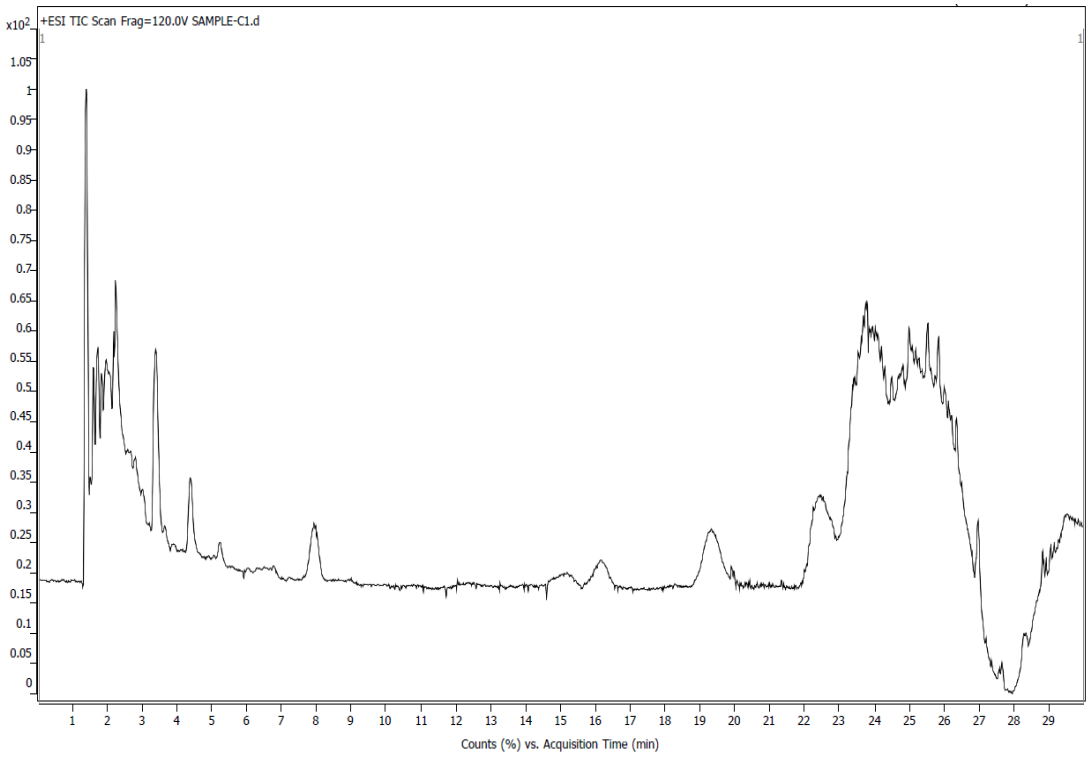


Fig. 4.50 LC-HRMS TIC Chromatogram for *Embelia subcoriacea*

Table 4.11 Compounds identified through LC-HRMS in *Embelia subcoriacea*

Sl.No	Retention Time (Minutes)	Compound Name	Molecular Weight (g/mol)	Chemical Formula
1.	1.96	Riboflavin	376.4	$C_{17}H_{20}N_4O_6$
2.	1.64	2-Hydroxy-6-oxo-6-phenylhexa-2,4-dienoate	217.2	$C_{12}H_9O_4^-$
3.	25.48	Prostaglandin B1	336.5	$C_{20}H_{32}O_4$
4.	2.34	2-Deoxy-D-ribose 1-phosphate	214.11	$C_5H_{11}O_7P$
5.	3.41	Lipoate	206.3	$C_8H_{14}O_2S_2$
6.	4.35	(R)-Mevalonate	147.15	$C_6H_{11}O_4^-$
7.	25.03	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$
8.	3.44	Deoxyuridine	228.2	$C_9H_{12}N_2O_5$
9.	2.28	Lancerin	406.3	$C_{19}H_{18}O_{10}$
10.	24.23	Mycolactone F	701	$C_{42}H_{68}O_8$
11.	3.46	(L-Seryl)adenylate	434.3	$C_{13}H_{19}N_6O_9P$
12.	2.02	Ipolamiide	406.4	$C_{17}H_{26}O_{11}$
13.	3.38	Naringenin 7-O-beta-D-glucoside	434.4	$C_{21}H_{22}O_{10}$
14.	26.97	Cannabidiolic acid	358.5	$C_{22}H_{30}O_4$
15.	23.70	Stearoylglycerone phosphate	436.5	$C_{21}H_{41}O_7P$
16.	23.76	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$
17.	25.30	Hexocyclium	317.5	$C_{20}H_{33}N_2O^+$
18.	2.19	Resistomycin	376.4	$C_{22}H_{16}O_6$
19.	1.62	Urate D-ribonucleotide	380.2	$C_{10}H_{13}N_4O_{10}P$
20.	1.79	4-Deoxy-4-thio-alpha-D-digitoxosyl-calicheamicin T0	730.9	$C_{30}H_{38}N_2O_{11}S_4$
21.	23.63	Betamethasone	392.5	$C_{22}H_{29}FO_5$
22.	23.70	Istamycin C1	431.5	$C_{19}H_{37}N_5O_6$
23.	2.19	Methylene blue	319.9	$C_{16}H_{18}ClN_3S$
24.	1.41	3-(3,4-Dihydroxyphenyl)lactate	198.17	$C_9H_{10}O_5$
25.	22.45	DTXSID50906063	731.7	$C_{40}H_{36}Cl_2N_8O_2$
26.	2.43	Chlorpheniramine maleate	390.9	$C_{20}H_{23}ClN_2O_4$
27.	23.81	Terpendole C	519.7	$C_{32}H_{41}NO_5$
28.	1.42	4-Maleylacetoacetate	200.14	$C_8H_8O_6$
29.	2.89	1-O-(1Z-Tetradecenyl)-2-(9Z-octadecenoyl)-sn-glycerol	550.9	$C_{35}H_{66}O_4$
30.	25.87	Prostaglandin F2alpha	354.5	$C_{20}H_{34}O_5$
31.	1.41	Aromatic aldehyde	106.12	C_7H_6O
32.	1.41	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate	127.14	$C_6H_9NO_2$
33.	2.51	1-O-(cis-9-Octadecenyl)-2-O-acetyl-sn-glycero-3-phosphocholine	549.7	$C_{28}H_{56}NO_7P$
34.	26.19	(15Z)-Tetracosenoic acid	366.6	$C_{24}H_{46}O_2$
35.	26.93	CBiol_001716	323.5	$C_{20}H_{37}NO_2$
36.	2.19	Berberine	336.4	$C_{20}H_{18}NO_4^+$
37.	27.61	Acetylleucyl-leucyl-norleucinal	383.5	$C_{20}H_{37}N_3O_4$
38.	23.90	Mancinellin	612.8	$C_{36}H_{52}O_8$
39.	24.00	L-Kynurenine	208.21	$C_{10}H_{12}N_2O_3$
40.	3.41	7,8-Dihydro-7,8-dihydroxykynurenate	222.17	$C_{10}H_8NO_5^-$
41.	1.67	Bergaptol	202.16	$C_{11}H_6O_4$
42.	2.26	Deoxy-5-methylcytidylate	321.22	$C_{10}H_{16}N_3O_7P$
43.	2.23	Tyr-OEt	209.24	$C_{11}H_{15}NO_3$
44.	24.03	Chitobiose	424.4	$C_{16}H_{28}N_2O_{11}$
45.	25.08	N-LAUROYLSARCOSINE	271.4	$C_{15}H_{29}NO_3$
46.	23.86	DTXSID80275032	565.1	$C_{28}H_{52}Cl_3N_5$
47.	24.08	Dicumarol	336.3	$C_{19}H_{12}O_6$
48.	25.85	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$
49.	1.84	alpha-Erythroidine	273.33	$C_{16}H_{19}NO_3$
50.	25.08	17a-Aza-D-homoandrost-5-en-3beta-ol	289.5	$C_{19}H_{31}NO$
51.	25.10	O-Decanoyl-L-carnitine	315.4	$C_{17}H_{33}NO_4$
52.	1.56	2-Bromoacetaldehyde	122.95	C_2H_3BrO

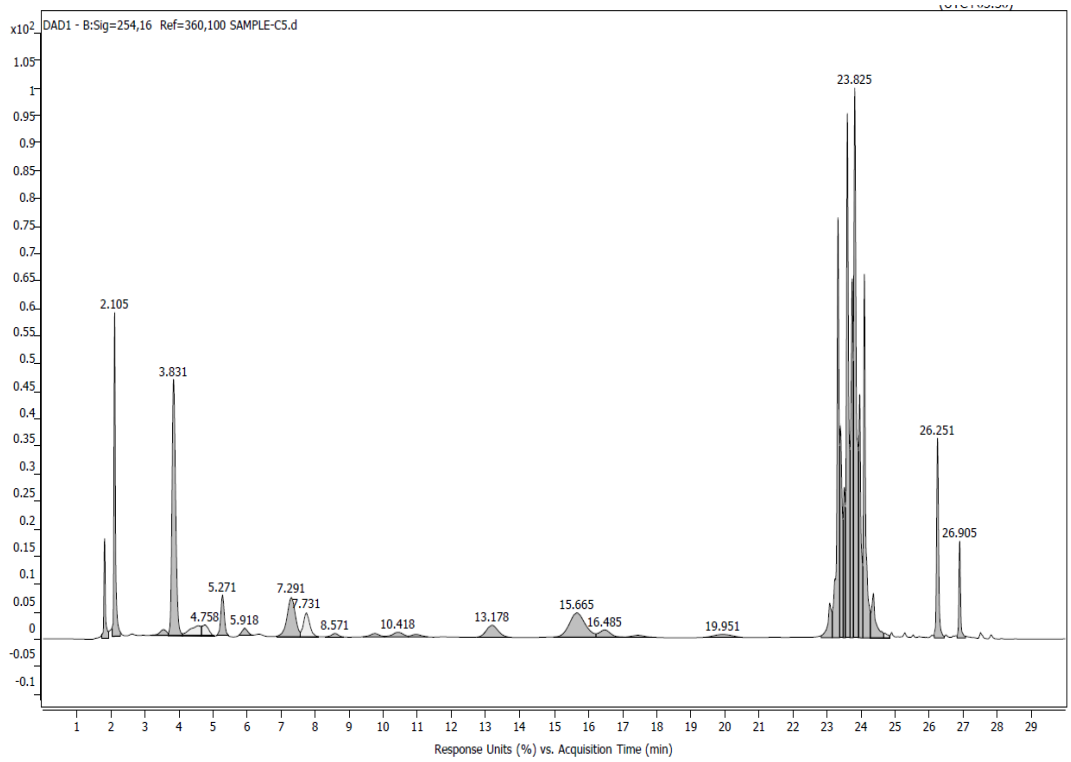


Fig. 4.51 LC-HRMS UV Chromatogram for *Terminalia chebula*

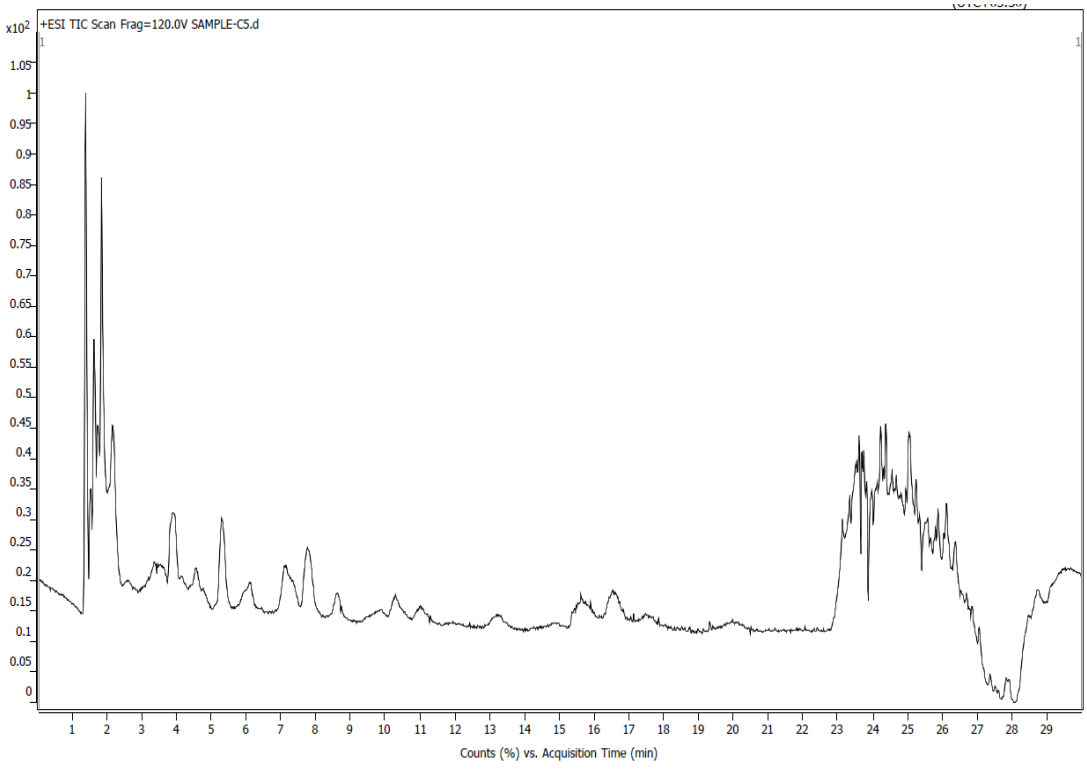


Fig. 4.52 LC-HRMS TIC Chromatogram for *Terminalia chebula*

Table 4.12 Compounds identified through LC-HRMS in *Terminalia chebula*

Sl.No	Retention Time (Minutes)	Compound Name	Molecular Weight (g/mol)	Chemical Formula
1.	25.09	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$
2.	2.105	Dihydro-heme d1	712.5	$C_{34}H_{32}FeN_4O_{10}$
3.	1.73	L-Proline	115.13	$C_5H_9NO_2$
4.	1.85	Thiamine acetic acid	279.34	$C_{12}H_{15}N_4O_2S^+$
5.	26.90	Isopropamide	353.5	$C_{23}H_{33}N_2O^+$
6.	25.11	17a-Aza-D-homoandrost-5-en-3beta-ol	289.5	$C_{19}H_{31}NO$
7.	1.80	Taurine	125.15	$C_2H_7NO_3S$
8.	1.63	Thiamine	265.36	$C_{12}H_{17}N_4OS^+$
9.	1.63	Deoxyadenosine	251.24	$C_{10}H_{13}N_5O_3$
10.	2.17	Sulindac	356.4	$C_{20}H_{17}FO_3S$
11.	23.69	Istamycin C1	431.5	$C_{19}H_{37}N_5O_6$
12.	1.40	3-(3,4-Dihydroxyphenyl)lactate	197.16	$C_9H_9O_5^-$
13.	23.61	Betamethasone	392.5	$C_{22}H_{29}FO_5$
14.	23.69	Stearoylglycerone phosphate	436.5	$C_{21}H_{41}O_7P$
15.	26.11	Sterol	248.4	$C_{17}H_{28}O$
16.	26.11	Estrone	270.4	$C_{18}H_{22}O_2$
17.	23.76	Netilmicin	475.6	$C_{21}H_{41}N_5O_7$
18.	24.22	Mycolactone F	701	$C_{42}H_{68}O_8$
19.	25.08	2S-Amino-tridecanoic acid	229.36	$C_{13}H_{27}NO_2$
20.	3.83	Deoxyuridine	228.2	$C_9H_{12}N_2O_5$
21.	23.61	Hydroxytamoxifen	387.5	$C_{26}H_{29}NO_2$
22.	26.06	L-Octanoylcarnitine	28739	$C_{15}H_{29}NO_4$
23.	23.76	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$
24.	2.15	N-Acetylmuramate	293.27	$C_{11}H_{19}NO_8$
25.	1.63	2-Hydroxy-6-oxo-6-phenylhexa-2,4-dienoate	217.2	$C_{12}H_9O_4^-$
26.	23.82	Terpendole C	519.7	$C_{32}H_{41}NO_5$
27.	23.67	Silymarin	482.4	$C_{25}H_{22}O_{10}$
28.	25.06	Hexocyclium	317.5	$C_{20}H_{33}N_2O^+$
29.	1.85	Orotate	156.1	$C_5H_4N_2O_4$
30.	2.14	alpha-Erythroidine	273.33	$C_{16}H_{19}NO_3$
31.	25.19	Dodine	287.44	$C_{15}H_{33}N_3O_2$
32.	26.38	Cystathionine	222.26	$C_7H_{14}N_2O_4S$
33.	1.70	Queuine	277.28	$C_{12}H_{15}N_5O_3$
34.	5.27	trans-Cinnamate	148.16	$C_9H_8O_2$
35.	1.40	5-Hydroxymethyluracil	142.11	$C_5H_6N_2O_3$
36.	24.37	Prunasin	295.29	$C_{14}H_{17}NO_6$
37.	24.56	Curcumin monoglucoside	530.5	$C_{27}H_{30}O_{11}$
38.	1.85	Lecanoric acid	318.28	$C_{16}H_{14}O_7$

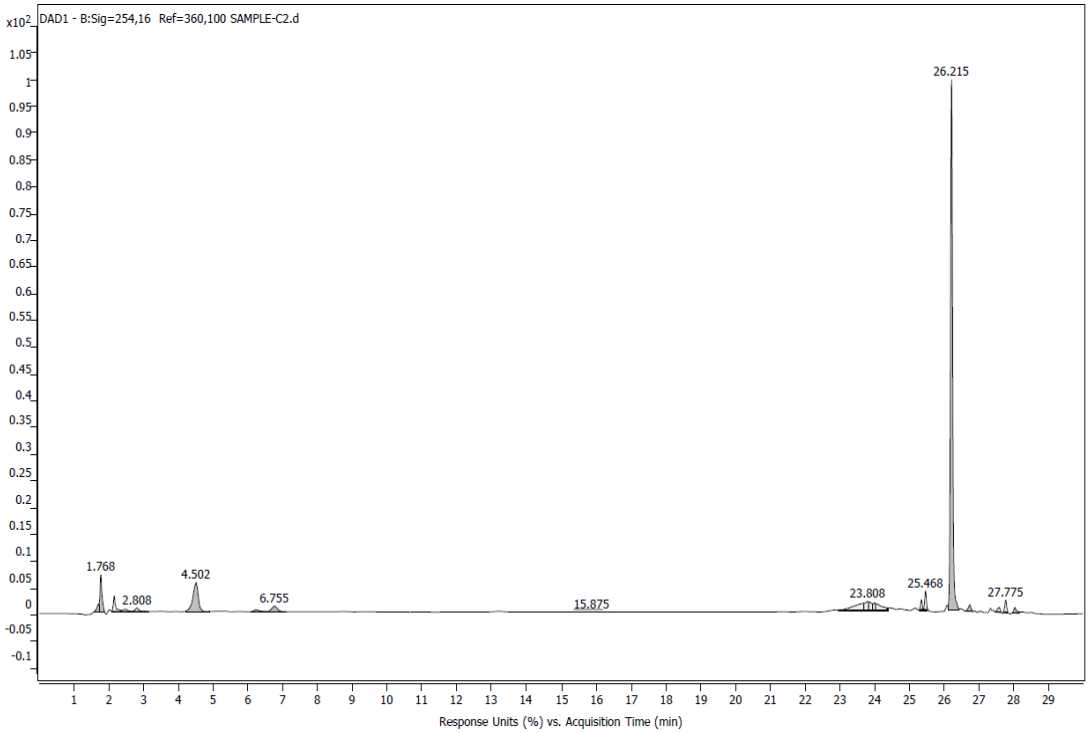


Fig. 4.53 LC-HRMS UV Chromatogram for *Garcinia kydia*

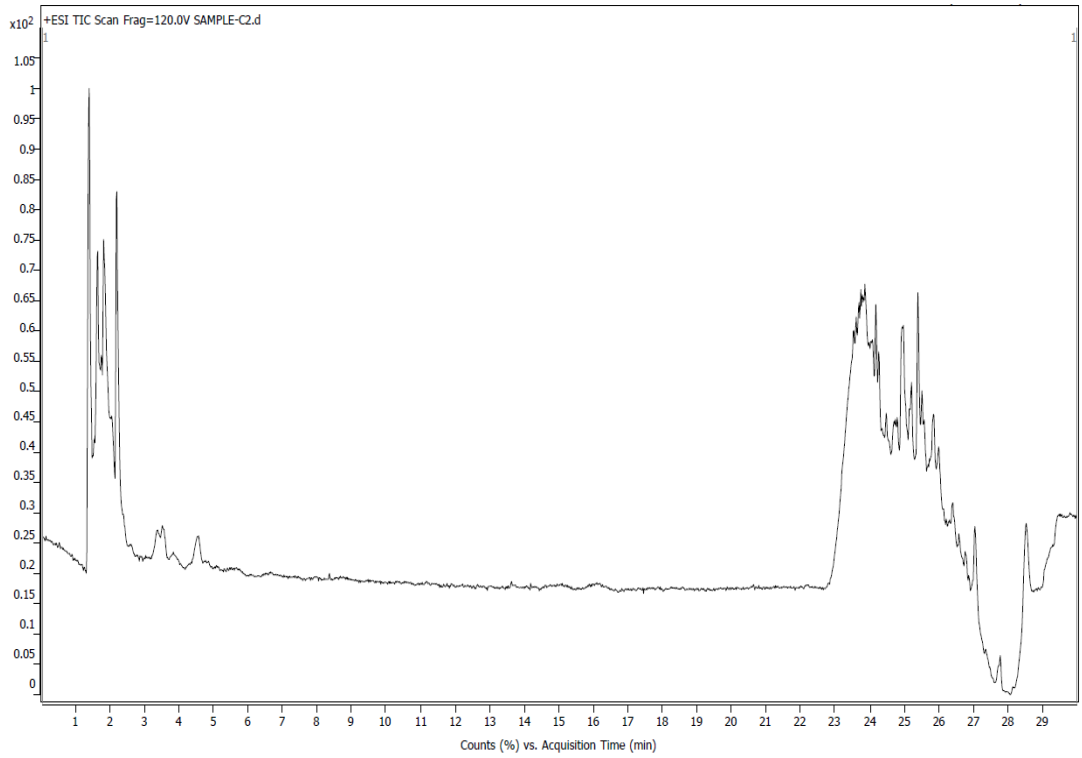


Fig. 4.54 LC-HRMS TIC Chromatogram for *Garcinia kydia*

Table 4.13 Compounds identified through LC-HRMS in *Garcinia kydia*

Sl. No	Retention Time (Minutes)	Compound Name	Molecular Weight (g/mol)	Chemical Formula
1.	25.54	Prostaglandin B1	336.5	$C_{20}H_{32}O_4$
2.	24.24	Mycolactone F	701	$C_{42}H_{68}O_8$
3.	1.76	Taurine	125.15	$C_2H_7NO_3S$
4.	23.77	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$
5.	23.72	Stearoylglycerone phosphate	436.5	$C_{21}H_{41}O_7P$
6.	2.13	Lophocerine	249.35	$C_{15}H_{23}NO_2$
7.	1.38	Aromatic aldehyde	106.12	C_7H_6O
8.	26.92	CBiol_001716	323.5	$C_{20}H_{37}NO_2$
9.	1.83	Tolmetin sodium	279.27	$C_{15}H_{14}NNaO_3$
10.	25.10	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$
11.	1.65	N-Benzoyl-4-hydroxyanthranilate	256.23	$C_{14}H_{10}NO_4^-$
12.	1.38	D-Erythrose 4-phosphate	200.08	$C_4H_9O_7P$
13.	23.87	Protoporphyrinogen IX	568.7	$C_{34}H_{40}N_4O_4$
14.	27.52	Garcinol	602.8	$C_{38}H_{50}O_6$
15.	1.65	N-Acetylmuramoyl-Ala	364.35	$C_{14}H_{24}N_2O_9$
16.	23.55	Gibberellin A1	348.4	$C_{19}H_{24}O_6$
17.	25.10	17a-Aza-D-homoandrost-5-en-3beta-ol	289.5	$C_{19}H_{31}NO$
18.	26.21	(15Z)-Tetracosenoic acid	366.6	$C_{24}H_{46}O_2$
19.	27.01	Acetylleucyl-leucyl-norleucinal	383.5	$C_{20}H_{37}N_3O_4$
20.	2.11	sn-Glycero-3-phosphocholine	257.22	$C_8H_{20}NO_6P$
21.	1.81	L-Valine	117.15	$C_5H_{11}NO_2$
22.	4.50	trans-Cinnamate	148.16	$C_9H_8O_2$
23.	27.06	Isopropamide	353.5	$C_{23}H_{33}N_2O^+$
24.	25.85	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$
25.	26.04	L-Octanoylcarnitine	287.39	$C_{15}H_{29}NO_4$
26.	24.04	Chitobiose	424.4	$C_{16}H_{28}N_2O_{11}$
27.	1.86	101418-42-2	747.4	$C_{26}H_{28}Cl_2N_8O_{14}$
28.	24.99	N,N,2,2-tetramethyl-1,5-diphenylpentan-1-amine	295.5	$C_{21}H_{29}N$
29.	24.24	DMPC;L-beta,gamma-Dimyristoyl-alpha-lecithin	678.9	$C_{36}H_{73}NO_8P^+$
30.	1.38	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate	127.14	$C_6H_9NO_2$
31.	1.60	Thiamine	265.36	$C_{12}H_{17}N_4OS^+$
32.	1.38	Adenine	135.13	$C_5H_5N_5$
33.	1.81	Lecanoric acid	318.28	$C_{16}H_{14}O_7$

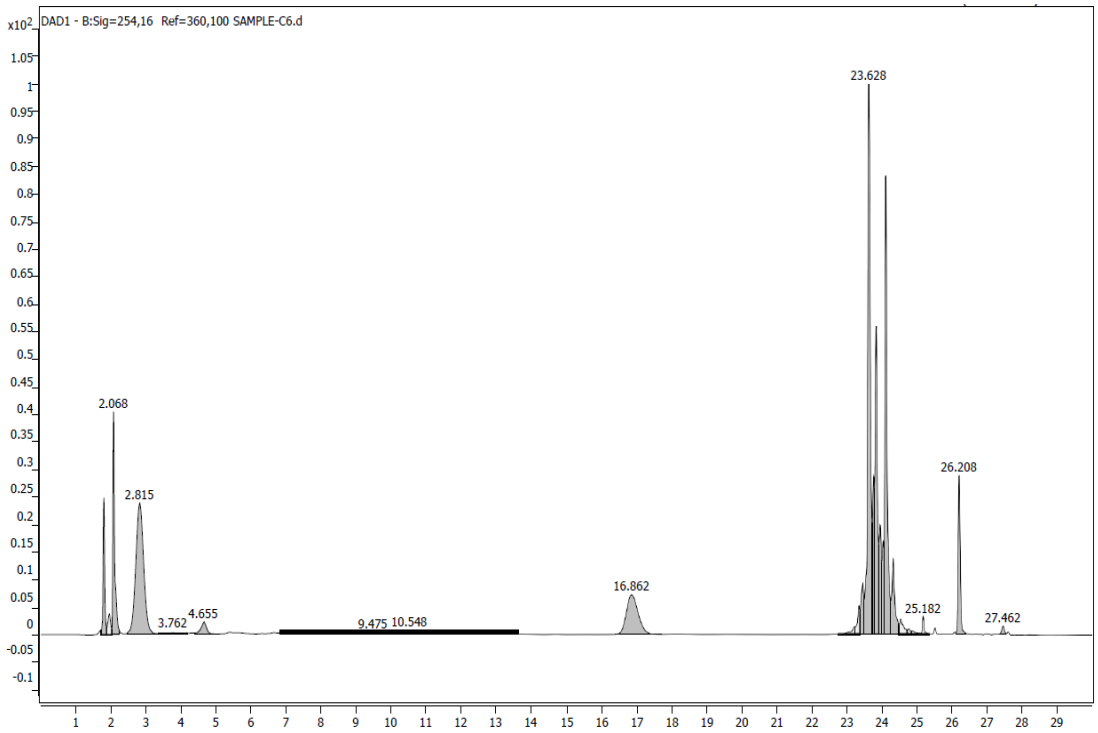


Fig. 4.55 LC-HRMS UV Chromatogram for *Emblica officinalis*

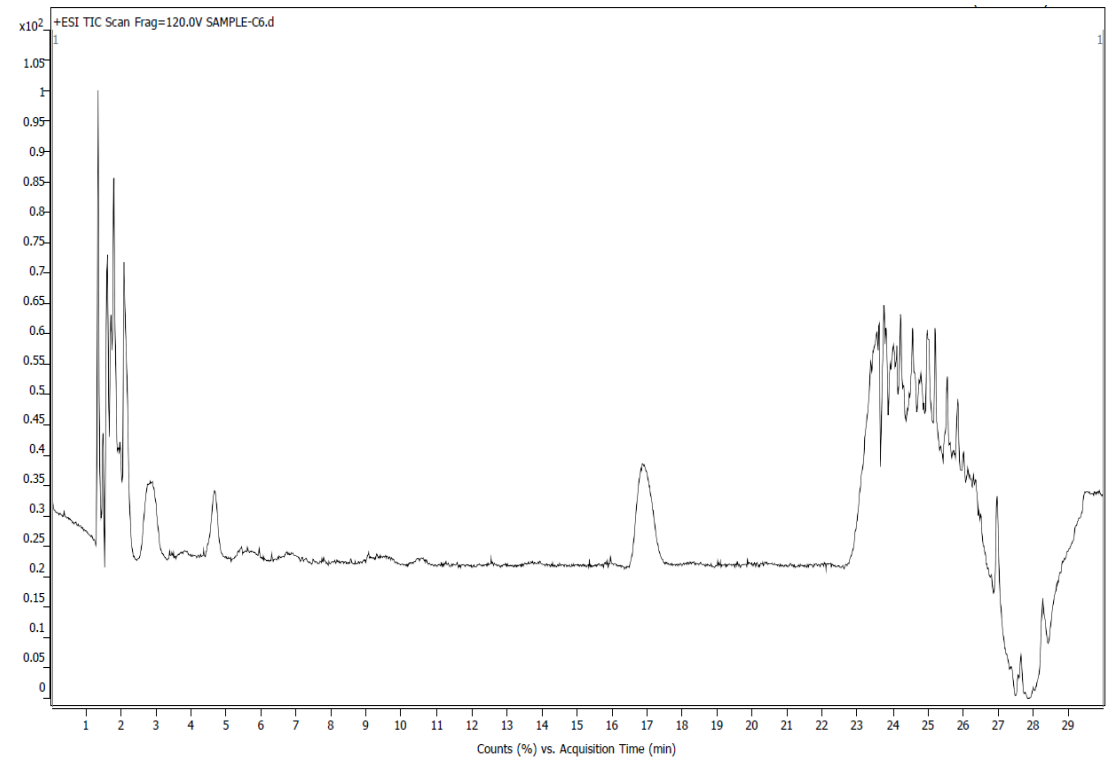


Fig. 4.56 LC-HRMS TIC Chromatogram for *Emblica officinalis*

Table 4.14 Compounds identified through LC-HRMS in *Emblica officinalis*

Sl. No	Retention Time (Minutes)	Compound Name	Molecular Weight (g/mol)	Chemical Formula
1.	25.05	N-LAUROYLSARCOSINE	271.4	$C_{15}H_{29}NO_3$
2.	25.03	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$
3.	1.77	D-Ribose 5-phosphate	230.11	$C_5H_{11}O_8P$
4.	24.21	Mycolactone F	701	$C_{42}H_{68}O_8$
5.	24.01	Sapelin A	474.7	$C_{30}H_{50}O_4$
6.	25.50	Prostaglandin B1	336.5	$C_{20}H_{32}O_4$
7.	25.40	Bis(3-dibutylaminopropyl)9-oxofluorene-2,7-dicarboxylate dihydrochloride hydrate	679.8	$C_{37}H_{56}Cl_2N_2O_5$
8.	1.87	Tyr-OEt	209.24	$C_{11}H_{15}NO_3$
9.	23.70	Stearoylglycerone phosphate	436.5	$C_{21}H_{41}O_7P$
10.	24.91	Tri(2-cyclohexylcyclohexyl)borate	554.7	$C_{36}H_{63}BO_3$
11.	23.61	Betamethasone	392.5	$C_{22}H_{29}FO_5$
12.	25.05	17a-Aza-D-homoandrost-5-en-3beta-ol	289.5	$C_{19}H_{31}NO$
13.	2.81	Lancerin	406.3	$C_{19}H_{18}O_{10}$
14.	1.66	Bergaptol	202.16	$C_{11}H_6O_4$
15.	25.03	SCHEMBL7294327	243.39	$C_{14}H_{29}NO_2$
16.	1.41	Aromatic aldehyde	106.12	C_7H_6O
17.	25.87	Prostaglandin F2alpha	354.5	$C_{20}H_{34}O_5$
18.	23.75	Netilmicin	475.6	$C_{21}H_{41}N_5O_7$
19.	26.98	CBiol_001716	323.5	$C_{20}H_{37}NO_2$
20.	1.62	L-Glutamate	147.13	$C_5H_9NO_4$
21.	25.23	Sphinganine	301.5	$C_{18}H_{39}NO_2$
22.	1.41	Isoflurophate	184.15	$C_6H_{14}FO_3P$
23.	1.71	Anthranilate	137.14	$C_7H_7NO_2$
24.	1.41	D-Erythrose 4-phosphate	200.8	$C_4H_9O_7P$
25.	23.68	Istamycin C1	431.5	$C_{19}H_{37}N_5O_6$
26.	23.83	Protoporphyrinogen IX	568.7	$C_{34}H_{40}N_4O_4$
27.	1.39	3-(3,4-Dihydroxyphenyl)lactate	198.17	$C_9H_{10}O_5$
28.	23.76	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$
29.	23.96	L-Kynurenine	208.21	$C_{10}H_{12}N_2O_3$
30.	23.78	Butaclamol	361.5	$C_{25}H_{31}NO$
31.	27.12	Isopropamide	353.5	$C_{23}H_{33}N_2O^+$
32.	28.48	3-Oxo-5beta-cholanate	373.5	$C_{24}H_{37}O_3^-$
33.	23.80	Terpendole C	519.7	$C_{32}H_{41}NO_5$
34.	1.39	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate	127.14	$C_6H_9NO_2$
35.	27.08	Acetylleucyl-leucyl-norleucinal	383.5	$C_{20}H_{37}N_3O_4$
36.	1.64	N-Acetylmuramate	293.27	$C_{11}H_{19}NO_8$
37.	23.55	Gibberellin A1	348.4	$C_{19}H_{24}O_6$
38.	1.79	Aciclovir	225.2	$C_8H_{11}N_5O_3$
39.	23.62	Hydroxytamoxifen	387.5	$C_{26}H_{29}NO_2$
40.	1.66	Nicotinamide D-ribonucleotide	334.22	$C_{11}H_{15}N_2O_8P$
41.	1.64	L-Adrenaline	183.2	$C_9H_{13}NO_3$
42.	25.00	2S-Amino-tridecanoic acid	229.36	$C_{13}H_{27}NO_2$
43.	24.78	L-Tryptophan	204.22	$C_{11}H_{12}N_2O_2$
44.	1.41	2-Deoxy-D-ribose 1-phosphate	214.11	$C_5H_{11}O_7P$
45.	25.83	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$
46.	4.54	trans-Cinnamate	148.16	$C_9H_8O_2$
47.	26.20	L-Octanoylcarnitine	287.39	$C_{15}H_{29}NO_4$
48.	1.69	L-Proline	115.13	$C_5H_9NO_2$
49.	26.78	(2-chlorophenyl)methyl-[2-[[8-[2-[(2-chlorophenyl)methyl-diethylazaniumyl]ethylamino]-8-oxooctanoyl]amino]ethyl]-diethylazanium	621.7	$C_{34}H_{54}Cl_2N_4O_2^{+2}$
50.	25.15	Talatizamine	421.6	$C_{24}H_{39}NO_5$
51.	2.06	Citrate	192.12	$C_6H_8O_7$
52.	1.64	Pterin	163.14	$C_6H_5N_5O$
53.	1.72	Macarpine	392.4	$C_{22}H_{18}NO_6^+$

4.4.1.7 In silico works

4.4.1.7.1 Molecular docking of bioactive compounds identified from the five underutilized fruits

Molecular docking was employed to determine the possibility of binding between the bioactive compounds and the receptor protein and the results were analysed. In this work, we identified significant bioactive compounds from *Elaeocarpus lanceifolius, Embelia subcoriacea, Terminalia chebula, Garcinia kydia and Emblica officinalis* which have proven to possess higher cytotoxicity towards the A549 cancer cells through MTT assay. The phytochemical analysis characterized molecules based on their potential picks obtained according to the retention time. The collected bioactive compounds docked on the active site of β' subunits of AKT1 and the analysis showed that Naringenin, 2-Deoxy-D-Ribose 1-Phosphate, Resistomysin, Berberine, Silymarin, Sulindac, 20-Hydroxyecdysone, Garcinol and had the highest binding affinity among the other selected molecule used for docking experiments respectively. The docking score of bioactive compounds was analysed in terms of binding affinity (Gibbs free energy ΔG) and the output of docking results including the standard drug shown in Table 4.15 to Table 4.19. The docking score against the target protein AKT1 a cancer drug target, some of the bioactive compounds showed the highest binding affinity and most of the compounds showed a considerable binding affinity with standard drug 5-fluorouracil. The highest binding affinity was found in the compounds Resistomysin (-10.1 kcal mol^{-1}), Berberine (-7.6 kcal mol^{-1}), Silymarin (-7.5 kcal mol^{-1}), Sulindac (-7.5 kcal mol^{-1}) followed by 20-Hydroxyecdysone (-7.0 kcal mol^{-1}) respectively. The docking pose of the molecules which showed a lesser ΔG score than 5-fluorouracil (-7.0 kcal/mol) is presented in Table 4.15 to Table 4.19. Analysis of docked poses of bioactive compounds was done using the structure visualization tool Pymol confirmed that all ligand molecules were docked at the same active pocket of the AKT1 protein receptor. The drug-binding domain of AKT1 is the hydrophobic cavity situated at the lower interface between the N- and C-lobes of the kinase domain. Further, molecular docking was analysed in terms of molecular interaction like hydrogen bonds, hydrophobic interaction, and amino acid residue contributed to the interaction.

Table 4.15. Molecular Docking results of phytocompounds identified from *Elaeocarpus lanceifolius*

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	Docking Score (kcal/mol)
1.	L-Valine	117.15	$C_5H_{11}NO_2$	-4.6
2.	Anthranilate	137.14	$C_7H_7NO_2$	-5.5
3.	2-Deoxy-D-Ribose 1-Phosphate	214.11	$C_5H_{11}O_7P$	-6.6
4.	N-Benzoyl-4-Hydroxyanthranilate	256.23	$C_{14}H_{10}NO_4^-$	-6.4
5.	4-Nitrophenol	139.11	$C_6H_5NO_3$	-5.3
6.	L-Kynurenine	208.21	$C_{10}H_{12}N_2O_3$	-5.5
7.	Dodine	287.44	$C_{15}H_{33}N_3O_2$	-4.2
8.	2-Deoxyadenosine	251.24	$C_{10}H_{13}N_5O_3$	-6.2
9.	Tyr-Oet	209.24	$C_{11}H_{15}NO_3$	-5.9
10.	Naringenin	272.25	$C_{15}H_{12}O_5$	-6.8
11.	N-Acetylmuramate	293.27	$C_{11}H_{19}NO_8$	-5.2
12.	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$	-5.0
13.	(S)-2,3,4,5-Tetrahydropyridine-2-Carboxylate	127.14	$C_6H_9NO_2$	-5.1
14.	Hydroxytamoxifen	387.5	$C_{26}H_{29}NO_2$	-6.3
15.	Isoflurophate	184.15	$C_6H_{14}FO_3P$	-4.2
16.	Netilmicin	475.6	$C_{21}H_{41}N_5O_7$	-6.1
17.	Thiamine	265.36	$C_{12}H_{17}N_4OS^+$	-5.1
18.	Aciclovir	225.2	$C_8H_{11}N_5O_3$	-5.2
19.	N-Acetylmuramoyl-Ala	364.35	$C_{14}H_{24}N_2O_9$	-5.6
20.	Thymine	126.11	$C_5H_6N_2O_2$	-4.8
21.	Fenpropimorph	303.5	$C_{20}H_{33}NO$	-6.2
22.	3-(3,4-Dihydroxyphenyl)Lactate	197.16	$C_9H_9O_5^-$	-5.9
23.	Citrate	192.12	$C_6H_8O_7$	-5.6
24.	Adenine	135.13	$C_5H_5N_5$	-4.7

Table 4.16. Molecular Docking results of phytocompounds identified from *Embelia subcoriacea*

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	Docking Score (kcal/ mol)
1.	Riboflavin	376.4	$C_{17}H_{20}N_4O_6$	-6.5
2.	Lipoate	206.3	$C_8H_{14}O_2S_2$	-4.7
3.	(R)-Mevalonate	147.15	$C_6H_{11}O_4^-$	-4.4
4.	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$	-4.3
5.	Deoxyuridine	228.2	$C_9H_{12}N_2O_5$	-5.6
6.	Lancerin	406.3	$C_{19}H_{18}O_{10}$	-6.7
7.	(L-Seryl)adenylate	434.3	$C_{13}H_{19}N_6O_9P$	-5.9
8.	Ipolamiide	406.4	$C_{17}H_{26}O_{11}$	-6.0
9.	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$	-6.8
10.	Hexocyclium	317.5	$C_{20}H_{33}N_2O^+$	-6.1
11.	Resistomycin	376.4	$C_{22}H_{16}O_6$	-10.1
12.	Betamethasone	392.5	$C_{22}H_{29}FO_5$	-7.2
13.	3-(3,4-Dihydroxyphenyl)lactate	198.17	$C_9H_{10}O_5$	-6.1
14.	4-Maleylacetoacetate	200.14	$C_8H_8O_6$	-5.4
15.	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate	127.14	$C_6H_9NO_2$	-5.1
16.	Berberine	336.4	$C_{20}H_{18}NO_4^+$	-7.6
17.	Mancinellin	612.8	$C_{36}H_{52}O_8$	-6.1
18.	L-Kynurenine	208.21	$C_{10}H_{12}N_2O_3$	-5.6
19.	7,8-Dihydro-7,8-dihydroxykynurenate	222.17	$C_{10}H_8NO_5^-$	-6.3
20.	Bergaptol	202.16	$C_{11}H_6O_4$	-6.4
21.	Deoxy-5-methylcytidylate	321.22	$C_{10}H_{16}N_3O_7P$	-6.2
22.	Tyr-OEt	209.24	$C_{11}H_{15}NO_3$	-5.9
23.	Chitobiose	424.4	$C_{16}H_{28}N_2O_{11}$	-5.1
24.	N-Lauroylsarcosine	271.4	$C_{15}H_{29}NO_3$	-4.6
25.	Dicumarol	336.3	$C_{19}H_{12}O_6$	-7.3
26.	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$	-4.6
27.	alpha-Erythroidine	273.33	$C_{16}H_{19}NO_3$	-6.2
28.	O-Decanoyl-L-carnitine	315.4	$C_{17}H_{33}NO_4$	-4.2
29.	2-Bromoacetaldehyde	122.95	C_2H_3BrO	-2.6

Table 4.17. Molecular Docking results of phytocompounds identified from *Terminalia chebula*

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	Docking Score (kcal/mol)
1.	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$	-4.1
2.	L-Proline	115.13	$C_5H_9NO_2$	-4.5
3.	Isopropamide	353.5	$C_{23}H_{33}N_2O^+$	-6.4
4.	Taurine	125.15	$C_2H_7NO_3S$	-3.7
5.	Thiamine	265.36	$C_{12}H_{17}N_4OS^+$	-5.4
6.	Deoxyadenosine	251.24	$C_{10}H_{13}N_5O_3$	-6.2
7.	Sulindac	356.4	$C_{20}H_{17}FO_3S$	-7.1
8.	3-(3,4-Dihydroxyphenyl) lactate	197.16	$C_9H_9O_5^-$	-5.9
9.	Netilmicin	475.6	$C_{21}H_{41}N_5O_7$	-5.8
10.	Deoxyuridine	228.2	$C_9H_{12}N_2O_5$	-5.6
11.	Hydroxytamoxifen	387.5	$C_{26}H_{29}NO_2$	-6.3
12.	L-Octanoylcarnitine	28739	$C_{15}H_{29}NO_4$	-4.8
13.	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$	-6.6
14.	N-Acetylmuramate	293.27	$C_{11}H_{19}NO_8$	-5.3
15.	2-Hydroxy-6-oxo-6-phenylhexa-2,4-dienoate	217.2	$C_{12}H_9O_4^-$	-6.2
16.	Silymarin	482.4	$C_{25}H_{22}O_{10}$	-7.5
17.	Hexocyclium	317.5	$C_{20}H_{33}N_2O^+$	-5.7
18.	Orotate	156.1	$C_5H_4N_2O_4$	-5.5
19.	alpha-Erythroidine	273.33	$C_{16}H_{19}NO_3$	-6.5
20.	Dodine	287.44	$C_{15}H_{33}N_3O_2$	-4.2
21.	Cystathionine	222.26	$C_7H_{14}N_2O_4S$	-4.8
22.	trans-Cinnamate	148.16	$C_9H_8O_2$	-5.5
23.	5-Hydroxymethyluracil	142.11	$C_5H_6N_2O_3$	-4.8
24.	Prunasin	295.29	$C_{14}H_{17}NO_6$	-5.6

Table 4.18. Molecular Docking results of phytocompounds identified from *Garcinia kydia*

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	Docking score (kcal/mol)
1.	Taurine	125.15	$C_2H_7NO_3S$	-3.6
2.	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$	-7.0
3.	Stearoylglycerone phosphate	436.5	$C_{21}H_{41}O_7P$	22
4.	Lophocerine	249.35	$C_{15}H_{23}NO_2$	-6.6
5.	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$	-4.4
6.	N-Benzoyl-4-hydroxyanthranilate	256.23	$C_{14}H_{10}NO_4^-$	-6.4
7.	Garcinol	602.8	$C_{38}H_{50}O_6$	-6.9
8.	N-Acetylmuramoyl-Ala	364.35	$C_{14}H_{24}N_2O_9$	-5.5
9.	sn-Glycero-3-phosphocholine	257.22	$C_8H_{20}NO_6P$	-4.3
10.	trans-Cinnamate	148.16	$C_9H_8O_2$	-5.8
11.	Isopropamide	353.5	$C_{23}H_{33}N_2O^+$	-5.7
12.	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$	-4.7
13.	L-Octanoylcarnitine	287.39	$C_{15}H_{29}NO_4$	-4.4
14.	N,N,2,2-tetramethyl-1,5-diphenylpentan-1-amine	295.5	$C_{21}H_{29}N$	-5.5
15.	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate	127.14	$C_6H_9NO_2$	-5.1
16.	Thiamine	265.36	$C_{12}H_{17}N_4OS^+$	-5.0
17.	Adenine	135.13	$C_5H_5N_5$	-4.7

Table 4.19. Molecular Docking results of phytocompounds identified from *Emblica officinalis*

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	Docking Score (kcal/mol)
1.	N-LAUROYLSARCOSINE	271.4	$C_{15}H_{29}NO_3$	-4.7
2.	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$	-4.2
3.	Bergaptol	202.16	$C_{11}H_6O_4$	-6.4
4.	Netilmicin	475.6	$C_{21}H_{41}N_5O_7$	-5.8
5.	Isoflurophate	184.15	$C_6H_{14}FO_3P$	-4.3
6.	Anthranilate	137.14	$C_7H_7NO_2$	-5.5
7.	3-(3,4-Dihydroxyphenyl)lactate	198.17	$C_9H_{10}O_5$	-6.1
8.	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$	-6.5
9.	L-Kynurenine	208.21	$C_{10}H_{12}N_2O_3$	-5.6
10.	Isopropamide	353.5	$C_{23}H_{33}N_2O^+$	-6.1
11.	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate	127.14	$C_6H_9NO_2$	-5.1
12.	N-Acetylmuramate	293.27	$C_{11}H_{19}NO_8$	-5.3
13.	Aciclovir	225.2	$C_8H_{11}N_5O_3$	-5.1
14.	Hydroxytamoxifen	387.5	$C_{26}H_{29}NO_2$	-6.3
15.	L-Adrenaline	183.2	$C_9H_{13}NO_3$	-5.5
16.	L-Tryptophan	204.22	$C_{11}H_{12}N_2O_2$	-6.3
17.	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$	-4.4
18.	trans-Cinnamate	148.16	$C_9H_8O_2$	-5.4
19.	L-Octanoylcarnitine	287.39	$C_{15}H_{29}NO_4$	-4.4
20.	L-Proline	115.13	$C_5H_9NO_2$	-4.5
21.	Citrate	192.12	$C_6H_8O_7$	-5.6
22.	Pterin	163.14	$C_6H_5N_5O$	-5.4

4.4.1.7.2 *In-silico ADME prediction for drug-likeness analysis of bioactive compounds*

An effective method for evaluating possible therapeutic compounds is the ADME properties study. The ADME study looks at a number of parameters, such as pharmacokinetics, lipophilicity, water solubility, physiochemical properties, drug similarity, and medicinal chemistry, to identify the bioactive chemicals that have the best chance of creating a potent treatment. All bioactive substances obtain adequate drug-like qualities in addition to the other drug-likeness factors, according to the ADME results shown in Table 4.20 to Table 4.24. All of the chosen compounds' physiochemical characteristics, such as their molecular weight (MW) being less than 500 Da, their number of H-bond donors (HBD) being less than 5, their number of H-bond acceptors (HBA) being less than 10, and their LogP value being less than 5, satisfied the five Lipinski rules. Depending on their structural characteristics, most molecules are very soluble in water, with others having moderate solubility and others having low solubility. Each molecule has a topological polar surface area (TPSA) of less than 140. Additionally, the logKp value, which represents skin permeability, is within the typical range of -8.0 to -1.0, suggesting that the molecules may be able to inhibit the enzymes. where physicochemical characteristics like as size, polarity, solubility, flexibility, saturation, and lipophilicity are examples that are taken into account. Based on ADME characteristics and the calculation of molecular features and bioactivity using the Lipinski rule of five, the majority of the compounds may be the subject of further research as possible medications; nevertheless, here we highlight the three molecules.

Table 4.20 Lipinski properties of phytochemical compounds from *Elaeocarpus lanceifolius* calculated with SwissADME

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	RB	HBA	HBD	MR	TPSA	Lipophilicity (Consensus)	Water solubility
42.	L-Valine	117.15	$C_5H_{11}NO_2$	2	3	2	30.63	63.32	-0.78	Highly soluble
43.	Anthranilate	137.14	$C_7H_7NO_2$	1	2	2	37.81	63.32	0.72	Very soluble
44.	2-Deoxy-D-Ribose 1-Phosphate	214.11	$C_5H_{11}O_7P$	3	7	4	39.52	126.26	-1.55	Highly soluble
45.	N-Benzoyl-4-Hydroxyanthranilate	256.23	$C_{14}H_{10}NO_4^-$	4	4	2	67.75	89.46	2.18	Soluble
46.	4-Nitrophenol	139.11	$C_6H_5NO_3$	1	3	1	37.29	66.05	0.69	Soluble
47.	L-Kynurenine	208.21	$C_{10}H_{12}N_2O_3$	4	4	3	55.13	106.41	-0.69	Highly soluble
48.	Dodine	287.44	$C_{15}H_{33}N_3O_2$	11	3	3	87.2	101.7	3.31	Soluble
49.	2-Deoxyadenosine	251.24	$C_{10}H_{13}N_5O_3$	2	6	3	61.51	119.31	-0.85	Very soluble
50.	Naringenin	272.25	$C_{15}H_{12}O_5$	1	5	3	71.57	86.99	1.84	Soluble
51.	N-Acetylmuramate	293.27	$C_{11}H_{19}NO_8$	6	8	5	63.31	145.55	-1.64	Highly soluble
52.	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$	10	4	4	70.99	137.46	-0.16	Very soluble
53.	(S)-2,3,4,5-Tetrahydropyridine-2-Carboxylate	127.14	$C_6H_9NO_2$	1	3	1	37.62	49.66	0.42	Very soluble
54.	Hydroxytamoxifen	387.5	$C_{26}H_{29}NO_2$	8	3	1	121.75	32.7	5.36	Poorly soluble
55.	Isoflurophate	184.15	$C_6H_{14}FO_3P$	4	4	0	41.84	45.34	1.66	Very soluble
56.	Netilmicin	475.6	$C_{21}H_{41}N_5O_7$	8	12	8	117.83	199.73	-2.2	Highly soluble
57.	Thiamine	265.36	$C_{12}H_{17}N_4OS^+$	4	3	2	73.26	104.15	0.53	Soluble
58.	Aciclovir	225.2	$C_8H_{11}N_5O_3$	4	5	3	55.68	119.05	-0.99	Very soluble
59.	N-Acetylmuramoyl-Ala	364.35	$C_{14}H_{24}N_2O_9$	9	9	6	80.73	174.65	-1.87	Very soluble
60.	Thymine	126.11	$C_5H_6N_2O_2$	0	2	2	32.65	65.72	0.15	Very soluble
61.	Fenpropimorph	303.5	$C_{20}H_{33}NO$	5	2	0	99.72	12.47	4.31	Moderately soluble
62.	Veatchine	343.5	$C_{22}H_{33}NO_2$	0	3	1	102.99	32.7	3.45	Moderately soluble
63.	3-(3,4-Dihydroxyphenyl)Lactate	197.16	$C_9H_9O_5^-$	3	5	4	48	97.99	-0.01	Very soluble
64.	Citrate	192.12	$C_6H_8O_7$	5	7	4	37.47	132.13	-1.51	Highly soluble
65.	Adenine	135.13	$C_5H_5N_5$	0	3	2	36.09	80.48	-0.2	Very soluble

Rotatable bonds (RB), H-bond acceptors (HBD), H-bond donors (HBD), Molar Refractivity (MR), Topological Polar surface area (TPSA)

Table 4.21 Lipinski properties of phytochemical compounds from *Embelia subcoriacea* calculated with SwissADME

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	RB	HBA	HBD	MR	TPSA	Lipophilicity (Consensus)	Water solubility
1.	Riboflavin	376.4	$C_{17}H_{20}N_4O_6$	5	8	5	96.99	161.56	-0.32	Very soluble
2.	Lipoate	206.3	$C_8H_{14}O_2S_2$	5	2	0	53.47	90.73	1.75	Very soluble
3.	(R)-Mevalonate	147.15	$C_6H_{11}O_4^-$	4	4	2	33.15	80.59	-0.56	Highly soluble
4.	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$	14	3	3	84.06	66.48	3.7	Soluble
5.	Deoxyuridine	228.2	$C_9H_{12}N_2O_5$	2	5	3	53.11	104.55	-0.85	Very soluble
6.	Lancerin	406.3	$C_{19}H_{18}O_{10}$	2	10	7	98.68	181.05	-0.4	Soluble
7.	(L-Seryl)adenylate	434.3	$C_{13}H_{19}N_6O_9P$	8	13	6	92	248.2	-3.59	Highly soluble
8.	Ipolamiide	406.4	$C_{17}H_{26}O_{11}$	5	11	6	88.6	175.37	-1.57	Very soluble
9.	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$	5	7	6	129.74	138.45	1.76	Soluble
10.	Hexocyclium	317.5	$C_{20}H_{33}N_2O^+$	4	2	1	105.39	23.47	0.96	Soluble
11.	Resistomycin	376.4	$C_{22}H_{16}O_6$	0	6	4	104.73	115.06	2.89	Moderately soluble
12.	Betamethasone	392.5	$C_{22}H_{29}FO_5$	2	6	3	101.96	94.83	2.14	Soluble
13.	3-(3,4-Dihydroxyphenyl)lactate	198.17	$C_9H_{10}O_5$	3	5	3	46.06	100.82	-0.17	Very soluble
14.	4-Maleylacetoacetate	200.14	$C_8H_8O_6$	6	6	2	44.04	108.74	-0.38	Very soluble
15.	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate	127.14	$C_6H_9NO_2$	1	3	1	37.62	49.66	0.42	Very soluble
16.	Berberine	336.4	$C_{20}H_{18}NO_4^+$	2	4	0	94.87	40.8	2.53	Moderately soluble
17.	Mancinellin	612.8	$C_{36}H_{52}O_8$	14	8	4	169.81	136.82	5.08	Poorly soluble
18.	L-Kynurenine	208.21	$C_{10}H_{12}N_2O_3$	4	4	3	55.13	106.41	-0.69	Highly soluble
19.	7,8-Dihydro-7,8-dihydroxykynurenate	222.17	$C_{10}H_8NO_5^-$	1	5	3	52.15	113.45	-0.84	Very soluble
20.	Bergaptol	202.16	$C_{11}H_6O_4$	0	4	1	54.28	63.58	1.77	Soluble
21.	Deoxy-5-methylcytidylate	321.22	$C_{10}H_{16}N_3O_7P$	4	8	4	70.56	166.94	-1.71	Highly soluble
22.	Tyr-OEt	209.24	$C_{11}H_{15}NO_3$	5	4	2	56.65	72.55	1.08	Very soluble
23.	Chitobiose	424.4	$C_{16}H_{28}N_2O_{11}$	12	11	8	92.26	215.11	-3.61	Highly soluble
24.	N-LAUROYLSARCOSINE	271.4	$C_{15}H_{29}NO_3$	13	3	1	79.09	57.61	3.37	Soluble
25.	Dicumarol	336.3	$C_{19}H_{12}O_6$	2	6	2	92.03	100.88	2.62	Soluble
26.	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$	10	4	4	70.99	137.46	-0.16	Very soluble
27.	alpha-Erythroidine	273.33	$C_{16}H_{19}NO_3$	1	4	0	78.37	38.77	1.43	Very soluble
28.	O-Decanoyl-L-carnitine	315.4	$C_{17}H_{33}NO_4$	14	4	0	87.32	66.43	0.82	Soluble
29.	2-Bromoacetaldehyde	122.95	C_2H_3BrO	1	1	0	19.8	17.07	0.62	Very soluble

Rotatable bonds (RB), H-bond acceptors (HBD), H-bond donors (HBD), Molar Refractivity (MR), Topological Polar surface area (TPSA)

Table 4.22 Lipinski properties of phytochemical compounds from *Terminalia chebula* calculated with SwissADME

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	RB	HBA	HBD	MR	TPSA	Lipophilicity (Consensus)	Water solubility
39.	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$	14	3	3	84.06	66.48	3.7	Soluble
40.	L-Proline	115.13	$C_5H_9NO_2$	1	3	2	32.52	49.33	-0.92	Highly soluble
41.	Isopropamide	353.5	$C_{23}H_{33}N_2O^+$	8	1	1	109.13	43.09	1.93	Moderately soluble
42.	Taurine	125.15	$C_2H_7NO_3S$	2	4	2	24.97	88.77	-1.43	Highly soluble
43.	Thiamine	265.36	$C_{12}H_{17}N_4OS^+$	4	3	2	73.26	104.15	0.53	Soluble
44.	Deoxyadenosine	251.24	$C_{10}H_{13}N_5O_3$	2	6	3	61.51	119.31	-0.85	Very soluble
45.	Sulindac	356.4	$C_{20}H_{17}FO_3S$	4	4	1	98.35	73.58	3.94	Moderately soluble
46.	3-(3,4-Dihydroxyphenyl)lactate	197.16	$C_9H_9O_5^-$	3	5	4	48	97.99	-0.01	Very soluble
47.	Netilmicin	475.6	$C_{21}H_{41}N_5O_7$	8	12	8	117.83	199.73	-2.2	Highly soluble
48.	Deoxyuridine	228.2	$C_9H_{12}N_2O_5$	2	5	3	53.11	104.55	-0.85	Very soluble
49.	Hydroxytamoxifen	387.5	$C_{26}H_{29}NO_2$	8	3	1	121.75	32.7	5.36	Poorly soluble
50.	L-Octanoylcarnitine	28739	$C_{15}H_{29}NO_4$	12	4	0	77.71	66.43	0.27	Soluble
51.	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$	5	7	6	129.74	138.45	1.76	Soluble
52.	N-Acetylmuramate	293.27	$C_{11}H_{19}NO_8$	6	8	5	63.31	145.55	-1.64	Highly soluble
53.	2-Hydroxy-6-oxo-6-phenylhexa-2,4-dienoate	217.2	$C_{12}H_9O_4^-$	4	4	1	56.89	77.43	1.4	Soluble
54.	Silymarin	482.4	$C_{25}H_{22}O_{10}$	4	10	5	120.55	155.14	1.59	Moderately soluble
55.	Hexocyclium	317.5	$C_{20}H_{33}N_2O^+$	4	2	1	105.39	23.47	0.96	Soluble
56.	Orotate	156.1	$C_5H_4N_2O_4$	1	4	3	34.64	103.02	-0.47	Very soluble
57.	alpha-Erythroidine	273.33	$C_{16}H_{19}NO_3$	1	4	0	78.37	38.77	1.43	Very soluble
58.	Dodine	287.44	$C_{15}H_{33}N_3O_2$	11	3	3	87.2	101.7	3.31	Soluble
59.	Cystathionine	222.26	$C_7H_{14}N_2O_4S$	7	6	4	52.31	151.94	-2.58	Highly soluble
60.	trans-Cinnamate	148.16	$C_9H_8O_2$	2	2	1	43.11	37.3	1.79	Soluble
61.	5-Hydroxymethyluracil	142.11	$C_5H_6N_2O_3$	1	3	3	33.81	85.95	-0.63	Highly soluble
62.	Prunasin	295.29	$C_{14}H_{17}NO_6$	4	7	4	69.51	123.17	-0.59	Very soluble

Rotatable bonds (RB), H-bond acceptors (HBD), H-bond donors (HBD), Molar Refractivity (MR), Topological Polar surface area (TPSA)

Table 4.23 Lipinski properties of phytochemical compounds from *Garcinia kydia* calculated with SwissADME

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	RB	HBA	HBD	MR	TPSA	Lipophilicity (Consensus)	Water solubility
1.	Taurine	125.15	$C_2H_7NO_3S$	2	4	2	24.97	88.77	-1.43	Highly soluble
2.	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$	5	7	6	129.74	138.45	1.76	Soluble
3.	Stearoylglycerone phosphate	436.5	$C_{21}H_{41}O_7P$	22	7	2	116.62	119.94	4.76	Moderately soluble
4.	Lophocerine	249.35	$C_{15}H_{23}NO_2$	3	3	1	78.43	32.7	2.72	Soluble
5.	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$	14	3	3	84.06	66.48	3.7	Soluble
6.	N-Benzoyl-4-hydroxyanthranilate	256.23	$C_{14}H_{10}NO_4^-$	4	4	2	67.75	89.46	2.18	Soluble
7.	Garcinol	602.8	$C_{38}H_{50}O_6$	10	6	3	180.06	111.9	7.35	Poorly soluble
8.	N-Acetylmuramoyl-Ala	364.35	$C_{14}H_{24}N_2O_9$	9	9	6	80.73	174.65	-1.87	Very soluble
9.	sn-Glycero-3-phosphocholine	257.22	$C_8H_{20}NO_6P$	8	6	2	55.73	108.86	-2.86	Highly soluble
10.	trans-Cinnamate	148.16	$C_9H_8O_2$	2	2	1	43.11	37.3	1.79	Soluble
11.	Isopropamide	353.5	$C_{23}H_{33}N_2O^+$	8	1	1	109.13	43.09	1.93	Moderately soluble
12.	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$	10	4	4	70.99	137.46	-0.16	Very soluble
13.	L-Octanoylcarnitine	287.39	$C_{15}H_{29}NO_4$	12	4	0	77.71	66.43	0.27	Soluble
14.	N,N,2,2-tetramethyl-1,5-diphenylpentan-1-amine	295.5	$C_{21}H_{29}N$	7	1	0	96.99	3.24	4.98	Moderately soluble
15.	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate	127.14	$C_6H_9NO_2$	1	3	1	37.62	49.66	0.42	Very soluble
16.	Thiamine	265.36	$C_{12}H_{17}N_4OS^+$	4	3	2	73.26	104.15	0.53	Soluble
17.	Adenine	135.13	$C_5H_5N_5$	0	3	2	36.09	80.48	-0.2	Very soluble

Rotatable bonds (RB), H-bond acceptors (HBD), H-bond donors (HBD), Molar Refractivity (MR), Topological Polar surface area (TPSA)

Table 4.24 Lipinski properties of phytochemical compounds from *Emblica officinalis* calculated with SwissADME

Sl. No	Compound Name	Molecular Weight (g/mol)	Chemical Formula	RB	HBA	HBD	MR	TPSA	Lipophilicity (Consensus)	Water solubility
54.	N-Lauroylsarcosine	271.4	$C_{15}H_{29}NO_3$	13	3	1	79.09	57.61	3.37	Soluble
55.	Hexadecasphinganine	273.45	$C_{16}H_{35}NO_2$	14	3	3	84.06	66.48	3.7	Soluble
56.	Bergaptol	202.16	$C_{11}H_6O_4$	0	4	1	54.28	63.58	1.77	Soluble
57.	Netilmicin	475.6	$C_{21}H_{41}N_5O_7$	8	12	8	117.83	199.73	-2.2	Highly soluble
58.	Isoflurophate	184.15	$C_6H_{14}FO_3P$	4	4	0	41.84	45.34	1.66	Very soluble
59.	Anthranilate	137.14	$C_7H_7NO_2$	1	2	2	37.81	63.32	0.72	Very soluble
60.	3-(3,4-Dihydroxyphenyl)lactate	198.17	$C_9H_{10}O_5$	3	5	3	46.06	100.82	-0.17	Very soluble
61.	20-Hydroxyecdysone	480.6	$C_{27}H_{44}O_7$	5	7	6	129.74	138.45	1.76	Soluble
62.	L-Kynurenine	208.21	$C_{10}H_{12}N_2O_3$	4	4	3	55.13	106.41	-0.69	Highly soluble
63.	Isopropamide	353.5	$C_{23}H_{33}N_2O^+$	8	1	1	109.13	43.09	1.93	Moderately soluble
64.	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate	127.14	$C_6H_9NO_2$	1	3	1	37.62	49.66	0.42	Very soluble
65.	N-Acetylmuramate	293.27	$C_{11}H_{19}NO_8$	6	8	5	63.31	145.55	-1.64	Highly soluble
66.	Aciclovir	225.2	$C_8H_{11}N_5O_3$	4	5	3	55.68	119.05	-0.99	Very soluble
67.	Hydroxytamoxifen	387.5	$C_{26}H_{29}NO_2$	8	3	1	121.75	32.7	5.36	Poorly soluble
68.	L-Adrenaline	183.2	$C_9H_{13}NO_3$	3	4	4	49.03	72.72	0.1	Very soluble
69.	L-Tryptophan	204.22	$C_{11}H_{12}N_2O_2$	3	3	3	57.36	79.11	0.17	Very soluble
70.	Pantetheine	278.37	$C_{11}H_{22}N_2O_4S$	10	4	4	70.99	137.46	-0.16	Very soluble
71.	trans-Cinnamate	148.16	$C_9H_8O_2$	2	2	1	43.11	37.3	1.79	Soluble
72.	L-Octanoylcarnitine	287.39	$C_{15}H_{29}NO_4$	12	4	0	77.71	66.43	0.27	Soluble
73.	L-Proline	115.13	$C_5H_9NO_2$	1	3	2	32.52	49.33	-0.92	Highly soluble
74.	Citrate	192.12	$C_6H_8O_7$	5	7	4	37.47	132.13	-1.51	Highly soluble
75.	Pterin	163.14	$C_6H_5N_5O$	0	4	2	42.36	97.55	-0.49	Very soluble

Rotatable bonds (RB), H-bond acceptors (HBD), H-bond donors (HBD), Molar Refractivity (MR), Topological Polar surface area (TPSA)

4.4.1.7.3 Molecular interaction analysis

Additionally, molecular docking was examined in terms of molecular interactions, such as hydrophobic and hydrogen bonding interactions, and the contributions of amino acid residues to the interaction shown in Table 4.25. Several highly advantageous hydrophobic interactions that were a component of the ligand binding mechanism in the AKT1 receptor's active region were examined and displayed in two dimensions. (Figure 4.57 to Figure 4.64). The 4 A° area around the ligand molecules was used to determine these hydrophobic interactions. The amino acid residues shown in Table # were important in the creation of hydrophobic contact or H-bonds; similarly, the same set of amino acid residues was implicated in the binding of 5-fluorouracil to the AKT1 protein. Where the involvement of amino acids like Lys20, Glu149, Tyr7, Asn9, His 183, Val153, Val156, Phe109, Ser102, Gln187, Asp193, Trp192, IIe186 were significant residues in the formation of H-bond or hydrophobic contact.

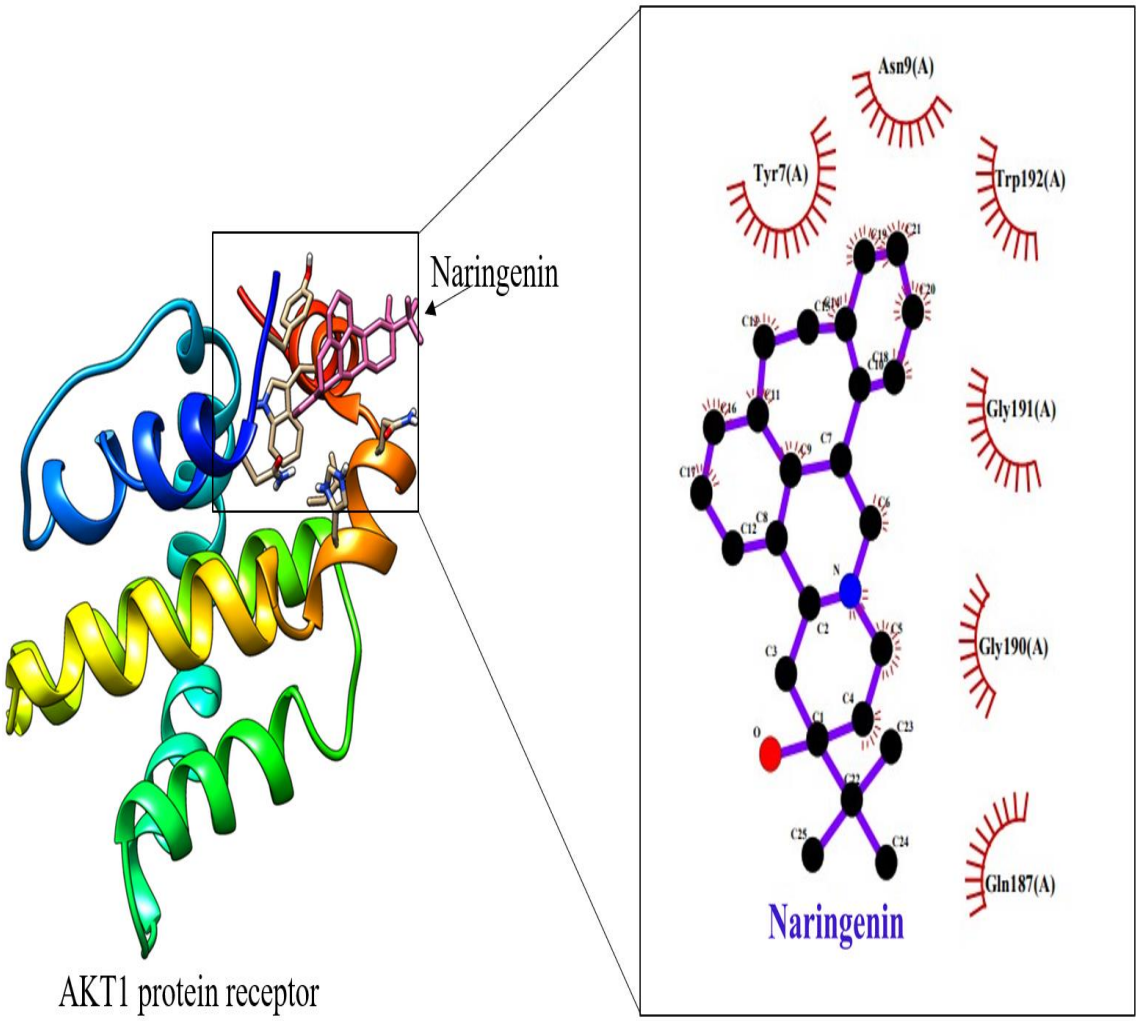


Figure 4.57 AKT1-Naringenin complex and their molecular interactions

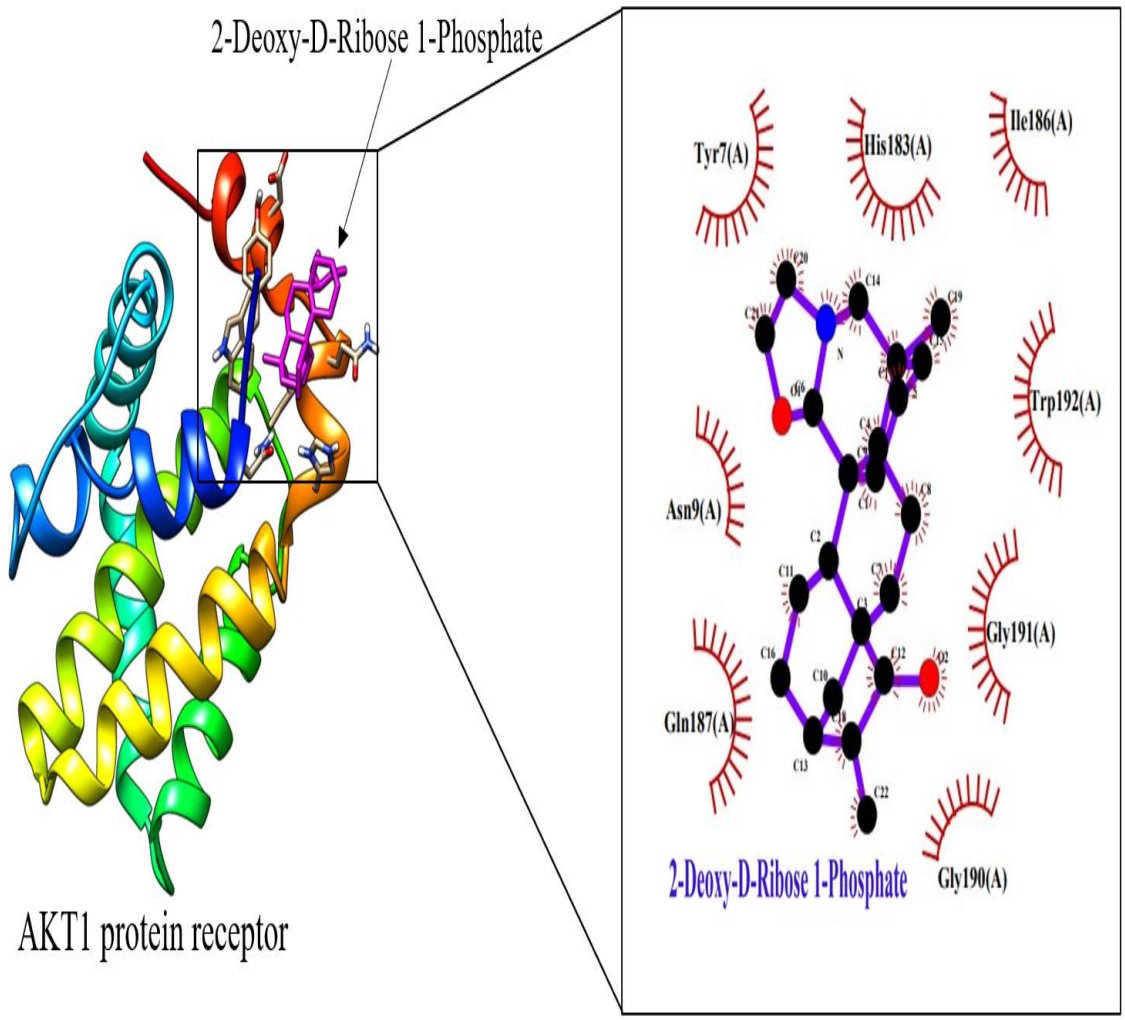


Figure 4.58 2-Deoxy-D-Ribose 1- Phosphate complex and their molecular interactions

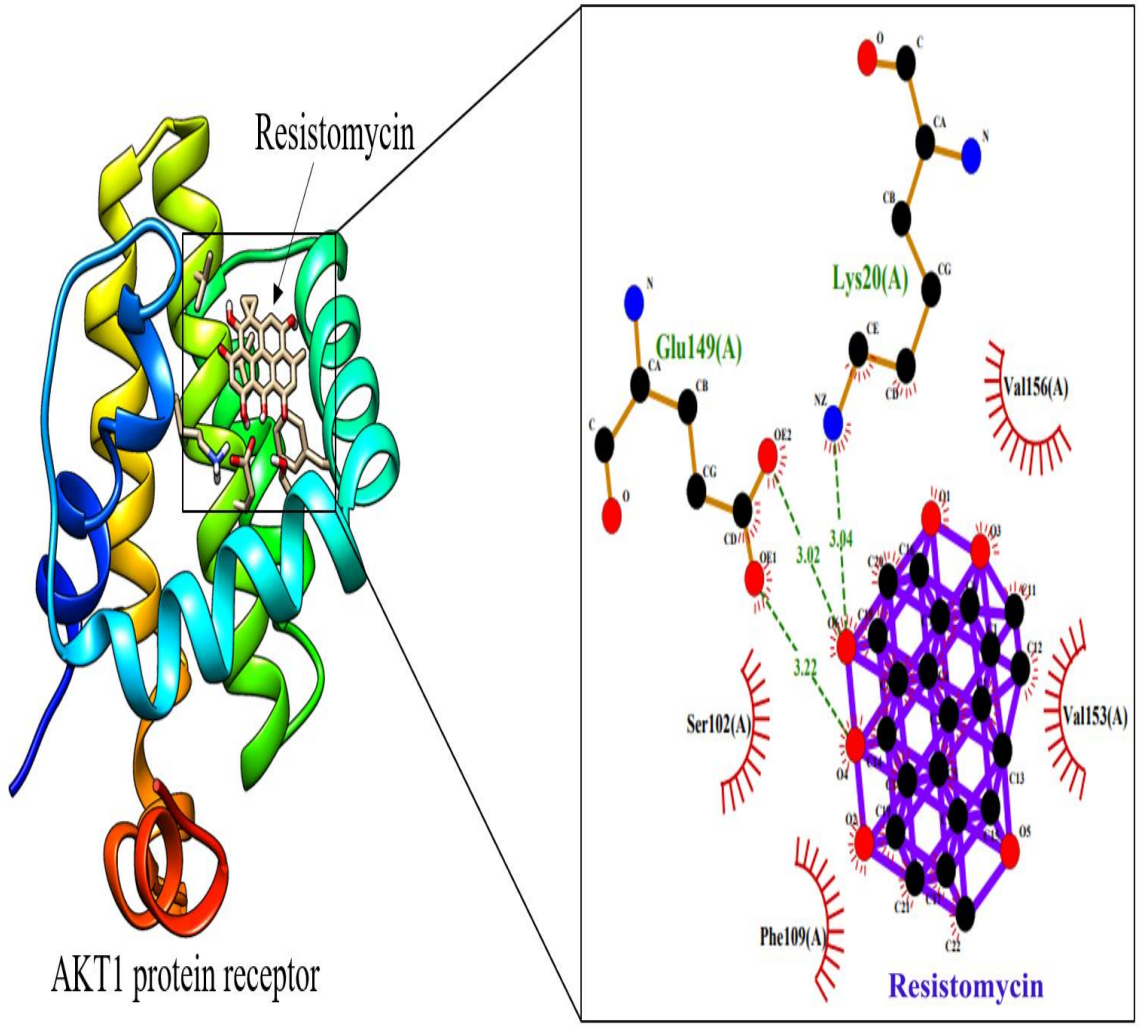


Figure 4.59 AKT1-Resistomycin complex and their molecular interactions

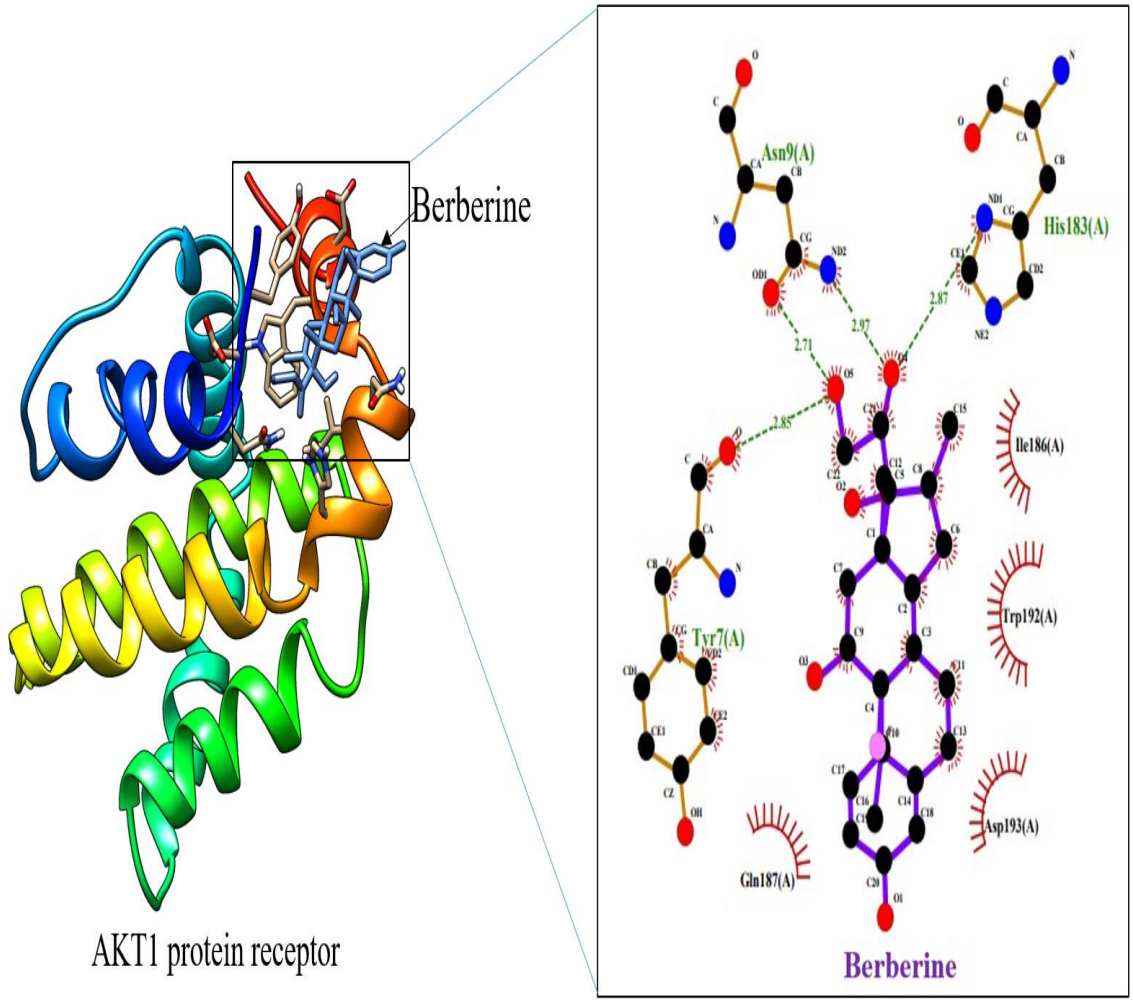


Figure 4.60 AKT1-Berberine complex and their molecular interactions

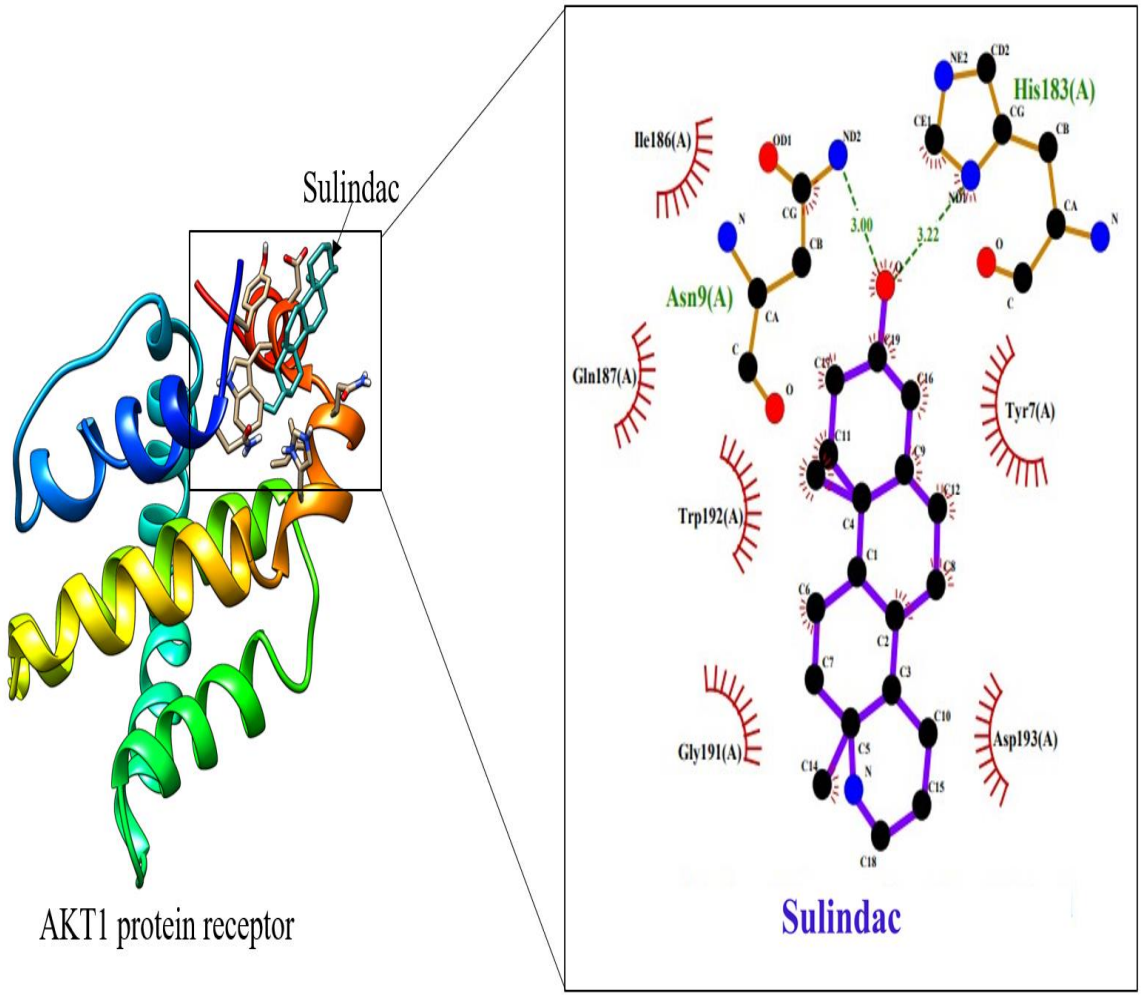


Figure 4.61 AKT1-Sulindac complex and their molecular interactions

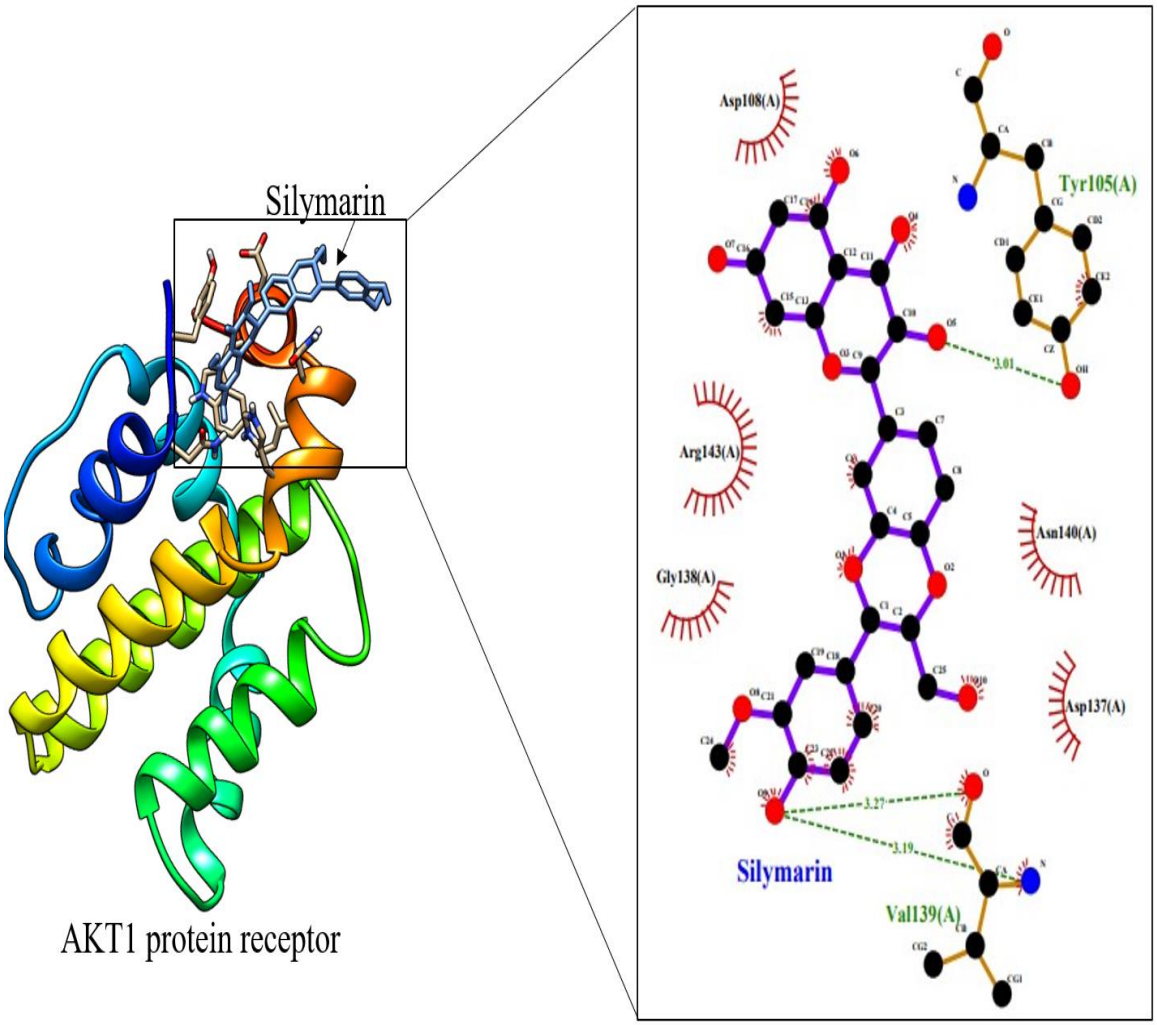


Figure 4.62 AKT1-Silymarin complex and their molecular interactions

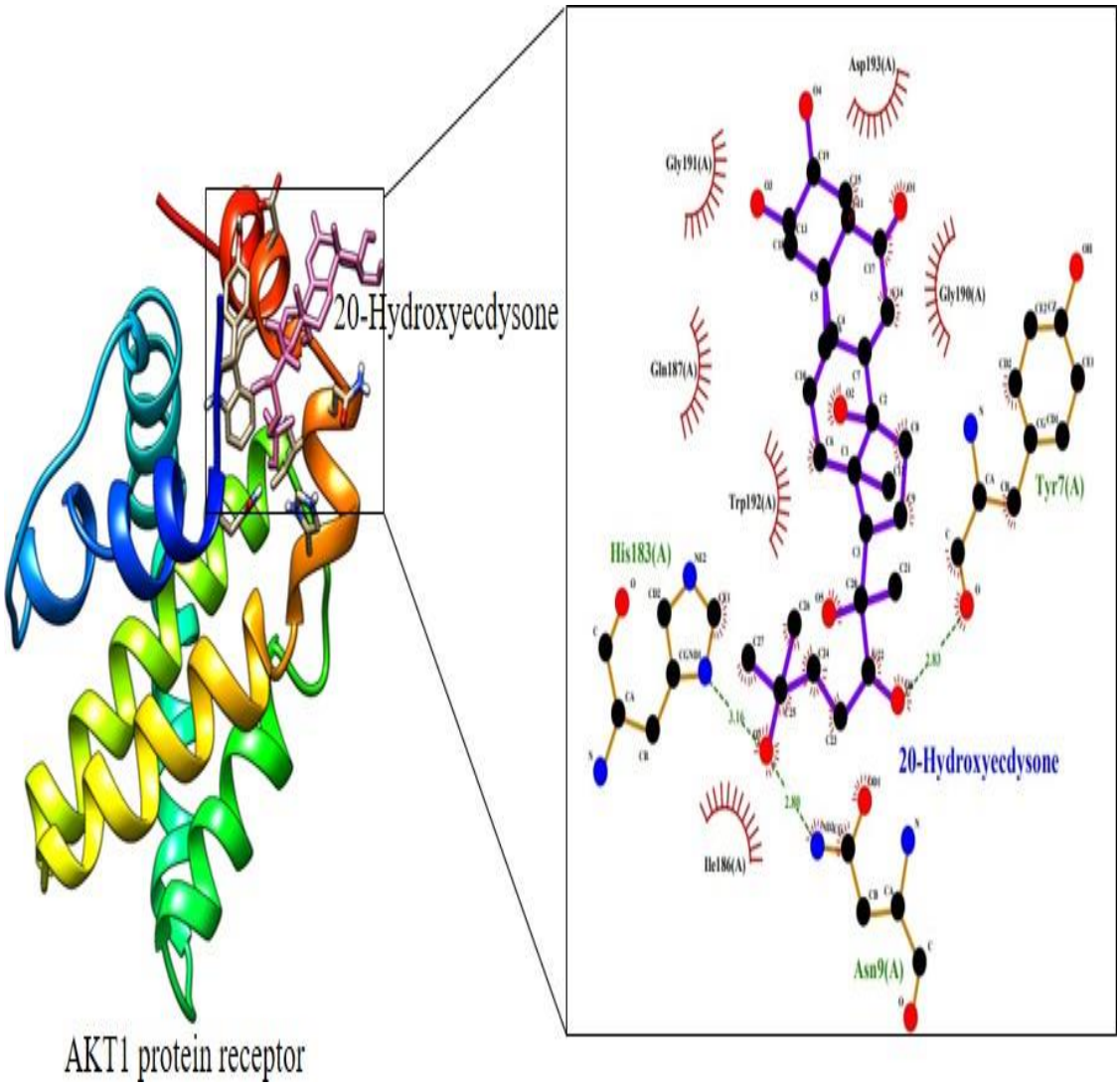


Figure 4.63 AKT1-20-Hydroxyecdysone complex and their molecular interactions

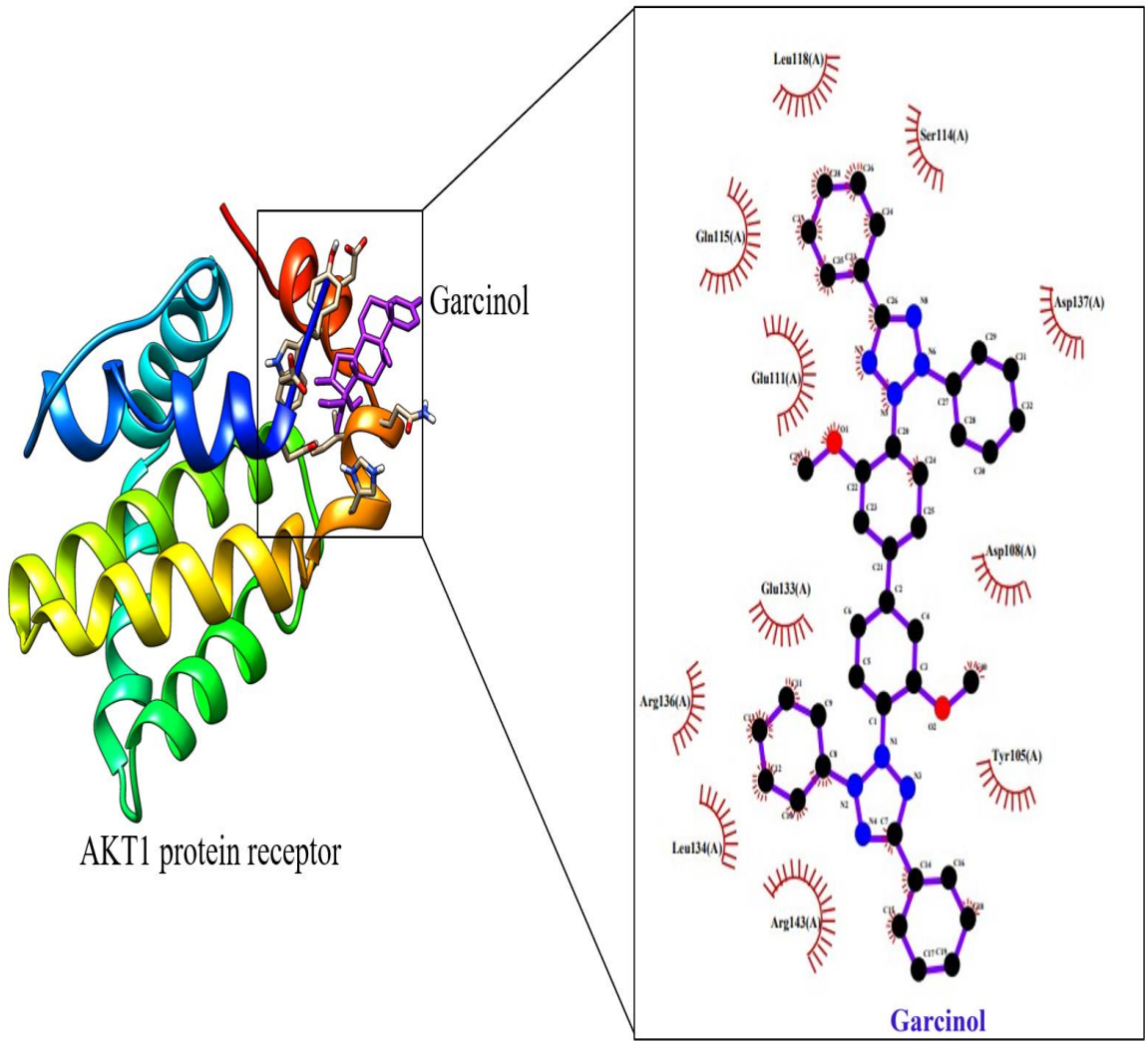


Figure 4.64 AKT1-Garcinol complex and their molecular interactions

Table 4.25 Molecular interaction analysis of bioactive compounds with AKT1 Protein

Protein Receptor	Ligand Molecules	H-bond Interaction	Hydrophobic Interaction
AKT1	Naringenin		Gln187, Gly190, Gly191, Trp192, Asn9, Tyr 7
	2-Deoxy-D-Ribose 1-Phosphate		Gly190, Gly191, Trp192, IIe186, His183, Tyr7, Asn9, Gln187
	Resistomycin	Glu149, Lys20	Val153, Val156, Phe109, Ser102
	Berberine	Tyr7, Asn9, His 183	Gln187, Asp193, Trp192, IIe186
	Sulindac	His183, Asn9	Asp193, Tyr7, IIe186, Gln 187, Trp192, Gly191
	Silymarin	Val39, Tyr105	Asp137, Asn140, Asp108, Arg1443, Gly138
	20-Hydroxyecdysone	His183, Asn 9, Tyr7	IIe186, Trp192, Gln187, Gly191, Asp193, Gly190
	Garcinol		Tyr105, Asp108, Asp137, Ser114, Leu118, Gln115, Glu111, Glu133, Arg136, Leu134, Arg 143

4.4.1.7.4 Molecular dynamic simulations to test the protein-ligand complex's stability.

To explore the binding stability of ligand molecules the protein-ligand complexes such as Naringenin-AKT1, Silymarin-AKT1, Resistomysin-AKT1, and 20-Hydroxyecdysone-AKT1 which possess higher binding affinity among the molecules docked were taken for MD simulation based on molecular docking results. Along with these complexes, the binding stability of 5-fluorouracil with AKT1 was also analysed in terms of root mean square deviation (RMSD), root mean square fluctuation (RMSF) and Radius of gyration (Rg), Solvent Accessible Surface Area (SASA), Hydrogen Bond (H-bond).

To assess the equilibration of MD trajectories and the stability of complex systems can be confirmed through RMSD analysis. To ascertain whether a substantial conformational shift occurred during the MD simulation. The generated MD trajectories were used to compare beneficial drug molecules' binding stability with a protein receptor. The simulated trajectories of protein-ligand complexes, as represented by the RMSD plot, examination of every trajectory demonstrates that the deviation in structure was found more from the early stages, which eventually stabilized throughout the simulation time (Figure 4.65)**.** Molecular binding of bioactive compounds like Naringenin, Silymarin, Resistomysin, and 20-Hydroxyecdysone binding was found to be stable with AKT1. The average deviation in the backbone of the protein is found near 0.25 Å, whereas the structural deviation in AKT1 was observed similar after binding with Silymarin, Resistomysin and the standard drug 5-fluorouracil compared to Naringenin and 20-Hydroxyecdysone. Whereas the binding of Naringenin and 20-Hydroxyecdysone shows the highest deviation initially and after 50 ns with minor fluctuation it got stable till 100 ns. As observed in Figure 4.65, the RMSD plot of the carbon backbone RMSD values reveals that all complexes change by less than 0.4 Å.

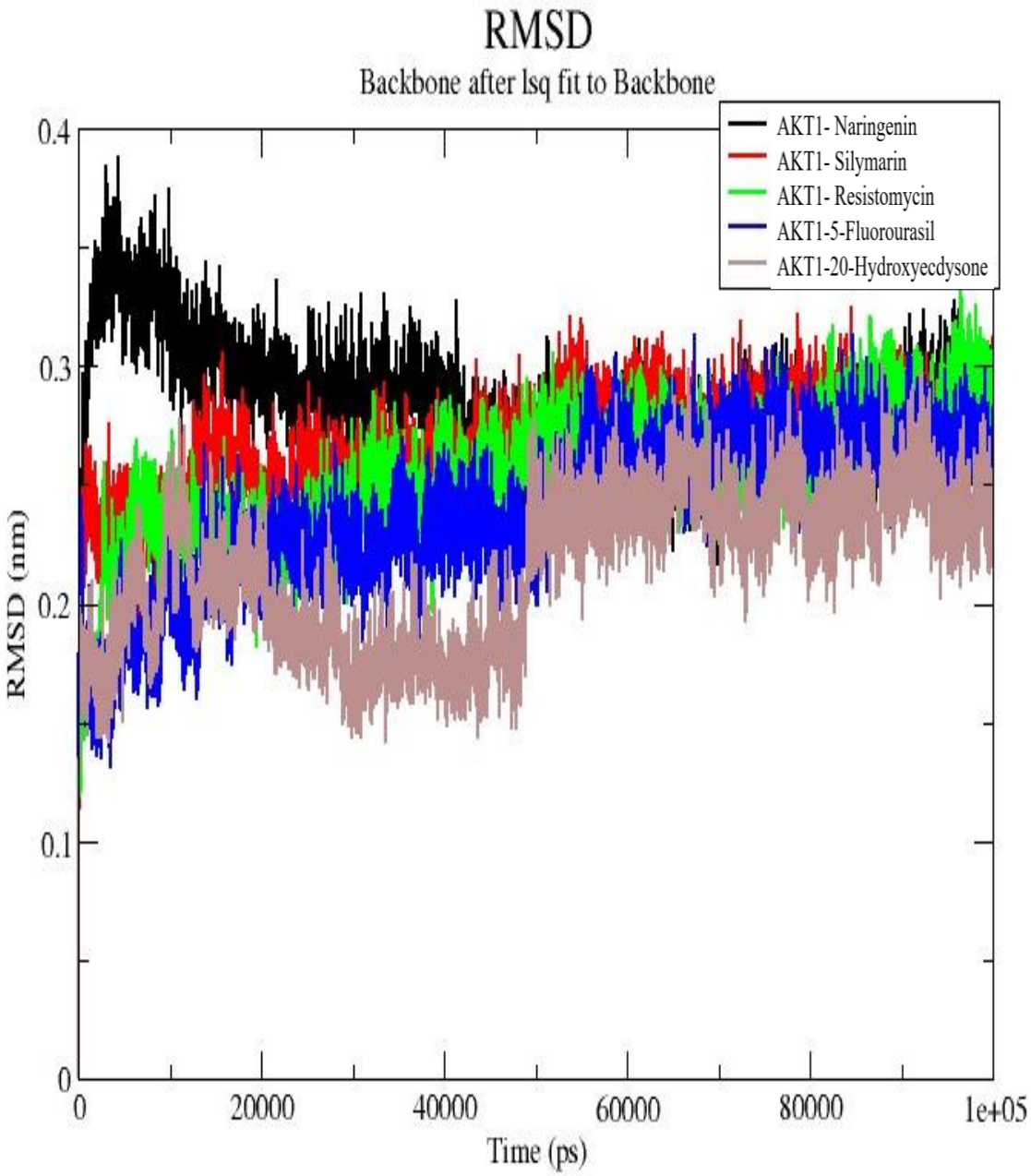


Fig. 4.65 Root mean square deviation (RMSD) plot of protein-ligand complexes along with 5-fluorouracil-AKT1 protein.

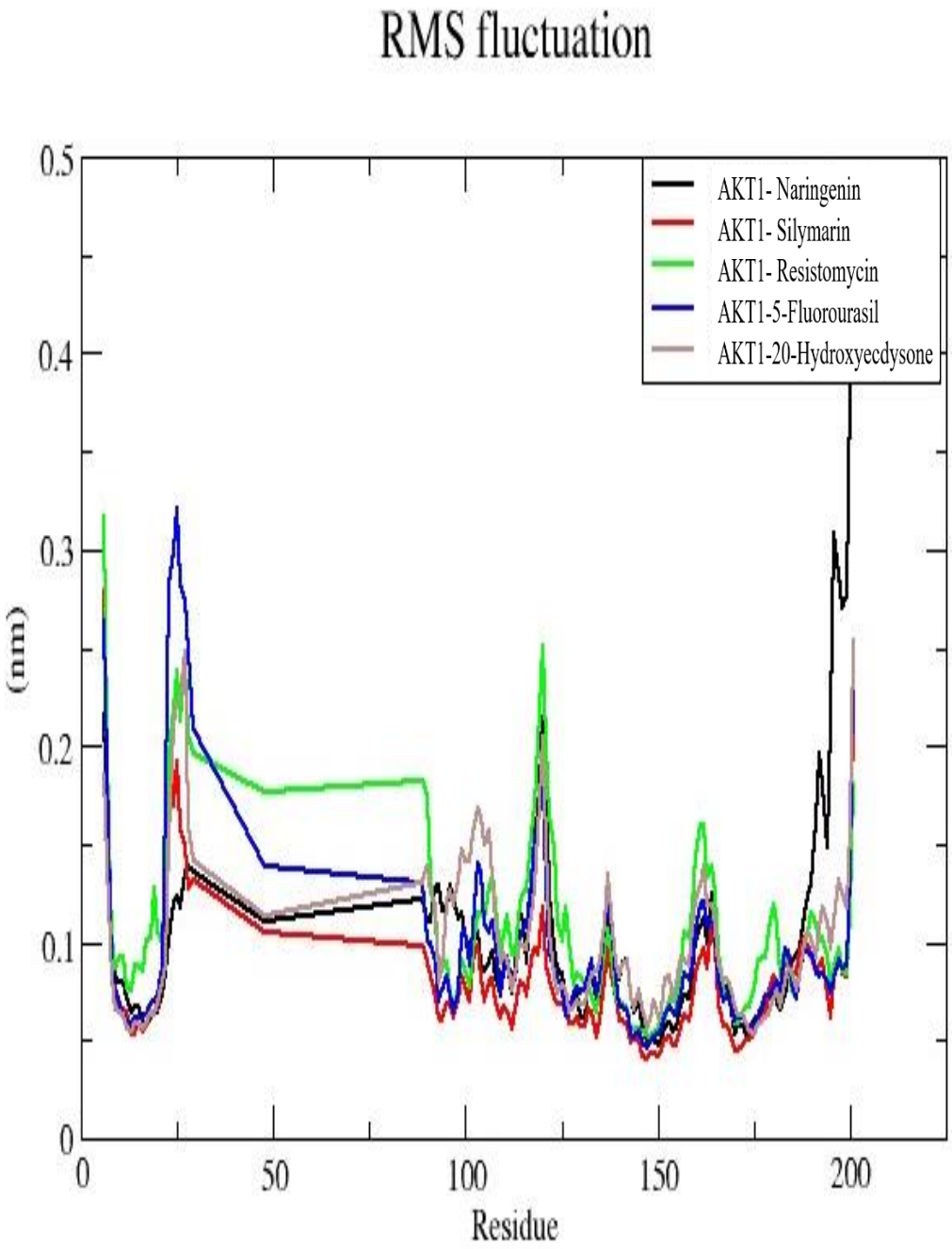


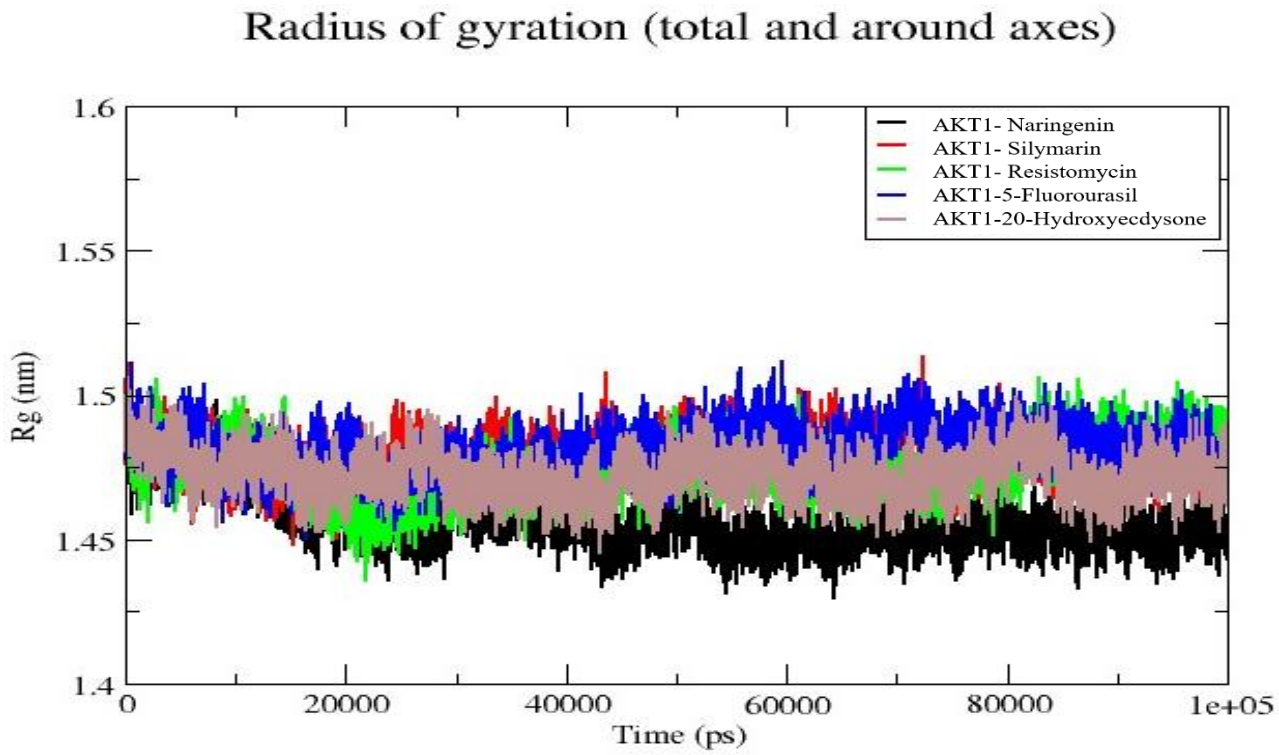
Fig. 4.66 Root mean square fluctuation (RMSF) plot of protein-ligand complexes along with apo-protein.

The flexibility of the protein backbone upon interaction with ligand molecules is revealed by RMSF analysis, which is represented at the residue level and the fluctuating behaviour shown in the plot displayed in Figure 4.66. The residual fluctuations in C-alpha atoms of AKT1 showed a similar pattern of RMS fluctuation that was observed throughout the simulation in all protein-ligand complexes including the 5-fluorouracil. The residual fluctuations in AKT1 after binding with Resistomycin showed slightly higher compared to, especially near residue number 20-30, and the fluctuation was observed till 0.32 nm during the simulation. The protein in complex with Silymarin, Resistomysin, and 20-Hydroxyecdysone showed similar RMS fluctuations at the same region but a little lower along with 5-fluorouracil. The RMSF plot of the complexes demonstrates that the bioactive compounds bind to the target protein steadily. Nonetheless, the complex with the lowest RMSF value, AKT1-20-Hydroxyecdysone complex was determined to be the most stable.

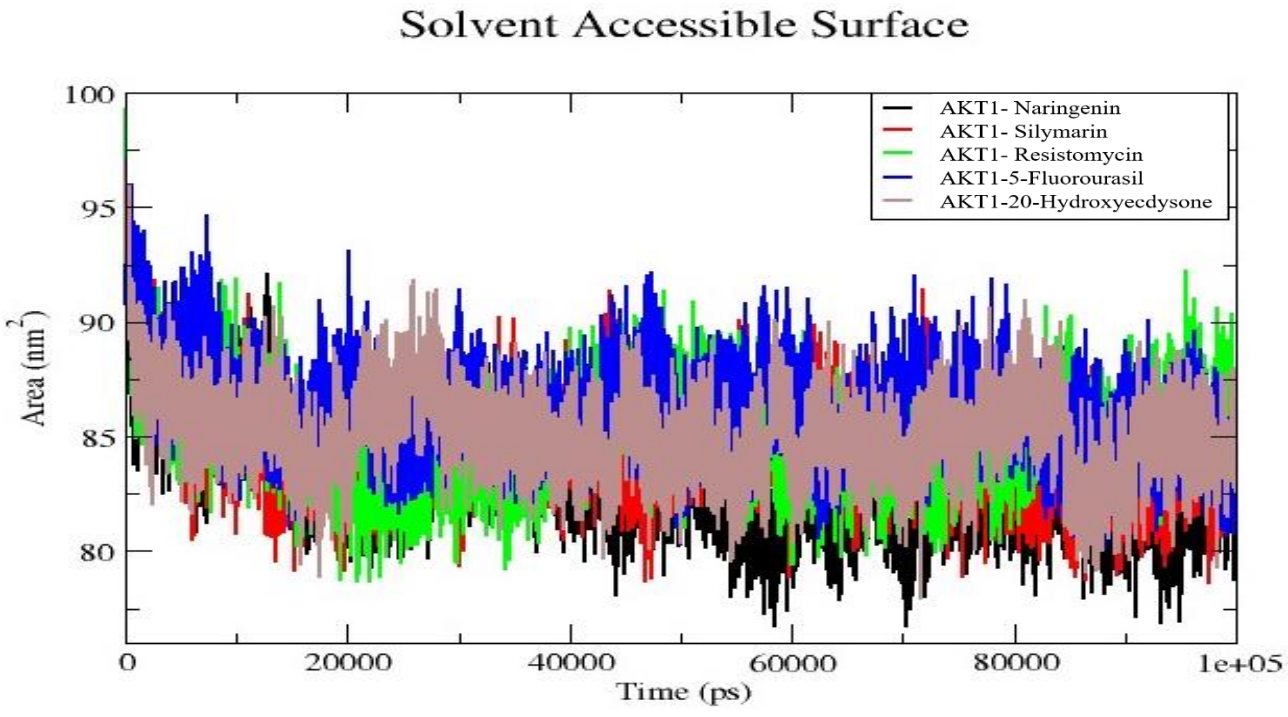
The system appears to be more rigid and compact when there is less variation and volatility during the simulation that can be measured through the radius of gyration (Rg) tool. The Rg values for each of the following complexes such as Naringenin-AKT1, Silymarin-AKT1, Resistomysin-AKT1, and 20-Hydroxyecdysone-AKT1 to ascertain the compactness in the protein are shown in figure 4. 67 (A). All the protein-ligand complexes show slight compaction till the end of the simulation compared to their initial state. Whereas the pattern of compaction in protein-ligand complexes is the same. The protein-ligand complexes showed almost similar and consistent patterns of fluctuation that decreased from 1.5 nm to 1.45 nm till the end of the simulation. Whereas, the structure fluctuation in 20-Hydroxyecdysone-AKT1 complex shows a little more decrease in compactness compared to other protein-ligand complexes that are around < 1.45 nm. It is confirmed that amino acid residues are less accessible to binding with surrounding solvents when there is greater compactness, which indicates less water interaction with the environment and may be measured in terms of the overall SASA score (Figure 4.67 B). Compared to protein-ligand all protein-ligand complexes the 20-Hydroxyecdysone-AKT1 showed higher instability during the entire period of

simulation but interaction from the surroundings decreased from 95 to 85 till the end of the simulation, it was stable with ≤ 85 hydrogen bond interaction. SASA research unequivocally demonstrates that protein structure may be considerably compressed by ligand binding. With minimal change expected during the simulations experiment, the higher SASA values indicate that the protein volume is increasing.

Since it is commonly known that hydrogen bonding can significantly affect a drug's specificity, absorption, and metabolisation, this property informs drug design concepts. In order to understand the binding strength of the ligand-protein complexes from generated trajectories, the number of hydrogen bonds was calculated during the simulation. During the whole simulation time, all protein-ligand complexes are unstable in terms of intermolecular hydrogen bonding. The average H-bond formed between Silymarin-AKT1, Resistomysin-AKT1, and 20-Hydroxyecdysone-AKT1 are 1, 2, 3 and 2 (Figure 4.68). During the simulation, the chosen bioactive compounds showed a higher number of hydrogen bonds based on their molecular ability, indicating a higher level of stability. (Ralte *et al.*, 2024).



(A)



(B)

Fig. 4.67 (A) Radius of gyration (Rg) and (B) Solvent accessible surface area (SASA) plot of protein-ligand complexes

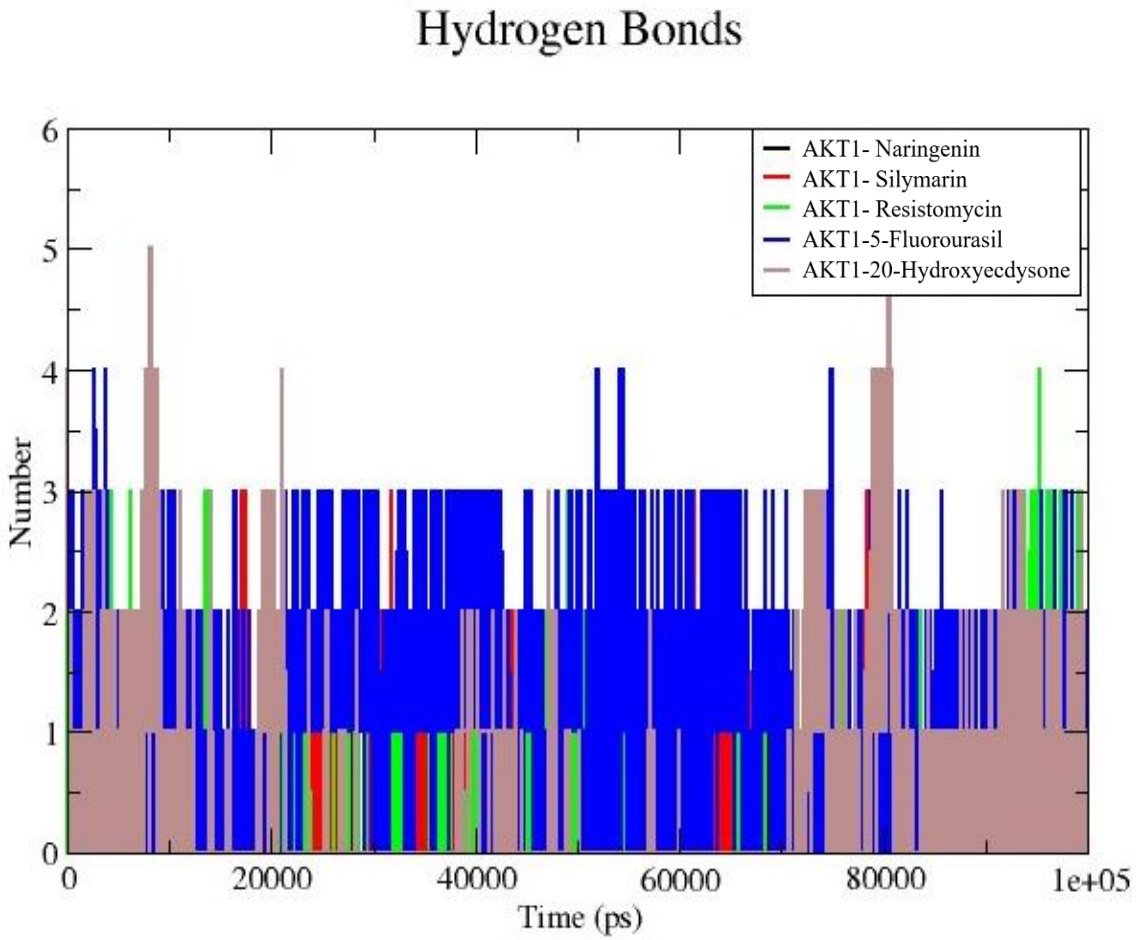


Fig 4.68 Hydrogen Bond (H-bond) plot of protein-ligand complexes.

4.4.2 Evaluation of antioxidant activity

Excessive free radicals generated during metabolic processes can be detrimental, as they exhibit a binding affinity for essential biomolecules such as DNA, proteins, and lipids, leading to the development of various diseases. To mitigate ROS-induced oxidative damage and its related diseases, the utilisation of natural antioxidants is strongly recommended. A substantial body of research indicates that antioxidant supplements may effectively mitigate oxidative stress and potentially inhibit or decelerate the advancement of disease complications (Badmus *et al*., 2011).

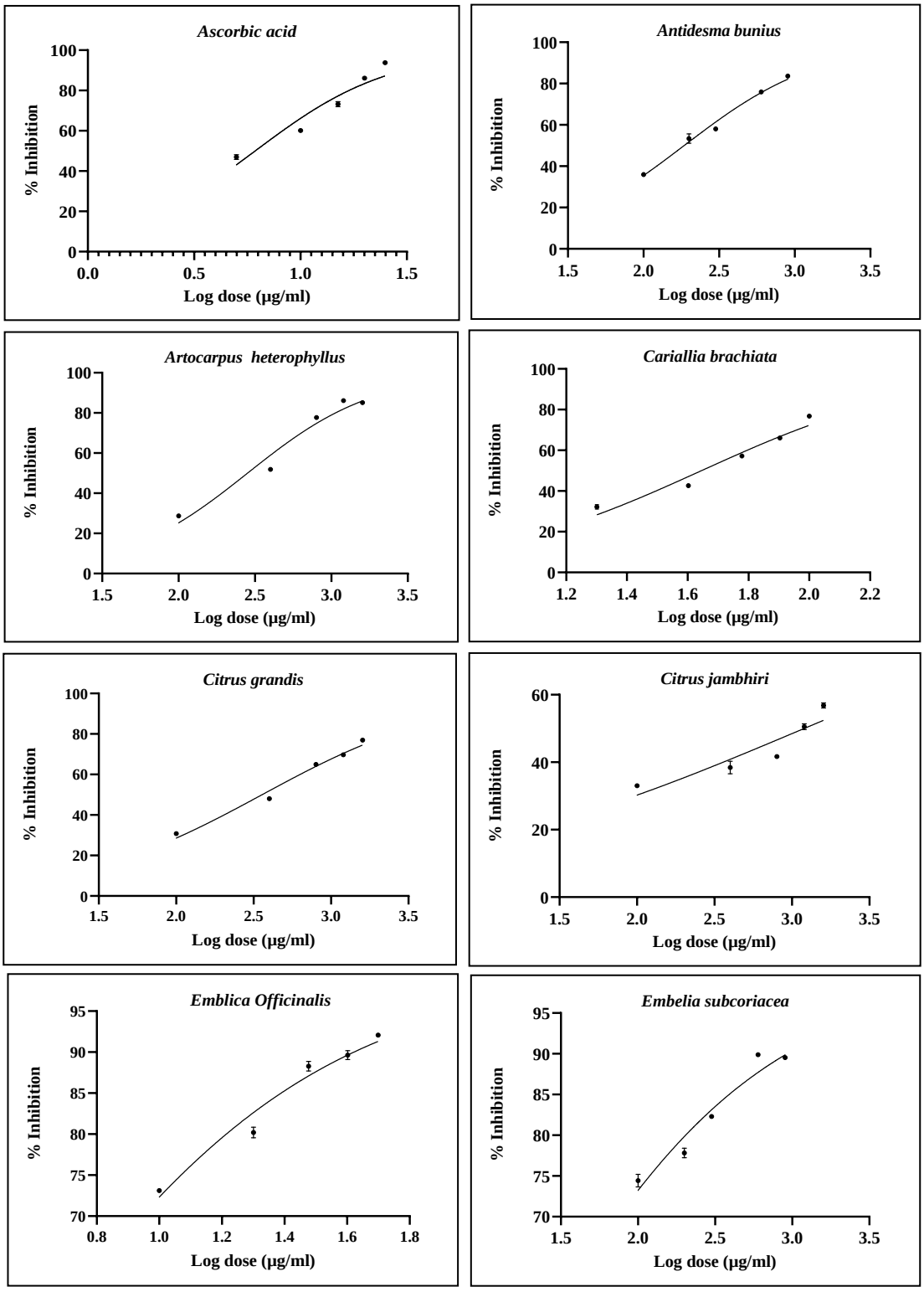
4.4.2.1 DPPH radical scavenging activity

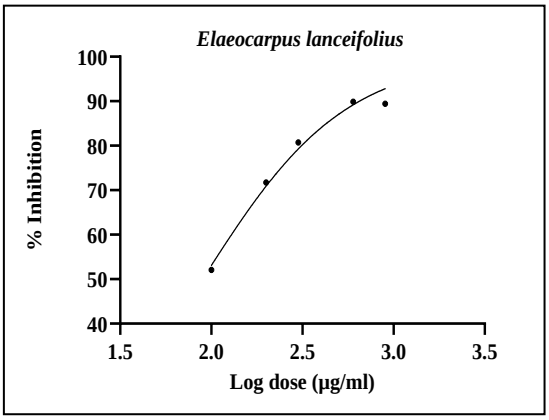
DPPH is a stable free radical that accepts an electron or hydrogen radical, resulting in the formation of a stable diamagnetic molecule. The phenomenon is predicated on the capacity of DPPH, a stable free radical, to undergo decolourisation in the presence of antioxidants. The methanolic extracts of 21 underutilised fruits demonstrated a dose-dependent enhancement in DPPH radical scavenging activity, as evidenced by the observed discolouration of DPPH. The logarithmic doses of diverse fruit extracts and standard ASA were plotted against DPPH inhibition percentages to determine the IC_{50} value (Figure 4.69). The inhibitory concentration (IC_{50}) for DPPH radical varied between 21.75 ± 0.21 µg/mL and 2921.67 ± 8.09 µg/ml. The mean scavenging activity of the methanolic extracts from 21 underutilised fruits was recorded at a concentration of 317.49 µg/mL (Figure 4.70). The antioxidant activity with the lowest IC_{50} value was recorded in *Emblica*

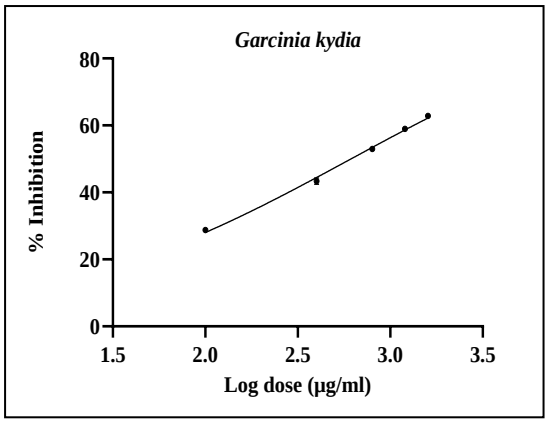
officinalis (3.29 ± 0.05 µg/ml), which is statistically comparable to *Terminalia chebula* (10.53 ± 0.24 µg/ml) and *Embelia subcoriacea* (15.39 ± 3.45 µg/ml). In contrast, the minimal antioxidant activity, as measured by DPPH, was recorded in *Citrus jambhiri* (1217.00 ± 7.59 µg/ml), which exhibited elevated IC_{50} values. Meanwhile, *Tamarindus indica* (1138.00 ± 7.55 µg/ml) and *Garcinia xanthochymus* (825.60 ± 0.95 µg/ml) demonstrated reduced antioxidant activity (Table 4.26).

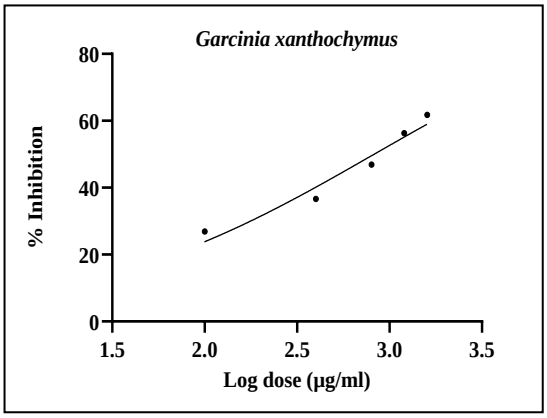
The antioxidant capabilities of natural compounds, including plant extracts, can be assessed by their capacity to convert methanolic DPPH solution into the non-radical form DPPH-H. DPPH represents a stable nitrogen-centered free radical commonly employed to evaluate the radical scavenging activity of plant extracts or compounds. The violet hue of the stable DPPH radical was diminished to the yellow shade of diphenylpicrylhydrazine radical upon the acceptance of an electron from the antioxidant molecule, a change that was assessed colourimetrically. Compounds that can facilitate this reaction may be classified as antioxidants and, consequently, as radical scavengers (Dehpour *et al*., 2009). The antioxidative potential of various fruit extracts was assessed based on their capacity to scavenge DPPH radicals, demonstrating their efficacy as active radical scavengers. Polyhydroxy aromatic compounds, cysteine, tocopherol, glutathione, and ASA are recognised for their capacity to reduce DPPH through hydrogenation (Blois, 1958; Moon *et al*., 2010).

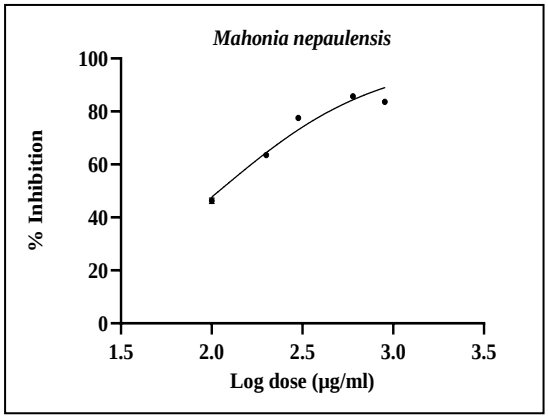
Fig. 4.69 Plots of log doses of methanolic extracts of different fruits against mean inhibition (%) of DPPH activity for the calculation of IC_{50}

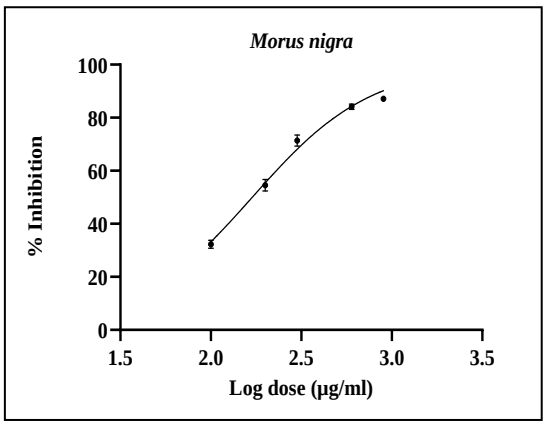


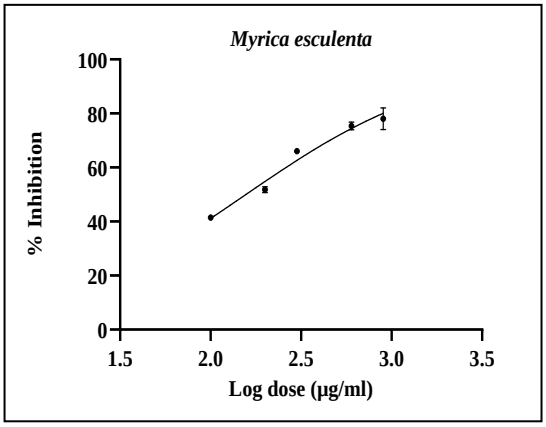

Elaeocarpus lanceifolius
% Inhibition
100
90
80
70
60
50
40
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)

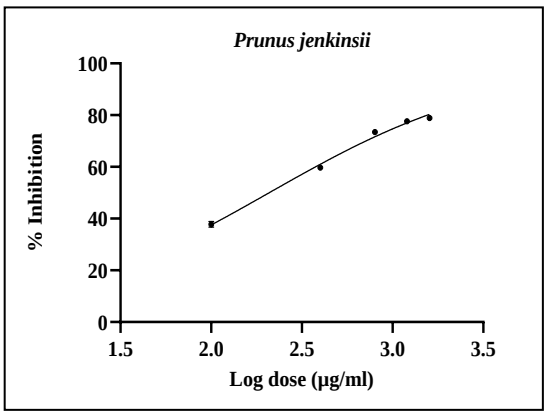

Garcinia kydia
% Inhibition
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)

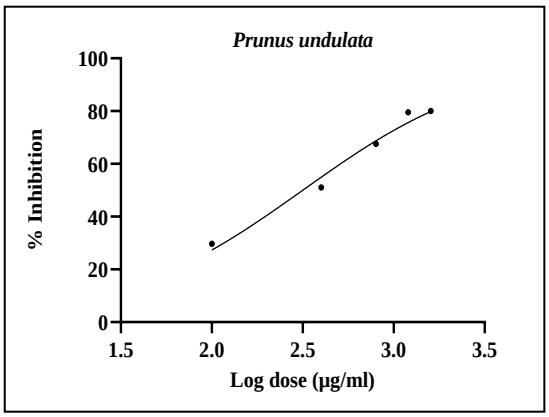

Garcinia xanthochymus
% Inhibition
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)

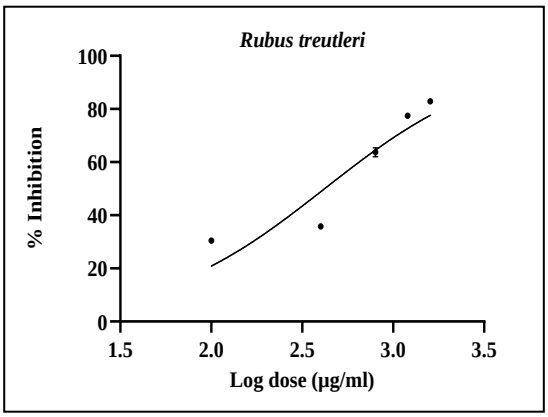

Mahonia nepaulensis
% Inhibition
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)

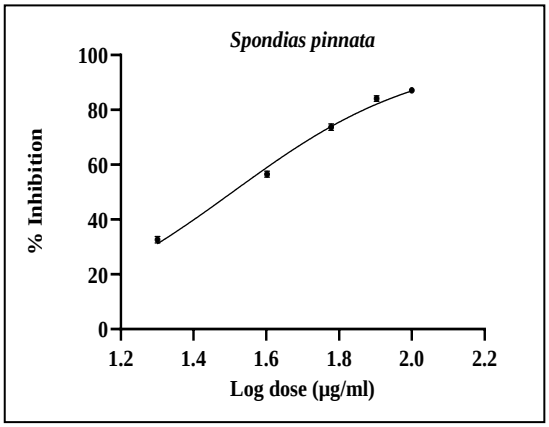

Morus nigra
% Inhibition
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)

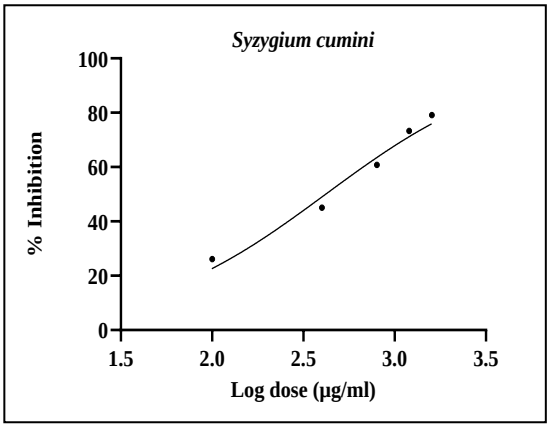

Myrica esculenta
% Inhibition
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)

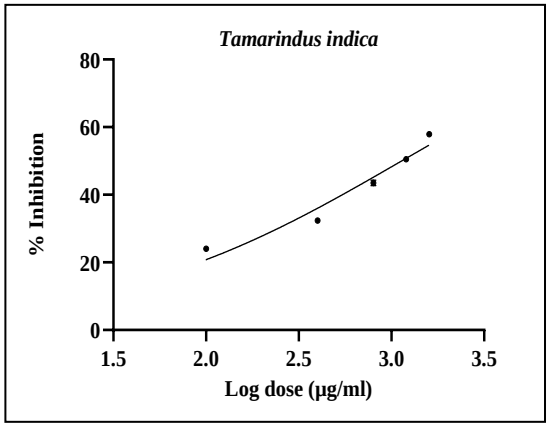

Prunus jenkinsii
% Inhibition
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)

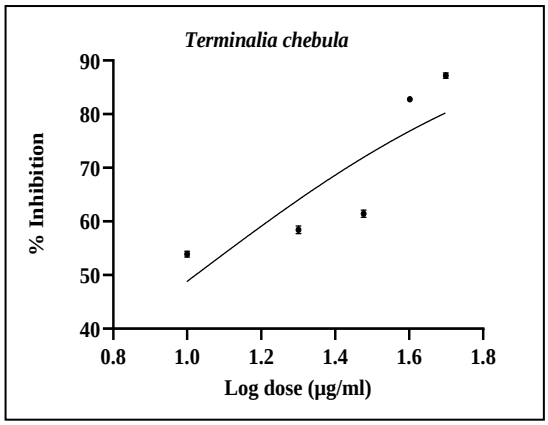

Prunus undulata
% Inhibition
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)

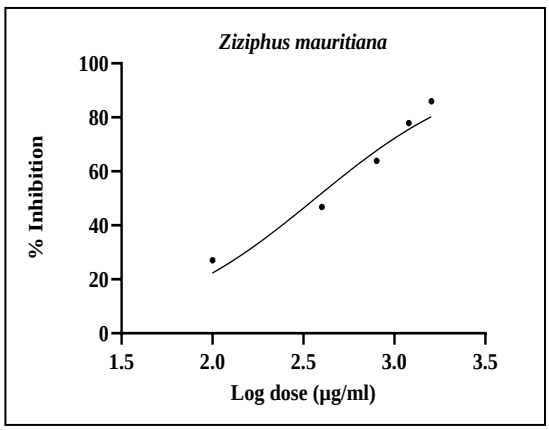

Rubus treutleri
% Inhibition
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)


Spondias pinnata
% Inhibition
100
80
60
40
20
0
1.2
1.4
1.6
1.8
2.0
2.2
Log dose (µg/ml)


Syzygium cumini
% Inhibition
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)


Tamarindus indica
% Inhibition
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)


Terminalia chebula
% Inhibition
90
80
70
60
50
40
0.8
1.0
1.2
1.4
1.6
1.8
Log dose (µg/ml)


Ziziphus mauritiana
% Inhibition
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log dose (µg/ml)

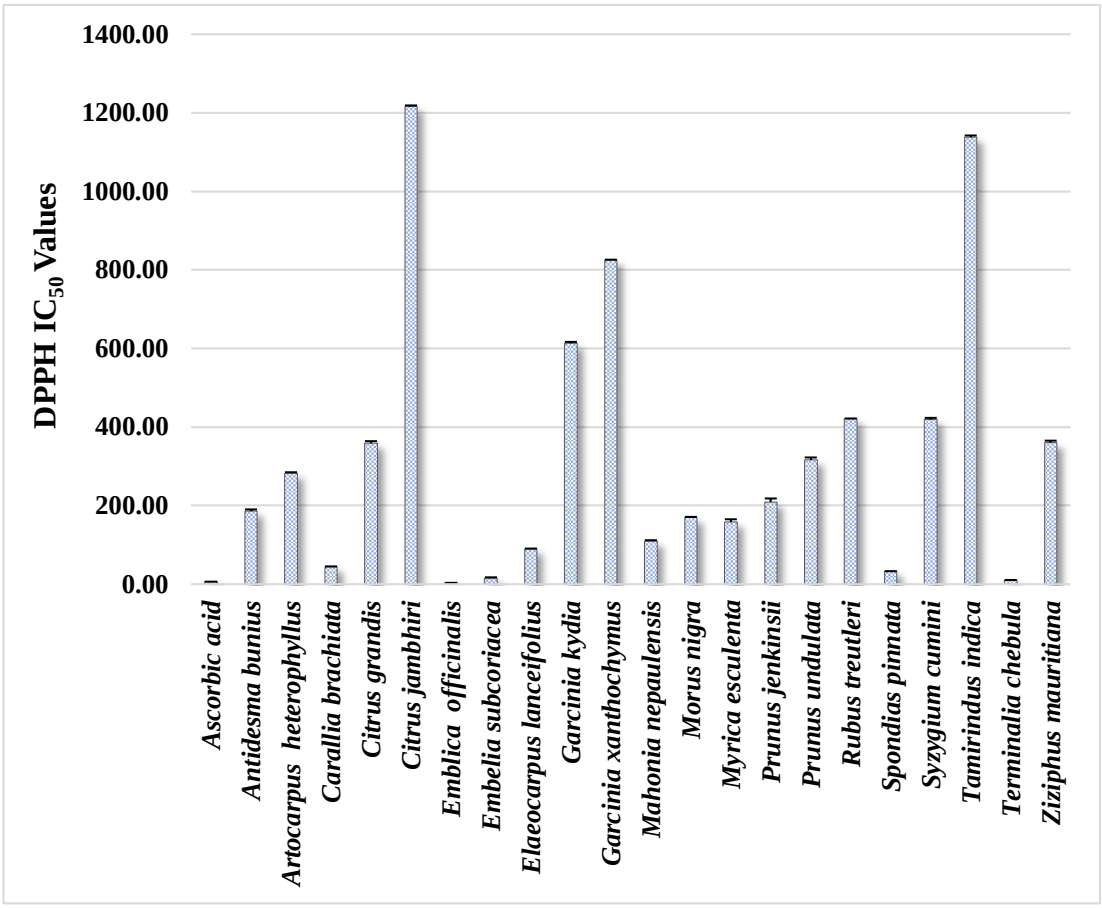


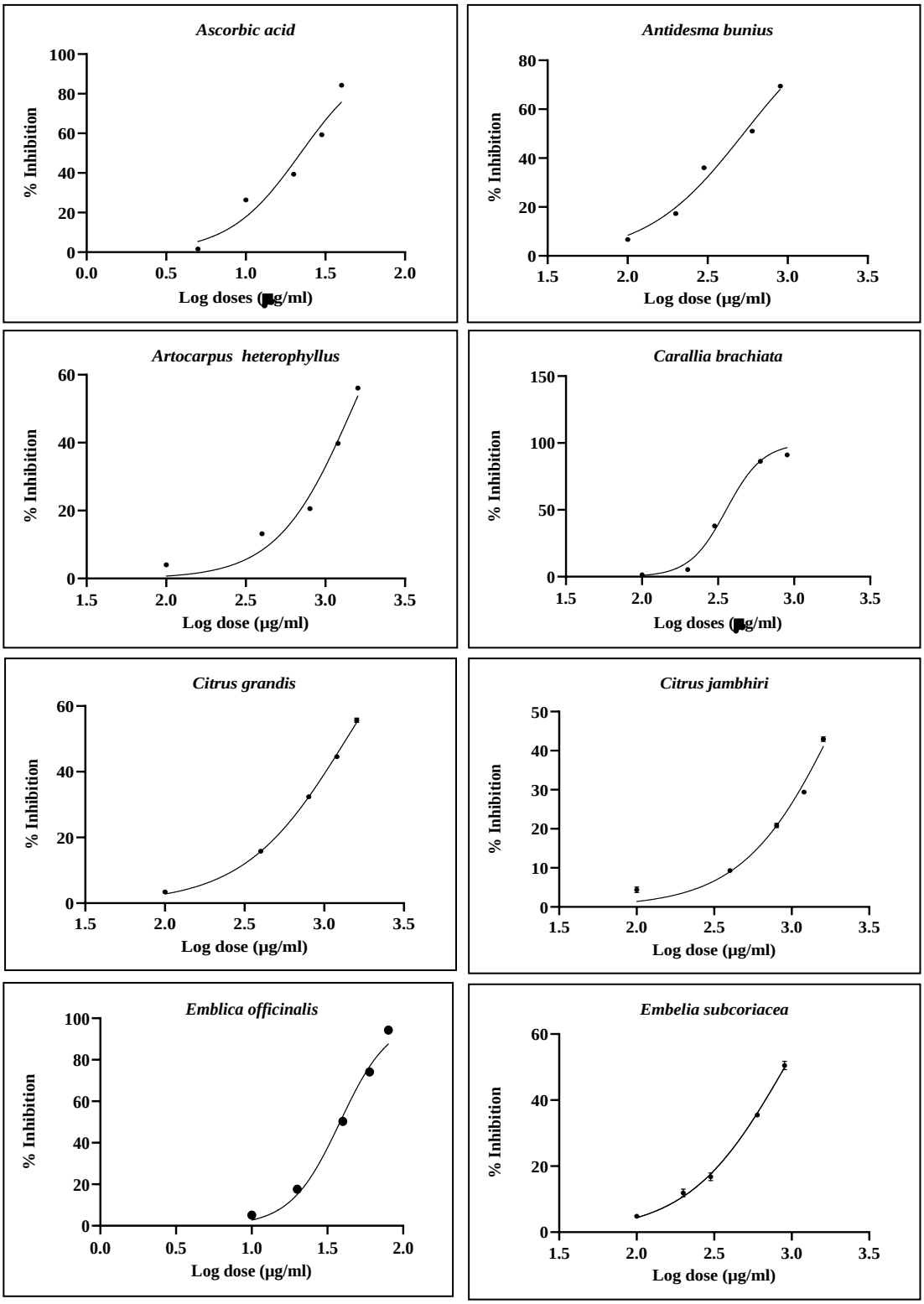
Fig. 4.70 IC_{50} Values for DPPH activity of the studied underutilised fruits

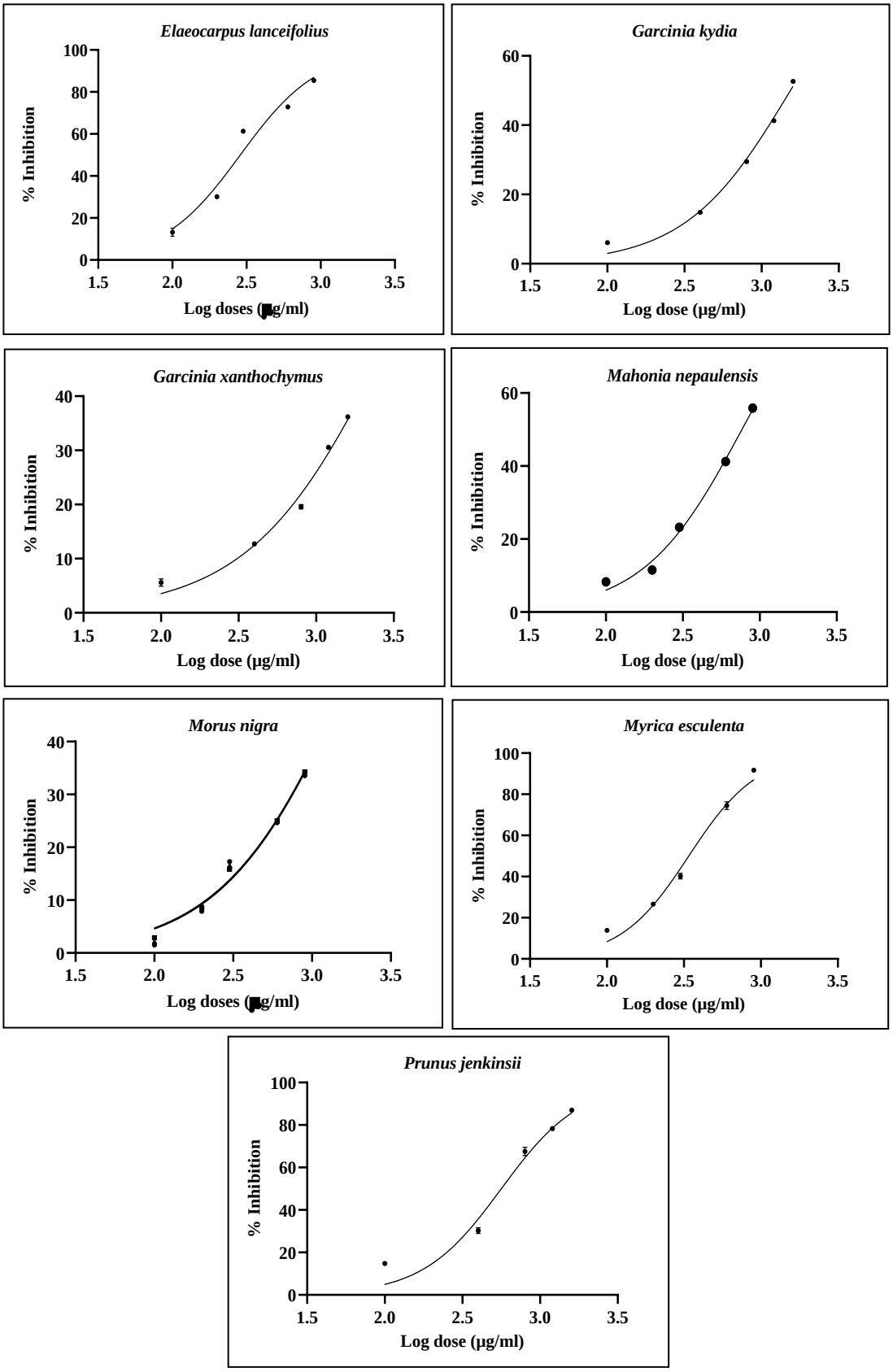
4.4.2.2 ABTS radical scavenging activity

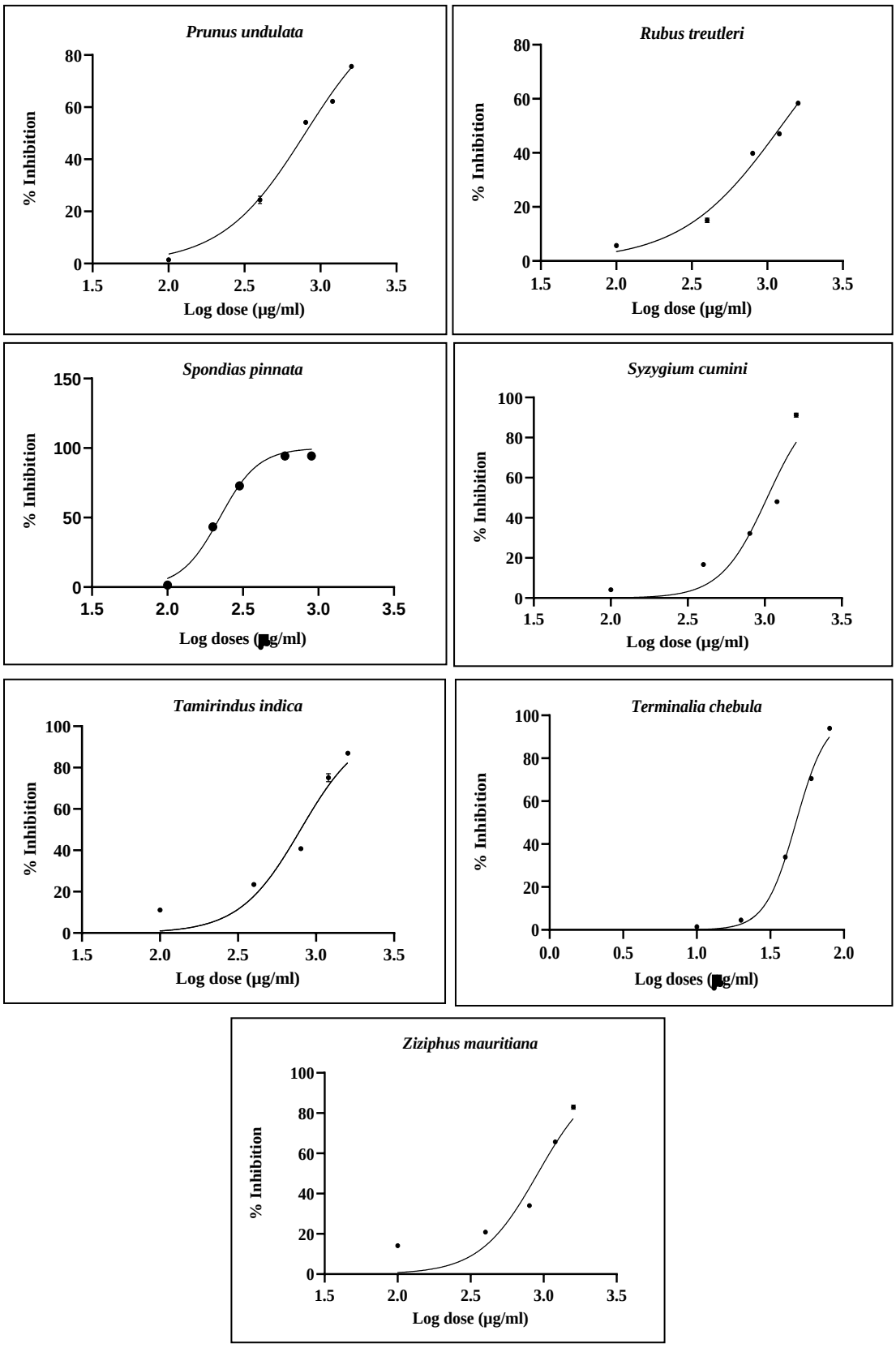
The ABTS assay serves as a robust method for assessing the antioxidant activity of hydrogen-donating antioxidants, particularly in their capacity to scavenge aqueous phase radicals, as well as chain-breaking antioxidants that target lipid peroxyl radicals. The $ABTS^{\bullet+}$ radical scavenging capacity of methanolic fruit extracts exhibited a concentration-dependent increase, as evidenced by the discolouration of the $ABTS^{\bullet+}$ solution. The log doses of different methanolic fruit extracts and standard Ascorbic acid (ASA) were plotted against $ABTS^{\bullet+}$ inhibition (%) to determine the IC_{50} value (Figure 4.71). The ABTS cation scavenging method yielded an inhibitory concentration (IC_{50}) ranging from 21.75 ± 0.21 µg/mL to 2921.67 ± 8.09 µg/mL (Figure 4.72). The mean scavenging activity was measured at 903.2 µg/mL, with *Emblica officinalis* exhibiting the highest ABTS radical scavenging activity, indicated by the lowest IC_{50} value of 38.33 ± 1.01 µg/mL. This value is statistically comparable to that of *Terminalia chebula*, which recorded an IC_{50} of 47.16 ± 0.33 µg/mL. Simultaneously, our current investigation revealed that *Garcinia xanthocymus* exhibited the lowest ABTS radical scavenging activity, as indicated by the highest IC_{50} value (2921.67 ± 8.09) (Table 4.26).

The antioxidative activity of diverse fruit extracts was assessed by evaluating their capacity to reduce the blue-coloured $ABTS^{\bullet+}$, generated through the interaction of ABTS and potassium ferricyanide, to ABTS. The efficacy of this conversion is contingent upon several factors, including the prevalence of aromatic rings, the nature of hydroxyl group substitutions, and the molecular weight of phenolic compounds (Hagerman *et al*., 1998). The research illustrates that extracts of *Emblica officinalis* and *Terminalia chebula* exhibit significant antioxidant properties, with their ABTS• + scavenging activity likely attributable to their phenolic compounds and vitamin content.

Fig. 4.71 Plots of log doses of methanolic extracts of different fruits against mean inhibition (%) of ABTS activity for the calculation of IC_{50}




Elaeocarpus lanceifolius
% Inhibition
Log doses (µg/ml)
Garcinia kydia
% Inhibition
Log dose (µg/ml)
Garcinia xanthochymus
% Inhibition
Log dose (µg/ml)
Mahonia nepaulensis
% Inhibition
Log dose (µg/ml)
Morus nigra
% Inhibition
Log doses (µg/ml)
Myrica esculenta
% Inhibition
Log dose (µg/ml)
Prunus jenkinsii
% Inhibition
Log dose (µg/ml)


Prunus undulata
% Inhibition
Log dose (µg/ml)
Rubus treutleri
% Inhibition
Log dose (µg/ml)
Spondias pinnata
% Inhibition
Log doses (µg/ml)
Syzygium cumini
% Inhibition
Log dose (µg/ml)
Tamirindus indica
% Inhibition
Log dose (µg/ml)
Terminalia chebula
% Inhibition
Log doses (µg/ml)
Ziziphus mauritiana
% Inhibition
Log dose (µg/ml)

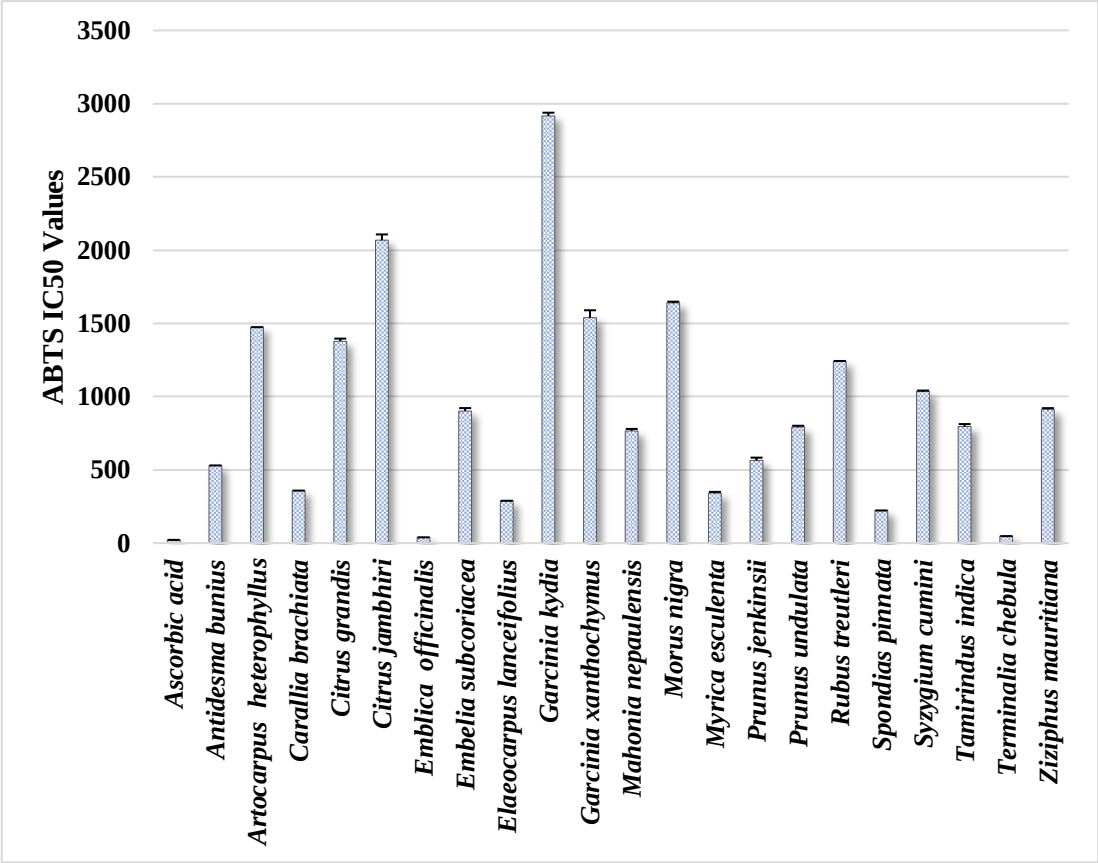


Figure 4.72 IC_{50} Values for ABTS activity of the studied underutilised fruits

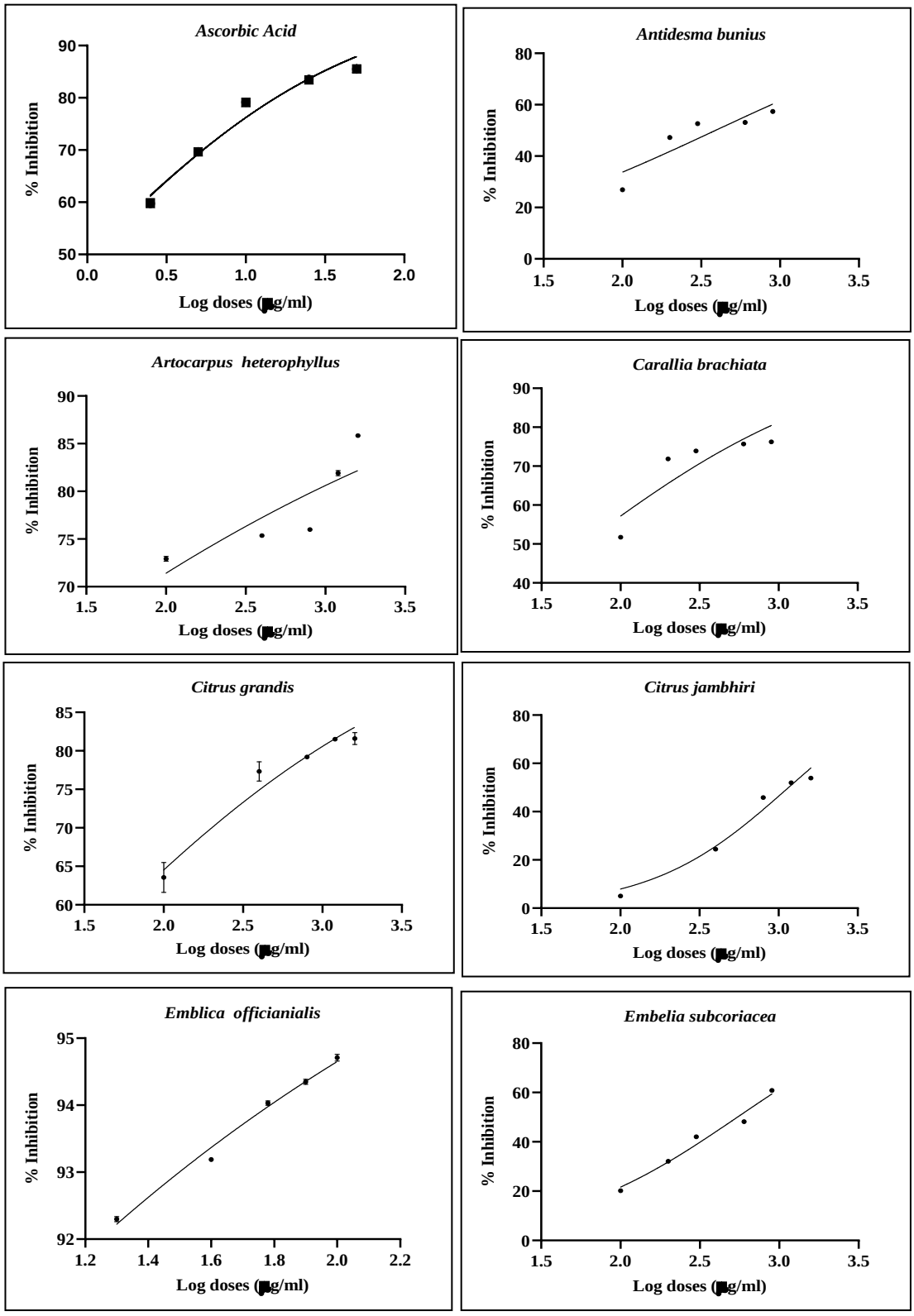
4.4.2.3 Superoxide anion radical scavenging activity

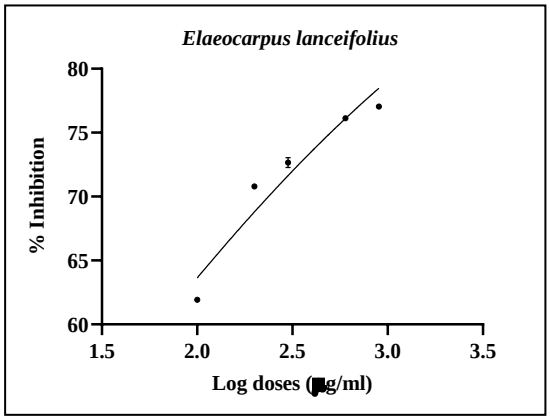
Antioxidants possess the ability to neutralise superoxide radicals, thereby safeguarding cells from their detrimental impacts. Different methanolic fruit extracts demonstrated a dose-dependent enhancement in the inhibition of superoxide radical ($O2^{\bullet-}$) production. The superoxide radical ($O_2^{\bullet-}$) constitutes a reactive oxygen species (ROS) produced through multiple biological mechanisms, notably electron leakage from the mitochondrial electron transport chain. Figure 4.73 illustrates the relationship between the logarithmic doses of different fruit extracts and standard ASA in relation to $O2^{\bullet-}$ scavenging activity, facilitating the determination of IC_{50} values. The superoxide anion radical scavenging method yielded an inhibitory concentration (IC_{50}) ranging from 0.65 ± 0.23 µg/mL to 1501.67 ± 2.01 µg/mL (Fig 4.74). The mean scavenging activity of $O2^{\bullet-}$ was measured at 282.27 µg/mL across the fruit extracts. The highest antioxidant activity, indicated by the lowest IC_{50} values, was recorded in *Terminalia chebula* (0.65 ± 0.23 µg/mL), which is statistically comparable to *Emblica officinalis* (0.92 ± 0.07 µg/mL), *Spondias pinnata* (1.02 ± 0.03 µg/mL), *Rubus treutleri* (1.09 ± 0.06 µg/mL), *Prunus jenkinsii* (1.30 ± 0.05 µg/mL), *Artocarpus heterophyllus* (1.64 ± 0.57 µg/mL), *Prunus undulata* (2.52 ± 0.02 µg/mL), and *Syzygium cumini* (3.21 ± 0.06 µg/mL). In contrast, *Garcinia xanthochymus* exhibited the lowest antioxidant activity, with the highest IC_{50} value recorded at 1501.67 ± 2.01 µg/mL. *Tamarindus indica* and *Citrus jambhiri* demonstrated lower antioxidant capacities, with IC_{50} values of 1240.33 ± 2.50 µg/mL and 1156.00 ± 1.54 µg/mL, respectively (Table 4.26).

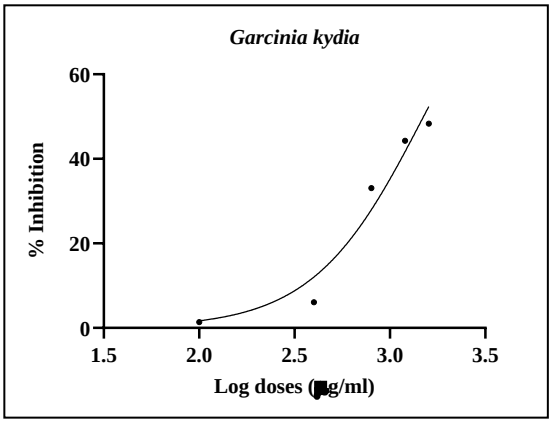
The superoxide anion radical ($O2^{\bullet-}$) is a highly reactive free radical produced through the reduction of molecular oxygen in aqueous environments, which can result in cellular damage by indirectly initiating lipid oxidation (Aruoma and Halliwell, 1987). Radicals are also produced in aerobic cells as a result of electron leakage from the electron transport chain. Activated phagocytes, including macrophages, monocytes, eosinophils, and neutrophils, generate superoxide radicals that serve as a defensive mechanism to eliminate pathogens through phagocytosis. The superoxide radical can decompose to generate more potent reactive oxygen

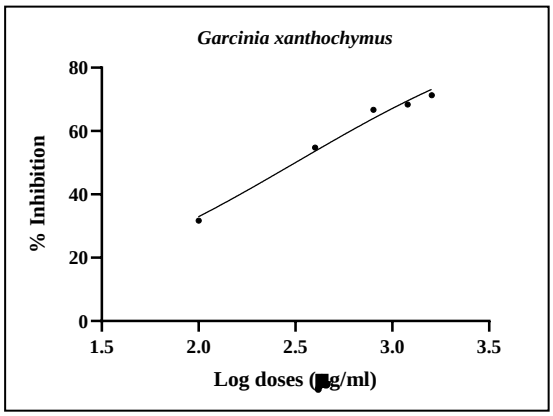
species (ROS), such as hydroxyl radicals and singlet oxygen. In the presence of metals, the superoxide anion generates hydrogen peroxide (H_2O_2), which subsequently leads to the formation of hydroxyl free radicals (Halliwell, 1995; Lushchak, 2014). Consequently, the scavenging of $O2^{\bullet-}$ may inhibit the generation of other reactive oxygen species and safeguard cells against oxidative damage. The current investigation indicates that the $O2^{\bullet-}$ radical scavenging activity of *Terminalia chebula* and *Emblica officinalis* surpasses that of the standard ASA. In contrast, *Spondias pinnata, Rubus treutleri, Prunus jenkinsii, Artocarpus heterophyllus, Prunus undulata,* and *Syzygium cumini* exhibit superior scavenging potential among the fruits analysed. The scavenging activity of $O2^{\bullet-}$ by the fruit extracts may be ascribed to their flavonoid content (Robak and Gryglewski, 1988).

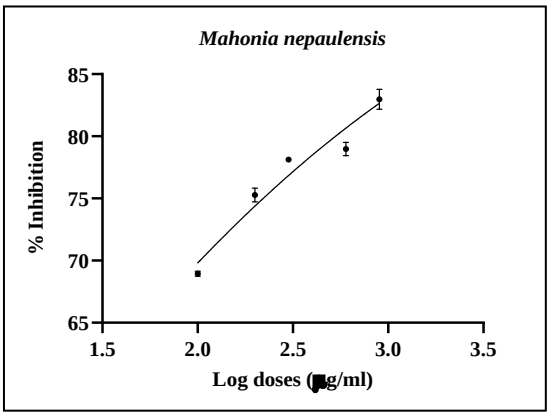
Fig. 4.73 Plots of log doses of methanolic extracts of different fruits against mean inhibition (%) of Superoxide anion radical activity for the calculation of IC_{50}

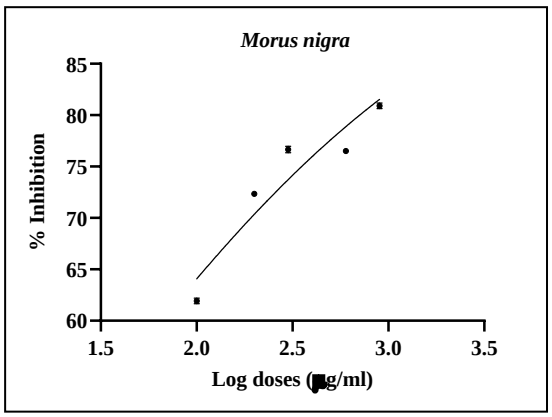


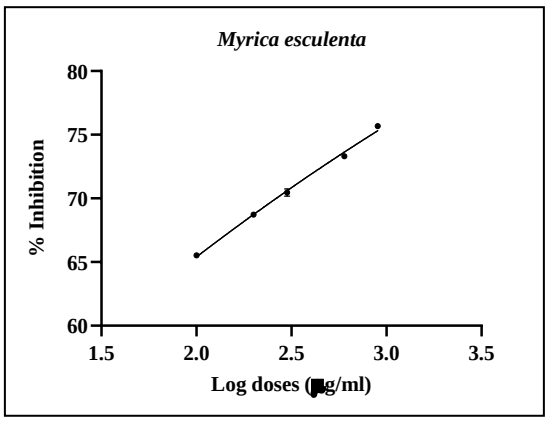

Elaeocarpus lanceifolius
% Inhibition
80
75
70
65
60
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)

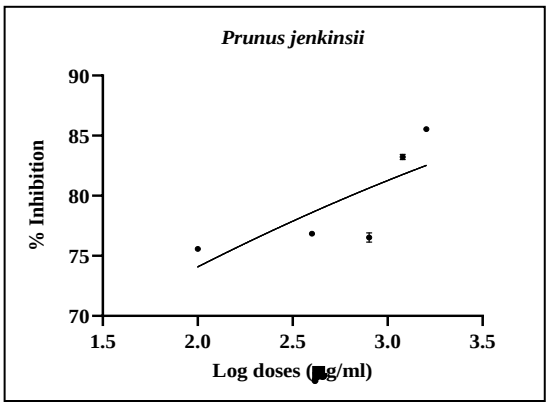

Garcinia kydia
% Inhibition
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)

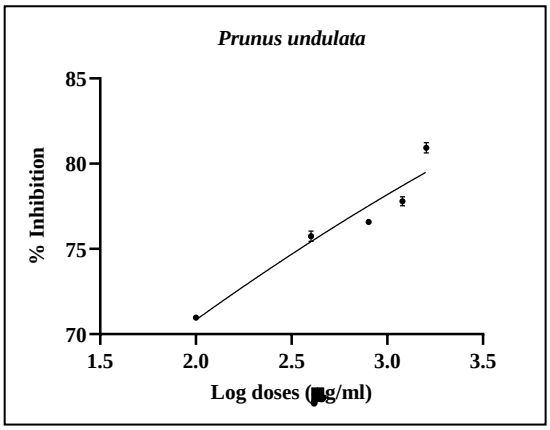

Garcinia xanthochymus
% Inhibition
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)

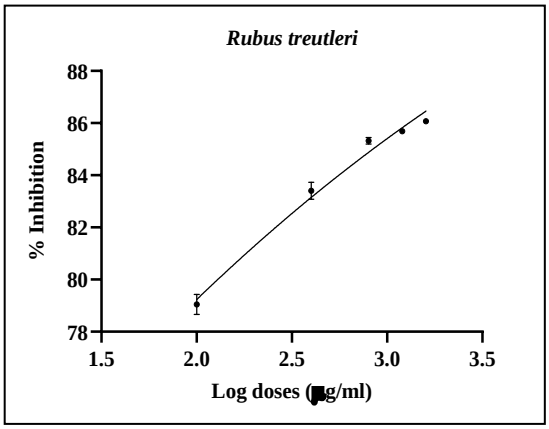

Mahonia nepaulensis
% Inhibition
85
80
75
70
65
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)

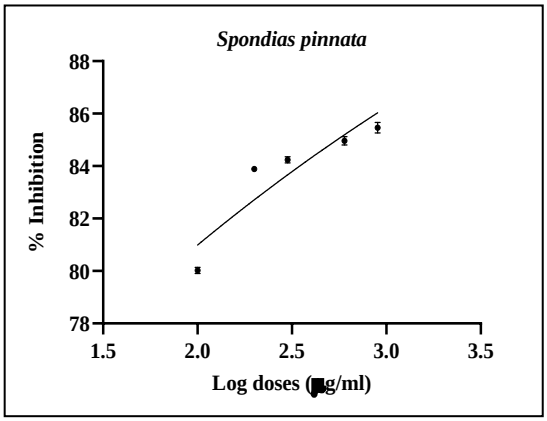

Morus nigra
% Inhibition
85
80
75
70
65
60
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)

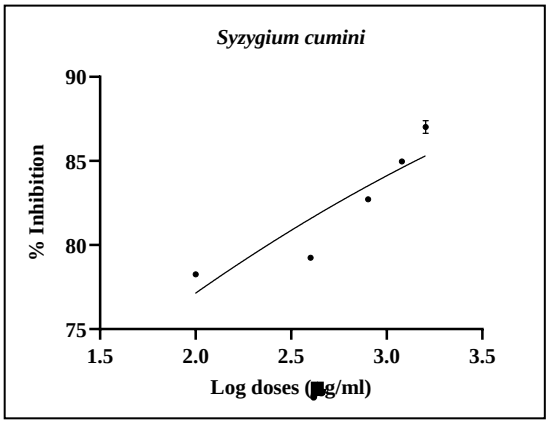

Myrica esculenta
% Inhibition
80
75
70
65
60
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)

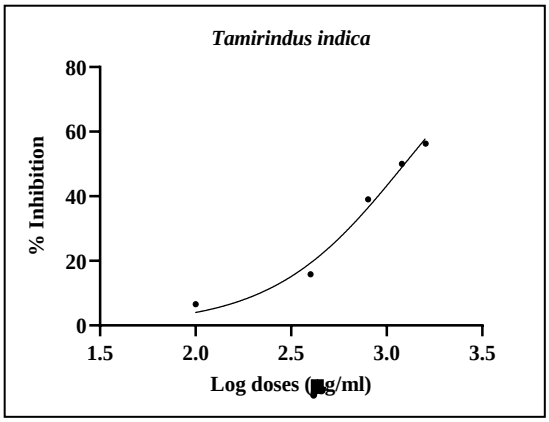

Prunus jenkinsii
% Inhibition
90
85
80
75
70
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)

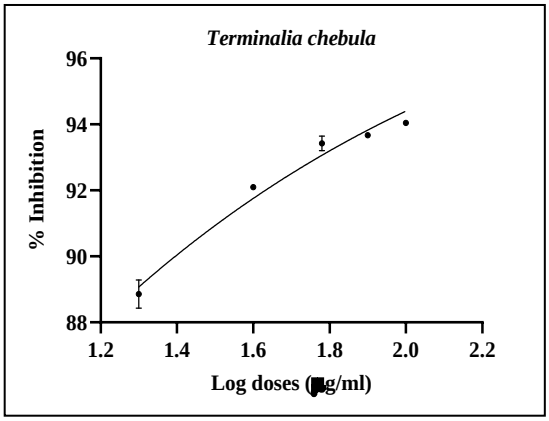

Prunus undulata
% Inhibition
85
80
75
70
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)

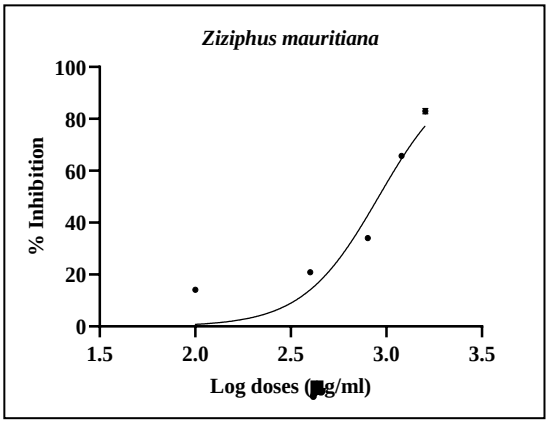

Rubus treutleri
% Inhibition
88
86
84
82
80
78
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)


Spondias pinnata
% Inhibition
88
86
84
82
80
78
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)


Syzygium cumini
% Inhibition
90
85
80
75
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)


Tamirindus indica
% Inhibition
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)


Terminalia chebula
% Inhibition
96
94
92
90
88
1.2
1.4
1.6
1.8
2.0
2.2
Log doses (µg/ml)


Ziziphus mauritiana
% Inhibition
100
80
60
40
20
0
1.5
2.0
2.5
3.0
3.5
Log doses (µg/ml)

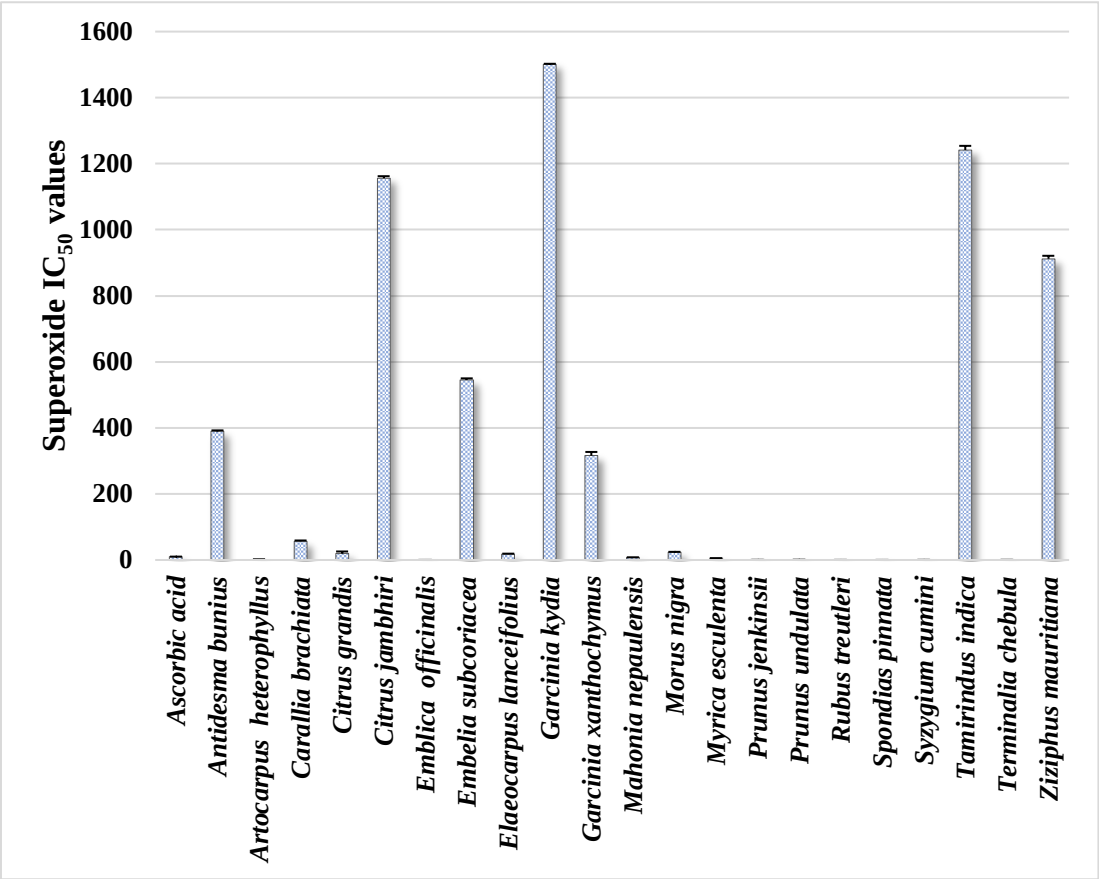


Fig. 4.74 IC_{50} Values for Superoxide anion radical scavenging activity of the studied underutilised fruits

4.4.2.4 Ferric-reducing antioxidant power (FRAP) assay

The Ferric-reducing antioxidant power (FRAP) assay evaluates the ability of antioxidants to convert the TPTZ-Fe (III) complex into the TPTZ-Fe (II) complex, resulting in the formation of a potent blue Fe2+-TPTZ complex. The IC_{50} values were derived from the Trolox standard graph (Figure 4.75). The FRAP antioxidant activity method yielded an inhibitory concentration (IC_{50}) ranging from 0.51 ± 0.08 µg/mL to 35.44 ± 7.16 µg/mL The mean FRAP-reducing activity observed among the fruit extracts was 9.15 µg/mL (Figure 4.76). The maximum reducing activity of fruit extracts was assessed through the measurement of the conversion of Fe3+ to Fe2+. It was found that *Emblica officinalis* exhibited the lowest IC_{50} values (0.51 ± 0.08 µg/mL), which were statistically comparable to those of *Terminalia chebula* (0.62 ± 0.08 µg/mL), *Rubus treutleri* (0.69 ± 0.05 µg/mL), *Prunus undulata* (1.37 ± 0.45 µg/mL), *Garcinia kydia* (1.65 ± 0.08 µg/mL), and Garcinia xanthochymus (4.07 ± 0.39 µg/mL). The lowest FRAP-reducing activity was recorded in *Embelia subcoriacea* (42.76 ± 0.63 µg/mL), which exhibited the highest IC_{50} values. Additionally, *Elaeocarpus lanceifolius* (31.23 ± 5.62 µg/mL) and *Morus nigra* (35.44 ± 7.16 µg/mL) demonstrated comparatively lower FRAP reducing activity (Table 4.26).

Several studies have indicated that the reduction power is linked to the presence of antioxidants, which donate hydrogen atoms to free radicals (Fejes *et al*., 2000). The correlation between reducing power and antioxidant activity suggests that the former may serve as a significant indicator of the latter (Oktay *et al*., 2003). Compounds exhibiting reducing power act as electron donors capable of reducing the

oxidised by products generated during lipid peroxidation processes, thereby serving as both primary and secondary antioxidants (Yen and Chen, 1995). The reduction of Fe (III) serves as an indicator of electron-donating activity, a fundamental mechanism underlying the action of phenolic antioxidants (Nabavi *et al*., 2009). Consequently, the reducing capacity of compounds functions as a marker for their antioxidant properties (Meir *et al*., 1995). The increased absorbance observed in the studied underutilised fruits at elevated concentrations indicates a significant reducing power potential of the extracts.

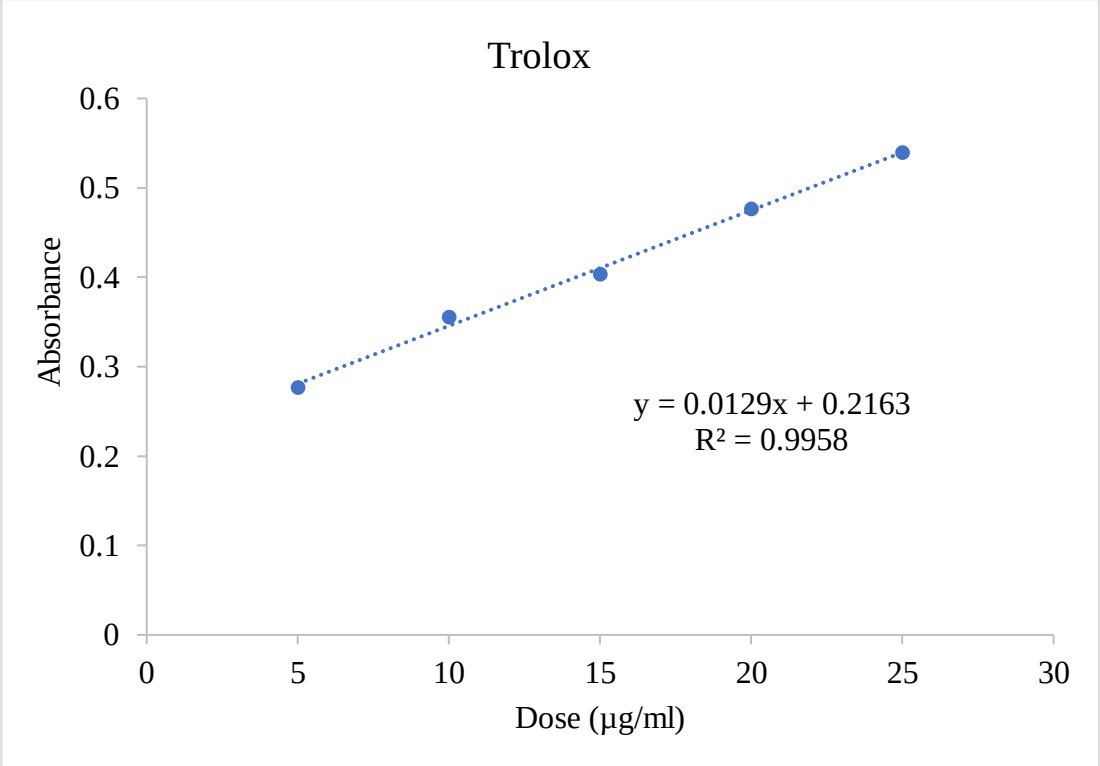


Fig. 4.75 Trolox standard curve for the calculation of IC_{50} values for FRAP

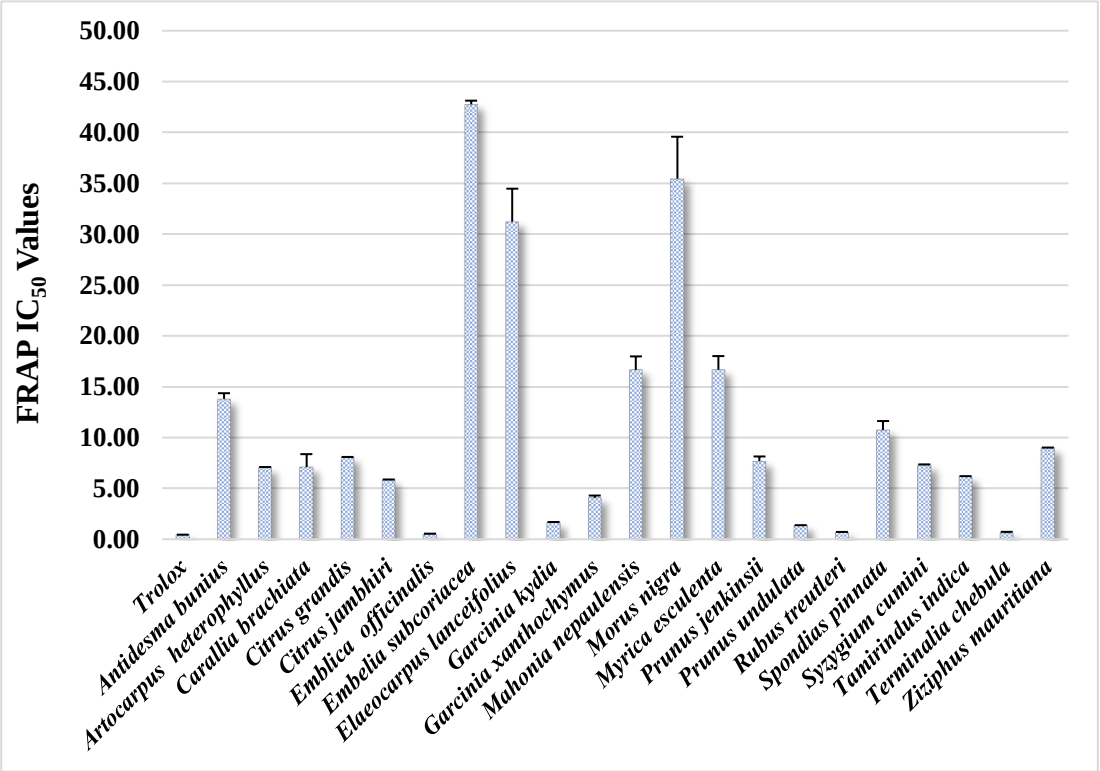


Fig. 4.76 IC_{50} Values for FRAP antioxidant activity of the studied underutilised fruits

Table 4.26 IC_{50} values for the different antioxidant activity of the studied underutilised fruits

Botanical Name	DPPH IC_{50} (µg/ml)	ABTS IC_{50} (µg/ml)	Superoxide IC_{50} (µg/ml)	FRAP IC_{50} (µg/ml)
Ascorbic acid	6.12 ± 0.30^{a}	21.75 ± 0.21^{a}	1.02 ± 0.01^{a}	-
Trolox	-	-		0.41 ± 0.08^{a}
Antidesma bunius	185.67 ± 7.95^{de}	529.10 ± 1.70^{e}	389.47 ± 5.25^{f}	13.80 ± 0.96^{ef}
Artocarpus heterophyllus	282.60 ± 3.90^{fg}	1473.00 ± 2.00^{k}	1.64 ± 0.57^{a}	7.05 ± 0.55^{bcd}
Carallia brachiata	44.16 ± 1.74^{ab}	357.77 ± 1.05^{d}	56.91 ± 3.63^{d}	7.10 ± 2.19^{bcd}
Citrus grandis	358.53 ± 9.60^{gh}	1379.33 ± 2.50^{j}	20.13 ± 2.03^{bc}	8.02 ± 0.12^{bcd}
Citrus jambhiri	1217.00 ± 7.59^{l}	2066.00 ± 6.51^{n}	1156.00 ± 1.54^{i}	5.85 ± 0.74^{bc}
Emblica officinalis	3.29 ± 0.05^{a}	38.33 ± 1.01^{a}	0.92 ± 0.07^{a}	0.51 ± 0.08^{a}
Embelia subcoriacea	15.39 ± 3.45^{a}	902.37 ± 3.94^{g}	545.40 ± 8.15^{g}	42.76 ± 0.63^{i}
Elaeocarpus lanceifolius	89.48 ± 1.86^{abc}	286.87 ± 3.06^{c}	18.76 ± 0.69^{bc}	31.23 ± 5.62^{g}
Garcinia kydia	613.43 ± 3.20^{i}	1538.67 ± 3.00^{l}	315.63 ± 2.65^{e}	1.65 ± 0.08^{a}
Garcinia xanthochymus	825.60 ± 0.95^{j}	2921.67 ± 8.09^{o}	1501.67 ± 2.01^{k}	4.07 ± 0.39^{ab}
Mahonia nepaulensis	109.87 ± 3.35^{bcd}	765.83 ± 2.65^{f}	7.85 ± 0.22^{ab}	16.65 ± 2.30^{f}
Morus nigra	170.10 ± 7.56^{cde}	1639.00 ± 7.35^{m}	24.62 ± 0.09^{c}	35.44 ± 7.16^{h}
Myrica esculenta	158.03 ± 2.40^{cde}	340.73 ± 6.01^{d}	5.36 ± 0.14^{ab}	16.66 ± 2.33^{f}
Prunus jenkinsii	209.07 ± 5.80^{ef}	563.40 ± 5.45^{e}	1.30 ± 0.05^{a}	7.69 ± 0.78^{bcd}
Prunus undulata	316.40 ± 10.6^{5g}	793.30 ± 3.10^{f}	2.52 ± 0.02^{a}	1.37 ± 0.45^{a}
Rubus treutleri	420.50 ± 19.65^{h}	1239.67 ± 5.51^{i}	1.09 ± 0.06^{a}	0.69 ± 0.05^{a}
Spondias pinnata	32.15 ± 1.49^{ab}	222.20 ± 0.40^{b}	1.02 ± 0.03^{a}	10.73 ± 1.54^{de}
Syzygium cumini	418.80 ± 7.90^{h}	1036.00 ± 9.00^{h}	3.21 ± 0.06^{a}	7.30 ± 2.52^{cd}
Tamirindus indica	1138.00 ± 7.55^{k}	796.53 ± 2.76^{f}	1240.33 ± 2.50^{j}	6.13 ± 1.07^{bc}
Terminalia chebula	10.53 ± 0.24^{a}	47.16 ± 0.33^{a}	0.65 ± 0.23^{a}	0.62 ± 0.08^{a}
Ziziphus mauritiana	360.10 ± 9.05^{gh}	912.17 ± 5.85^{g}	912.17 ± 5.85^{h}	8.98 ± 0.57^{cd}

4.4.3 Correlation between antioxidant activity along with the phenol and flavonoid composition of the underutilised fruits.

In our present investigation as presented in Table 4.27 correlations between the antioxidant activity with phenol and flavonoid were identified by examining linear regression correlations, as phenol and flavonoid are one of the major contributors to the antioxidant activity of fruits. The antioxidant activity estimated by DPPH scavenging activity exhibited high positive correlations with phenol (r = 0.73) followed by flavonoid (r = 0.38). The ABTS radical scavenging activity showed positive correlations with both phenol (r = 0.43) and flavonoid (r = 0.26) showing a higher correlation with phenol. The present investigation showed a high positive correlation of superoxide anion radical scavenging activity with phenol (r = 0.76) followed by flavonoid (r = 0.48) while FRAP estimation showed positive correlations with both phenol (r = 0.10) and flavonoid (r = 0.17) having higher correlations with flavonoid.

Table 4.27 Correlation between the antioxidant activity with phenol and flavonoid

	DPPH	***ABTS***	***Superoxide***	***FRAP***	***Phenol***	***Flavonoid***
DPPH	1					
ABTS	**0.66****	1				
Superoxide	**0.77****	**.59****	1			
FRAP	**-0.29***	-0.003	-0.051	1		
Phenol	**0.73****	**0.43****	**0.76****	**0.10****	1	
Flavanoid	**0.38****	**0.26***	**0.48****	**0.17****	**0.70****	1

***. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*

4.4.4. GCMS analysis for the identification of major compounds present in the studied fruits.

Gas Chromatography–Mass Spectrometry (GC–MS) represents a sophisticated analytical approach that integrates two fundamental methodologies. A sample is vaporised and subsequently injected into a gas chromatograph in gas chromatography (GC). The components of the sample are segregated according to their volatility and interaction with the stationary phase within a capillary column. Mass spectrometry identifies and quantifies compounds through the measurement of their mass-to-charge ratio. The process involves the ionisation of the isolated compounds followed by the analysis of their mass spectra.

The present study identified several significant compounds from the methanolic extract of 21 underutilised fruits, which are illustrated alongside their corresponding peaks. These compounds may demonstrate additional significance in elucidating their properties and potential applications across various disciplines.

4.4.4.1 *Antidesma bunius*

From the GC-MS analysis of *Antidesma bunius in* our present investigation, 12 major compounds were isolated (Table 4.28) and represented by their retention time (R.T) (Figure 4.77) and out of which the compounds which had high and larger peak area were identified and included Orotyl amide (R.T= 9.754), 1-methyl-2-oxocyclohex-3-enecarboxylic acid, methyl ester (R.T= 9.843), Thiophene (R.T= 15.023), Thiophene-2-acetic acid (R.T= 15.123) and 2-pyrazoline (R.T= 22.782).

4.4.4.2 *Artocarpus heterophyllus*

As depicted in Table 4.29, from the GC-MS analysis of *Artocarpus heterophyllus* 17 compounds were isolated and represented by their retention time (R.T) out of which the major compounds detected based on their peaks were Oxazole (R.T= 5.299), Benzenepropanamide (R.T= 6.505), Chromium (R.T= 6.690), Perylene (R.T= 9.0.86), 3,5-pyridinedicarboxylic acid (R.T= 9.426) and 1-hepten-3-one (R.T= 22.834) (Figure 4.78).

4.4.4.3 *Carallia brachiata*

From the underutilised fruit *Carallia brachiata,* 12 compounds were isolated (Table 4.30) in the GC-MS analysis out of which the compound Tetrahydrocyclopenta [1,3] Dioxin-4-One (R.T= 9.851), 2-Cyclohexen-1-One (R.T= 9.951), Succinic Acid, (R.T= 10.006), 2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One- (R.T= 10.034), 6-Azabicyclo[3,2,1] Octane (R.T= 22.543), Methyl P-Coumarate (R.T= 24.092) and Propofol (R.T= 28.919) were found to obtain higher peaks and areas when compared to the other isolated compounds (Figure 4.79).

4.4.4.4 *Citrus grandis*

The results represented in Table 4.31 highlighted that 12 major compounds were isolated from the underutilised fruit *Citrus grandis* out of which the compounds with higher peaks and areas were 1,3-Dioxolane (R.T= 9.875), Thiophene (R.T= 15.013), 5-Hydroxymethylfurfural (R.T= 15.198) and 4-Undecene, 6-Methyl- (R.T= 22.601) (Figure 4.80).

4.4.4.5 *Citrus jambhiri*

As depicted in table 4.32 the GC-MS analysis of Citrus jambhiri reported 5 major compounds out of which the compounds having higher peaks and areas were Tetrahydrocyclopenta[1,3]Dioxin-4-One (R.T=9.716), 2,5-Di(Trifluoromethyl)Benzoic Acid (R.T= 17.134), 2-Propylphenyl Ester and 4-Methyl-2,4-Bis(P-Hydroxyphenyl)Pent-1-ene (R.T = 24.342) (Figure 4.81).

4.4.4.6 *Emblica officinalis*

From the GC-MS analysis of *Emblica officinalis in* our present investigation, 12 major compounds were isolated (Table 4.33) and represented by their respective retention time (R.T) (Figure 4.70) and out of which the compounds which had high and larger peak areas were identified and this compound included 3,5-Dimethylphentlsilyloxybenzene (R.T=17.134) and Hexamethylene Diacrylate (R.T= 22.701) (Figure 4.82).

4.4.4.7 *Embelia subcoriacea*

The results represented in Table 4.34 highlighted that 13 major compounds were isolated from the underutilised fruit *Embelia subcoriacea* out of which the compounds with higher peaks and areas were Tetrahydrocyclopenta[1,3]Dioxin-4-One (R.T= 9.875) and N-Heptyl Acrylate (R.T= 15.013) (Figure 4.83).

4.4.4.8 *Elaeocarpus lanceifolius*

From the underutilised fruit *Elaeocarpus heterophyllus*, 19 compounds were isolated (Table 4.35) in the GC-MS analysis out of which the compound Methyl P-

coumarate (R.T= 3.013), Benzothiophene-3-Carboxamide (R.T= 5.434), Succinic Acid (R.T= 9.821), 2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One- (R.T=10.431), Thymine (R.T= 12.880), 1-Methyl-5-Fluorouracil (R.T= 13,583), Thiophene, 2-Propyl- (R.T= 15.02), Trans-2.4-Dimethylthiane-S,S-Dioxide (R.T= 22.541) and Phthalic Acid (R.T= 24.077) were found to obtain higher peaks and areas when compared to the other isolated compounds (Figure 4.84).

4.4.4.9 *Garcinia Kydia*

As depicted from our present investigation in Table 4.36, from the GC-MS analysis of *Garcinia kydia* 14 compounds were isolated and represented by their retention time (R.T) out of which the major compounds detected based on their peaks were Orotylamide (R.T= 9.723), 1,3-Benzenediol, O-Acryloyl-O'-Dichloroacetyl- (R.T=13.008), 2-Butenedioic Acid (Z)-Dimethyl Ester (R.T= 14.354), Dodecyl Acrylate (R.T= 22.532) and 2,2,2-Triflouroethyl Acrylate (R.T= 24.192) (Figure 4.85).

4.4.4.10 *Garcinia xanthochymus*

The findings in our present study as represented in Table 4.37, 7 compounds were isolated from *Garcinia xanthochymus* out of which the compounds with higher peaks and areas were (+)-3,4-Dehydroproline Amide (R = 9.771), Succinic Acid, 3Methylbut-3-Enyl Propyl Ester (R = 10.421), Thiophene, 2-Propyl- (R = 15.034) and Trans-2,4-Dimethylthiane, S, S-Dioxide (R = 22.552) (Figure 4.86).

4.4.4.11 *Mahonia nepaulensis*

From the GC-MS analysis of *Mahonia nepaulensis in* our present investigation, 14 major compounds were isolated (Table 4.38) and represented by their respective retention time (R.T) (Figure 4.87) and out of which the compounds which had high and larger peak areas were identified and this compound included N-(4-Methylcyclohexyl)Acetamide, Cis- (R.T=3.013), Oxazole,4,4-Dimethyl-2-(2-Methylselanylphenyl)-4,5-Dihydro- (R.T= 5.384), 1,3-Dioxolane, 2-Cyclohexyl-4,5-Dimethyl- (R.T=10.416), Molinate (R.T = 12.848), 1,3-Dioxolane,4-Ethyl-2-Pentadecyl- (R.T=13.563), Thiophene,2-Propyl- (R.T=15.033) and 4-Undecene, 6-Methyl- (R.T=22.632).

4.4.4.12 *Morus nigra*

From the underutilised fruit *Elaeocarpus heterophyllus*, 19 compounds were isolated (Table 4.39) in the GC-MS analysis out of which the compound 2-Propenoic Acid, Oxiranylmethyl Ester (R.T= 5.259), Benzene, 1,3-Dimethyl- (R.T= 7.715), D-Alanine, N-Propargloxycarbonyl-,Decyl Ester (R.T=12.876) and Trans-2,4-Dimethylthiane, S,S-Dioxide (R.T=22.543), were found to obtain higher peaks and areas when compared to the other isolated compounds (Figure 4.88).

4.4.4.13 *Myrica esculenta*

As depicted in Table 4.40, from the GC-MS analysis of *Myrica esculenta* 17 compounds were isolated and represented by their retention time (R.T) out of which the major compounds detected based on their peaks were 4-Methylpiperidine-1-Carboxylic Acid, Phenyl Ester (R.T= 12.937), N-Decanoic Acid (R.T= 24.112),

Dodecanoic Acid (R.T= 24.142), Ethanol, 2-(9-Octadecenyloxy)-, (E) (R.T= 25.052) and 33-Octanol, 3,7-Dimethyl- (R.T= 26.373) (Figure 4.89).

4.4.4.14 *Prunus jenkinsii*

The results represented in Table 4.41 highlighted that 16 major compounds were isolated from the underutilised fruit *Prunus jenkinsii* out of which the compounds with higher peaks and areas were 3-Methyl-2-(2-Oxopropyl) Furan (R.T= 3.041), 2,4,5-Trichlorophenyl Propenoate (R.T= 5.434), 1,3-Dioxolane,2-Cyclohexyl-4,5-Dimethyl- (R.T= 10.436), Thymine (R.T= 12.928), 1,3-Dioxolane,4-Ethyl-2-Pentadecyl- (R.T= 13.643), Thiophene,2-Propyl- (R.T= 15.083) and 1-Hepten-3-One (R.T= 22.676) (Figure 4.90).

4.4.4.15 *Prunus undulata*

The findings in our present study as represented in Table 4.42, Seventeen (17) compounds were isolated from *Prunus undulata* out of which the compounds with higher peaks and areas were Isobutyl Acrylate (R = 9.854), 1,3-Dioxolane, 2-Cyclohexyl-4,5-Dimethyl- (R = 10.436), Thymine (R = 12.917), 1,3-Dioxepane, 2-Heptyl- (R = 13.589), Thiophene, 2-Propyl (R = 15.058), Trans-2,4-Dimethylthiane, S,S-Dioxide (R = 22.546), Phthalic Acid, Hexyl Heptyl Ester (R = 24.072) and Anthracene, 9-Ethyl-9,10-Dihydro-10-Trimethylsilyl- (R = 28.234) (Figure 4.91).

4.4.4.16 *Rubus treutleri*

The results from our present investigation represented in Table 4.43 highlighted that nineteen (19) major compounds were isolated from the underutilised fruit *Rubus treutleri* out of which the compounds with higher peaks and areas were

1,3-Dioxolane, 2-Cyclohexyl-4,5-Dimethyl- (R.T= 10.431), L-Alanine, N-Propargyloxycarbonyl-,Dodecyl Ester (R.T= 12.892), 2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One (R.T= 13.563), Thiophene, 2-Propyl- (R.T= 15.003), 5-Undecene, 6-Methyl- (R.T= 22.541) and Phthalic Acid, Butyl Ester, Ester with Butyl Glycolate (R.T= 24. 087) (Figure 4.92).

4.4.4.17 *Spondias pinnata*

The GC-MS analysis of Spondias pinnata in our present investigation isolated sixteen (16) major compounds (Table 4.44) and represented by their respective retention time (R.T) (Figure 4.93) and out of which the compounds which had high and larger peak areas were identified and this compound included Ethyl 3A,4,5,6A-Tetrahydrofuro[2,3-B] Furan-2-Carboxylate (R.T=12.998), Cyclooctyle Ethylphosphonofluoridoate (R.T= 14.258), Thiophene, 2-Propyl- (R.T=14.968), Isobutyl Acrylate (R.T = 22.641), AnthranilicAcid, N-(2-Carboxyphenylmethylene)- (R.T=24.487), Thiophene,2-Propyl- (R.T=15.033) and 4-Undecene, 6-Methyl- (R.T=22.632).

4.4.4.18 *Syzygium cumini*

As the results depicted in Table 4.45, *Syzygium cumini* highlighted seven (7) compounds which were isolated from the GC-MS analysis out of which the compound Maleic Anhydride (R.T=7. 908) and Tetrahydrocyclopenta [1,3]Dioxin-4-One (R.T=9.701) (Figure 4.94).

4.4.4.19 *Tamarindus indica*

From the present investigation, as represented in Table 4.46, there were fifteen (15) compounds isolated from *Tamarindus indica* in the GC-MS analysis out of which the compounds which were having higher peaks and areas were Thiophene, 2-Propyl- (R.T= 15.098), Trans-2,4-Dimethylthiane, S, S-Dioxide (R.T=22.547), Myo-Inositol, 4-C-Methyl- (R.T=22.882) and Benzeneacetic Acid, Alpha-(7-T-Butyldimethylsilyloxyheptanoyloxy)-, Methyl Ester (R.T=24.097) (Figure 4.95)

4.4.4.20 *Terminalia chebula*

Terminalia chebula highlighted ten (10) major compounds (Table 4.47) from the GC-MS analysis in the present study, out of which the compounds which were having higher peaks and areas were Bisphenol C (R.T=17.159), and 4,4'-Bi-4H-Pyran, 2,2',6,6'-Tetrakis(1.1-Dimethylethyl)-4,4'-Dimethyl- (R.T=24.842) (Figure 4.96).

4.4.4.21 *Ziziphus mauritiana*

The results represented in Table 4.48 highlighted that fifteen (15) major compounds were isolated from the underutilised fruit *Ziziphus mauritiana* out of which the compounds with higher peaks and areas were 32(3H)-Furanone, Dihydro-3-Methylene-5, 5-Diphenyl- (R.T= 9.836), 26-Azabicyclo[3,2,1]Octane (R.T= 9.865), Thymine (R.T= 12.882), 1,3-Dioxolane,4-Ethyl-2-Pentadecyl- (R.T= 13.583), Thiophene,2-Propyl- (R.T= 15.083), 1H-Pyrazole, 4,5-Dihydro-3-Methyl-1-Propyl- (R.T= 22.543), 1,2-Benzenedicarboxylic Acid, Dihexyl Ester (R.T= 24.082), and Heptasiloxane (R.T= 25.763) (Figure 4.97).

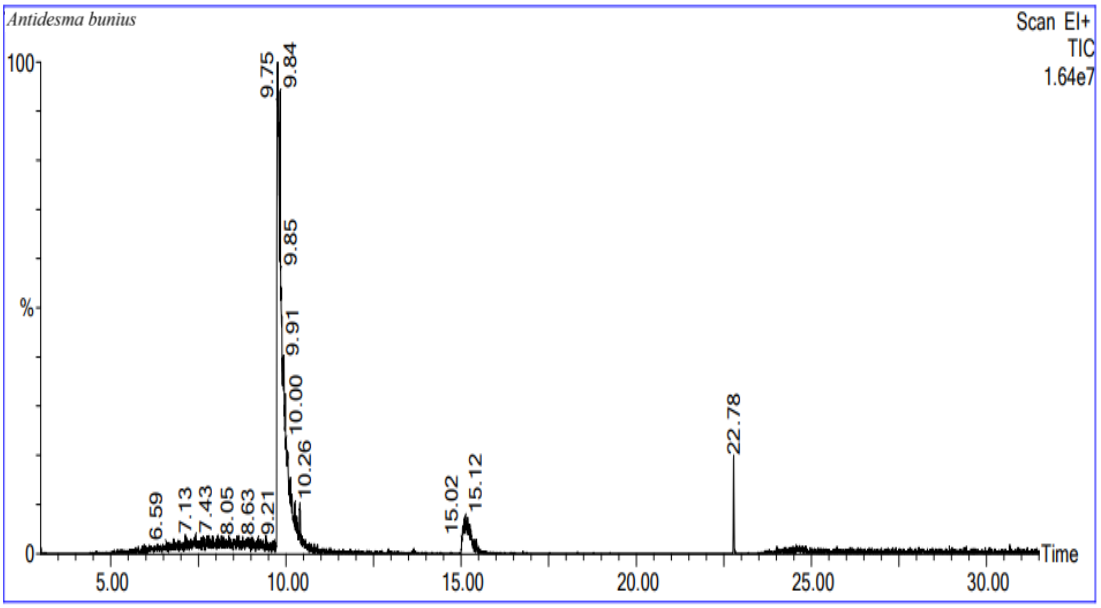


Fig. 4.77 GCMS chromatogram for *Antidesma bunius*

Table 4.28 Compounds identified using GCMS in *Antidesma bunius*

SL. No.	Retention Time (Minutes)	Compound Name	Molecular weight	Chemical Formula	Peak Area	Area %
1	7.750	3-ethoxy-6-(1-tetrahydropyranoxy-5-methyl-4-hexenyl)-2-cycloh	336	$C_{20}H_{32}O_4$	14,128.2	0.306
2	8.531	1-methyl-2, 5-dichloro-1,6-diazaphenalene	250	$C_{12}H_8N_2Cl_2$	16,182.2	0.350
3	9.754	Orotyl amide	155	$C_5H_5O_3N_3$	1,197,069.4	25.915
4	9.843	1-methyl-2-oxocyclohex-3-enecarboxylic acid, methyl ester	168	$C_9H_{12}O_3$	20,694.6	0.448
5	10.256	2(3h)-furanone, dihydro-3-methylene-5,5-diphenyl-	250	$C_{17}H_{14}O_2$	97,439.0	2.109
6	10.401	1,3-dioxolane, 4,5-dimethyl-2-pentadecyl-	312	$C_{20}H_{40}O_2$	161,468.8	3.496
7	15.023	Thiophene, 2-Propyl-	126	$C_7H_{10}S$	225,908.1	4.891
8	15.123	Thiophene-2-acetic acid, 2,3-dichlophenyl ester	286	$C_9H_{13}ON_5S$	1,047,560.6	22.678
9	15.163	Ethanol, 1-(2-methyl-2h-tetrazol-5-yll)-2-[(thiophen-2-ylmethyl)amino-]	239	$C_7H_{10}S$	18,530.2	0.401
10	15.253	Trophene, 2-propyl-	126	$C_{12}H_8O_2Cl_2S$	17,253.2	0.374
11	15.283	2-t-butyl-cyclopropanecarboxylic acid, 2,6-di-t-butyl-4-methyl-p	344	$C_7H_{14}N_2$	22,959.4	0.497
12	22.782	2-pyrazoline, 5-ethyl-1,4-dimethyl-	126	$C_{23}H_{36}O_2$	63,186.3	1.368

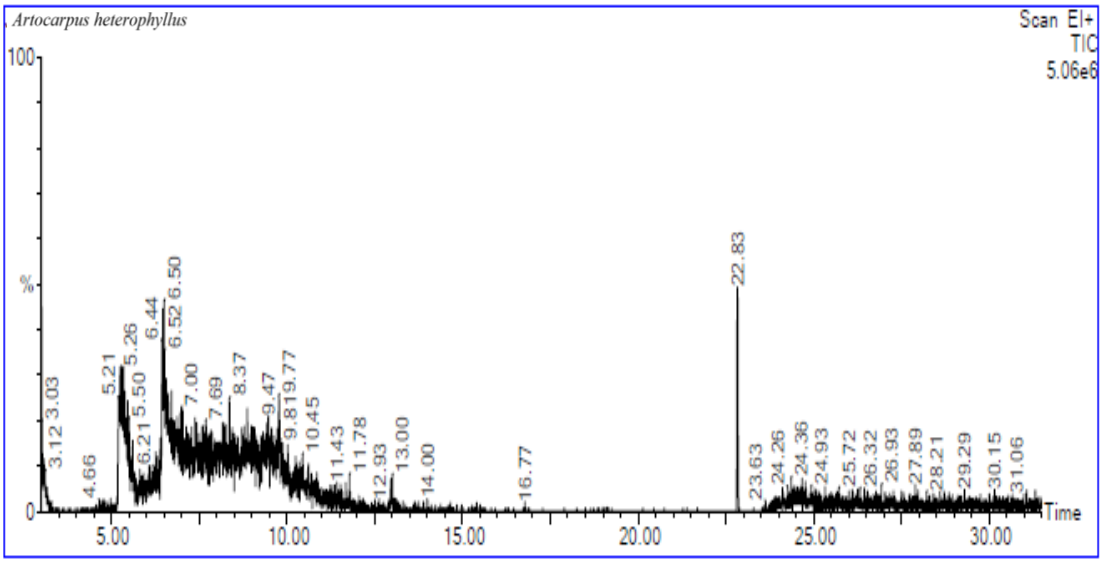


Fig. 4.78 GCMS chromatogram for *Artocarpus heterophyllus*

Table 4.29 Compounds identified using GCMS in *Artocarpus heterophyllus*

SL. No	Retention Time (Minutes)	Compound Name	Molecular weight	Chemical Formula	Peak Area	Area %
1	3.013	Butanoic Acid,4-Chloro-	122	$C_4H_7O_2CI$	48,681.6	1.673
2	5.259	2,4,5-Trichlorophenyl Propenoate	250	$C_9H_5O_2CI_3$	20,377.2	0.700
3	5.299	Oxazole, 4,4-Dimethyl-2-(2-Methylselanylphenyl)-4,5-Dihydro-	269	$C_{12}H_{15}ONSe$	97,959.0	3.367
4	5.349	Estra-4,9,11-Trien-3-One, 17-[[(Cyclohexylmethoxy)Carbolnyl]Oxy]-,	410	$C_{26}H_{34}O_4$	40,215.0	1.382
5	5.469	2,4,5-Trichlorophenyl Propenoate	250	$C_9H_5O_2CI_3$	51,113.8	1.757
6	5.504	Cinnamoylcymantrene	334	$C_{17}H_{11}O_4Mn$	23,104.1	0.794
7	6.475	1-Methyl-2,5-Dichloro-1,6-Diazaphenalene	250	$C_{12}H_8N_2CI_2$	27,117.5	0.932
8	6.505	Benzenepropanamide, Alpha-[[13-(Dimethylamino)Phenyl]Imino)-Bet	371	$C_{23}H_{21}O_2N_3$	96,649.2	3.322
9	6.565	5-Methyl-4'-Hydroxy-2-Benzylidene-Coumaran-3-One	252	$C_{16}H_{12}O_3$	28,301.4	0.973
10	6.625	3-(Gamma-Methylaminopropyl)-5-(4-Bromophenyl)-2-Methyl-2h-Pyr	307	$C_{14}H_{18}N_3Br$	24,207.3	0.832
11	6.690	Chromium,(.Eta 5-2,4-Cyclopentadien-1-Yl)[(1,2,3,4,5-.Eta.)-1,2,3,4,5-Pe	252	$C_{15}H_{20}Cr$	84,468.5	2.904
12	7.690	3-Ethoxy-6-(1-Tetrahydropyranoxy-5-Methyl-4-Hexenyl)-2-Cyclohexenone	336	$C_{20}H_{32}O_4$	24,943.9	0.857
13	9.086	Perylene	252	$C_{20}H_{12}$	66,482.9	2.285
14	9.426	3,5-Pyridinedicarboxylic Acid, 1,4-Dihydro-2,6-Dimethyl-4-Phenyl-	329	$C_{19}H_{23}O_4N$	69,453.9	2.387
15	9.866	5-Methyl-4'-Hydroxy-2-Benzylidene-Coumaran-3-One	252	$C_{16}H_{12}O_3$	22,740.1	0.782
16	9.886	Benzo[A]Pyrene	252	$C_{20}H_{12}$	22,356.2	0.768
17	22.834	1-Hepten-3-One	112	$C_7H_{12}O$	57,568.6	1.979

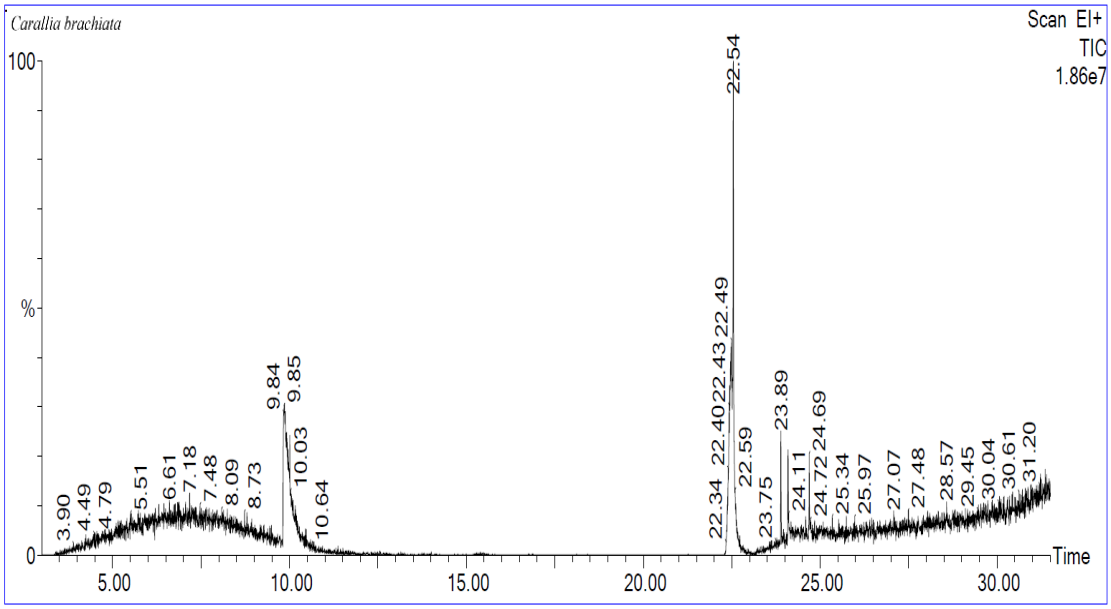


Fig. 4.79 GCMS chromatogram for *Carallia brachiata*

Table 4.30 Compounds identified using GCMS in *Carallia brachiata*

Sl. No	Retention Time (Minutes)	Compound Name	Molecular weight	Chemical Formula	Peak Area	Area %
1	3.013	3-Methyl-2-(2-Oxopropyl) Furan	138	$C_8H_{10}O_2$	76,719.1	0.687
2	9.851	Tetrahydrocyclopenta [1,3] Dioxin-4-One	142	$C_7H_{10}O_3$	675,039.6	6.045
3	9.906	Isobutyl 3-Methylbut-3-ENYL Carbonate	186	$C_{10}H_{18}O_3$	83,968.9	0.752
4	9.951	2-Cyclohexen-1-One	96	C_6H_8O	84,723.2	0.759
5	10.006	Succinic Acid,	282	$C_{12}H_{17}O_4$	749,969.1	6.716
6	10.034	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One-	144	$C_6H_8O_4$	756,039.8	6.770
7	22.543	6-Azabicyclo[3,2,1] Octane	111	$C_7H_{13}N$	125,918.8	1.128
8	22.593	N-Heptyl Acrylate	170	$C_{10}H_{18}O_2$	74,490.6	0.667
9	24.092	Methyl P-Coumarate	250	$C_{13}H_{18}O_3Si$	179,759.4	1.610
10	27.299	Pyrimidine	250	$C_6H_7N_2BrS_2$	73,602.0	0.659
11	28.919	Propofol	250	$C_{15}H_{26}OSi$	84,424.4	0.756
12	29.645	Methyl M-Hydroxycinnamate	250	$C_{13}H_{18}O_3Si$	76,313.3	0.683

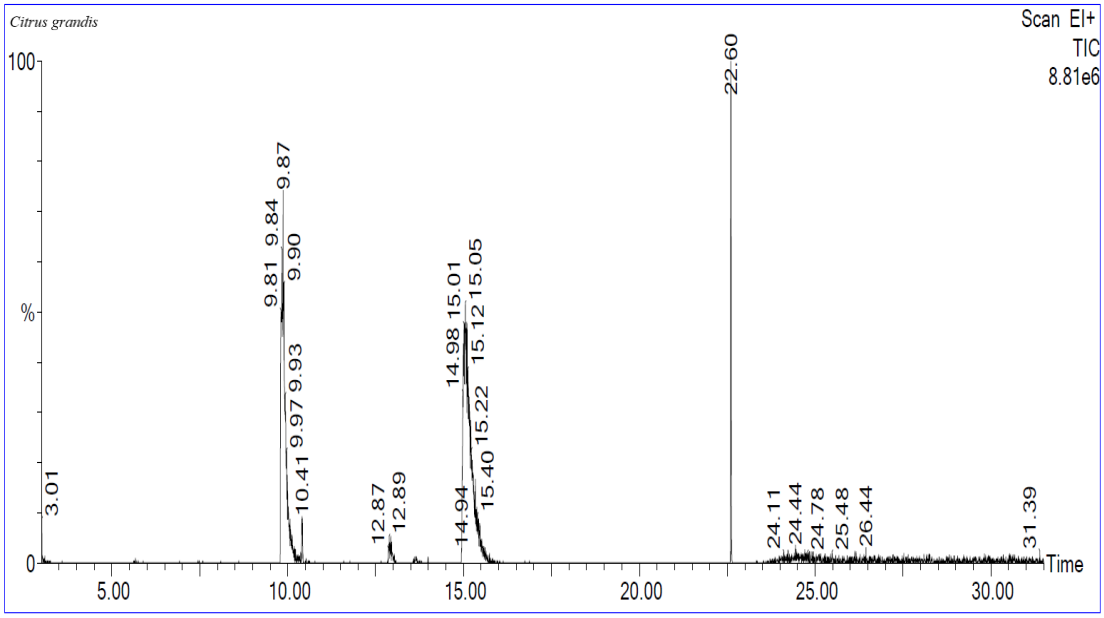


Fig. 4.80 GCMS chromatogram for *Citrus grandis*

Table 4.31 Compounds identified using GCMS in *Citrus grandis*

Sl. No	Retention Time (Minutes)	Compound Name	Molecular weight	Chemical Formula	Area	Area %
1	9.875	1,3-Dioxolane	184	$C_{11}H_{20}O_2$	528,384.4	23.082
2	12.887	Thymine	126	$C_5H_6O_2N_2$	131,477.8	5.743
3	12.932	7-Chloro-4-[P-[1-Dimethylaminocyclohexylmethyl]Anilino]Quinidin	393	$C_{24}H_{28}N_3Cl$	19,157.3	0.837
4	12.957	Prolintane	217	$C_5H_6O_2N_2$	13,889.6	0.607
5	15.013	Thiophene	126	$C_7H_{10}S$	22,319.7	0.975
6	15.053	Propennitrile	257	$C_{10}H_{11}O_3NS_2$	11,340.6	0.495
7	15.198	5-Hydroxymethylfurfural	126	$C_7H_{10}S$	229,091.1	10.007
8	15.318	1H-Pyrazole, 4,5-Dihydro-3-Methyl-1-Propyl-	126	$C_7H_{14}N_2$	8,212.7	0.359
10	15.428	Furazan-3-Carboxylic acid	267	$C_{10}H_{13}O_2N_5S$	187,593.7	8.195
11	22.601	4-Undecene, 6-Methyl-	168	$C_{12}H_{24}$	455,721.2	19.907
12	24.567	Silicic Acid, Diethyl Bis(Trimethylsilyl)Ester	296	$C_{10}H_{28}O_4Si_3$	172,178.3	7.521

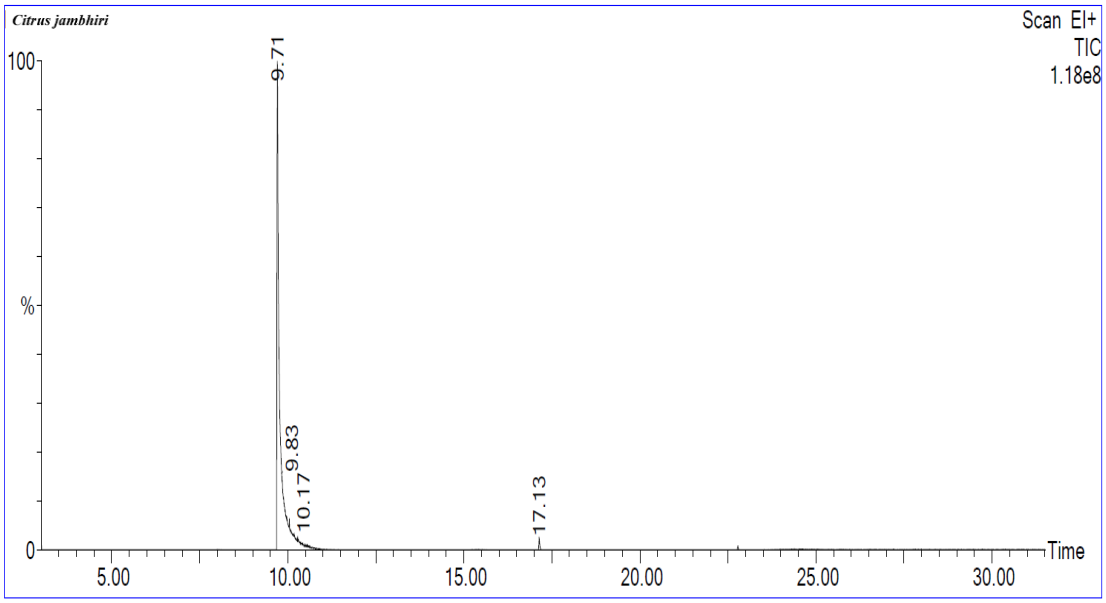


Fig. 4.81 GCMS chromatogram for *Citrus jambhiri*

Table 4.32 Compounds identified using GCMS in *Citrus jambhiri*

Sl. No	Retention Time (Minutes)	Name	Molecular weight	Chemical Formula	**Area**	**Area %**
1	3.013	1-Propanone, 1-[2-(1,1-Dimethylethyl)Cyclopropyl]-2,2-Dimethyl-, Trans-	182	$C_{12}H_{22}O$	6,431.9	0.056
2	9.716	Tetrahydrocyclopenta[1,3]Dioxin-4-One	142	$C_7H_{10}O_3$	9,531,595.0	83.051
3	10.076	1-Methyl-2-Oxocyclohex-3-Enecarboxylic Acid, Methyl Ester	168	$C_9H_{12}O_3$	47,051.9	0.410
4	17.134	2,5-Di(Trifluoromethyl)Benzoic Acid, 2-Propylphenyl Ester	376	$C_{18}H_{14}O_2F_6$	891,757.4	7.770
5	24.342	4-Methyl-2,4-Bis(P-Hydroxyphenyl)Pent-1-ene, 2TMS Derivative	412	$C_{24}H_{36}O_2Si_2$	126,485.5	1.102

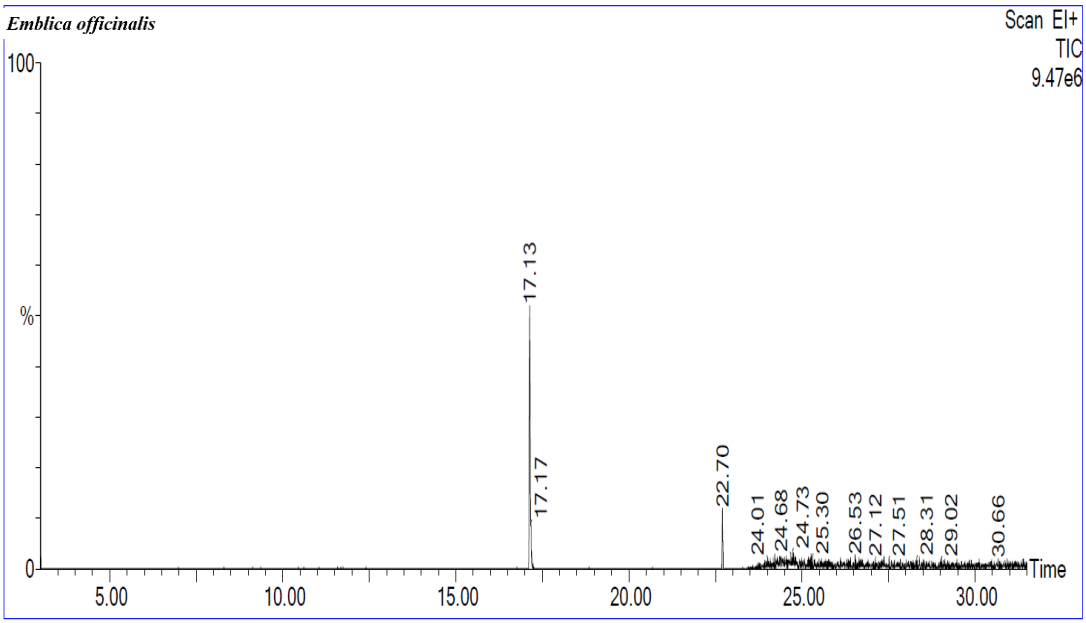


Fig. 4.82 GCMS chromatogram for *Emblica officinalis*

Table 4.33 Compounds identified using GCMS in *Emblica officinalis*

Sl. No	Retention Time (Minutes)	Compound Name	Molecular weight	Chemical Formula	Area	Area %
1	3.013	1,2-Dioxolan-3-One, 5,5-Diethyl-4-Methylene	156	$C_8H_{12}O_3$	47,370.0	6.758
2	17.134	3,5-Dimethylphentlsilyloxybenzene	256	$C_{16}H_{20}OSi$	168,921.5	24.100
3	22.701	Hexamethylene Diacrylate	226	$C_{12}H_{18}O_4$	24,177.3	3.449
4	24.007	4-Methyl-2,4-Bis(P-Hydroxyphenyl)Pent-1-Ene, 2TMS Derivative	412	$C_{24}H_{36}O2Si-2$	7,136.2	1.018
5	24.317	Silicic Acid, Diethyl Bis(Trimethylsilyl) Ester	296	$C_{10}H_{28}O_4Si_3$	3,172.2	0.453
6	24.442	Tricyclo[4,2,1,0(2,5)] Non-7-Ene, 3,4-Di(Tris(Trimethylsilyloxy)Silyl)-	708	$C_{27}H_{64}O_6Si_8$	4,764.3	0.680
7	24.642	1H-Purine-2,6-Dione, 3,7-Dihydro-1,3-Dimethyl-7-[2-[(1-Methyl-2-Phenylethyl)Amino]Ethyl]-	341	$C_{18}H_{23}O_2N_5$	5,766.1	0.823
8	25.548	(2-Phenyl-1-Benzimidazolyl)Acetic Acid	252	$C_{15}H_{12}O_2N_2$	3,246.3	0.463
9	25.753	4-Tert-Octylphenol, TMS Derivative	278	$C_{24}H_{36}O_2Si_2$	3,418.5	0.488
10	27.513	2-T-Butyl-5-(Dimethoxy-Phosphoryl)-3-Methyl-4-Oxoimidazolidine-1	364	$C_{15}H_{29}O_6N_2P$	3,463.6	0.494
11	27.839	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$	3,966.5	0.566
12	28.524	4-(Hydroxyphenyl)-4-Methyl-2-Pentanone, TMS Derivative	264	$C_{15}H_{24}O_2Si$	3,013.2	0.430

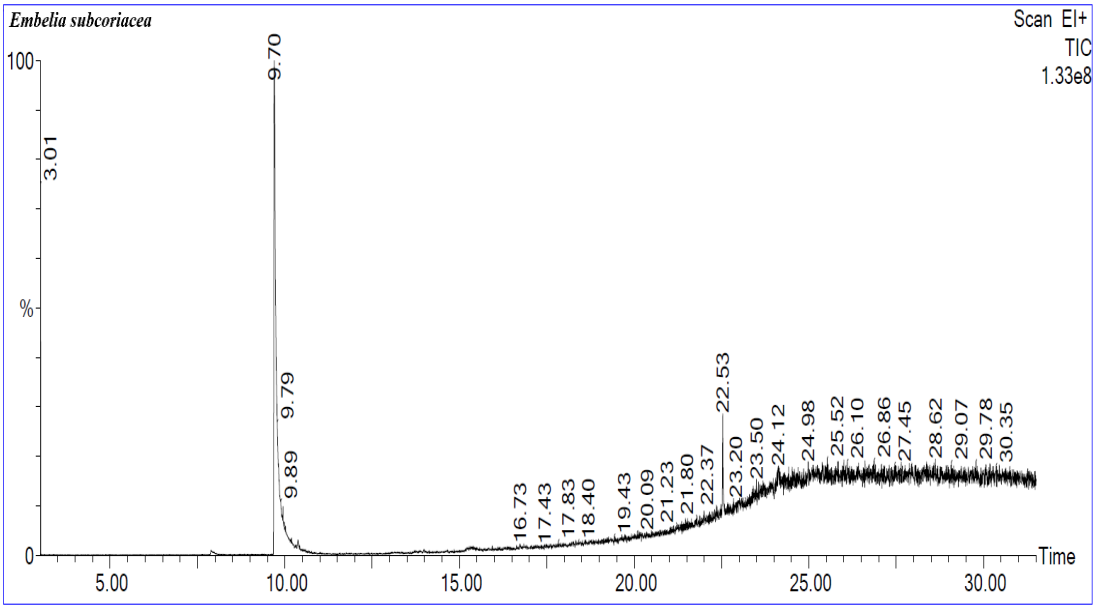


Fig. 4.83 GCMS chromatogram for *Embelia subcoriacea*

Table 4.34 Compounds identified using GCMS in *Embelia subcoriacea*

Sl. No.	Retention Time (Minutes)	Name	Molecular weight	Chemical Formula	Area	Area %
1	3.013	3-Methyl-2-(2-Oxopropyl)Furan	138	$C_8H_{10}O_2$	503,225.4	1.129
2	7.890	Carbonocyanidic Acid, Ethyl Ester	99	$C_4H_5O_2N$	415,782.3	0.933
3	9.701	Tetrahydrocyclopenta[1,3]Dioxin-4-One	142	$C_7H_{10}O_3$	10,906,412.0	24.461
4	9.806	Isobutyl 3-MethylBut-3-Enyl Carbonate	186	$C_{10}H_{18}O_3$	265,346.8	0.595
5	9.951	2-Cyclohexen-1-One	96	C_6H_8O	154,416.7	0.346
6	10.176	Succinic Acid, 1,1,1-Trifluoroprop-2-YL 3-Methylbut-3-EN-1-YL ESTE	282	$C_{12}H_{17}O_4F_3$	203,214.1	0.456
7	10.382	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	144	$C_6H_8O_4$	644,335.5	1.445
8	10.326	6-Azabicyclo[3,2,1]Octane	111	$C_7H_{13}N$	158,141.6	0.355
9	22.532	N-Heptyl Acrylate	170	$C_{10}H_{18}O_2$	1,440,837.2	3.232
10	24.092	Methyl P-Coumarate, TMS Derivative	250	$C_{13}H_{18}O_3Si$	158,818.0	0.356
11	27.078	Pyrimidine, 5-Bromo-2,4-Bis(Methylthio)-	250	$C_{13}H_{18}O_3Si$	202,233.7	0.454
12	27.299	Benzenepropanamide, Alpha-[[3-(Dimethylamino)Phenyl]Imino]-Bet	371	$C_{23}H_{21}O_2N_3$	144,606.6	0.324
13	28.919	Propofol, Trimethylsilyl Ether	250	$C_{15}H_{26}OSi$	146,548.3	0.329

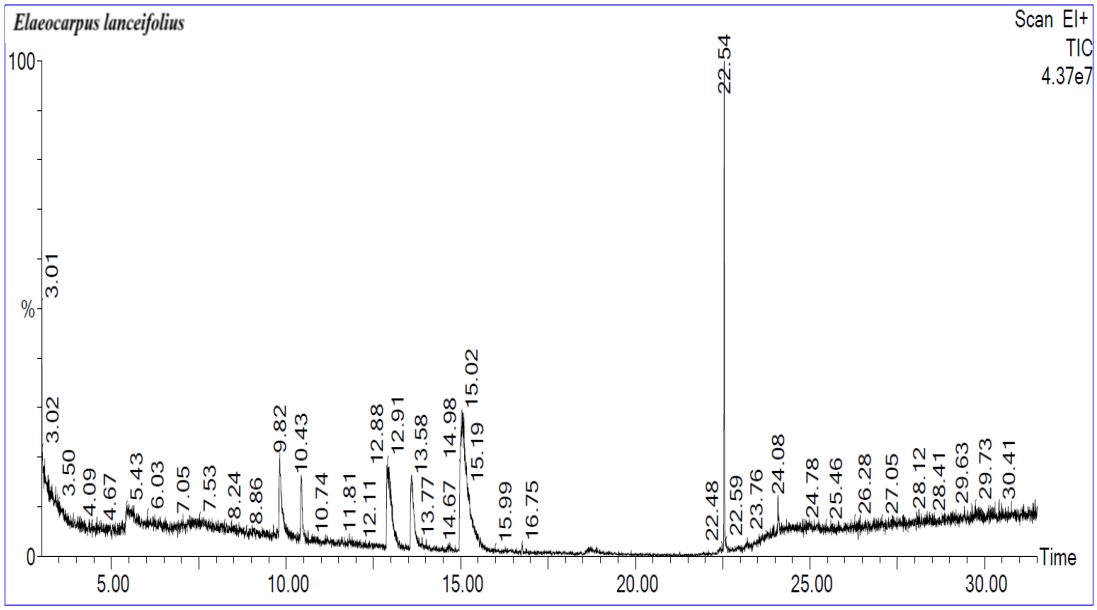


Fig.4.84 GCMS chromatogram for *Elaeocarpus lanceifolius*

Table 4.35 Compounds identified using GCMS in *Elaeocarpus lanceifolius*

SL. No	Retention Time	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	3.013	Methyl P-coumarate. TMS Derivative	250	$C_{13}H_{18}O_3Si$	1,214,290.1	5.487
2	3.304	[2,6-Bis(1-Methylethyl)Phenoxy](Trimethyl)Silane	250	$C_{15}H_{26}OSi$	438,612.1	1.982
3	3.304	1H-Indole-3-Acetic Acid, 5-Chloro-2-Methyl-1-(Trimethylsilyl)-, Trimethylsilyl Ester	367	$C_{17}H_{26}O_2NClSi_2$	97,873.9	0.442
4	3.594	9.9-Dichloro-9-Silafluorene	250	$C_{12}H_8Cl_2Si$	101,107.7	0.457
5	3.459	Benzenepropanamide, Alpha-[[3-(Dimethylamino)Phenyl]Imino]-Beta-Oxo-N-Phenyl-	371	$C_{23}H_{21}O_2N_3$	94,348.1	0.426
6	3.554	5-Methyl-4'-Hydroxy-2-Benzylidene-Coumaran-3-One	252	$C_{16}H_{12}O_3$	177,538.5	0.802
7	3.634	Fluvalinate	502	$C_{26}H_{22}O_3N_2ClF_3$	149,375.5	0.675
8	5.434	Benzothiophene-3-Carboxamide, 4,5,6,7-Tetrahydro-5-Tert-Butyl-2-Cyclohexanoylamino-	362	$C_{20}H_{30}O_2N_2S$	507,545.8	2.293
9	9.821	Succinic Acid, 1,1,1-Trifluoroprop-2-YL 3-Methylbut-3-En-1-YL Este	282	$C_{12}H_{17}O_4F_3$	241,856.4	1.093
10	10.441	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	144	$C_6H_8O_4$	624,226.1	2.821
11	12.880	Thymine	126	$C_5H_6O_2N_2$	513,981.1	2.322
12	12.953	1-[[3'S-Hydroxy-2'R-Butoxy]Methyl]Thymine, 1'-Ethyl Hydrogenphosphate	352	$C_{12}H_{21}O_8N_2P$	439,315.4	1.985
13	13.048	Benzo[A]Pyrene	252	$C_{20}H_{12}$	321,625.1	1.453
14	13.583	1-Methyl-5-Fluorouracil	144	$C_5H_5O_2N_2F$	1,035,416.6	4.678
15	15.028	Thiophene, 2-Propyl-	126	$C_7H_{10}S$	696,691.3	3.148
16	15.078	1-Hexyne, 3-Ethoxy-3,4-Dimethyl	154	$C_{10}H_{18}O$	154,667.9	0.699
17	22.541	Trans-2.4-Dimethylthiane-S,S-Dioxide	162	$C_7H_{14}O_2S$	1,733,828.4	7.834
18	24.077	Phthalic Acid,Monoamide,N-Ethyl-N-Phenyl-, Undecyl Ester	423	$C_{27}H_{37}O_3N$	709,906.7	3.208
19	24.857	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O3Si_4$	268,740.4	1.214

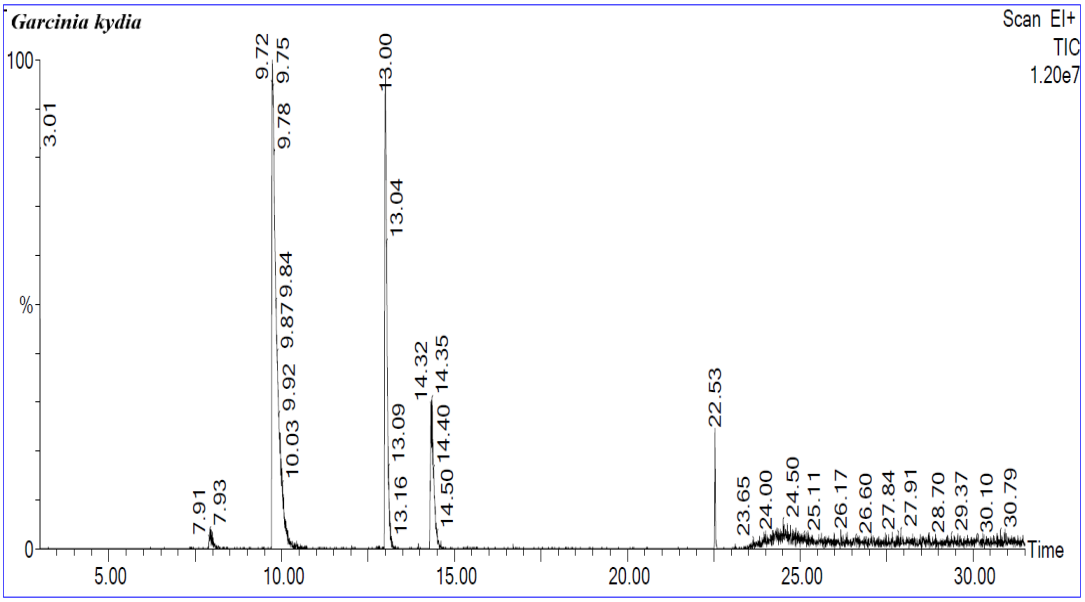


Fig. 4.85 GCMS chromatogram for *Garcinia kydia*

Table 4.36 Compounds identified using GCMS in *Garcinia kydia*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	3.013	1,5-Heptadiene, (Z)-	96	C_7H_{12}	14,304.0	0.329
2	9.723	Orotylamide	155	$C_5H_5O_3N_3$	48,942.1	1.126
3	13.008	1,3-Benzenediol, O-Acryloyl-O'-Dichloroacetyl-	274	$C_{11}H_8O_4Cl_2$	1,816,837.1	41.800
4	14.354	2-Butenedioic Acid (Z)-,Dimethyl Ester	144	$C_6H_8O_4$	58,825.0	1.353
5	22.532	Dodecyl Acrylate	240	$C_{15}H_{28}O_2$	241,585.9	5.558
6	24.167	Cyclotetrasiloxane, Octamethyl-	296	$C_8H_{24}O_4SI_4$	10,023.7	0.231
7	24.192	2,2,2-Triflouroethyl Acrylate	154	$C_5H_5O_2F_3$	827,923.2	19.048
8	24.517	Silicic Acid, Diethyl Bis(Trimethylsilyl) Ester	296	$C_{10}H_{28}O_4SI_3$	9,228.4	0.212
9	24.632	4-Methyl-2,4-Bis(P-Hydroxyphenyl)Pent-1-Ene, 2TMS Derivative	412	$C_{24}H_{36}O_2Si_2$	14,450.3	0.332
10	24.772	Tricyclo[4,2,1,0(2,5)]Non-7-Ene, 3,4-Di(Tris(Trimethylsilyloxy)Silyl)-	708	$C_{27}H_{64}O_6Si_8$	10,825.7	0.249
11	26.553	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$	9,141.5	0.210
12	26.603	4-Tert-Octylphenol, TMS Derivative	278	$C_{17}H_{30}OSI$	17,150.8	0.395
13	26.173	4-(4-Hydroxyphenyl)-4-Methyl-2-Pentanone,TMS Derivative	264	$C_{15}H_{24}O_2SI$	9,003.1	0.207
14	28.589	2'-Hydroxy-5'-Methylacetophenone, TMS Derivative	222	$C_{12}H_{18}O_2SI$	12,651.3	0.291

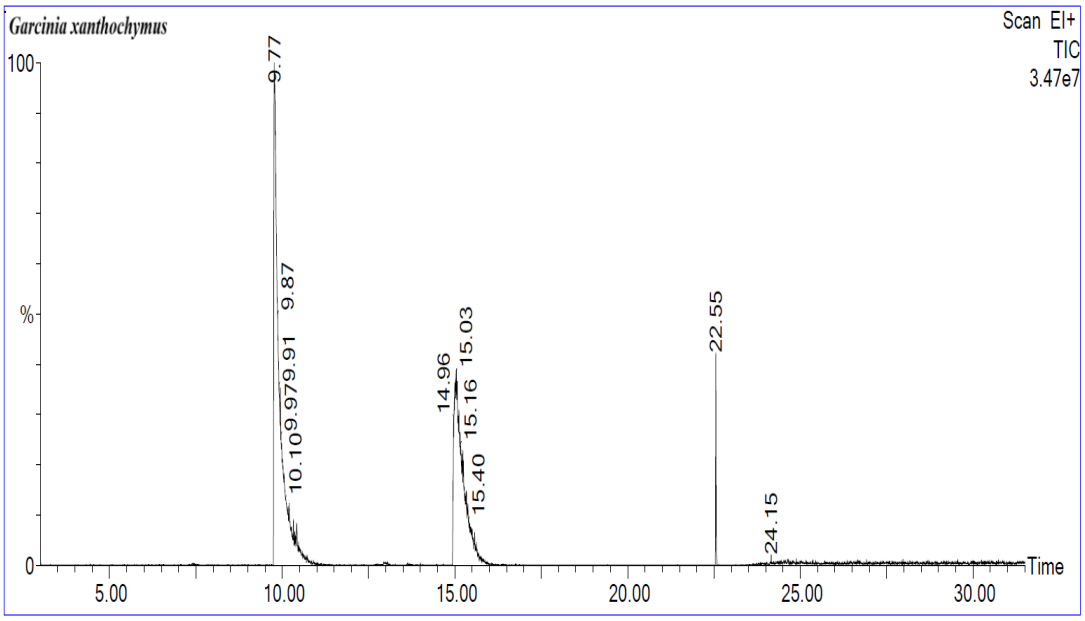


Fig. 4.86 GCMS chromatogram for *Garcinia xanthochymus*

Table 4.37 Compounds identified using GCMS in *Garcinia xanthochymus*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	9.771	(+)-3,4-Dehydroproline Amide	112	$C_5H_8ON_2$	5,783,382.0	50.531
2	10.421	Succinic Acid, 3Methylbut-3-Enyl Propyl Ester	228	$C_{12}H_{20}O_4$	62,115.2	0.543
3	10.472	Meropenem, O,O'-Bis(Trimethylsilyl)-	527	$C_{23}H_{41}O_5N_3SSi_2$	51,164.0	0.447
4	10.622	Isobutyl 3-Methylbut-3-Enyl Carbonate	186	$C_{10}H_{18}O_3$	107,935.9	0.943
5	15.034	Thiophene, 2-Propyl-	126	$C_7H_{10}S$	3,899,114.5	34.068
6	15.574	1H-Pyrazole,4,5-Dihydro-3-Methyl-1Propyl-	126	$C_7H_{14}N_2$	82,322.2	0.719
7	22.552	Trans-2,4-Dimethylthiane, S,S-Dioxide	162	$C_7H_{14}O_2S$	210,954.0	1.843

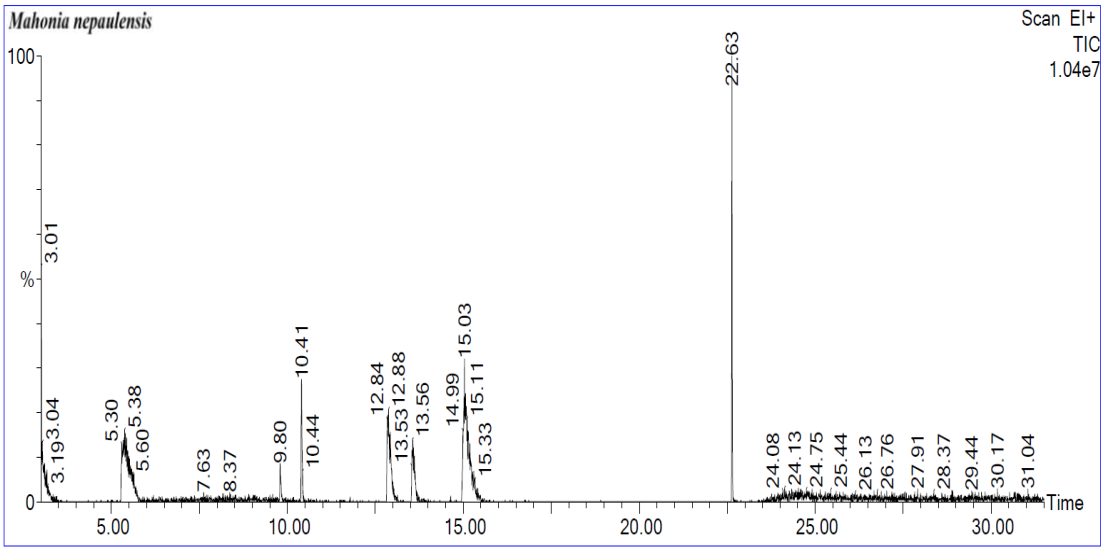


Fig. 4.87 GCMS chromatogram for *Mahonia nepaulensis*

Table 4.38 Compounds identified using GCMS in *Mahonia nepaulensis*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	3.013	N-(4-Methylcyclohexyl)Acetamide, Cis-	155	$C_9H_{17}ON$	48,876.4	2.064
2	5.384	Oxazole,4,4-Dimethyl-2-(2-Methylselanylphenyl)-4,5-Dihydro-	269	$C_{12}H_{15}ONSE$	131,517.5	5.555
3	5.464	2,4,5-Trichlorophenyl Propenoate	250	$C_9H_5O_2CI_3$	37,332.3	1.577
4	10.416	1,3-Dioxolane, 2-Cyclohexyl-4,5-Dimethyl-	184	$C_{11}H_{20}O_2$	165,560.8	6.993
5	12.877	Thymine	126	$C_5H_6O_2N_2$	34,856.7	1.472
6	12.848	Molinate	187	$C_9H_{17}ONS$	64,560.0	2.727
7	13.563	1,3-Dioxolane,4-Ethyl-2-Pentadecyl-	312	$C_{20}H_{40}O_2$	58,457.4	2.469
8	13.588	2-Acetyl-2,3,5,6-Tetrahydro-1,4-Thiazine	145	$C_6H_{11}ONS$	22,772.7	0.962
9	13.613	2-(3-Carboxypropionylamino)Thiazole	202	$C_7H_{10}O_3N_2S$	27,247.7	1.151
10	15.033	Thiophene,2-Propyl-	126	$C_7H_{10}S$	87,765.8	3.707
11	15.128	1H-Pyrazole, 4,5-Dihydro-3-Methyl-1-Propyl-	126	$C_7H_{14}N_2$	54,982.6	2.322
12	15.188	4-Octen-3-One	126	$C_8H_{14}O$	37,979.5	1.604
13	22.632	4-Undecene, 6-Methyl-	168	$C_{12}H_{24}$	145,513.1	6.146
14	15.033	4-Ethyl-2-Hydroxycyclopent-2-En-1-One	126	$C_7H_{10}O_2$	22,804.8	0.963

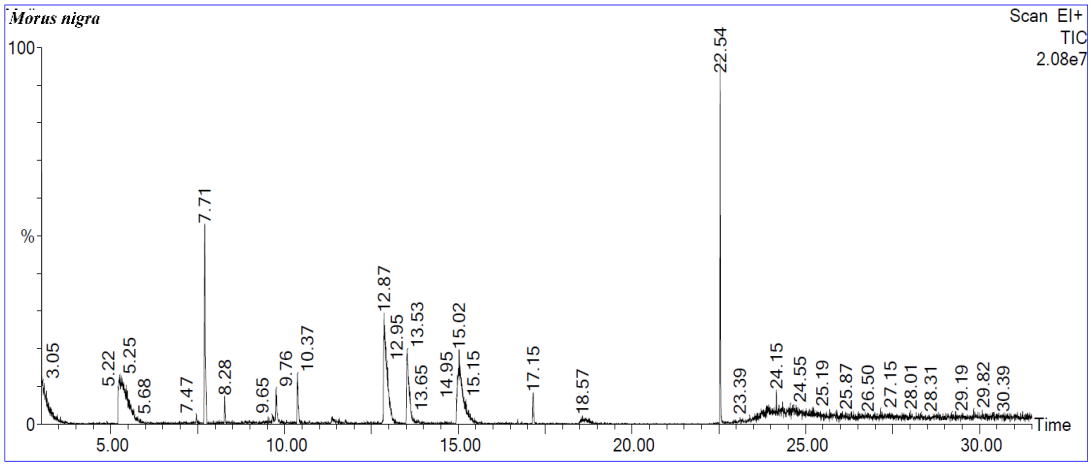


Fig. 4.88 GCMS chromatogram for *Morus nigra*

Table 4.39 Compounds identified using GCMS in *Morus nigra*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1.	3.013	Isobutyl Acrylate	128	$C_7H_{12}O_2$	156,095.3	2.358
2.	3.093	Pentanoic Acid	102	C5H10o2	133,592.1	2.018
3.	3.173	Benserazide	257	$C_{10}H_{15}O_5N_3$	47,484.3	0.717
4.	3.218	Butanoic Acid	88	$C_4H_8O_2$	46,881.5	0.708
5.	5.259	2-Propenoic Acid, Oxiranylmethyl Ester	128	$C_6H_8O_3$	350,739.0	5.298
6.	7.715	Benzene, 1,3-Dimethyl-	106	C_8H_{10}	520,125.9	7.856
7.	8.286	4,6-Octadiyn-3-Onr, 2-Methyl-	134	$C_9H_{10}O$	154,121.5	2.328
8.	9.756	Methylamine, N-Cyclopentylidene-	97	$C_6H_{11}N$	40,254.6	0.608
9.	10.371	Octanoic Acid	144	$C_8H_{16}O_2$	35,561.6	0.537
10.	12.876	D-Alanine, N-Propargloxycarbonyl-,Decyl Ester	311	$C_{17}H_{29}O_4N$	390,603.4	5.900
11.	13.533	2,4-Dihydroxy-2-5-Dimethyl-3(2H)-Furan-3-One	144	$C_6H_8O_4$	58,141.4	0.878
12.	15.018	1-Ethyl-3-Methylcyclohexane (C.T)	126	C_9H_{18}	68,754.5	1.038
13.	17.159	L-Proline, 2TMS Derivative	259	$C_{11}H_{25}O_2NSi_2$	102,073.8	1.542
14.	22.543	Trans-2,4-Dimethylthiane, S,S-Dioxide	162	$C_7H_{14}O_2S$	659,882.3	9.967

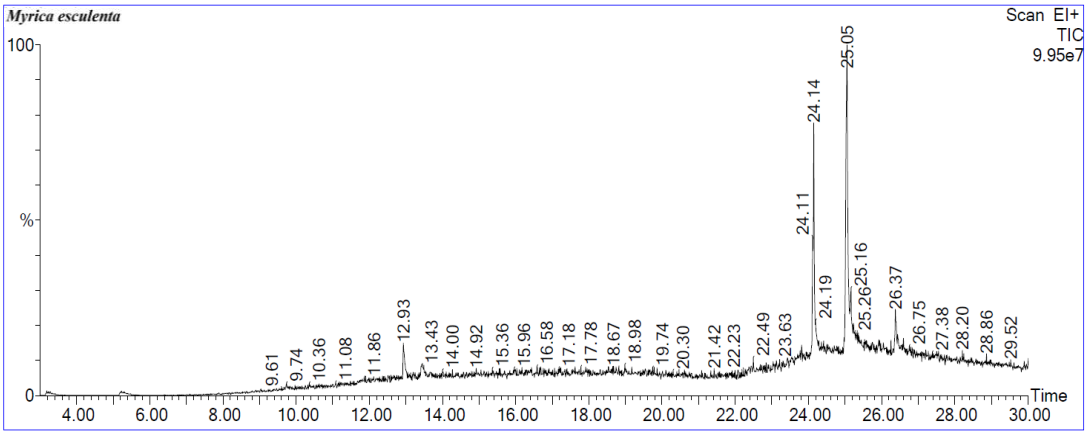


Fig. 4.89 GCMS chromatogram for *Myrica esculenta*

Table 4.40 Compounds identified using GCMS in *Myrica esculenta*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1.	9.736	Fluvalinate	502	$C_{26}H_{22}O_3N_2ClF_3$	451,897.8	0.730
2.	9.826	Benzenepropanamide, Alpha-[[3-(Dimethylamino)Phenyl]Imino]-Bet	371	$C_{23}H_{21}O_2N_3$	469,624.5	0.759
3.	9.966	5-Methyl-4'-Hydroxy-2-Benzylidene-Coumaran-3-One	252	$C_{16}H_{12}O_3$	774,725.8	1.252
4.	11.897	1-Methyl-2,5-Dichloro-1,6-Diazaphenalene	250	$C_{12}H_8N_2Cl_2$	484,246.7	0.782
5.	11.487	3-Ethoxy-6-(1-Tetrahydropyranoxy-5-Methyl-4-Hexenyl)-2-Cycloh	336	$C_{20}H_{32}O_4$		
6.	12.932	4-Methylpiperidine-1-Carboxylic Acid, Phenyl Ester	219	$C_{13}H_{17}O_2N$	1,100,786.6	1.779
7.	13.048	Perylene	252	$C_{20}H_{12}$	625,679.2	1.011
8.	16.679	Pyrimidine, 5-Bromo-2,4-Bis(Methylthio)-	250	$C_6H_7N_2BrS_2$	638,680.7	1.032
9.	22.491	Benzothiophene-3-Carboxamide, 4,5,6,7-Tetrahydro-5-Tert-Butyl-2-Cyclohexanoylamino-	362	$C_{20}H_{30}O_2N_2S$	655,066.7	1.058
10.	24.112	N-Decanoic Acid	172	$C_{10}H_{20}O_2$	1,070,042.4	1.729
11.	24.142	Dodecanoic Acid	200	$C_{10}H_{20}O_2$	3,304,476.2	5.340
12.	25.052	Ethanol, 2-(9-Octadecenyloxy)-, (E)	312	$C_{20}H_{40}O_2$	5,546,213.5	8.962
13.	25.162	3-Dodecanol, 3,7,11-Trimethyl-	228	$C_{15}H_{32}O$	863,475.4	1.395
14.	26.373	3-Octanol, 3,7-Dimethyl-	158	$C_{10}H_{22}O$	1,080,196.5	1.745
15.	27.213	[2,6-Bis(1-Methylethyl)Phenoxy](Trimethyl)Silane	250	$C_{15}H_{26}OSi$	563,694.8	0.911
16.	27.553	Anthracene, 9,10-Dihydro-9,10-Bis(Trimethylsilyl)-	324	$C_{20}H_{28}Si_2$	478,445.9	0.773
17.	26.173	4-Bromo-2-(Trifluoromethoxy)Thiophenol, S-Trimethylsilyl	344	$C_{10}H_{12}OBrF_3SSi$	585,136.7	0.945

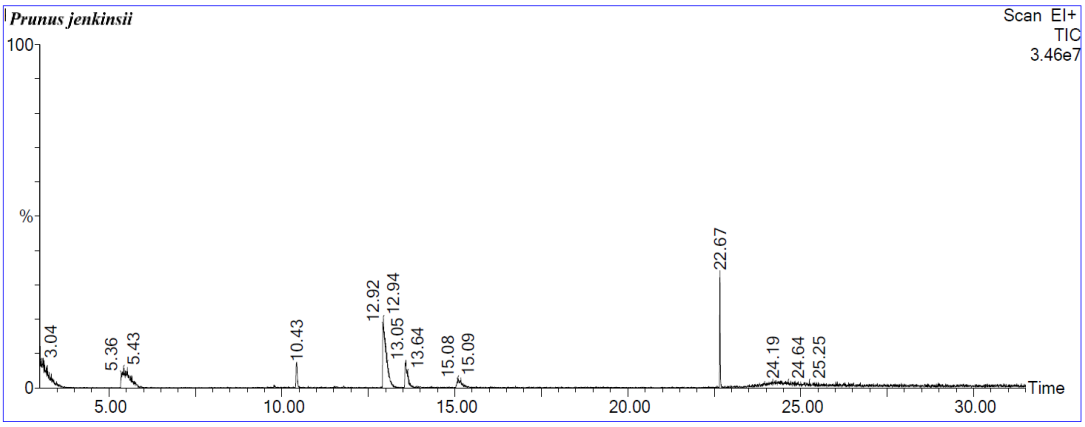


Fig. 4.90 GCMS chromatogram for *Prunus jenkinsii*

Table 4.41 Compounds identified using GCMS in *Prunus jenkinsii*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	3.041	3-Methyl-2-(2-Oxopropyl)Furan	138	$C_8H_{10}O_2$	278,396.8	6.214
2	5.434	2,4,5-Trichlorophenyl Propenoate	250	$C_9H_5O_2CI_3$	110,696.4	2.471
3	5.589	2,4,5-Trichlorophenyl Propenoate	250	$C_9H_5O_2CI_3$	71,774.8	1.602
4	5.705	Oxazole,4,4-Dimethyl-2-(-2-Methylselanyphenyl)-4,5-Dihydro-	269	$C_{12}H_{15}ONSe$	40,433.2	0.903
5	5.820	Estra-4,9,11-Trien-3-One,17-[[(Cyclohexylmethoxy)Carbonyl]Oxy]-,(	410	$C_{26}H_{34}O_4$	50,378.5	1.124
6	10.416	2,4-Dihydroxy-2,5-Dimethyl-3(2h)-Furan-3-One	144	$C_6H_8O_4$	46,015.7	1.027
7	10.436	1,3-Dioxolane,2-Cyclohexyl-4,5-Dimethyl-	184	$C_{11}H_{20}O_2$	218,964.4	4.887
8	12.928	Thymine	126	$C_5H_6O_2N_2$	77,688.0	1.734
9	13.588	1-Methyl-5-Fluorouracil	144	$C_5H_5O_2N_2F$	35,653.4	0.796
10	13.553	Succinic Acid, Cyclobutyl Propyl Ester	214	$C_{11}H_{18}O_4$	44,578.4	0.995
11	13.578	1-Methyl-5-Fluorouracil	144	$C_5H_5O_2N_2F$	47,379.7	1.058
12	13.603	1,3-Dioxepane,2-Heptyl-	200	$C_{12}H_{24}O_2$	59,071.4	1.319
13	13.633	1,3-Dioxolane, 4-Ethyl-2-Pentadecyl-	312	$C_{20}H_{40}O_2$	59,639.2	1.331
14	13.643	1,3-Dioxolane,4-Ethyl-2-Pentadecyl-	312	$C_20H_{40}O_2$	52,603.0	1.174
15	15.083	Thiophene,2-Propyl-	126	$C_7H_{14}N_2$	110,065.2	2.457
16	15.138	1h-Pyrazole,4,5-Dihydro-3-Methyl-1-Propyl-	126	$C_7H_{14}N_2$	81,593.0	1.821
17	22.676	1-Hepten-3-One	112	$C_7H_{12}O$	793,784.0	17.718

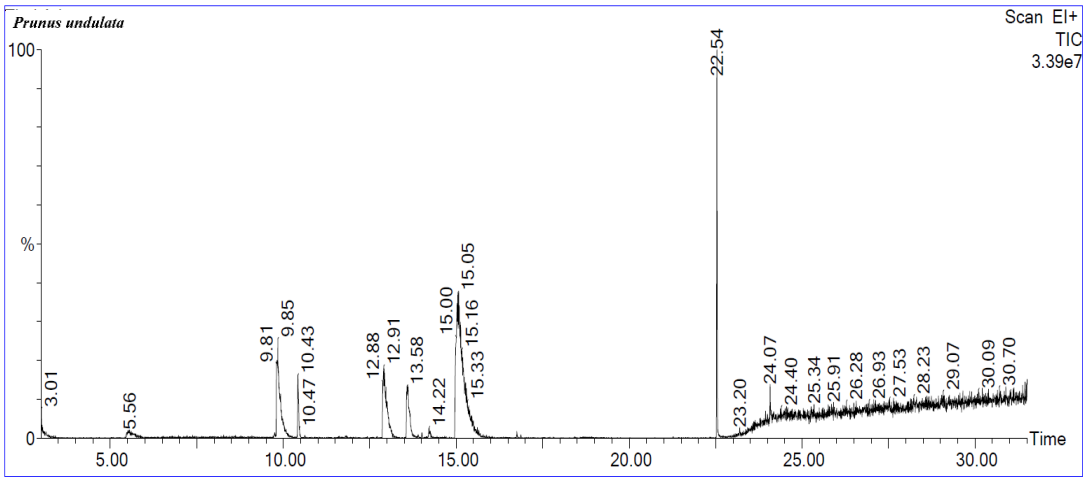


Fig. 4.91 GCMS chromatogram for *Prunus undulata*

Table 4.42 Compounds identified using GCMS in *Prunus undulata*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	9.854	Isobutyl Acrylate	128	$C_7H_{12}O_2$	447,424.9	4.090
2	10.436	1,3-Dioxolane, 2-Cyclohexyl-4,5-Dimethyl-	184	$C_{11}H_{20}O_2$	888,818.2	8.124
3	12.917	Thymine	126	$C_5H_6O_2N_2$	427,262.0	3.905
4	13.002	Prolintane	217	$C_{15}H_{23}N$	51,543.7	0.471
5	13.589	1,3-Dioxepane, 2-Heptyl-	200	$C_{12}H_{24}O_2$	847,932.2	7.751
6	15.058	Thiophene, 2-Propyl	126	$C_7H_{10}S$	1,531,237.2	13.996
7	15.067	5-Hydroxymethylfurfural	126	$C_6H_6O_3$	167,143.5	1.528
8	15.278	Fueazan-3-Carboxylic Acid, 4-Amino-,[2-[(Thiophen-2-Ylmethyl)Amino]Ethyl]Amide	256	$C_{10}H_{13}O_2N_5S$	67,394.7	0.616
9	15.298	1-Hexyne, 3-Ethoxy-3-4-Dimethyl-	154	$C_{10}H_{18}O$	78,222.1	0.715
10	15.363	Propennitttrile, 3-Ethoxy-2-(2-Thienylmethylsulfonyl)-	257	$C_{10}H_{11}O_3NS_2$	78,222.1	0.715
11	15.393	1-Hexyne, 3-Ethoxy-3,4-Dimethyl-	154	$C_{10}H_{18}O$	104,028.4	0.951
12	15.533	1H-Pyrazole, 4,5-Dihydro-3-Methyl-1-Propyl-	126	$C_7H_{14}N_2$	65,545.9	0.599
13	22.546	Trans-2,4-Dimethylthiane, S,S-Dioxide	162	$C_7H_{14}O_2S$	593,054.4	5.421
14	24.072	Phthalic Acid, Hexyl Heptyl Ester	348	$C_{21}H_{32}O_4$	256,503.2	2.345
15	24.812	1,3-Dioxolane, 2-Pentadecyl-	284	$C_{18}H_{36}O_2$	42,098.4	0.385
16	28.229	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$	107,140.4	0.979
17	28.234	Anthracene, 9-Ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$	191,516.9	1.751

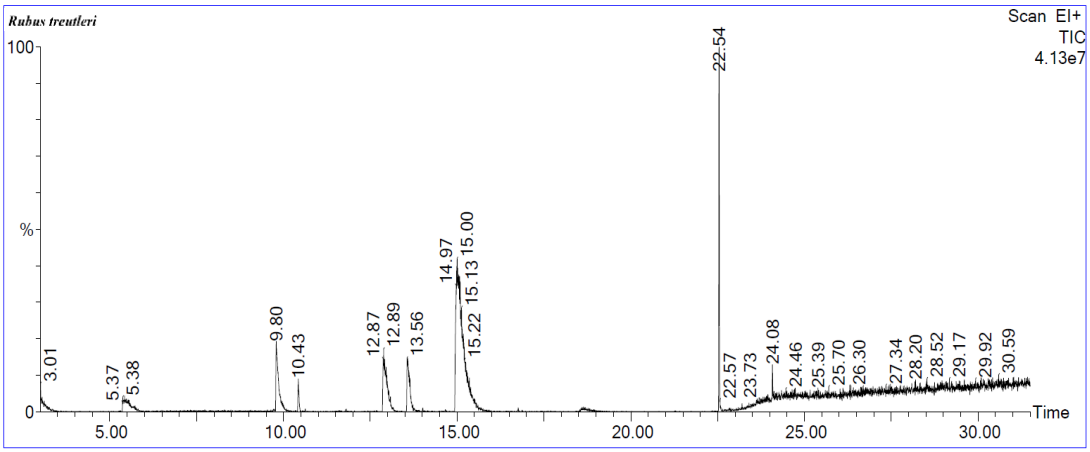


Fig. 4.92 GCMS chromatogram for *Rubus treutleri*

Table 4.43 Compounds identified using GCMS in *Rubus treutleri*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	3.013	1-Octadecanamine, N-Hydroxy-N-Methyl-	299	$C_{19}H_{41}ON$	51,910.8	0.446
2	3.153	Pentanoic Acid	102	$C_5H_{10}O_2$	66,867.9	0.575
3	3.263	Butanoic Acid	88	$C_4H_8O_2$	53,569.5	0.460
6	5.584	2,4,5-Trichlorophenyl Propenoate	250	$C_9H_5O_2Cl_3$	60,673.1	0.521
7	5.790	Estra-4,9,11-Trien-3-One, 17-[[(Cyclohexylmethoxy)Carbonyl]Oxy]-,	410	$C_{26}H_{34}O_4$	62,552.7	0.537
8	10.431	1,3-Dioxolane, 2-Cyclohexyl-4,5-Dimethyl-	184	$C_{11}H_{20}O_2$	605,015.4	5.198
9	10.441	1,3-Dioxolane, 4,5-Dimethyl-2-Pentadecyl-	312	$C_{20}H_{40}O_2$	54,161.5	0.465
10	12.887	Thymine	126	$C_5H_6O_2N_2$	114,441.7	0.983
11	12.892	L-Alanine, N-Propargyloxycarbonyl-,Dodecyl Ester	339	$C_{19}H_{33}O_4N$	689,502.8	5.924
12	12.977	3-Acetyl Thymine	168	$C_7H_8O_3N_2$	60,931.7	0.524
13	13.083	Prolintane	217	$C_{15}H_{23}N$	65,671.4	0.564
14	13.563	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	144	$C_6H_8O_4$	385,039.6	3.308
15	13.643	1,3-Dioxepane, 2-Heptyl-	200	$C_{12}H_{24}O_2$	152,310.2	1.309
16	15.003	Thiophene, 2-Propyl-	126	$C_7H_{10}S$	3,295,977.0	28.319
17	15.284	5-Hydroxymethylfurfural	126	$C_6H_6O_3$	57,200.5	0.491
18	22.541	5-Undecene, 6-Methyl-	168	$C_{12}H_{24}$	694,789.4	5.970
19	24.087	Phthalic Acid, Butyl Ester, Ester with Butyl Glycolate	336	$C_{18}H_{24}O_6$	673,569.6	5.787
20	28.524	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$	95,323.7	0.819
21	30.144	Heptalene, 7,7'-Dihydro-6,6'-Bis(Trimethylsilyl)Methyl-	354	$C_{22}H_{34}Si_2$	48,065.7	0.413

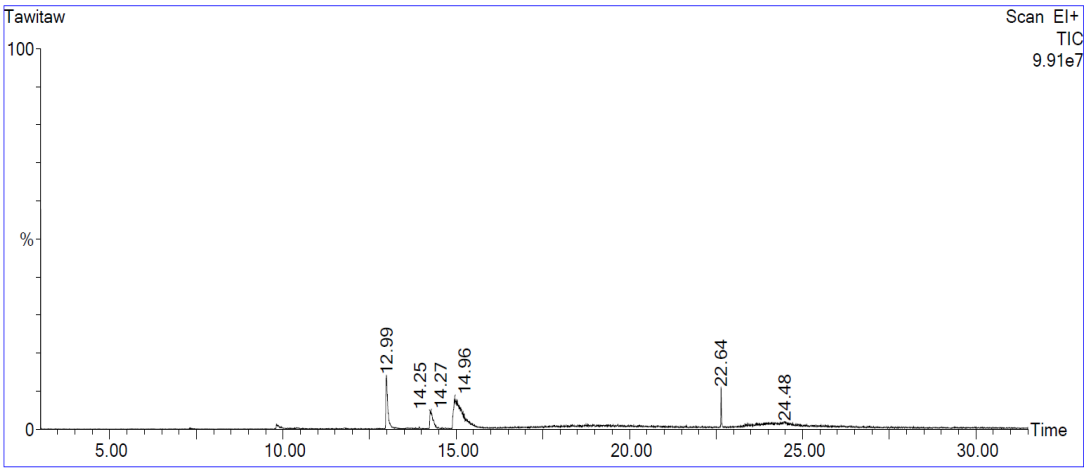


Fig.4.93 GCMS chromatogram for *Spondias pinnata*

Table 4.44 Compounds identified using GCMS in *Spondias pinnata*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	3.013	3-Methyl-2-(2-Oxopropyl)Furan	138	$C_8H_{10}O_2$	208,726.9	2.048
2	9.816	Fumaric Acid, Heptyl 3-Methylbut-3-Enyl Ester	282	$C_{16}H_{26}O_4$	35,872.9	0.352
3	12.998	Ethyl 3A,4,5,6A-Tetrahydrofuro[2,3-B] Furan-2-Carboxylate	184	$C_9H_{12}O_4$	903,530.1	8.867
4	14.258	Cyclooctyle Ethylphosphonofluoridoate	222	$C_{10}H_{20}O_2FP$	479,041.8	4.701
5	14.968	Thiophene, 2-Propyl-	126	$C_7H_{10}S$	751,003.1	7.370
6	15.028	1-Hexyne, 3-Ethoxy-3,4-Dimethyl-	154	$C_{10}H_{18}O$	993,386.2	9.748
7	15.238	5-Hydroxymethylfurfural	126	$C_6H_6O_3$	364,886.1	3.581
8	15.383	4-Octen-3-One	126	$C_8H_{14}O$	100,451.6	0.986
9	15.484	4-Ethyl-2-Hydroxycyclopent-2-En-1-One	126	$C_7H_{10}O_2$	75,257.1	0.739
10	18.885	Benzenepropanamide, Alpha-[[3-(Dimethylamino)Phenyl]Imino]-Bet	371	$C_{23}H_{21}O_2N_3$	35,753.3	0.351
11	19.240	Fluvalinate	502	$C_{26}H_{22}O_3N_2ClF_3$	41,586.4	0.408
12	19.670	1-Methyl-2,5-Dichloro-1,6-Diazaphenalene	250	$C_{12}H_8N_2Cl_2$	43,966.9	0.431
13	22.641	Isobutyl Acrylate	128	$C_7H_{12}O_2$	498,590.4	4.893
14	23.657	2,4,6-Cycloheptatrien-1-One, 3,5-Bis-Trimethylsilyl-	250	$C_{13}H_{22}Osi_2$	39,529.4	0.388
15	24.102	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$	44,910.7	0.441
16	24. 485	AnthranilicAcid, N-(2-Carboxyphenylmethylene)-	269	$C_{15}H_{11}O_4N$	63,254.7	0.621

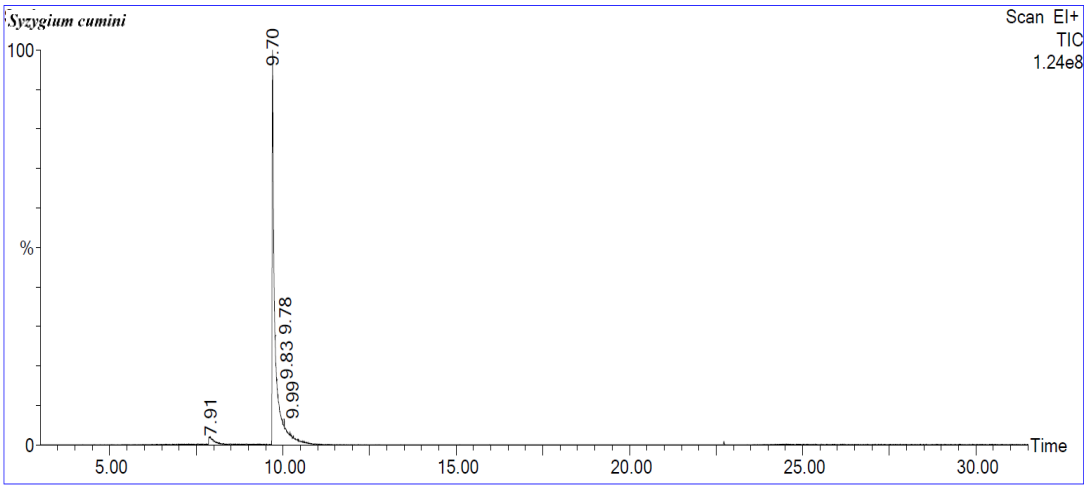


Fig. 4.94 GCMS chromatogram for *Syzygium cumini*

Table 4.45 Compounds identified using GCMS in *Syzygium cumini*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	7. 908	Maleic Anhydride	98	$C_4H_2O_3$	675,137.9	5.447
2	8.626	Perylene	252	$C_{20}H_{12}$	180,730.4	1.458
3	9.701	Tetrahydrocyclopenta[1,3]Dioxin-4-One	142	$C_7H_{10}O_3$	9,927,829.0	80.093
4	10.046	1-Methyl-2-oxocyclohex-3-Enecarboxylic Acid, Methyl Ester	168	$C_9H_{12}O_3$	22,971.6	0.185
5	10.276	7-Oxabicyclo[2,2,1]Hept-5-En-2-One	110	$C_6H_6O_2$	11,168.6	0.090
6	10.642	2(3H)-Furanone,Dihydro-3-Methylene-5,5-Diphenyl	250	$C_{17}H_{14}O_2$	8,575.1	0.069
7	22.721	1-Penten-3-One	84	C_5H_8O	239,472.5	1.932

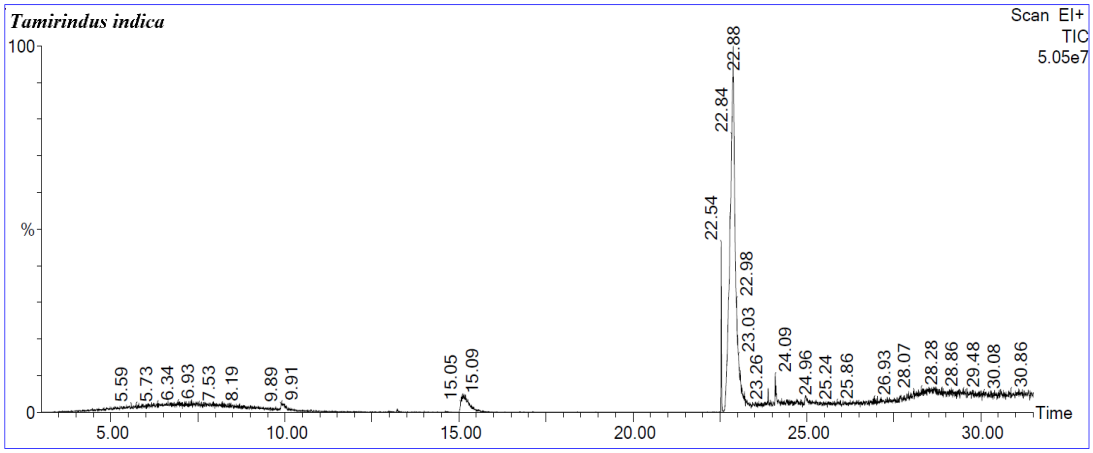


Fig. 4.95 GCMS chromatogram for *Tamirindus indica*

Table 4.46 Compounds identified using GCMS in *Tamirindus indica*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	9.906	2(3H)-Furanone, Dihydro-3-Methylene-5,5-Diphenyl-	250	$C_{17}H_{14}O_2$	63,532.6	0.362
2	15.098	Thiophene, 2-Propyl-	126	$C_7H_{10}S$	349,590.1	1.993
3	15.259	5-Hydroxymethylfurfural	126	$C_6H_6O_3$	94,106.0	0.536
4	22.547	Trans-2,4-Dimethylthiane, S,S-Dioxide	162	$C_7H_{14}O_2S$	313,616.9	1.788
5	22.882	Myo-Inositol, 4-C-Methyl-	194	$C_7H_{14}O_6$	8,457,497.0	48.211
6	23.087	Scyllo-Inositol, 1-C-Methyl-	194	$C_7H_{14}O_6$	73,858.9	0.421
7	23.202	1,3-Dioxolane, 2-Pentadecyl-	284	$C_{18}H_{36}O_2$	77,305.2	0.441
8	23.237	3-Pentanol, 3-Methyl-	102	$C_6H_{14}O$	185,531.5	1.058
9	24.087	Benzeneacetic Acid, Alpha-(7-T-Butyldimethylsilyloxyheptanoyloxy)-, Methyl Ester	408	$C_{22}H_{36}O_5Si$	58,144.3	0.331
10	24.112	3-Dodecanol, 3,7,11-Trimethyl-	228	$C_{15}H_{32}O$	60,035.2	0.342
11	24.958	Cyclopentylcarboxylic Acid	114	$C_6H_{10}O_2$	65,932.2	0.376
12	28.354	3-Octanol, 3,6-Dimethyl-	158	$C_{10}H_{22}O$	58,768.1	0.335
13	28.504	Isopropyl 5, 11-Dihydroxy-3,7,11-Trimethyl-2-Dodecenoate	314	$C_{18}H_{34}O_4$	71,332.0	0.407
14	28.589	Decane, 3-Methoxy-	172	$C_{11}H_{24}O$	55,861.6	0.318
15	28.804	3-Heptanol, 3,5-Dimethyl-	144	$C_9H_{20}O$	61,868.6	0.353

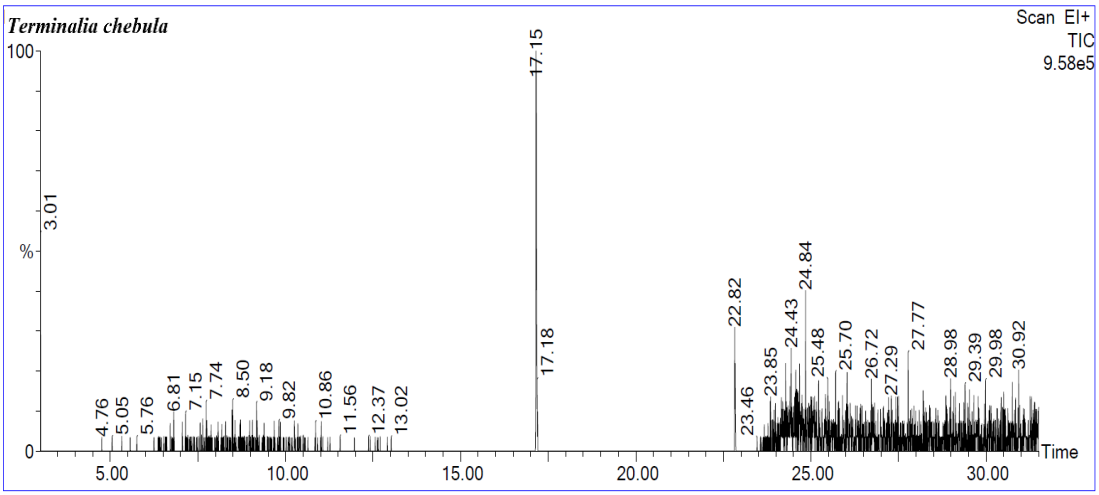


Fig. 4.96 GCMS chromatogram for *Terminalia chebula*

Table 4.47 Compounds identified using GCMS in *Terminalia chebula*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	17.159	Bisphenol C	256	$C_{17}H_{20}O_2$	21,624.9	6.074
2	24.167	4-Methyl-2,4Bis(P-Hydroxphenyl)Pent-1-Ene,2TMS Derivative	412	$C_{24}H_{36}O_2Si_2$	2,615.2	0.735
3	24.397	4-Tert-Octylphenol, Tms Derivative	278	$C_{17}H_{30}OSi$	2,601.8	0.731
4	24.562	Silicic Acid, Diethyl Bis(Trimethylsilyl)Ester	296	$C_{10}H_{28}O_4Si_3$	2,207.7	0.620
5	24.627	Cyclotrisiloxane, Hexamethyl-	222	$C_6H_{18}O_3Si_3$	2,040.2	0.573
6	24.842	4,4'-Bi-4H-Pyran, 2,2',6,6'-Tetrakis(1.1-Dimethylethyl)-4,4'-Dimethyl-	414	$C_{28}H_{46}O_2$	5,367.8	1.508
7	27.879	Tricyclo[4.2.1.0(2,5)]Non-7-Ene,3,4-Di(Tris(Trimethylsilyloxy)Silyl)-	708	$C_{27}H_{64}O_6Si_8$	1,972.8	0.554
8	28.439	N,N-Dimethyl-4-Nitroso-3-(Trimethylsilyl)Aniline	222	$C_{11}H_{18}ON_2Si$	4,098.5	1.151
9	28.439	1H-[1,2,3]Triazole-4-Carboxylic Acid,1-(4-Dimethylamino-6-Methoxy-[	307	$C_{12}H_{17}O_3N_7$	2,936.8	0.825
10	30.980	4-Methyl-2,4-Bis(P-Hydroxphenyl)Pent-1-Ene,2TMS Derivative	412	$C_{24}H_{36}O_2Si_2$	4,279.5	1.202

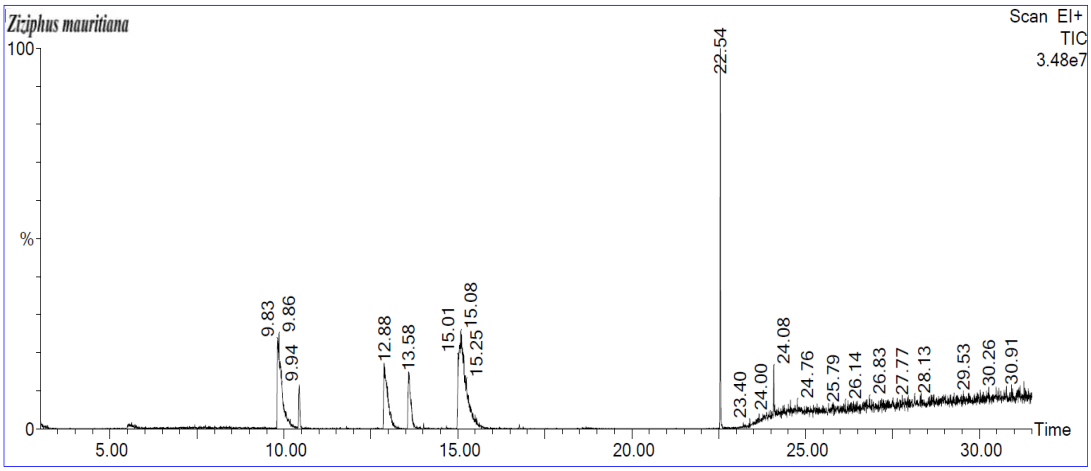


Fig. 4.97 GCMS chromatogram for *Ziziphus mauritiana*

Table 4.48 Compounds identified using GCMS in *Ziziphus mauritiana*

Sl. No.	Retention Time (Minutes)	Compound Name	Molecular Weight	Chemical Formula	Peak Area	Area %
1	9.836	2(3H)-Furanone, Dihydro-3-Methylene-5, 5-Diphenyl-	250	$C_{17}H_{14}O_2$	309,102.7	3.260
2	9.865	6-Azabicyclo[3,2,1]Octane	111	$C_7H_{13}N$	762,881.3	8.045
3	10.441	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	144	$C_6H_8O_4$	74,682.4	0.788
4	12.882	Thymine	126	$C_5H_6O_2N_2$	436,132.2	4.599
5	12.983	Prolintane	217	$C_{15}H_{23}N$	150,423.7	1.586
6	13.583	1,3-Dioxolane, 4,5-Dimethyl-2-Pentadecyl-	312	$C_{20}H_{40}O_2$	507,956.4	5.357
7	15. 083	Thiophene, 2-Propyl-	126	$C_7H_{10}S$	243,372.0	2.567
8	15.308	Propennitrile, 3-Ethoxy-2-(2-Thienylmethylsulfonyl)-	257	$C_{10}H_{11}O_3NS_2$	47,140.9	0.497
9	15.429	1-Hexyne, 3-Ethoxy-3,4-Dimethyl	154	$C_{10}H_{18}O$	61,420.3	0.648
10	22.543	1H-Pyrazole, 4,5-Dihydro-3-Methyl-1-Propyl-	126	$C_7H_{14}N_2$	1,774,898.1	18.718
11	24.082	1,2-Benzenedicarboxylic Acid, Dihexyl Ester	334	$C_{20}H_{30}O_4$	228,888.9	2.414
12	24.502	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$	39,013.2	0.411
13	25.763	Heptasiloxane,	504	$C_{14}H_{44}O_6Si_7$	559,712.1	5.903
14	26.663	Anthracene, 9-Ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$	75,545.6	0.797
15	27.929	4-(4-Hydroxyphenyl)-4-Methyll-2-Pentanone, TMS Derivative	264	$C_{15}H_{24}O_2Si$	43,605.0	0.460

4.5. Principal Component Analysis (PCA)

PCA facilitates the reduction of variables or features within a dataset, preserving a significant portion of the original information.

4.5.1. Nutritional Compositions

To evaluate the relationships between the nutritional components of the studied fruits, principal component analysis (PCA) was conducted using the nutritional and mineral characteristics of the fruits, as outlined in Table 4.49. Out of 28 variables, eight principal components with eigenvalues greater than 1.0 were identified. These components collectively explained 84.60% of the observed variance among the twenty-one (21) underutilised fruits. The remaining principal components, with eigenvalues less than 0.5, were not subjected to further analysis.

The first principal component (PC1) accounted for 20.01% of the overall variance and mainly represented total sugar (0.89), energy (0.88), non-reducing sugar (0.88), reducing sugar (0.73), TSS (0.70) and carbohydrate (0.63). Principal Component 2 (PC2), accounted for 17.85% of the observed variance and was predominantly characterised by fruit attributes such as crude fibre (0.94), lignin (0.86) and dry matter (0.81). PC3 was primarily defined by Zn (0.87) and Cu (0.84), contributing to 11.18 % of the variance.

Components PC4 encompassed Fe (0.94), Co (0.90) and fat (0.82), accounting for 9.76% of the overall variance. Principal Component 5 (PC5) accounted for 8.21% of the variance, indicated by protein (0.77) and cellulose (0.76). PC6 included P (0.89) totalling 7.67% of the variance. Principal Component 7 and

Principal Component 8 contributed to 5.23% and 4.69% of variance predominantly characterised by Na (0.47) and total ash (0.29) respectively.

The loading plot delineates the impact of factors and their interrelations, specifically the proximity of angles, while providing a synthesis of the similarities and differences among the samples and the nutritional characteristics examined. This also illustrates the positioning of twenty-one underutilised fruits and the distribution of quality attributes within a space characterised by the first and second PCA dimensions, as depicted in Figure 4.98.

Overall, the PCA analysis uncovered substantial differences in the nutritional characteristics of the twenty-one underutilised fruits, emphasising noteworthy variations. The analysis of various nutritional features of the fruits revealed eight significant components, which accounted for 84.60% of the overall variance.

Table 4.49 Principal components (PC) and component loadings extracted from nutrient parameters analysed from 21 underutilised fruits were used to interpret the PC

	PRINCIPAL COMPONENT							
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Eigen Value	5.60	5.00	3.13	2.73	2.30	2.15	1.46	1.31
Percentage of Variance	20.01	17.85	11.18	9.76	8.21	7.67	5.23	4.69
Cumulative Percentage	20.01	37.86	49.05	58.81	67.01	74.68	79.91	84.60
FACTOR LOADING/EIGENVECTOR								
Total sugar (%)	**0.89**	0.22	-0.18	-0.03	0.10	-0.14	0.15	0.13
Energy (kcal)	**0.88**	0.02	0.28	0.04	0.07	0.07	-0.07	0.17
Non reducing sugar (%)	**0.88**	0.01	-0.16	-0.09	0.20	-0.24	0.06	-0.02
Reducing sugar (%)	**0.73**	0.25	-0.29	0.01	-0.15	-0.40	-0.01	-0.09
TSS°B	**0.70**	-0.35	0.21	-0.16	-0.08	-0.28	0.06	0.26
Carbohydrate (%)	**0.63**	-0.26	0.39	0.15	-0.28	0.20	0.36	-0.17
Crude fibre (%)	-0.02	**0.94**	0.11	-0.13	0.02	-0.02	-0.01	0.01
Lignin (%)	-0.12	**0.86**	0.09	-0.07	-0.14	-0.11	-0.20	-0.14
Moisture (%)	-0.17	-0.81	-0.40	0.00	-0.07	0.01	-0.08	-0.22
Dry matter (%)	0.17	**0.81**	0.39	-0.01	0.06	0.02	0.06	0.23
Starch (%)	-0.11	-0.51	0.10	-0.26	0.47	-0.33	0.18	-0.33
Zn (mg100 g^{-1})	0.02	0.21	**0.87**	0.24	0.07	-0.07	0.04	0.10
Cu (mg100 g^{-1})	-0.06	0.28	**0.84**	0.00	-0.11	-0.10	-0.20	-0.02
Hemicellulose (%)	0.08	0.39	0.61	-0.09	-0.52	-0.27	-0.09	0.08
Fe (mg100 g^{-1})	-0.06	-0.05	0.06	**0.94**	-0.11	-0.15	0.00	0.04
Co (mg100 g^{-1})	-0.08	-0.19	0.11	**0.90**	0.01	-0.12	-0.12	-0.04
Fat (%)	0.08	0.07	0.01	**0.82**	0.09	0.23	0.24	-0.07
Protein (%)	0.35	-0.23	0.19	-0.26	**0.77**	-0.18	-0.10	0.04
Cellulose (mg 100 g^{-1})	-0.09	0.06	-0.18	0.26	**0.76**	-0.13	0.15	0.12
Na (mg100 g^{-1})	0.06	0.19	-0.04	-0.27	0.55	0.26	**0.47**	-0.05
Total Ash (%)	-0.43	-0.37	0.02	-0.29	-0.44	0.43	-0.07	**0.29**
P (%)	-0.17	0.02	-0.08	0.05	-0.05	**0.89**	-0.07	0.10
N (%)	-0.25	-0.19	-0.25	-0.15	-0.21	0.57	0.00	0.03
Ca (mg100 g^{-1})	-0.27	0.19	0.53	-0.12	0.08	0.57	-0.03	-0.26
Acidity (%)	-0.31	0.12	0.03	0.13	-0.02	0.09	-0.81	0.04
Mn (mg 100 g^{-1})	-0.09	-0.07	-0.10	0.30	0.18	-0.09	0.71	0.02
K (%)	0.23	0.08	0.08	-0.07	0.07	0.04	-0.07	0.92
Mg (mg 100 g^{-1})	-0.12	0.46	-0.07	0.03	-0.05	0.11	0.50	0.58

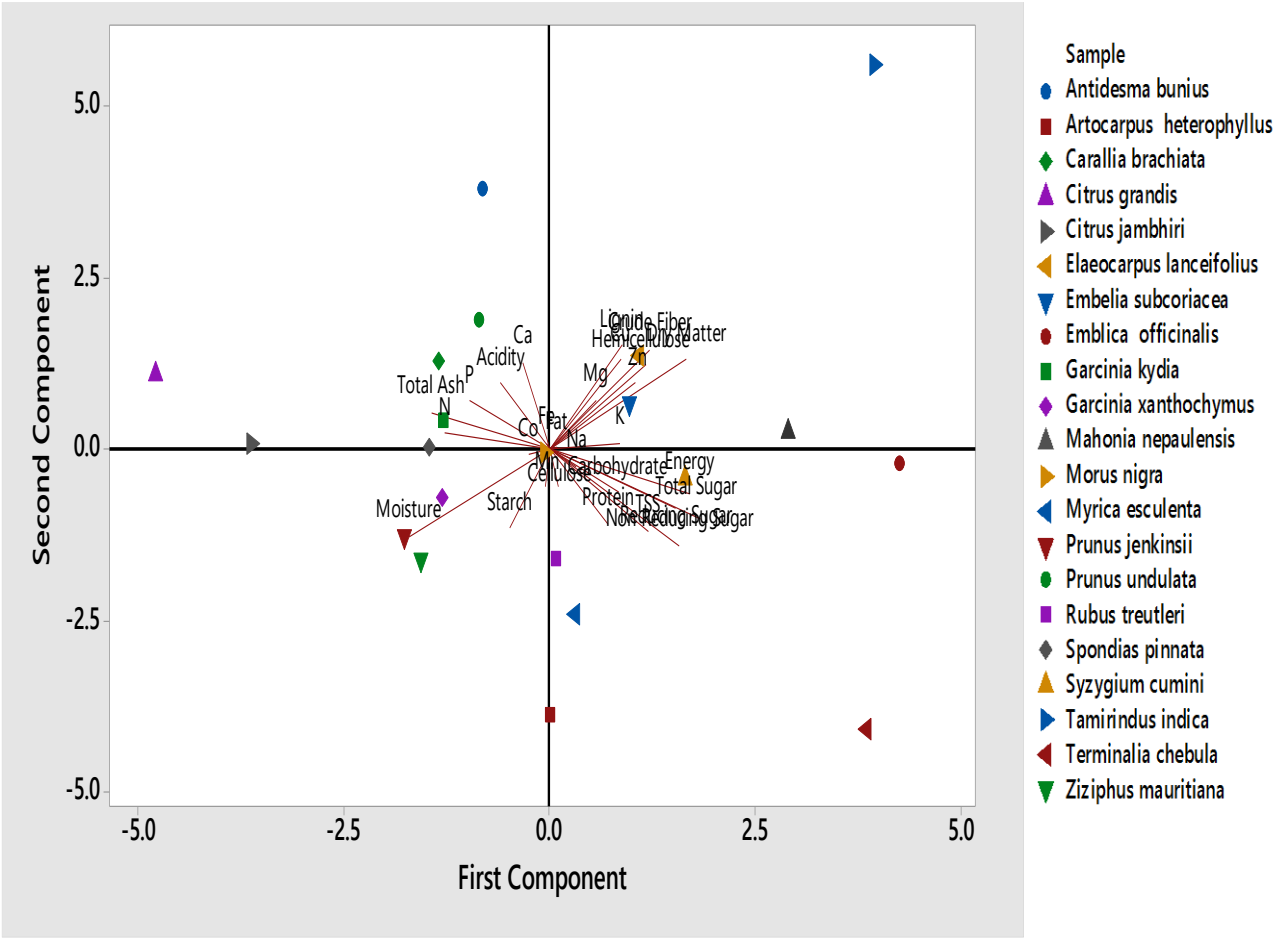


Fig. 4.98 Principal Component Analysis (PCA) Bi-plot (score and loading plot) of the nutritional component of the 21 underutilised fruits highlighting substantial differences in the nutritional characteristics

4.5.2. Bioactive Compositions

To evaluate the relationships between the bioactive of the studied fruits, principal component analysis (PCA) was conducted using the bioactive characteristics of the fruits, as outlined in Table 4.50 Out of 9 variables, three principal components with eigenvalues greater than 1.0 were identified. These components collectively explained 68.63% of the observed variance among the twenty-one (21) underutilised fruits. The remaining principal components, with eigenvalues less than 0.5, were not subjected to further analysis.

The first principal component (PC1) accounted for 35.43% of the overall variance and mainly represented anthocyanin (0.90), saponin (0.65) and alkaloid (0.55). Principal Component 2 (PC2), accounted for 21.38% of the observed variance, was predominantly characterised by fruit attributes such as vitamin C (0.88), phenol (0.74) and flavanoid (0.56). PC3 was primarily defined by total carotenoid (0.73), total chlorophyll (0.71) and vitamin E (0.66), contributing to 11.82 % of the variance.

Overall, the PCA analysis uncovered substantial variations in the bioactive components of the twenty-one underutilised fruits as depicted in the bi-plot graph (Figure 4.99) where *Emblica officinalis, Terminalia chebula, Spondias pinnata, Embelia subcoriacea* and *Prunus undulata* were found to possess higher amounts of the bioactive compounds emphasising their noteworthy variations. The analysis of various nutritional features of the fruits revealed three significant components, which together accounted for 68.63% of the overall variance.

Table 4.50 Principal components (PC) and component loadings extracted from the bioactive parameters analysed from 21 underutilised fruits were used to interpret the PC

	PRINCIPAL COMPONENT		
	PC1	**PC2**	**PC3**
Eigen Value	3.19	1.92	1.06
Percentage of Variance	35.43	21.38	11.82
Cumulative Percentage	35.43	56.81	68.63
FACTOR LOADING/EIGEN VECTOR			
Anthocyanin (mg100 g^{-1})	**0.90**	0.18	0.02
Saponin (mg DE 100 g^{-1})	**0.65**	0.03	0.32
Alkaloid (mg AE 100 g^{-1})	**0.55**	-0.32	0.43
Vitamin C (mg 100 g^{-1})	-0.12	**0.88**	-0.25
Phenol (mg 100 g^{-1})	0.44	**0.74**	0.20
Flavonoid (mg 100 g^{-1})	0.54	**0.56**	0.16
Total Carotenoid (mg 100 g^{-1})	0.39	-0.04	**0.73**
Total Chlorophyll (mg100 g^{-1})	0.20	-0.07	**0.71**
Vitamin E (mg100 g^{-1})	-0.19	0.49	**0.66**

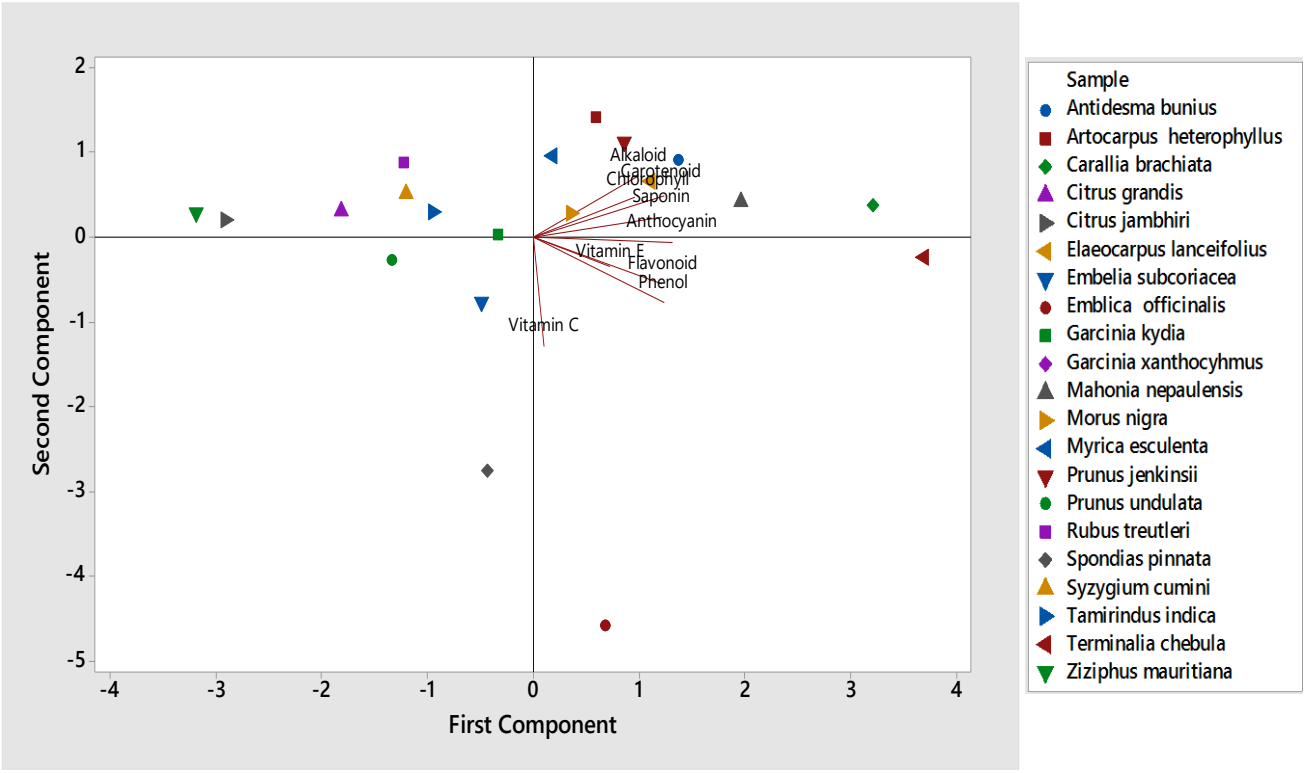


Fig. 4.99 Principal Component Analysis (PCA) Bi-plot (score and loading plot) of the bioactive components of the 21 underutilised fruits highlighting the variations of bioactive components

4.5.3. Antinutritional Component

To evaluate the relationships between the bioactive of the studied fruits, principal component analysis (PCA) was conducted using the bioactive characteristics of the fruits, as outlined in Table 4.50 Out of 3 variables, three principal components with eigenvalues greater than 1.0 were identified. These components collectively explained 83.42% of the observed variance among the twenty-one (21) underutilised fruits. The remaining principal components, with eigenvalues less than 0.5, were not subjected to further analysis.

The first principal component (PC1) accounted for 48.03% of the overall variance and mainly represented phytic (0.92) and tannin (0.66). Principal component 2 (PC2), which accounted for 35.39% of the observed variance, was predominantly characterised by fruit attributes oxalate (0.3) contributing to 35.39% of the variance.

The PCA analysis uncovered variations in the anti-nutritional components of the twenty-one underutilised fruits, emphasising the higher composition of tannin in *Emblica officinalis and Terminalia chebula* from the bi-plot graph (Figure 4.100). The analysis of various nutritional features of the fruits revealed two significant components, which together accounted for 83.42% of the overall variance.

The PCA analysis performed for the twenty-one underutilised fruits identifies the principal components that capture the majority of the variance in the data accumulated from the present investigation, and project high-dimensional data onto a lower-dimensional space. This enables effective visualization of the data and helps identify patterns, clusters, and relationships. This exploratory procedure may help us to visually analyse the importance of underutilized fruit in the region, and further find its application in post harvest processing, value addition, neutraceutical and food industries.

Table 4.50 Principal components (PC) and component loadings extracted from the anti-nutritional parameters analysed from 21 underutilised fruits were used to interpret the PC

	PRINCIPAL COMPONENT	
	PC1	**PC2**
Eigen Value	1.44	1.06
Percentage of Variance	48.03	35.39
Cumulative Percentage	48.03	83.42
FACTOR LOADING/EIGEN VECTOR		
Phytic (mg100 g–1)	**0.916**	-0.14
Tannin (mg100 g–1)	**-0.663**	-0.57
Total oxalate (mg100 g–1)	-0.074	**0.93**

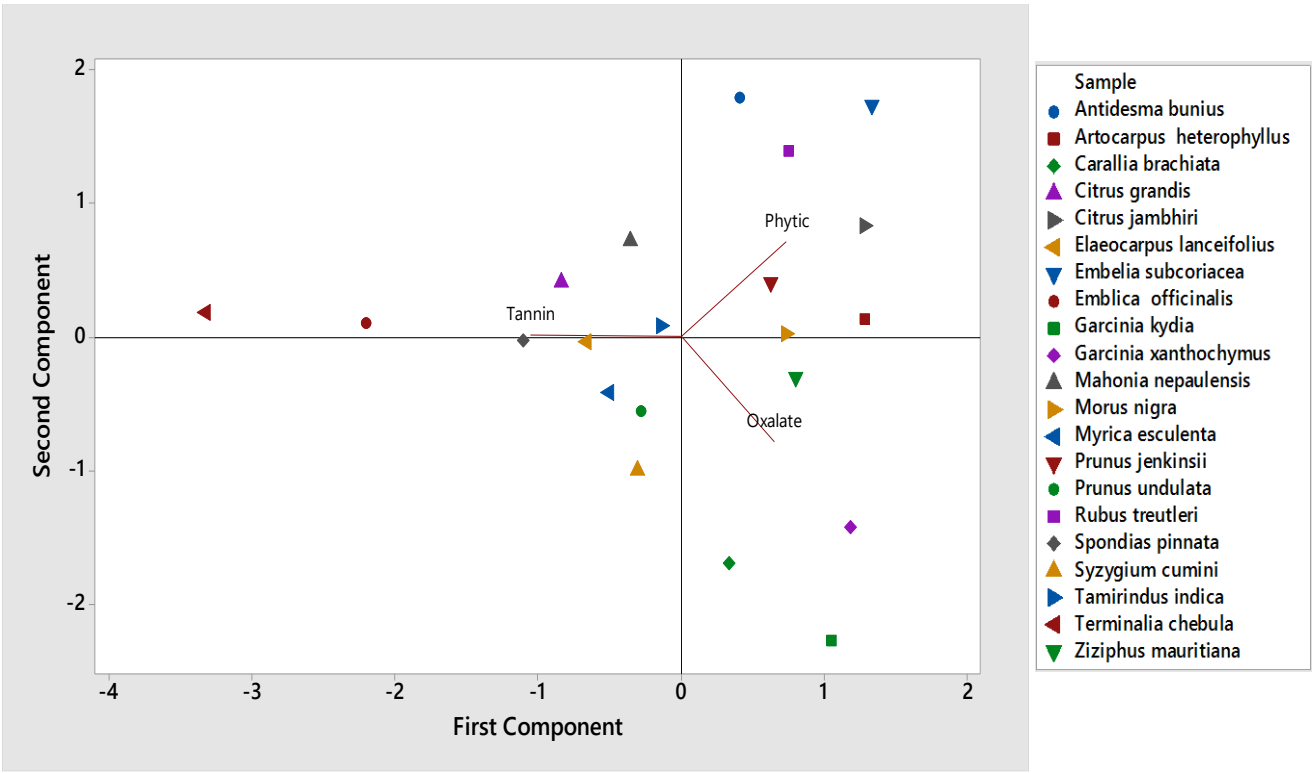


Fig. 4.100 Principal Component Analysis (PCA) Bi-plot (score and loading plot) of the anti-nutritional components of the 21 underutilised fruits highlighting the variations of bioactive components

4.6. Cluster Analysis

4.6.1. Nutritional Compositions

On the basis of the performance of different nutritional characters, the clustering pattern of 21 diverse species of underutilised fruits using the Wards method is presented in Figure 4.101. All the 21 species were grouped into 3 clusters. The maximum number of species was accommodated in cluster-II (15), followed by cluster-I (4) and cluster-III (2) (Table 4.51 and 4.52). The cluster mean by Ward's method for various nutritional characters is presented in Table 4.53. Cluster-I recorded the highest mean for crude fibre (15.65 %), acidity (3.90 %), lignin (8.13 %), P (0.23 %), Ca (392.98 mg 100 g^{-1}), Na (52.56 mg 100 g^{-1}), Cu (9.28 mg 100 g^{-1}) and Zn (5.98 mg 100 g^{-1}). Cluster II recorded the highest mean value for moisture (81.03 %), total ash (3.84 %), fat (2.15 %), starch (46.44 mg 100 g^{-1}), N (4.75 %), Mg (219.82 mg 100 g^{-1}), Co (0.44 mg 100 g^{-1}), Fe (9.10 mg 100 g^{-1}) and Mn (9.13 mg 100 g^{-1}). Out of the 21 diverse underutilised fruits under study, *Emblica officinalis* and *Terminalia chebula* came under major cluster III. The unique nutritional composition may be the reason for being under a separate cluster from the rest of the 19 fruit species. This cluster reported the highest mean value for dry matter (27.17 %), carbohydrate (38.60 %), protein (121.90 %), energy (652.49 kcal 100 g^{-1}), total sugar (27.44 %), reducing sugar (13.32 %), non-reducing sugar (13.41 %), TSS (22.82 °Brix) and the lowest mean value for the traits moisture (72.83 %), total ash (1.90 %), fat (1.17 %), starch (5.98 mg 100 g^{-1}), N (3.23 %), P (0.14 %), Ca (15.18 mg 100 g^{-1}), Na (50.23 mg 100 g^{-1}), Cu (1.51 mg 100 g^{-1}), Zn (3.98 mg 100 g^{-1}).

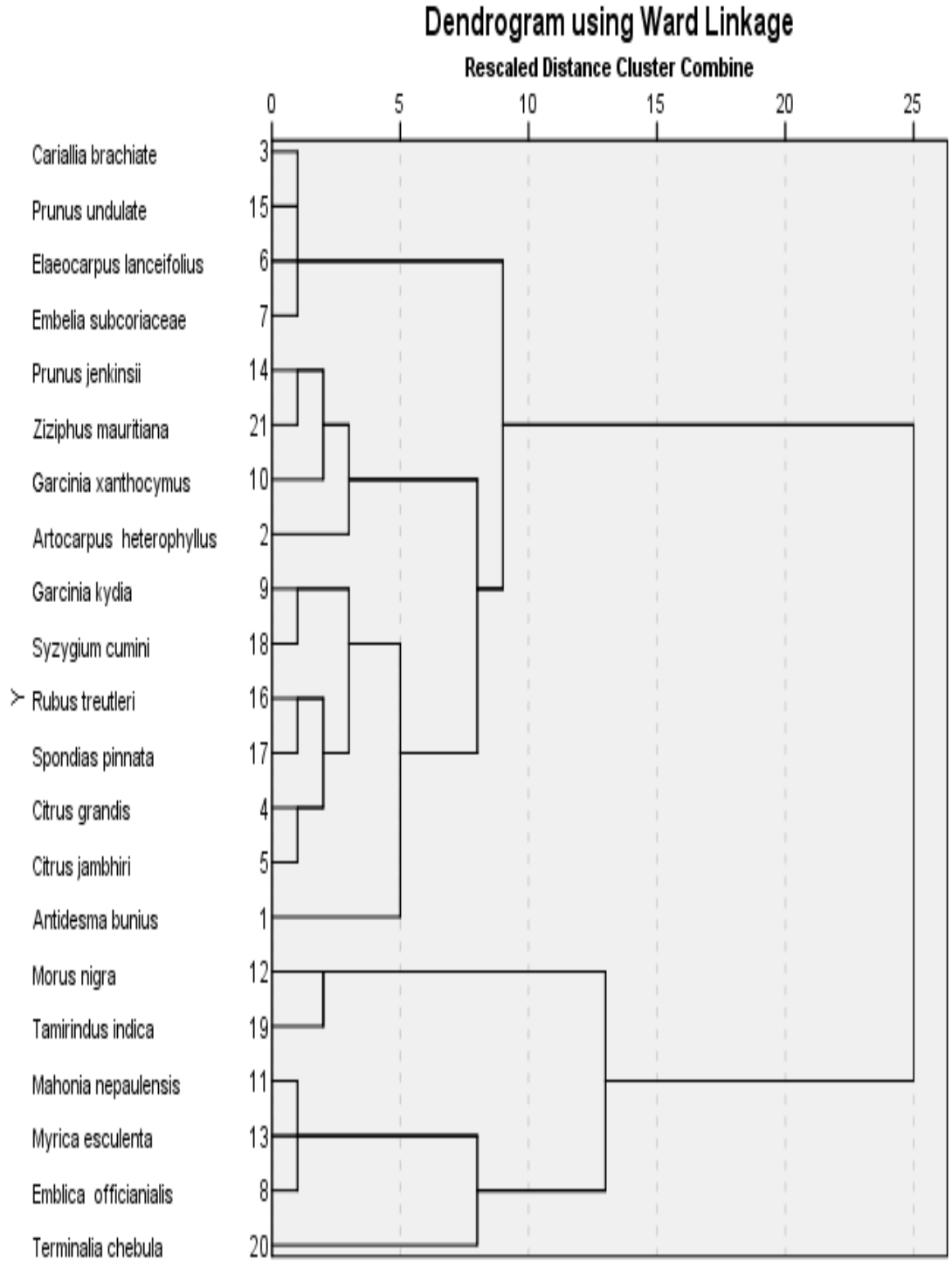


Fig. 4.101 UPGMA-based dendrogram generated by Wards method for fruit nutritional characteristics

Table 4.51 Number of cases in each cluster

Cluster	**1**	**4.00**
	2	**15.00**
	3	**2.00**
Valid		**21.00**
Missing		**0.00**

Table 4.52 Clustering pattern of 21 underutilized fruit species by Wards method based on fruit nutritional characteristics

Cluster I	Cluster II	Cluster III
Antidesma bunius	***Artocarpus heterophyllus***	***Emblica officinalis***
Citrus jambhiri	***Carallia brachiata***	***Terminalia chebula***
Morus nigra	***Citrus grandis***	
Tamirindus indica	***Elaeocarpus lanceifolius***	
	Embelia subcoriacea	
	Garcinia kydia	
	Garcinia xanthochymus	
	Mahonia nepaulensis	
	Myrica esculenta	
	Prunus jenkinsii	
	Prunus undulata	
	Rubus treutleri	
	Spondias pinnata	
	Syzygium cumini	
	Ziziphus mauritiana	

Table 4.53 Cluster mean of fruit nutritional characteristics as per Wards method

Sr.No	Traits	Cluster		
		I	II	III
1.	Moisture (%)	75.24	**81.03**	72.83
2.	Dry Matter (%)	24.76	19.15	**27.17**
3.	Total Ash (%)	3.42	**3.84**	1.90
4.	Crude Fiber (%)	**15.65**	9.23	13.42
5.	Carbohydrate (%)	36.60	36.08	**38.60**
6.	Protein (%)	56.67	45.78	**121.90**
7.	Fat (%)	1.67	**2.15**	1.17
8.	Energy (kcal 100 g^{-1})	289.09	238.64	**652.49**
9.	Starch (mg 100 g^{-1})	25.03	**46.44**	5.98
10.	Total Sugar (%)	3.55	8.94	**27.44**
11.	Reducing Sugar (%)	2.24	6.51	**13.32**
12.	Non Reducing Sugar (%)	1.94	3.88	**13.41**
13.	Acidity (%)	**3.90**	2.69	3.13
14.	TSS °B	8.94	12.38	**22.82**
15.	Lignin (%)	**8.13**	5.17	5.86
16.	Cellulose (mg100 g^{-1})	50.49	53.85	**57.45**
17.	Hemicellulose (%)	**5.86**	4.02	4.35
18.	N (%)	4.05	**4.75**	3.23
19.	P (%)	**0.23**	0.18	0.14
20.	K (%)	1.48	2.01	**4.19**
21.	Ca (mg100 g^{-1})	**392.98**	87.10	50.18
22.	Mg (mg100 g^{-1})	168.54	**219.82**	188.67
23.	Na (mg100 g^{-1})	**52.56**	51.05	50.23
24.	Co (mg100 g^{-1})	0.10	**0.44**	0.12
25.	Cu (mg100 g^{-1})	**9.28**	2.58	1.51
26.	Fe (mg100 g^{-1})	4.74	**9.10**	5.08
27.	Mn (mg100 g^{-1})	4.84	**9.13**	5.40
28.	Zn (mg100 g^{-1})	**5.98**	4.24	3.98

4.6.2. Bioactive Compositions

Based on the performance of different nutritional characters, the clustering pattern of 21 diverse species of underutilised fruits using the Wards method is presented in Figure 4.102. All the 21 species were grouped into 3 clusters. A maximum number of species were accommodated in cluster-II (13), followed by cluster-III (5) and cluster-II (2) (Table 4.54 and 4.55). The cluster mean by Ward's method for various nutritional characters is presented in Table 4.56 Out of the 21 diverse underutilised fruits under study, *Emblica officinalis* and *Spondias pinnata* come under major Cluster-I. The unique nutritional composition may be the reason for being under a separate cluster from the rest of the 19 fruit species. This cluster recorded the highest mean for total phenol (584.3 mg 100 g^{-1}), vitamin C (700 mg 100 g^{-1}) and vitamin E (27.59 mg 100 g^{-1}) and the lowest mean value for total carotenoid (0.46 mg 100 g^{-1}), total chlorophyll (0.03 mg 100 g^{-1}) and alkaloid (92.64 mg 100 g^{-1}). Cluster II recorded the highest mean value for total carotenoid (0.72 mg 100 g^{-1}). Cluster III reported the highest mean value for total flavonoid (400.28 mg 100 g^{-1}), total chlorophyll (0.20 mg 100 g^{-1}), anthocyanin (591.36 mg 100 g^{-1}), alkaloid (161.47 mg 100 g^{-1}) saponin (9.86 mg 100 g^{-1}).

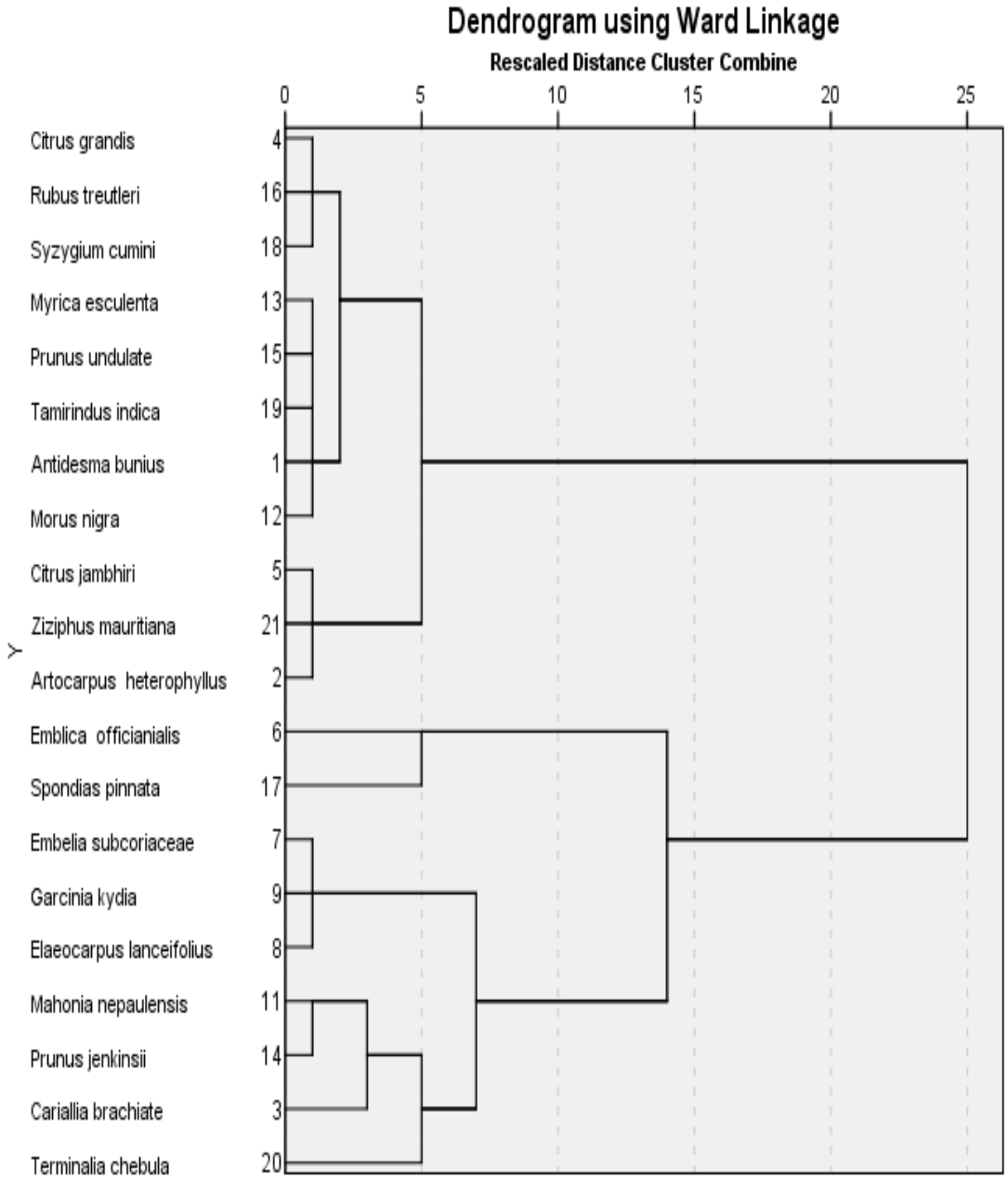


Fig. 4.102 UPGMA-based dendrogram generated by Ward's method for fruit bioactive characteristics

Table 4.54 Number of cases in each cluster

Cluster	**1**	**2.00**
	2	**13.00**
	3	**5.00**
Valid		**20.00**
Missing		**1.00**

Table 4.55 Clustering pattern of 21 underutilized fruit species by Wards method based on fruit bioactive characteristics

Cluster I	Cluster II	Cluster III
Emblica officinalis	***Antidesma bunius***	***Carallia brachiata***
Spondias pinnata	***Artocarpus heterophyllus***	***Elaeocarpus lanceifolius***
	Citrus grandis	***Mahonia nepaulensis***
	Citrus jambhiri	***Prunus jenkinsii***
	Embelia subcoriacea	***Terminalia chebula***
	Garcinia kydia	
	Garcinia xanthochymus	
	Morus nigra	
	Myrica esculenta	
	Prunus undulata	
	Rubus treutleri	
	Syzygium cumini	
	Tamirindus indica	
	Ziziphus mauritiana	

Table 4.56 Cluster mean of fruit's bioactive characteristics as per Wards method

Sr.No	Traits	Cluster		
		I	II	III
1.	Total Phenol	**548.30**	194.26	426.15
2.	Total Flavonoid	336.88	111.03	**400.28**
3.	Total Carotene (mg100 g^{-1})	0.46	**0.72**	1.50
4.	Total Chlorophyll (mg100 g^{-1})	0.03	0.08	**0.20**
5.	Anthocyanin (mg100 g^{-1})	382.92	229.19	**591.36**
6.	Vitamin C (mg100 g^{-1})	**700**	92.72	111.79
7.	Vitamin E (mg100 g^{-1})	**27.59**	24.08	22.86
8.	Alkaloid (mg100 g^{-1}AE)	92.64	133.15	**161.47**
9.	Saponin (mg100 g^{-1} DE)	6.50	6.43	**9.86**

4.6.3 Antinutritional composition.

On the basis of the performance of different antinutritional characters, the clustering pattern of 21 diverse species of underutilised fruits using the Wards method is presented in Figure 4.103. All the 21 species of underutilised fruits were grouped into 3 clusters. The maximum number of species was accommodated in cluster-I (18), followed by cluster-II (2) and cluster-III (1) (Table 4.57 & 4.58). The cluster mean by Ward's method for various nutritional characters is presented in Table 4.59. Cluster-I recorded the highest mean for phytic acid (4.58 mg 100 g^{-1}) and total oxalate (7.13 mg 100 g^{-1}). Cluster III recorded the highest mean value for tannin (483.79 mg 100 g^{-1}).

The clustering patterns showed that the fruit species collected from the same geographical area or same family did not necessarily belong to the same cluster due to genetic factors (Wangchu *et al*., 2017).

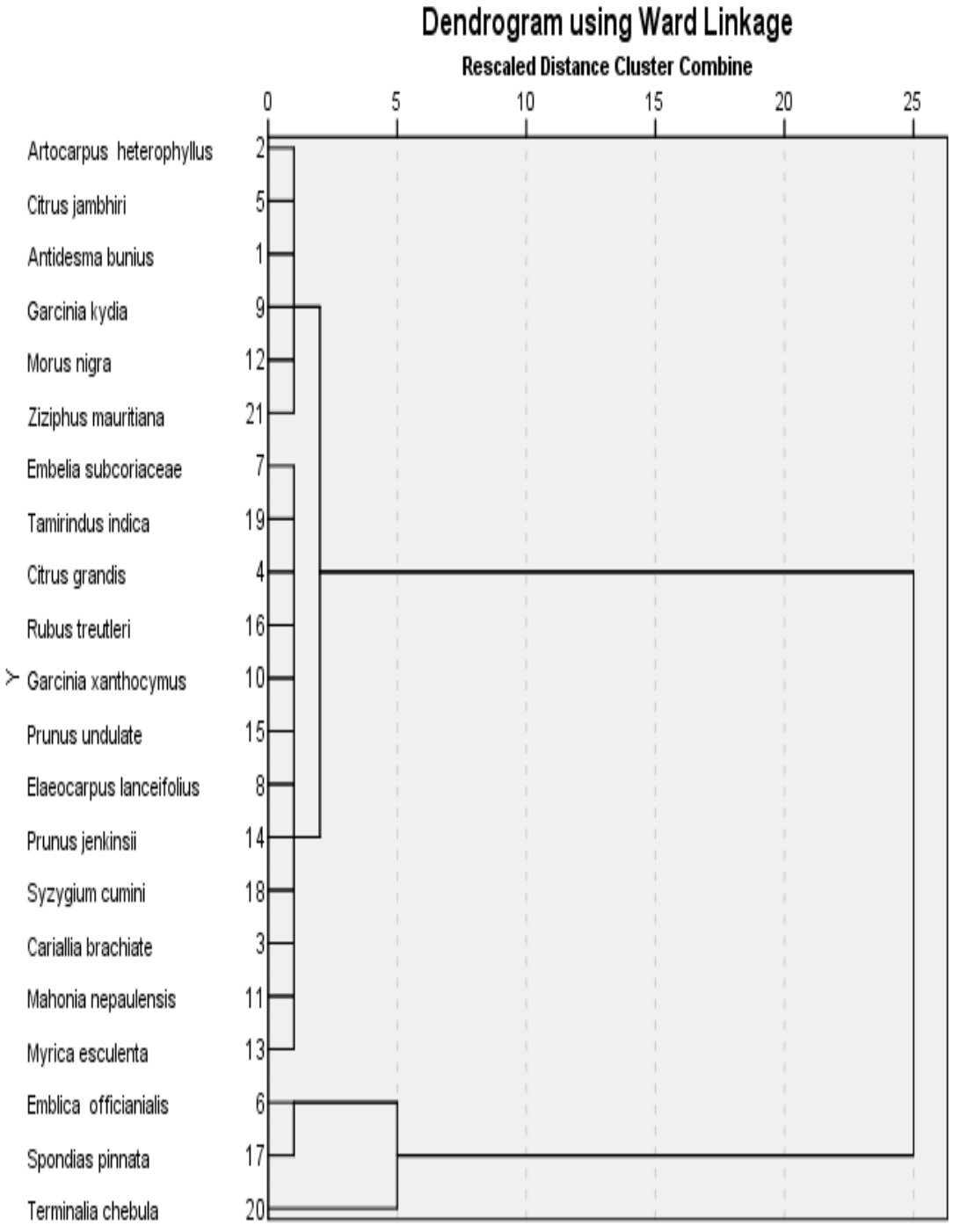


Fig. 4.103 UPGMA-based dendrogram generated by Ward's method for fruit Anti-nutritional characteristics

Table 4.57 Number of cases in each cluster

Cluster	**1**	**18**
	2	**2**
	3	**1**
Valid		**21**
Missing		**0**

Table 4.58 Clustering pattern of 21 underutilized fruit species by Ward's method based on fruit anti-nutritional characteristics

Cluster I	Cluster II	Cluster III
Emblica officinalis	***Antidesma bunius***	***Terminalia chebula***
Spondias pinnata	***Artocarpus heterophyllus***	
	Carallia brachiata	
	Citrus grandis	
	Citrus jambhiri	
	Embelia subcoriacea	
	Elaeocarpus lanceifolius	
	Garcinia kydia	
	Garcinia xanthochymus	
	Mahonia nepaulensis	
	Morus nigra	
	Myrica esculenta	
	Prunus jenkinsii	
	Prunus undulata	
	Rubus treutleri	
	Syzygium cumini	
	Tamirindus indica	
	Ziziphus mauritiana	

Table 4.59 Cluster mean of fruit anti-nutritional characteristics as per Wards method

Sr. No	Traits	Cluster		
		I	II	III
1.	Phytic (mg 100 g^{-1})	**4.58**	3.68	3.61
2.	Total oxalate (mg 100 g^{-1})	**7.13**	4.84	4.18
3.	Tannin (mg 100 g^{-1})	84.46	264.55	**483.78**

Chapter V

SUMMARY AND CONCLUSION

Summary and Conclusion

The present investigation entitled "Nutrient profiling, functional food compounds and antioxidant properties of some underutilised fruits of Mizoram" was carried out at the Department of Horticulture Aromatic and Medicinal Plants, Mizoram University, Tanhril, Mizoram during the year 2021-2023 The twenty-one (21) selected underutilised fruits were collected from eight (8) districts of Mizoram, for fruit nutrient analysis, their antioxidant and anticancer properties to acquire information on its physical characteristics, nutritional and anti-nutritional compositions. The salient findings of the study are summarised and concluded below.

5.1 Fruit Nutrient Analyses

5.1.1 Proximate composition

5.1.1.1 The highest moisture percentage was found in *Citrus jambhiri* (95.43±0.97 %) and the lowest was recorded in *Tamarindus indica* (59.40±1.12 %).

5.1.1.2 *Tamarindus indica* exhibited maximum dry matter content (40.60±1.12 %) and *Citrus jambhiri* recorded the minimum dry matter content (4.57±1.68 %).

5.1.1.3 The highest total ash percentage was recorded in *Citrus grandis* (9.21±0.06 %) and the lowest was observed in *Emblica officinalis* (1.16±0.12 %).

5.1.1.4 Crude fiber percentage was found to be highest in *Antidesma bunius* (24.27±1.43 %) and the lowest was recorded in *Prunus jenkinsii* (3.43±0.46 %).

5.1.1.5 The highest carbohydrate was observed in *Myrica esculenta* (79.84±3.74 %)**,** while the lowest was observed in *Antidesma bunius* (5.91±0.04 %)**.**

5.1.1.6 *Terminalia chebula* possessed maximum protein content (89.16±3.41 %), while the lowest was observed in *Artocarpus heterophyllus* (1.88±0.04 %).

5.1.1.7 The highest Fat percentage was observed in *Garcinia kydia* (6.23±0.06 %), while lowest was recorded in *Ziziphus mauritiana* (0.15±0.05 %).

5.1.1.8 *Terminalia chebula* reported the highest energy kcal 100 g^{-1} (780.31±8.01 kcal 100 g^{-1}) while the lowest Energy kcal was recorded in *Antidesma bunius* (59.00±0.58 kcal 100 g^{-1}).

5.1.1.9 Starch content was observed maximum in *Artocarpus heterophyllus* (222.67±6.83 mg100 g^{-1}), while the lowest was recorded in *Antidesma bunius* (3.12±0.004 mg100 g^{-1}).

5.1.1.10 Total sugar percentage was highest in *Emblica officinalis* (28.13±1.33 %) while the lowest was observed *in Antidesma bunius* (2.24±0.25 %).

5.1.1.11 Reducing sugar percentage was observed highest in *Garcinia xanthochymus* (14.61±1.76 %) and the lowest was observed in *Citrus jambhiri* (1.35±0.42 %).

5.1.1.12 *Terminalia chebula* was found to be maximum in non-reducing sugar (13.83±2.75 %) and the lowest was observed in *Prunus undulata* (0.40±0.08 %).

5.1.1.13 *Citrus jambhiri* reported the highest titratable acidity (6.19±0.37 %) and the lowest was recorded in *Syzygium cumini* (0.74±0.25 %)

5.1.1.14 The highest TSS was observed in *Terminalia chebula* (20.77±0.32˚B) while the lowest was recorded in *Antidesma bunius* (3.27±0.15 ˚B)

5.1.1.15 Lignin percentage was recorded highest in *Tamarindus indica* (13.69±0.01%), and the lowest was obtained in *Terminalia chebula* (2.06±0.01 %).

5.1.1.16 Cellulose was found to be maximum in *Embelia subcoriacea* (75.43±1.51 mg 100 g^{-1}) while it was lowest in *Mahonia nepaulensis* (37.72±0.31 mg 100 g^{-1}).

5.1.1.17 The hemicellulose content was highest in *Tamarindus indica* (12.09±0.01 %) and was lowest in *Cariallia brachiate* (1.87±0.01 %).

5.1.2 Mineral composition

5.1.2.1 Nitrogen percentage was observed to be maximum in *Citrus grandis* (12.14±0.49 %) and the lowest was observed in *Embelia subcoriacea* (0.41±0.02 %).

5.1.2.2 Phosphorus percentage was recorded highest n *Citrus grandis* (0.46±0.02 %) and lowest was recorded in *Garcinia xanthochymus* (0.04±0.01 %).

5.1.2.3 The highest potassium percentage was recorded in *Terminalia chebula* (4.37±0.05 %) and the lowest was recorded in *Garcinia xanthochymus* (0.03±0.01 %).

5.1.2.4 *Morus nigra* was recorded to be the highest in calcium (477.75±3.00 mg 100 g–1) and the lowest amount of calcium was recorded in *Terminalia chebula* (7.40±3.42 mg 100 g–1).

5.1.2.5 Magnesium content was recorded highest in *Prunus undulata* (377.33±3.45 mg 100 g^{-1}) and the lowest was recorded in *Artocarpus heterophyllus* (28.33±15.04 mg 100 g^{-1}).

5.1.2.6 Sodium was highest in *Rubus treutleri* (136.05±1.18 mg 100 g–1) and was lowest in *Garcinia xanthochymus* (4.94 ± 0.02 mg 100 g^{-1}).

5.1.2.7 Cobalt content was found to be maximum in *Garcinia kydia* (3.95±0.10 mg 100 g^{-1}) while the lowest was recorded in *Elaeocarpus lanceifolius* (0.02 ± 0.01 mg 100 g^{-1}).

5.1.2.8 The copper was reported to be richest in *Tamarindus indica* (32.94±1.62 mg 100 g^{-1}) and the lowest was observed in *Morus nigra* (0.45±0.04 mg 100 g^{-1}).

5.1.2.9 Iron content was found to be the highest in *Garcinia kydia* (43.09±1.56 mg 100 g^{-1}) while it was lowest in *Artocarpus heterophyllus* (1.13±0.11 mg 100 g^{-1}).

5.1.2.10 Manganese content was obtained to be maximum in *Myrica esculenta* (27.66±1.04 mg 100 g^{-1}) and the lowest was observed in *Citrus grandis* (0.50±0.04 mg 100 g^{-1}).

5.1.2.11 The highest zinc content was found in *Tamarindus indica* (11.43±0.71 mg 100 g^{-1}) and the lowest was reported in *Spondias pinnata* (1.97±0.01 mg 100 g^{-1})

5.2 Bioactive components of the fruits

5.2.1 The highest phenol content was observed in *Terminalia chebula* (792.28±0.32 mg 100 g^{-1}) and the lowest was recorded in *Ziziphus mauritiana* (38.95±0.97 mg 100 g^{-1}).

5.2.2 Flavonoid was obtained to be the highest in *Carallia brachiata* (632.62±0.65 mg 100 g^{-1}) and the lowest in *Tamarindus indica* (22.41±0.24 mg 100 g^{-1}).

5.2.3 *Prunus jenkinsii* was found to be highest in total carotenoid (1.97±0.21 mg 100 g^{-1}) and the lowest was recorded in *Syzygium cumini* (0.03±0.005 mg 100 g^{-1})

5.2.4 Total chlorophyll content was observed to be maximum in *Artocarpus heterophyllus* (0.507±0.053 mg 100 g^{-1}) and the lowest was observed in *Citrus grandis* (0.004±0.001 mg 100 g^{-1}).

5.2.5 Anthocyanin content was reported to be the richest in *Carallia brachiata*

(816.67±5.28 mg 100 g^{-1}) and the lowest was recorded in *Artocarpus heterophyllus* (14.21±1.05 mg 100 g^{-1}).

5.2.6 Vitamin C was recorded highest in *Emblica officinalis* (756.00±4.00 mg 100 g^{-1}) while the lowest was observed in *Artocarpus heterophyllus* (38.33±3.51 mg 100g^{-1}).

5.2.7 Highest vitamin E content was observed in *Antidesma bunius* (36.69±0.80 mg 100 g^{-1}) and the lowest was in *Prunus jenkinsii* (19.13±0.55 mg 100 g^{-1}).

5.2.8 *Terminalia chebula* (192.84±4.84 mg 100 g^{-1}) reported the highest alkaloid content, while it was lowest in *Garcinia xanthochymus* (41.67±2.17 mg 100 g^{-1}).

5.2.9 Saponin content was reported to be maximum in *Carallia brachiata* (13.70±0.50 mg 100 g^{-1}) and the lowest was observed in *Ziziphus indica* (0.98±0.38 mg 100 g^{-1}).

5.3 Antinutrient composition.

5.3.1 Phytic acid was found highest in *Embelia subcoriacea* (7.60±0.16mg100 g^{-1}) while the lowest was observed in *Garcinia Kydia* (4.52±0.04 mg 100 g^{-1})

5.3.2 The highest total oxalate was found in *Garcinia kydia* (13.42±1.10 mg 100 g^{-1}) and the lowest was observed in *Citrus grandis* (2.64±0.44 mg 100 g^{-1})

5.3.2 *Terminalia chebula* recorded the highest tannin content (483.78±7.49 mg 100 g^{-1}) and the lowest was observed in *Garcinia kydia* (46.44±0.39 mg 100 g^{-1}).

5.4 Anticancer and antioxidant properties

5.4.1 Anticancer properties:

5.4.1.1 The methanolic extract of *Elaeocarpus lanceifolius* (29.16±2.38 µg/ml) reported the lowest IC_{50} for MTT Assay followed by *Embelia Subcoriacea (31.54±2.83 µg/ml) and Terminalia chebula* (39.93±1.64 µg/ml) which have the highest cytotoxicity form the results of MTT assay for the twenty one (21) underutilized fruit.

5.4.1.2 Methanolic extract of *Elaeocarpus lanceifolius* induced the best cytotoxic activity through apoptosis against A549 cell at 50 µg/ml followed by *Embelia subcoriacea* and *Terminalia chebula* which were statistically at par.

5.4.1.3 The fruit extracts *Elaeocarpus lanceifolius, Embelia subcoriacea and Terminalia chebula* significantly decreased glutathione (GSH) concentration and the activities of glutathione-s-transferase (GST) and superoxide dismutase (SOD) when compared to the untreated control at 50 µg/ml.

5.4.1.4 Among the five (5) fruit extracts studied, *Elaeocarpus lanceifolius* extract induced the highest efficacy with an up-regulation of pro-apoptotic genes such as Bax and Bid followed by *Embelia subcoriacea* when compared to the untreated control and induced significant down-regulation of anti-apoptotic genes Bcl-X_L and BCl-2.

5.4.1.5 Among the five (5) extracts, *Elaeocarpus lanceifolius* induced the highest Caspase-3 activity followed by *Embelia subcoriacea* and *Terminalia chebula* which were statistically at par with 5-Fluorourasil (5FU).

5.4.1.6 Among the five (5) extracts, *Elaeocarpus lanceifolius induced the highest* Caspase-6 activity followed by *Embelia subcoriacea and Terminalia chebula* which were statistically at par with 5FU.

5.4.1.7 LC-HRMS was carried out on five (5) fruit species which were selected on the basis of their cytotoxicity against A549 cells from MTT assay. In *Elaeocarpus lanceifolius* Tyr-Oet, Isoflurophate,Uh-301, L-Kynurenine, Dodine, Estrone, Brn 0575813, Soyasapogenol B 3-O-D-Glucuronide were found to be prominent. In *Embelia subcoriacea* Bergaptol, Resistomycin, (R)-Mevalonate, Chitobiose, Prostaglandin B1, (15Z)-Tetracosenoic acid, Acetylleucyl-leucyl-norleucinal were found to be prominent. In *Terminalia chebula* Dihydro-heme d1, Deoxyuridine, trans-Cinnamate, Terpendole C and Isopropamide were found to be prominent. In *Garcinia kydia,* Taurine,trans-Cinnamate and (15Z)-Tetracosenoic acid were found to be prominent. In *Emblica officinalis* in the present study, out of which Citrate, Lancerin, Hydroxytamoxifen and L-Octanoylcarnitine were found to be prominent.

5.4.1.8 Molecular docking was employed to determine the possibility of binding between the bioactive compounds and the receptor protein and the results were analysed. In this work, we identified significant bioactive compounds from *Elaeocarpus lanceifolius, Embelia subcoriacea, Terminalia chebula, Garcinia kydia and Emblica officinalis* which have proven to possess higher cytotoxicity

towards the A549 cancer cells through MTT assay where several compounds were identified and further analysed through molecular docking to see the interaction of the compounds and its interaction with the protein AKT1 which is a cancer drug target. The results demonstrated that out of the compound identified the highest binding affinity was found in the compounds Resistomysin (-10.1 kcal mol^{-1}), Berberine (-7.6 kcal mol^{-1}), Silymarin (-7.5 kcal mol^{-1}), Sulindac (-7.5 kcal mol^{-1}) followed by 20-Hydroxyecdysone (-7.0 kcal mol^{-1}) respectively. The docking pose of the molecules which showed a lesser ΔG score than 5-fluorouracil (-7.0 kcal/mol).

5.4.2 Antioxidant activity

5.4.2.1 The highest DPPH antioxidant activity having the lowest IC_{50} value was observed in *Emblica officinalis* (3.29±0.05 µg/ml) and the lowest antioxidant activity using DPPH was observed in *Citrus jambhiri* (1217.00±7.59 µg/ml) having a higher IC_{50} value.

5.4.2.2 *Emblica officinalis* possessed the highest ABTS scavenging activity (lowest IC_{50}; 38.33±1.01 µg/mL) while the lowest ABTS scavenging activity with the highest IC_{50} values was observed in *Garcinia xanthochymus* (2921.67±8.09 µg/mL).

5.4.2.3 Maximum antioxidant activity using superoxide anion radical scavenging activity with the lowest IC_{50} values was observed in *Terminalia chebula* (0.65±0.23 µg/mL). Conversely, the lowest superoxide scavenging activity having the highest IC_{50} values was observed in *Garcinia xanthochymus* (1501.67±2.01 µg/mL).

5.4.2.4 The maximum ferric reducing activity of fruit extracts was observed in *Emblica officinalis* having the lowest IC_{50} values (0.51±0.08 µg/mL) the lowest ferric-reducing antioxidant activity was observed in *Embelia subcoriacea* (42.76±0.63) having the highest IC50 values.

5.4.2.5 GCMS was carried out for 21 underutilised fruit species to identify the major compounds present. All the species have different types of compounds present which was identified from the National Institute of Standards and Technology (NIST) library in the GCMS.

5.4.3 Correlations, principal component analysis and cluster analysis.

5.4.3.1 The correlation conducted among the twenty one fruits in their nutritional, bioactive and anti-nutritional properties showed high correlations among each other which could help us in accessing the fruit's nutritional profile.

5.4.3.2 The principal component analysis identifies several components and variances in the nutritional, bioactive and antinutritional data of the 21 underutilised fruits. This could help us identify the important components for the selection of highly nutritional fruits.

5.4.3.3 The cluster analysis divided the studied 21 underutilised fruits into 3 main clusters according to their nutritional, bioactive and antinutritional characteristics which help us cluster the studied underutilised fruits accordingly.

Conclusion

This study provides a comprehensive report and documentation of the extensive nutritional potential of the underutilised fruits of Mizoram, northeast India. It highlights that these species represent a significant and advantageous source of essential nutrients, encompassing carbohydrates, fats, proteins, sugars, vitamins, pigments, antioxidants, and additional components. These fruits may be regarded as nutritionally comparable to the prevalent commercial fruits of today. Research indicates that each fruit possesses distinct potential, characterised by a rich nutritional profile, functional food properties, and significant anticancer and antioxidant attributes.

Gaining comprehensive insights into the health-promoting properties of these underutilised fruits may lead to an improved understanding of their benefits, particularly in the contexts of functional foods, nutraceuticals, and ethnomedicine. The year-round availability of these indigenous fruit species presents considerable potential for enhancing food and nutritional security during times of food scarcity. The creation of a nutritional composition database will inform the community about the benefits of consuming locally sourced and nutritious foods, thereby preserving their cultural heritage and facilitating targeted domestication efforts for future generations. Recognising the nutraceutical properties of these fruits and their potential as value-added products in local, national, and global markets is crucial for highlighting their importance in nutrition and the conservation of natural resources, while promoting health and well-being. The promotion of the consumption and commercialisation of underutilised fruits has the potential to enhance food security, generate income, and preserve traditional knowledge within the region. The current results and findings may assist breeders in developing superior germplasm from diverse underutilised edible fruits and improving their nutritional profiles.

Among the fruit studied *Terminal chebula* was observed to be highest in TSS, Non Reducing Sugar, Potassium, Phenol, Alkaloid, Saponin, Tannin, and MTT cytotoxic assay. *Rubus treutleri* have higher concentration of Mg, Fe, Zn and Total Ash. *Tamarindus indica* possess the highest concentration of Crude fibre, Mn, Lignin,

Hemicellulose. *Morus nigra* was a rich source of Fat, Energy, Ca. *Emblica officianialis* is a significant source of Vitamin C, Total Sugar and DPPH antioxidant activity. *Mahonia nepaulensis* was observed to have a higher concentration in Carbohydrate, Co andAnthocyanin. *Citrus jambhiri* was a good source of Acidity and Moisture while *Citrus grandis* was shown to be a good source of Nitrogen and Phosphorus.

From the parameters we can conclude that many underutilized fruits could have a huge potential for their nutrients and phytochemical compounds. These underutilized fruit could be immensely promoted only if people are made aware of the potential of these fruits as many fruits under these underutilised fruits are not palatable.

5.6 Future Scope Of Work:

1. Further exploration, collection and documentation of more underutilised fruit species endemic to Mizoram exploring their nutritional and medicinal values and identifying promising species for ethnomedicine, pharmaceutical and nutraceutical properties.
2. Diversity analysis of each of these fruit species within the region can be carried out and standardisation of scientific agro-techniques for the promising underutilised fruit species needs to be done.
3. Identification of promising underutilised fruit species by registering them under geographical indication (GI tag) to aid in their popularity in the market as well as in conservation.

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APPENDICES

BRIEF BIODATA

Name **:** Rody Ngurthankhumi

Date of Birth **:** 05.11.1992

Gender **:** Female

Father's name **:** Mr. Lalrinmawia (L)

Mother's name **:** Mrs. F. Rohmingthangi

Permanent address **:** H/N:B-16, Tuikual South, Aizawl, Mizoram

Academic profiles

Qualification	Passing year	University/ Institution	Percentage	Division
M.Sc. Horticulture (Fruit Science)	2018	Mizoram University	81.9 %	Distinction
B.Sc. (Hons.) Horticulture	2016	Central Agricultural University-Imphal	83.4 %	Distinction
HSSLC	2011	MBSE	54.4 %	2nd
HSLC	2009	MBSE	72.4 %	1st

List of Publications:

1. **Rody Ngurthankhumi**, T.K. Hazarika, Zothansiama and Esther Lalruatsangi. 2024. Nutritional composition and anti-nutritional properties of wild edible fruits of northeast India. *Journal of Agriculture and Food Research*, **16,** p.101221.
2. **Rody Ngurthankhumi**, T.K. Hazarika, Esther Lalruatsangi. T. Lalnunsangi and H. P. Lalhmangaihzuali. 2024. Bioactive constituents and health promoting compounds of few wild edible fruits of North-East India. *International Journal of Food Properties*, **27(1),** pp.927-950.
3. **Rody Ngurthankhumi**, T.K. Hazarika, Esther Lalruatsangi, Zothansiama, H Lalhmachhuani and Panthor Debbarma. 2024. Anticancer Screening of Few Wild Edible Fruits in Mizoram, Northeast India using MTT Assay. *Indian Journal of Agricultural Research*, **1,** p.6.
4. Malsawmkimi, Hazarika, T.K., Debbarma, P. and **Ngurthankhumi, R**., 2024. Effects of bio-fertiliser enriched organic amendments on flowering and fruiting behaviour, yield and quality attributes of Khasi mandarin (*Citrus reticulata* Blanco). *Biological Agriculture & Horticulture*, pp.1-15.
5. Hazarika, T.K., Malsawmkimi and **Ngurthankhumi, R.,** 2024. Biodynamics enriched organic amendments in improving leaf and soil nutrition of Khasi mandarin (*Citrus reticulata* Blanco). *Journal of Plant Nutrition*, **47(6):** pp.868-880.
6. Hazarika, T.K., Devi, L.S., Ningombam, L., Debbarma, P. and **Ngurthankhumi, R.**, 2024. Unravelling the genetic diversity of *Garcinia pedunculata* Roxb. with multivariate analysis. *Genetic Resources and Crop Evolution*, **71(6):** pp.2375-2397.
7. Hazarika, T.K., Lalduhsangi, R.C. and **Ngurthankhumi, R**., 2023. Morpho-physico-biochemical characterisation of Avocado (*Persea americana*) for selection of elite types. *The Indian Journal of Agricultural Sciences*, **93(1)**, pp.78-83.

8. Talukdar, P., Bhuyan, R., **Ngurthankhumi, R**. and Talukdar, D., 2023. Nutritional Evaluation of Spent Mushroom Substrate of Fibrous Agricultural Residues at Different Phases of Mushroom Harvest.

9. Tridip Kumar Hazarika, Basik Tayeng, **Rody Ngurthankhumi**, Esther Lalruatsangi, Kalidas Upadhyaya and Nicolee Lyngdoh, 2022. Unlocking wild edible fruits of indo-burma biodiversity hot spot, Arunachal Pradesh, India, to support food security and sustainable rural livelihood. *Sustainability*, **14(23):** p.16088.

10. Hazarika, T.K., Malsawmkimi and **Ngurthankhumi, R.,** 2022. Phenological attributes, fruit set, fruit drop, yield and quality of Khasi mandarin orange as influenced by application of organic amendments and biodynamic preparations. *Biological Agriculture & Horticulture*, **38(4):** pp.235-246.

11. Hazarika, T.K., Lalhriatpuia, C., **Ngurthankhumi, R**., Lalruatsangi, E. and Lalhmachhuani, H., 2023. Edible coatings in extending the shelf life of fruits: a review. *Indian Journal of Agricultural Research*, **57(5):** pp.555-558.

12. Hazarika, T.K. and **Ngurthankhumi, R**., 2021. Genetic variability of star gooseberry in North East India. *Indian Journal of Horticulture*, **78(03):** pp.244-250.

Seminar/Symposium/Training attended:

1. Attended the Webinar (13th October 2020) organized by the Department of Horticulture, Aromatic and Medicinal Plants (HAMP), Mizoram University, Aizawl, on the Topic entitled "Recent Trends in Post Harvest Preservation of Fruits & Vegetables".
2. Oral presentation at International E-conference on 'Advances and Future Outlook in Biotechnology and crop improvement for sustainable productivity' organized by UHS, Bhagalkot and COH, Bangalore from 24th to 27th November 2020.
3. Participated in the DST, Government of India Sponsored training program on "Climate Change Adaptation for Natural Resource Management for the State of Mizoram" during March 11-15, 2021at Mizoram University, Mizoram.
4. E- Poster presentation on 'National Online Training on Conservation, Management & Utilization of Horticultural Genetic Resources for Livelihood and Nutritional Security' organised on November 22-26, 2021by ICAR-IIHR, Bengaluru.
5. Attended National Workshop on "Hydroponic Cultivation" under NAHEP-IG ICAR, New Delhi in Virtual Mode held on 28-29, March, 2022 organized by the College of Horticulture and Forestry, Jhalawar Rajasthan.
6. Best Oral Presentation at National Seminar on 'Fruit Production on Eastern Tropical Region of India-Challenges and Opportunity" organized by Central Horticultural Experiment Station (ICAR-IIHR), Bhubaneshwar in collaboration with Society for Promotion of Horticulture, ICAR-IIHR, Bengaluru during March 24-26, 2022.
7. Participated in the three days National Seminars on "Recent Trends in Seed Production, Processing and Marketing: An Entrepreneurship Development" organised by the College of Horticulture and Forestry, Central Agricultural University (Imphal), Pasighat, Arunachal Pradesh, under Instructional Development plan of National Agricultural Higher Education Project held on 26th to 29th March 2022
8. Participated in the National Seminar on Value Addition and Marketing of Horticultural Crops, Medicinal and Aromatic Plants: Opportunities and

Challenges & Training on suitable Aromatic Crops for Mizoram under Aroma Mission-III on 15th and 16th May 2023, jointly organised by Department of Horticulture, Aromatic and Medicinal Plants (HAMP), Mizoram University, Aizawl and CSIR- Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, U.P. India.

9. Best oral presentation on the occasion of two day National Seminar on "Utilization and Conservation of Plant Resources for Sustainable Development" organised by Biodiversity Research Centre, Department of Environmental Science, Mizoram University on 1st -2nd June, 2023

10. Oral presentation at the International Conference on "Climate Change and Natural Resource Management for Sustainable Development" hosted by the School of Earth Science & Natural Resource Management, Mizoram University, from 13-15th March. 2024

11. Best oral presentation at the National Seminar on "Biodiversity, Conservation, Utilization and Commercialization of Medicinal and Aromatic Plants" on February 08-09, 2024, organised by Department of Botany, Mizoram University, Aizawl.

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE:	**Rody Ngurthankhumi**
DEGREE:	**Doctor of Philosophy**
DEPARTMENT:	**Department of Horticulture, Aromatic & Medicinal Plants**
TITLE OF THESIS:	**Nutrient Profiling, Functional Food Compounds and Antioxidant Properties of Some Underutilized Fruits of Mizoram**
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DEPARTMENT OF HORTICULTURE,

AROMATIC AND MEDICINAL PLANTS

ABSTRACT

NUTRIENT PROFILING, FUNCTIONAL FOOD COMPOUNDS AND ANTIOXIDANT PROPERTIES OF SOME UNDERUTILIZED FRUITS OF MIZORAM

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DEPARTMENT OF HORTICULTURE, AROMATIC AND MEDICINAL PLANTS
SCHOOL OF EARTH SCIENCES AND NATURAL RESOURCES MANAGEMENT
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ABSTRACT

NUTRIENT PROFILING, FUNCTIONAL FOOD COMPOUNDS AND ANTIOXIDANT PROPERTIES OF SOME UNDERUTILIZED FRUITS OF MIZORAM

BY

RODY NGURTHANKHUMI

DEPARTMENT OF HORTICULTURE, AROMATIC AND MEDICINAL PLANTS

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SUBMITTED

In partial fulfillment of the requirement of the degree of Doctor of Philosophy in Horticulture, Aromatic and Medicinal Plants of Mizoram University, Aizawl.

Wild, underutilized fruits are an untapped wealth of nutrients and minerals, and huge numbers are found in the vast wilderness of northeast India, including the hilly state of Mizoram. However, their nutritional properties still need to be explored. These fruits possess market value but are not extensively cultivated and are seldom available in mainstream markets. These fruits have deep cultural roots in specific regions and are cherished as traditional crops. They play a vital role in the lives of local and rural communities, serving as a primary source of traditional medicine and essential nutrients, such as vitamins and micronutrients. These fruits are highly valued in areas where access to commercially available nutrient-rich fruits is limited and often unaffordable. Upon conducting a thorough nutritional analysis of these underutilized fruits, it becomes evident that their nutritional quality often matches and, in certain instances, surpasses that of domesticated varieties of fruits that require extensive cultivation. They provide affordable yet nutritious options for a wide range of individuals living in both urban and rural regions. Numerous underutilized fruits remain undiscovered or underutilized despite their significant nutritional benefits. Many of these underutilized fruits are abundant in northeastern India. The north-eastern hill region is renowned for its exceptional biodiversity, vibrant ethnic diversity, and traditional culture.

In light of the aforementioned details, the present investigation study, titled "Nutrient profiling, functional food compounds and antioxidant properties of some underutilized fruits of Mizoram," was conducted at the Department of Horticulture Aromatic and Medicinal Plants, Mizoram University, Tanhril, Mizoram, from 2021 to 2023. The underutilized fruits were sourced from various districts in Mizoram. The findings revealed that these underutilized fruits possessed a varying source of nutritional, functional food components, anticancer and antioxidant properties revealing their immense untapped nutritional profile.

The experiment was carried out in seven districts of Mizoram: Aizawl, Kolasib, Lunglei, Serchhip, Champhai, Saitual, and Khawzawl, each representing distinct agro-climatic zones. Fruit samples were obtained from several sites

according to their seasonal availability. A total of 21 underutilized fruit species were gathered. These species were represented by 15 families, with the Rosaceae family having the largest representation with 3 species. The Phyllanthaceae, Rutaceae, and Clusiaceae families each had 2 species, and so on. The fruit samples were sent to the department of Horticulture Aromatic and Medicinal Plants Mizoram University Aizawl, Mizoram for the analysis of nutritional and biochemical characteristics along with their antioxidant and antinutritional properties.

The twenty-one (21) underutilized fruits were initially cleaned with an aqueous ethanol solution and subsequently rinsed with distilled water. This method was implemented to eliminate any impurities or particles present on the surface of the fruits. After washing, the fruits were dried through absorption using blotting techniques. The fruits were subsequently divided into smaller segments. The specimen underwent desiccation in a controlled atmosphere within an oven, with the temperature regulated between 50-60˚C. It was then ground into a fine powder and filtered using a 20 µm mesh. Subsequently, the specimens were stored in hermetically sealed containers to safeguard against external contamination. The samples underwent rotary evaporation with a Buchi R-300 to yield methanol extracts, subsequently stored in a tightly sealed container. Subsequently, the samples were analysed for evaluation.

The analysis was carried out following AOAC 2019 and the Biochemical Methods by S. Sadasivam and A. Manickam along with several methods from previously published papers from reputable journals

From the present investigation, significant observations were made in regard to the nutritional characteristics where *Terminalia chebula* was reported to have maximum protein (89.16±3.41 %), energy (780.31±8.01 kcal 100 g^{-1}), non-reducing sugar (13.83±2.75 %) and TSS (20.77±0.32˚B). *Tamarindus indica* was found to be a rich source of dry matter (40.60±1.12 %), lignin (13.69±0.01%) and hemicellulose (12.09±0.01 %). *Citrus jambhiri* was observed to be a good source of moisture

(95.43±0.97 %) and acidity (6.19±0.37 %). *Antidesma bunius* had the highest fiber content (24.27±1.43 %) while *Artocarpus heterophyllus* was reported to be a good source of starch (222.67±6.83 mg100 g^{-1}). The highest ash content was observed in *Citrus grandis* (9.21±0.06 %) and the total sugar was found to be highest in *Emblica officinalis* (28.13±1.33 %). The highest cellulose content was reported in Embelia subcoriacea *subcoriacea* (75.43±1.51 mg 100 g^{-1}) and *maximum fat* content was recorded in *Garcinia kydia* (6.23±0.06 %). The highest reducing sugar and carbohydrates was observed in *Garcinia xanthochymus* (14.61±1.76 %) and *Myrica esculenta* (79.84±3.74 %) respectively.

From the reported mineral composition among the 21 underutilized fruit *Citrus grandis* reported the highest N (12.14±0.49%) and P (0.46±0.02%) content while *Tamarindus indica* exhibited the maximum values for Cu (32.94±1.62 mg 100 g^{-1}) and Zn (11.43±0.71 mg 100 g^{-1}). *Garcinia kydia* was reported to have a higher amount of the microelements Co (3.95±0.10 mg 100 g^{-1}) and Fe (43.09±1.56 mg 100 g^{-1}) among the studied underutilized fruits. The highest Ca, Na and Mn content was recorded in *Morus nigra* (477.75±3.00 mg 100 g–1), *Rubus treutleri* (136.05±1.18 mg 100 g–1) and *Myrica esculenta* (27.66±1.04 mg 100 g^{-1}) respectively. Mg was reported to be the highest in *Prunus undulata* (377.33±3.45 mg 100 g^{-1}) and *Terminalia chebula* was observed to be the highest in the K level (4.37±0.05%).

In the present study the bioactive composition has highlighted that *Carallia brachiata* was observed to exhibit the highest values for Flavonoid (632.62±0.65 mg 100 g^{-1}), Anthocyanin (816.67±5.28 mg 100 g^{-1}) and saponin 13.70±0.50 mg 100 g^{-1}). The highest phenol content was observed in *Terminalia chebula* (792.28±0.32 mg 100 g^{-1}) and at the same time *Prunus jenkinsii* was found to be highest in total carotenoid (1.97±0.21 mg 100 g^{-1}). Total chlorophyll content was observed to be maximum in *Artocarpus heterophyllus* (0.507±0.053 mg 100 g^{-1}) and vitamin C was recorded highest in *Emblica officinalis* (756.00±4.00 mg 100 g^{-1}). The highest vitamin E and alkaloid content was observed in *Antidesma bunius*

(36.69±0.80 mg 100 g^{-1}) and *Terminalia chebula* (192.84±4.84 mg 100 g^{-1}).

In aspects of the anti-nutritional composition, Phytic acid was found highest in *Embelia subcoriacea* (7.60±0.16mg100 g^{-1}) while the highest total oxalate was found in *Garcinia kydia* (13.42±1.10 mg 100 g^{-1}) and *Terminalia chebula* recorded the highest tannin content (483.78±7.49 mg 100 g^{-1})

The methanolic extract of *Elaeocarpus lanceifolius* (29.16±2.38 µg/ml) reported the lowest IC_{50} for MTT Assay followed by *Embelia Subcoriacea (31.54±2.83 µg/ml) and Terminalia chebula* (39.93±1.64 µg/ml) which have the highest cytotoxicity form the results of MTT assay for the twenty-one (21) underutilized fruit.

Methanolic extract of *Elaeocarpus lanceifolius* induced the best cytotoxic activity through apoptosis against A549 cells at 50 µg/ml followed by *Embelia subcoriacea* and *Terminalia chebula* which were statistically at par.

The fruit extracts *Elaeocarpus lanceifolius, Embelia subcoriacea and Terminalia chebula* significantly decreased glutathione (GSH) concentration and the activities of glutathione-s-transferase (GST) and superoxide dismutase (SOD) when compared to the untreated control at 50 µg/ml.

Among the five (5) fruit extracts studied, *Elaeocarpus lanceifolius* extract induced the highest efficacy with an up-regulation of pro-apoptotic genes such as Bax and Bid followed by *Embelia subcoriacea* when compared to the untreated control and induced significant down-regulation of anti-apoptotic genes Bcl-X_L and BCl-2.

Among the five (5) extracts, *Elaeocarpus lanceifolius* induced the highest Caspase-3 activity followed by *Embelia subcoriacea* and *Terminalia chebula* which were statistically at par with 5-Fluorourasil (5FU).

Among the five (5) extracts, *Elaeocarpus lanceifolius induced the highest* Caspase-6 activity followed by *Embelia subcoriacea and Terminalia chebula* which were statistically at par with 5FU.

LC-HRMS was carried out on five (5) fruit species which were selected on the basis of their cytotoxicity against A549 cells from MTT assay. In *Elaeocarpus lanceifolius* Tyr-Oet, Isoflurophate,Uh-301, L-Kynurenine, Dodine, Estrone, Brn 0575813, Soyasapogenol B 3-O-D-Glucuronide were found to be prominent. In *Embelia subcoriacea* Bergaptol, Resistomycin, (R)-Mevalonate, Chitobiose, Prostaglandin B1, (15Z)-Tetracosenoic acid, Acetylleucyl-leucyl-norleucinal were found to be prominent. In *Terminalia chebula* Dihydro-heme d1, Deoxyuridine, trans-Cinnamate, Terpendole C and Isopropamide were found to be prominent. In *Garcinia kydia,* Taurine,trans-Cinnamate and (15Z)-Tetracosenoic acid were found to be prominent. In *Emblica officinalis* in the present study, out of which Citrate, Lancerin, Hydroxytamoxifen and L-Octanoylcarnitine were found to be prominent.

Molecular docking was employed to determine the possibility of binding between the bioactive compounds and the receptor protein and the results were analysed. In this work, we identified significant bioactive compounds from *Elaeocarpus lanceifolius, Embelia subcoriacea, Terminalia chebula, Garcinia kydia and Emblica officinalis* which have proven to possess higher cytotoxicity towards the A549 cancer cells through MTT assay where several compounds were identified and further analysed through molecular docking to see the interaction of the compounds and its interaction with the protein AKT1 which is a cancer drug target. The results demonstrated that out of the compound identified the highest binding affinity was found in the compounds Resistomysin (-10.1 kcal mol^{-1}), Berberine (-

7.6 kcal mol^{-1}), Silymarin (-7.5 kcal mol^{-1}), Sulindac (-7.5 kcal mol^{-1}) followed by 20-Hydroxyecdysone (-7.0 kcal mol^{-1}) respectively. The docking pose of the molecules which showed a lesser ΔG score than 5-fluorouracil (-7.0 kcal/mol) .

The highest DPPH antioxidant activity having the lowest IC_{50} value was observed in *Emblica officinalis* (3.29±0.05 µg/ml) and the lowest antioxidant activity using DPPH was observed in *Citrus jambhiri* (1217.00±7.59 µg/ml) having a higher IC_{50} value.

Emblica officinalis possessed the highest ABTS scavenging activity (lowest IC_{50}; 38.33±1.01 µg/mL) while the lowest ABTS scavenging activity with the highest IC_{50} values was observed in *Garcinia xanthochymus* (2921.67±8.09 µg/mL).

Maximum antioxidant activity using superoxide anion radical scavenging activity with the lowest IC_{50} values was observed in *Terminalia chebula* (0.65±0.23 µg/mL). Conversely, the lowest superoxide scavenging activity having the highest IC_{50} values was observed in *Garcinia xanthochymus* (1501.67±2.01 µg/mL).

The maximum ferric-reducing activity of fruit extracts was observed in *Emblica officinalis* having the lowest IC_{50} values (0.51±0.08 µg/mL) the lowest ferric-reducing antioxidant activity was observed in *Embelia subcoriacea* (42.76±0.63) having the highest IC50 values.

GCMS was carried out for 21 underutilized fruit species to identify the major compounds present. All the species have different types of compounds present which was identified from the National Institute of Standards and Technology (NIST) library in the GCMS.

The correlation conducted among the twenty-one fruits in their nutritional, bioactive and anti-nutritional properties showed high correlations among each other which could help us in accessing the fruit's nutritional profile.

The principal component analysis identifies several components and variance in the nutritional, bioactive and antinutritional data of the 21 underutilised fruits. This could help us identify the important components for the selection of highly nutritional fruits.

The cluster analysis divided the studied 21 underutilised fruits into 3 main clusters according to their nutritional, bioactive and antinutritional characteristics which help us cluster the studied underutilised fruits accordingly.

This study provides a comprehensive report and documentation of the extensive nutritional potential of the underutilized fruits of Mizoram, northeast India. It highlights that these species represent a significant and advantageous source of essential nutrients, encompassing carbohydrates, fats, proteins, sugars, vitamins, pigments, antioxidants, and additional components. These fruits may be regarded as nutritionally comparable to the prevalent commercial fruits of today. Research indicates that each fruit possesses distinct potential, characterized by a rich nutritional profile, functional food properties, and significant anticancer and antioxidant attributes. Gaining comprehensive insights into the health-promoting properties of these underutilized fruits may lead to an improved understanding of their benefits, particularly in the contexts of functional foods, nutraceuticals, and ethnomedicine. The year-round availability of these indigenous fruit species presents considerable potential for enhancing food and nutritional security during times of food scarcity. The creation of a nutritional composition database will inform the community about the benefits of consuming locally sourced and nutritious foods, thereby preserving their cultural heritage and facilitating targeted domestication efforts for future generations.