PHYTOCHEMICAL INVESTIGATION OF ANTIMALARIAL PLANTS IN MIZORAM

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PHYTOCHEMICAL INVESTIGATION OF ANTIMALARIAL PLANTS IN MIZORAM

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Submitted

In partial fulfillment of the requirement of the degree of Doctor of Philosophy in Forestry of Mizoram University, Aizawl.

SUPERVISOR'S CERTIFICATE

I certify that the thesis entitled "**Phytochemical Investigation of Antimalarial Plants in Mizoram**," submitted to Mizoram University in partial fulfillment of the requirements for the Doctor of Philosophy degree in the Department of Forestry by **Lalngaihmanawmi** is a bona fide record of original research conducted under my supervision and guidance during the period 2019–2024. I confirm that this work has not been previously submitted for any degree, diploma, associateship, fellowship, or any other similar title at this University or any other University or institution of higher learning.

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DECLARATION

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MAY 2025

I **Lalngaihmanawmi**, 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.

This is being submitted to the Mizoram University for the **Degree of Doctor of Philosophy in Forestry.**

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ABBREVIATIONS

µg : Microgram

A. scholaris : *A. scholaris*

ASP : *A. scholaris* petroleum ether extract

ASC : *A. scholaris* chloroform extract

ASM : *A. scholaris* methanol extract

ASaq : *A. scholaris* aqueous extract

BHT : Butylated hydroxytoluene

BSI : Botanical Survey of India

Conc : Concentration

DMSO : Dimethyl sulfoxide

DPPH : 2,2-diphenyl-1-picrylhydrazyl

DPX : Distyrene plasticizer xylene

EDTA: Ethylenediaminetetraacetic acid

$FeCl_3$: Ferric chloride

ANOVA : Analysis of Variance

g : Gram

GC-MS : Gas chromatography-mass spectrometry

H_2SO_4 : Sulphuric acid

HCL : Hydrochloric acid

mg : Milligram

ml : Milliliter

nm : Nanometer

M : Mole

NIST : National Institute of Standards and Technology

BC : Before Christ

cm : Centimeter

et al., : *et alii* : and others

etc : *et ceteri or cetera* : and others

E : East

N : North

pp : *pegina* : page or pages

UV : Ultra-violet

WHO : World Health Organization

P. falciparium : *Plasmodium falciparium*

CQ : Chloroquine

hrs : hours

PBS : Phosphate-buffered saline

CHAPTER 1

INTRODUCTION

Malaria is one of the oldest and most widespread infectious diseases in human history. Its origins can be traced back to ancient times, with evidence found in Egyptian mummies over 4,000 years old. The name “malaria” comes from the Italian words “*mal*” (bad) and “*aria*” (air), reflecting the incorrect belief that the disease was caused by bad air in marshy areas (Tropical Medical Bureau Reports, 2023). In 2010, scientists identified traces of malaria parasites in the blood of an ancient mummy (Britannica, 2024). It is believed that human malaria originated in Africa and spread to regions such as the Nile Valley, Mesopotamia, India, and China through human migration. Ancient Chinese texts are among the earliest records of a disease resembling malaria, detailing symptoms like fever, chills, and headaches. The Nei-Ching, an ancient medical text, described types of fevers associated with malaria and the link to enlarged spleens (Cox, 2004). Notable historical figures have succumbed to malaria, including Alexander the Great, who died in 323 BC near the Euphrates River, and several others, such as Pope Innocent III in 1216, the poet Dante in 1321, and the artist Raphael in 1520. In Italy, malaria also claimed the lives of Pope Leo X in 1521 and former Holy Roman Emperor Charles V in 1559. Efforts to control malaria date back to ancient Greek times when swamp drainage was attempted. However, a specific treatment wasn’t available until the 1630s when cinchona bark was introduced to Spain from Peru. The bark, used by English physician Thomas Sydenham, helped distinguish malaria from other fevers, marking the beginning of targeted drug therapy. Quinine, the active ingredient in cinchona, was isolated in the mid-19th century, and the Dutch began cultivating the tree in Java. There were no major advances in understanding malaria until the 1870s when Louis Pasteur and Robert Koch’s research laid the groundwork for modern microbiology. In 1880, French military physician Alphonse Laveran, working in Algeria, identified parasites in the red blood cells of malaria patients, earning him the 1907 Nobel Prize in Physiology or Medicine. In 1897, British Army surgeon Ronald Ross discovered the transmission of malarial parasites in mosquitoes in India, for which he received the 1902 Nobel Prize in Physiology or Medicine while Giovanni Grassi and his team in

Rome found the parasite in *Anopheles* mosquitoes in 1898 (Britannica encyclopaedia, 2024).

Malaria is a life-threatening disease transmitted to humans through certain types of mosquitoes, belonging to the genus *Anopheles* that are predominantly in tropical regions (WHO, 2023). According to the World Malaria Report, there were 249 million malaria cases in 2022, up from 244 million in 2021, with deaths totaling 608,000 in 2022, slightly down from 610,000 in 2021 (WHO, 2023). The disease significantly affects communities' health and economic progress in tropical and subtropical areas (Ribeiro *et al*., 2023).

Malaria is caused by a parasite transmitted through the bites of infected female *Anopheles* mosquitoes. There are over 150 blood parasites of the *Plasmodium* genus, but only five species cause significant human disease. *Plasmodium falciparum*, the most common, leads to the majority of severe cases and deaths. *P. vivax* is responsible for relapsing malaria, causing both blood and liver infections with recurrent symptoms. Less common species include *P. ovale*, *P. malariae*, and *P. knowlesi* (Medicine for Malaria Venture, 2023). *P. vivax* and *P. malariae* are more common in the Americas and Europe, while *P. falciparum* dominates in Africa (Cartwright *et al*., 2006). The WHO African Region bears the highest burden of malaria globally, accounting for about 94% of cases and 95% of deaths in 2022. Children under five make up approximately 78% of malaria-related deaths in the region, with rural, impoverished populations being the most affected (WHO, 2024). Four African nations—Nigeria (26.8%), the Democratic Republic of the Congo (12.3%), Uganda (5.1%), and Mozambique (4.2%) account for over half of global malaria deaths (WHO, 2023). Factors like climate change, humanitarian crises, and conflicts are displacing populations in malaria-endemic countries, increasing their vulnerability to the disease (WHO, 2024). In 2021, India represented about 79% of malaria cases in its region, with *P. vivax* responsible for 40% of cases. Over the past two decades, India has seen a 76% reduction in malaria cases, equating to a decrease of 17.4 million cases by 2021 (WHO, 2022).

The history of malaria treatment began with quinine, an alkaloid derived from the bark of the cinchona tree in South America. Introduced to Europe in the 1600s, it became a standard treatment after its chemical isolation and commercial production in 1820 by French chemists. In 1934, German scientists synthesized chloroquine (Resochin) and sontochin, new antimalarial drugs from the four-amino quinoline class. Chloroquine's development accelerated during World War II when American researchers modified captured German drugs. Proguanil, a pyrimidine derivative, was developed during World War II, leading to the discovery of pyrimethamine. The US Army, WHO, and Hoffman-La Roche collaborated on the development of mefloquine in the 1970s. Chinese scientists in 1972 isolated artemisinin from sweet wormwood (Qinghao), showing it to be highly effective against malaria, comparable to quinine and chloroquine. Testing on humans followed, with findings published in 1979 (US Institute of Medicine Report, 2004).

There are now several drugs available for treating malaria, including artemether/lumefantrine, artesunate, atovaquone, atovaquone/proguanil, chloroquine, hydroxychloroquine sulfate, mefloquine, primaquine, pyrimethamine, quinidine, quinine, and tafenoqui. Artemether/lumefantrine combination works by inhibiting nucleic acid and protein synthesis in the parasite, possibly by blocking beta-hematin formation or through endoperoxide activity. Artesunate contains an endoperoxide bridge that reacts with heme iron, causing oxidative stress and inhibiting protein and nucleic acid synthesis, which impairs parasite growth. Atovaquon disrupts the electron transport chain at the cytochrome bc1 complex, damaging the parasite's mitochondrial membrane. In Atovaquone/proguanil combination, atovaquone disrupts the parasite's mitochondrial function, while proguanil inhibits dihydrofolate reductase, an enzyme crucial for the parasite's reproduction. Chloroquine acts on the erythrocytic forms of the malarial parasite, though the precise mechanism is unclear. Hydroxychloroquine sulfate's exact mechanism against *Plasmodium* is not well understood, but it may interfere with the parasite's acid vesicles and inhibit heme polymerization and essential enzymes. Mefloquine is a quinine analog that kills schizonts in the blood, possibly by altering the pH within the parasite. Primaquine targets the parasite's mitochondria, causing its death. Pyrimethamine blocks the

enzyme dihydrofolate reductase in the parasite, reducing the production of folic acid required for nucleic acid synthesis. Quinidine accumulates in the parasite's food vacuole, forming a complex with heme and starving the parasite. Quinine's mechanism action is unclear, but it may disrupt DNA processes and interfere with hemoglobin digestion, starving the parasite. Tafenoquine is active as a metabolite against both the liver and blood forms of the parasite, preventing the development of malaria and reducing the risk of relapse (Sruthi, 2022).

Traditional antimalarials

Traditional medicines are generally cheaper than modern pharmaceuticals, and they are likely the only natural cures available and accessible in distant rural areas in underdeveloped nations (Popovic *et al*., 2016). Plant medicine has been used for a long time, particularly by some African tribes with a rich history (Petrovska, 2012). According to studies, the indigenous populations in Eastern Uganda's Tororo District have used herbal remedies to treat malaria, and its symptoms were made using a total of forty-five plant species from 26 families and 44 genera. *Vernonia amygdalina, Chamaecrista nigricans, Aloe nobilis, Warburgia ugandensis, Kedrostis foetidissima, Senna occidentalis, Azadirachta indica,* and *Mangifera indica* were the plant species most often named. The leaf was the most often utilized plant element (67.3%), while maceration was the most common method for preparing herbal remedies (56%). There were discrepancies in the recommended posology for oral administration, which was the most common way of administration (Tabuti *et al*., 2023). On the other hand, Papua Island in Indonesia has 72 kinds of medicinal plants from 67 genera and 40 families that were traditionally used to cure malaria. *Physalis minima, Carica papaya, Andrographis paniculata,* and *A. scholaris* are the most often utilized medicinal herbs in traditional antimalarial concoctions. Most plant components utilized to prepare the various traditional concoctions were the leaves, in line with prior ethnobotany studies (Mery Budiarti *et al*., 2022). One of the most common illnesses in the nation is malaria, which has several herbal remedies available. The residents of Acera's Osudoku area have long used these remedies. The majority of them employed neem tree therapies, which included drying the leaves, brewing a tea solution, and inhaling the cooked leaves' vapor. Others would

purchase the bottled herbal concoctions, which contain a variety of herbs, that are marketed to treat malaria. *Vernonia amygdalina, Momordica foetida, Zanthoxylum chalybeum, Lantana Camara,* and *Mangifera indica* were the most often suggested. The drugs derived from these plants were made as water extracts from a single species and were given at different times and in different quantities. Most of the members prepared the herbal treatment they used to treat malaria by combining different parts, leaves, and herbs from many plants. The most popular method of preparation used by the participants was boiling neem tree leaves and breathing in the vapors via a towel. Additionally, some people would combine salt and bitter herbs and drink the resulting mixture. Some participants ingested the concoction for treating malaria by burning pineapple, *Momordica foetida*, and *pawpaw* leaves; they felt that this was an excellent malaria therapy (Appiah *et al*., 2020).

Importance of medicinal plants: A world scenario

For thousands of years, people used plants as medicine without scientific understanding or competent instruction. Plant-based medicine is considered a natural healing medical system. Flowers, roots, stems, leaves, fruits, seeds, and complete plants have all been scientifically proven to have therapeutic effects. However, certain plants are harmful to human health due to poisonous chemicals (Wink, 2010). An essential part of conventional medical systems is the use of medicinal herbs. The earliest documents indicate that herbal remedies have been utilized and recorded for around 5000 years in Chinese, Indian, Egyptian, Greek, and Roman medical systems. Japan, the Arab and American nations, and other ancient societies have also long used traditional herbal treatment. Indian classical traditional medicine systems such as the Rigveda, Atherveda, Charak Samhita, and Sushruta Samhita are transcribed. Another vital source for the indigenous healthcare system is folk (tribal) remedies. India is renowned for having a vast collection of traditional medicinal herbs from past civilizations. Many aromatic and therapeutic plants are mostly found in India's woods (Debbarma *et al*., 2010; Kamboj 2000; Mukherjee, 2008).

Herbal medicine is extensively practiced across the world. For decades, people have resorted to natural treatments to treat common diseases such as colds, allergies, upset

stomachs, and toothaches, and this trend is only growing. As a result, there has been a worldwide movement from synthetic to herbal treatments, which we might call a 'Return to Nature' approach to illness prevention and treatment. Medicinal plants have come from nature. The World Health Organisation (WHO) claimed that 4 billion people (80% of the world's population) utilize herbal medicines for some part of primary care (Fabricant *et al*., 2001). Human civilization has utilized a variety of plants for food, medicine, clothing, and shelter from the beginning of time. High concentrations of many "supernutrients," including disease-prevention antioxidants, phytochemicals, and micronutrients, can be found in vegetarian diets. Pharmacological functions of plants include antiviral, anticancer, antibacterial, antifungal, and antiparasitic properties. Flavonoids, phenolics, anthocyanins, and vitamins are among the chemicals found in plants that scavenge free radicals and have antioxidant-like properties (Chopra *et al*., 2002). Youyou Tu, a Chinese pharmacologist, used *Artemisia annua*, a plant known for its sweet wormwood, to discover and create "Artemisinin," a novel herbal antimalarial medication. Anthocyanins, or chemical compounds produced from plant sources, are employed in contemporary medicine. Examples of these substances include quinine, digoxin, aspirin, ephedrine, atropine, and colchicine (Ram *et al*., 2015; Moteriya *et al*., 2015). According to botanical studies, there are around 2,50,000–3,50,000 distinct plant species in the globe. However, there are only 35,000 different species that are used worldwide to treat a range of injuries (Kong *et al*., 2003). According to a Globe News Wire report, the global market for herbal medicines is expected to reach 550 billion dollars by 2030. In 2005, the industry for medications derived from plants was estimated to be worth 18 billion dollars. By 2019, that amount had increased to 83 billion dollars (Saklani and Kutty, 2008; Willison and Andrews, 2004). It is hardly surprising that China and India control the majority of the global herbal trade business. China is estimated to export over 120,000 tonnes of herbal medicines annually, with India following closely behind with about 32,000 tonnes. In contrast, Europe is the world's biggest importer of medicinal plants, bringing in over 400,000 tonnes annually from various European countries to satisfy domestic demand for herbal treatments (Briskin, 2000; Khan, 2014). Notably, Germany and France are the two top nations in herbal treatments (De Smet, 2005). To be more specific, 50% of

Germans believe in herbal remedies as a cure for a range of diseases. Similarly, the use of herbal formulations as home remedies is common in many Asian and African countries (Gilani and Rahman, 2005). In the present era of medicinal engineering, various compounds extracted or synthesized from medicinal plants now have a large market share in every area of the world (Ndhlala *et al*., 2011). Around 50,000 plant species are thought to be utilized in traditional medicine globally, the bulk of which are found in Asian remedies (Wangchuk, 2004; Gewali, 2008). Indians are said to use no more than 20.0% of their total plant flora for medicinal purposes; China, Vietnam, Sri Lanka, and Thailand follow with 18.9%, 17.1%, and 15.5%, respectively. Despite having a large number of plant species, countries like the USA, Australia, Indonesia, and Malaysia hardly use them as therapeutic herbs. 7.8% of Australia's higher plant flora has been used medicinally, and Aboriginal groups continue to use this knowledge (Gott, 2008). More than 5,603 higher plant species have been identified in Bhutan; the majority are thought to provide therapeutic benefits (Wangchuk and Tashi Tobgay, 2015). More than a thousand medicinal plants are listed in Tibetan textbooks and Bhutanese traditional medicine (BTM), of which 600 are known to grow in Bhutan (Wangchuk, 2004). Of these, 300 are being utilized daily to prepare multi-ingredient formulations at Manjong Sorig Pharmaceuticals, which is part of the Ministry of Health in Bhutan (Wangchuk *et al*., 2011).

Scenario in India

In India, traditional medical knowledge has been passed down from generation to generation, usually within specific geographical areas or ethnic tribes (Dey *et al*., 2017). This ancient wisdom has its roots in Indian traditional medical systems such as Ayurveda and Siddha, which are also gaining favour in the Western world (Modak *et al*., 2015). India has a long history of traditional medicine. Materia Medica of India has a wealth of knowledge about folkloric practices and traditional characteristics of medicinally essential natural ingredients. Each of these ancient systems has its own set of ideas and practices, but the use of natural goods, mostly herbs, runs through them all. Indian traditional remedies have stood the test of time and have long been utilized for personal health care (Mukherjee *et al*., 2014)

Ayurvedic and Siddha systems employ formulations produced from suitable plant components to treat a variety of diseases. Past evidence reveals that herbal medicine has been practiced for about 5000 years in India, Syria, Egypt, and China. The Rigveda, Atharva Veda, Charaka Samhita, and Sushruta Samhita are traditional Indian books on human medicine (Kamboj, 2000). Approximately 40% of the medications in use are either entirely or partly derived from plants (Kumar and Tewari, 2018). For over three decades, several ethnomedicinal plants described in Ayurveda and Siddha health systems have been systematically investigated (Sharma and Patki, 2010). Scientific examination of ethnomedicinal plants offers evidence-based alternative medicines that serve as the foundation for the herbal medication business and the development of drug targets in the pharmaceutical sector (Patwardhan, 2005). The World Health Organisation (WHO) estimates that around 75% of the world's population receives medical treatment from traditional practitioners, mostly herbal remedies. The most widely accepted traditional medicine systems are Ayurveda and Chinese medicine, which have a significant body of study on pharmacognosy, chemistry, pharmacology, and clinical treatments (Sidhu and Pannu, 2010; Ayyanar and Ignacimuthu, 2011).

India is home to about 100 plant genera that are employed in ancient medical practices across the world. India produces the highest quality and quantity of medicinal plants and ranks second in exports. It is recognized as one of the world's 12 mega biodiversity hotspots (Prasathkumar *et al*., 2021). India contains 15 agroclimatic zones and 17000-18000 flowering plant species, with an estimated 6000-7000 being used medicinally in traditional and recorded medical systems such as Siddha, Ayurveda, Homoeopathy, and Unani. There are around 960 species of medicinal plants in commerce, with 178 species having annual consumption levels of more than 100 metric tonnes. Medicinal plants not only serve as a key supply for the traditional medicine and herbal industries, but they also make available sources of income and health security for a huge portion of the Indian people. A significant portion of the Indian population depends on medicinal plants for their livelihood and health security, in addition to serving as a vital resource base for the traditional medicine and herbal industries. The AYUSH industry's domestic commerce is

estimated to be worth between Rs. 80 and 90 billion (USD 1.3 and 1.5 billion). The export of Indian medicinal plants and their derivatives is estimated to be worth over Rs. 10 billion. The traditional and alternative healthcare systems are seeing a global comeback, which has led to 120 billion dollars in global herbal commerce which is predicted to grow to 7 trillion dollars by 2050 (Mukherjee *et al*., 2014).

Traditional herbal remedies with a variety of phytoconstituents and therapeutic qualities have been utilized for a long time to treat a variety of illnesses (Calixto, 2000). Because they are naturally occurring, effective, and have fewer adverse effects than pharmaceuticals, herbal treatments are thought to be inherently safe (Prabhatar *et al*., 2014). India has a long history of using medicinal herbs to control diabetes. World ethnobotanical information has recorded the use of over 800 plants for the treatment of diabetes mellitus, of which only 410 have been experimentally confirmed to have anti-diabetic effects, with the whole mechanism of action accessible for only about 109 species. Diabetes therapy with herbs is more beneficial and has fewer negative effects. Herbal medications protect β-cells during diabetes and lower blood glucose levels in several ways (Jeeva, 2014). Several medicinal plants, particularly those found in India, have long been used to prevent and treat cancer. However, just a few medicinal plants have piqued scientists' attention in researching their potential as a treatment for neoplasms (Shaikh *et al*., 2014). Several plants have been employed in ancient Ayurvedic medicine to treat and control various inflammatory illnesses and wound-healing processes (Shaikh *et al*., 2016). Approximately 200 plants spread across the world have been recommended in the treatment of eye problems, and several plant species have been traditional herbal remedies with the ability to overcome the limitations associated with current medications. As a result, many attempts have been made to find novel medicinal plants from various sources due to their efficacy, particularly because they are readily available locally and simply consumed raw or in simple therapeutic formulations. The demand for herbal pharmaceuticals is increasing as people become more aware of the benefits of plant-based therapies (Srikanth, 2014). Herbal medications, which are inexpensive, are increasingly being preferred for their ophthalmic benefits in Traditional Indian Medicine (Pooja, 2014). They are

overspending on pricey contemporary medications (Nafees *et al*., 2022). Malaria is treated using the bioactive chemicals artemisinin, quinine, and quinoline, which come from *Artemisia annua* and *Cinchona ledgeriana*, respectively. The medications digoxin and digitoxin, derived from *Digitalis purpurea*, are used to treat cardiac problems. Caffeine derived from *Coffea canephora* is mostly used as a stimulant medication to treat brain diseases. Diosgenin from *Dioscorea mexicana* and stigmasterol from *Glycine max* are used to treat sexual issues. Atropine from *Atropa belladonna* and hyoscyamine from *Hyoscyamus niger* demonstrated efficacy in treating nervous system-related diseases (Chakrabarty, 2018).

Many of India's medicinal plant needs are met by the forests of Himachal Pradesh, which is thought to be the birthplace of Ayurveda. According to one estimate, these forests provide up to 80% of all Ayurvedic medications, 46% of Unani medications, and 33% of allopathic medications created in India (Aryal, 1993).

The potential of herbal medicine in Mizoram

Mizoram, a small state in the Northeastern region of India, is one of the world's 25 mega biodiversity hotspots, home to over 400 species of medicinal plants (Rai and Lalramnghinglova, 2010). This state is renowned for its stunning natural beauty, diverse landscapes, and rich flora and fauna. Almost all tropical trees and plants thrive in Mizoram, and the hills are beautifully green (Anonymous, 2014). Located at the tail end of the Himalayan range, Mizoram lies between 21°58' and 24°35' N latitudes and 92°16' E and 93°29' E longitudes. It shares international borders with Myanmar to the east and southwest, Bangladesh to the southwest, and is bordered by Tripura to the west, Assam to the north, and Manipur to the northeast. The Tropic of Cancer runs through the southern edge of Aizawl, the state capital. Mizoram experiences a tropical monsoon climate with three distinct seasons: summer, monsoon, and winter. Summers are warm, with temperatures between 20 and 30°C, while winters are mild, with temperatures ranging from 11 to 21°C. The state receives heavy rainfall during the monsoon season, from May to September, averaging between 2,500 mm and 3,000 mm annually (Lalramnghinglova, 2003). Consequently, the state's forests include tropical wet evergreen, tropical semi-

evergreen, tropical moist deciduous and sub-tropical broadleaved hill forests (The Indian State of Forest Report, 2011).

Since ancient times, the Mizo people, despite lacking scientific and medical knowledge, have developed their own methods for treating various ailments (Lalruatpuii *et al*., 2024). The Mizo people possess extensive expertise in utilizing native plants, known as "*ramhmul damdawi*," to prevent and treat a wide range of diseases, a skill honed through their long history of battling illnesses. Within the Mizo community, knowledge of medicinal plants is widespread, resulting in a rich collection of remedies for disease prevention and treatment (Ralte *et al*., 2024). The use of these plants is deeply ingrained in their daily lives, culture, and customs, particularly in the treatment of various ailments. Some studies have applied quantitative methods to ethnobotanical data to explore the relationship between plant species and human practices (Turner, 1988). The knowledge of using plants for medicinal, ritual, cultural, or traditional purposes is deeply embedded among the native ethnic people of Mizoram, passed down orally from their ancestors (Singh *et al*., 2022).

Global landscape of antimalarial plants

The use of wild plants as medicinal herbs has been deeply rooted in various cultural traditions for centuries, with this knowledge being passed down through generations across the world (Ranjandeep, 2017). People trust herbal plants for their safety, effectiveness, cultural significance, and constant availability. The medicinal properties of these plants are often identified through intuition or a process of trial and error. Traditional healers worldwide employ numerous medicinal plants to treat malaria, although this practice is not fully recognized by modern medicine (Shooraj *et al*., 2022). Many plant species have been identified as having antimalarial properties, with ethnobotanical and ethnopharmacological research highlighting their potential. Indigenous communities continue to use a wide variety of herbs for treating diseases, including malaria. Studies in ethnomedicine and ethnobotany have opened up opportunities for developing and synthesizing new drugs, many of which are derived from traditional and folklore-based medicine (Ranjandeep, 2017).

Traditional medicinal plants have been the primary source of antimalarial drugs, with quinine and artemisinin being two of the most widely used treatments for malaria globally (Wright and Phillipson, 1990; Thomson, 1993). Quinine was derived from the bark of the Cinchona tree *Cinchona succiruba* (Rubiaceae) found in Peru, while artemisinin was obtained from the Qinghao plant *Artemisia annua* (Asteraceae). Both drugs were developed using the chemical structures in these plants as templates. The chemical modification of artemisinin has led to the development of several potential antimalarial drugs, including artemether, artesunate, and arteether, which are now used in combination with conventional medicines for malaria treatment (Simon *et al*., 1990; Nicholas, 1994).

According to Willcox (2008), 1,277 plant species from 160 families can be used to treat malaria (Ballou, 2009). The disease is a significant regional concern in Africa and a national issue in Kenya due to the high rates of morbidity and mortality caused by resistant strains of *P. falciparum* to many existing drugs, such as chloroquine. In Kenya, alternative medicine, particularly herbal remedies, is commonly employed to treat malaria. In this region, 27 plant species from 24 genera across 20 families have been reported for malaria treatment. Some of the most commonly used species include *Aloe deserti*, *Launea cornuta*, *Ocimum bacilicum* L, *Teclea simplicifolia*, *Verdoonia lobata*, *Gerranthus lobatus*, *Grewia hexaminta*, *Canthium glaucum*, *Amaranthus hybridus* L., *Combretum padoides*, *Senecio syringitolius*, *Ocimum suave*, *Aloe macrosiphon*, and *Laudolphia buchananii*, which are documented in this region for the first time for malaria treatment (Nguta *et al*., 2010). In Latin America, over 1,000 plant species have been used to treat malaria, with more than 600 species being cited only once. The genera with the highest number of antimalarial species include *Aspidosperma*, *Solanum*, *Piper*, *Croton*, and *Aristolochia*. The most widely used genera in terms of geographic distribution are *Aspidosperma*, *Momordica*, *Cinchona*, *Senna*, and *Stachytarpheta* (Milliken *et al*., 2021). Nigeria is known for its rich flora diversity and has numerous plant species used for medicinal purposes, including malaria treatment. Some species used across all ethnic and cultural groups in Nigeria include *Abrus precatorius*, *Adansonia digitata*, *Afzelia africana*, *Ageratum conyzoides*, *Alchornea cordifolia*, *Allanblackia floribunda*, *Allium sativum*, *Alstonia*

boonei, *Blighia sapida*, *Bridelia ferruginea*., *Bridelia micrantha*, *Cajanus cajan*, *Canna indica*, *Carica papaya*, *Cassia occidentalis*, *Daniellia ogea*, *Daniellia oliveri*, *Elaeis guineensis*, *Enantia chlorantha*, *Erythrina senegalensis*, and *Eupatorium odoratum* (Adebayo and Krettli, 2010)

Approximately 80% of Ethiopia's population, particularly those in rural areas, continue to rely on traditional medicinal plants to treat various diseases (Fullas, 2007). A total of 200 plant species from 71 families have been identified for traditional malaria treatment across the country. Some of the most frequently cited species include *Allium sativum*, *Carica papaya*, *Vernonia amygdalina*, *Croton macrostachyus*, *Lepidium sativum*, *Justicia schimperiana*, *Phytolacca dodecandra*, *Dodonaea angustifolia*, *Melia azedarach*, *Clerodendrum myricoides*, *Aloe* spp., *Azadirachta indica*, *Brucea antidysenterica*, *Calpurnia aurea*, *Eucalyptus globulus*, *Ajuga integrifolia*, *Carissa spinarum*, *Artemisia afra*, *Moringa stenopetala*, *Ruta chalepensis*, *Salvadora persica*, and *Tamarindus indica* (Alebie *et al*., 2017). In Mozambique, scientific studies have investigated the antimalarial potential of plants used in traditional medicine, particularly against malaria and fever. Out of 58 extracts from 15 plant species, several demonstrated significant inhibition against a chloroquine-sensitive *P. falciparum* strain (3D7). The most notable species include *Trichilia emetica*, *Tabernaemontana elegans*, *Schefflera actinophylla*, *Senna didymobotrya*, *Plumbago auriculata*, *Parkinsonia aculeata*, *Acacia karroo*, *Aloe parvibracteata*, *Bridelia cathartica*, *Cassia abbreviata*, *Cassia occidentalis*, *Crossopteryx febrifuga*, *Leonotis leonurus*, and *Momordica balsamina* (Ramalhete *et al*., 2008)

In Central Kenya, 58 species across 54 genera and 33 families have been identified as antimalarial herbal remedies. The most common species in this region include *Caesalpinia volkensii*, *Strychnos henningsii*, *Ajuga remota*, *Warburgia ugandensis*, and *Olea europaea* (Njoroge, 2006). In Bangladesh, malaria is prevalent, particularly in the southeast, north, and northeast regions, with about 15% of the population affected, especially among tribal communities (Haque *et al*., 2011). In these regions, 11 plant species from 10 families are traditionally used to treat malaria (Ramatullah

et al., 2012). In the islands of São Tomé and Príncipe (STP) in the Gulf of Guinea, the antimalarial activity of 13 medicinal plants used in traditional medicine has been recorded (Madureira *et al*., 2002). In Nigeria, 59 medicinal plants are utilized as antimalarial remedies by Omu Aran, Ogbomoso, Ado Ekiti, and Sagamu communities. The most commonly cited species include *Azadirachta indica*, *Mangifera indica*, *Carica papaya*, *Cymbopogon citratus*, *Cassia fistula*, *Morinda lucida*, *Anacardium occidentale*, *Vernonia amygdalina*, *Helianthus annuus*, *Enantia chlorantha*, *Moringa oleifera*, *Chromolaena odorata*, and *Psidium guajava*. The frequent citation of a plant species indicates its efficacy and has attracted attention in pharmacological research, potentially leading to the discovery of new antimalarial drugs (Oladeiji *et al*., 2020). In addition, 41 plant species from 27 families, including *Alstonia boonei*, *Azadirachta indica*, *Carica papaya*, *Cymbopogon citratus*, *Enantia chlorantha*, *Mangifera indica*, and *Morinda lucida*, have been recognized in antimalarial traditional recipes in Nigeria (Ishola *et al*., 2014). In Ghana, some of the commonly used plants for treating malaria include *Moringa oleifera*, *Sarcocophalus lalifolius*, and species are from the *Poaceae*, *Sapindaceae*, *Arecaceae*, *Annonaceae*, and *Solanaceae* families (Appiah *et al*., 2022). Phytochemical studies have identified more than 252 compounds in *Agrimonia pilosa* Ledeb, such as flavonoids, isocoumarins, lignans, m-benzotrienols, organic acids, pentacyclic triterpenoids, phenols, tannins, volatile oils, among others. The extracts and derivatives from *Agrimonia pilosa* are known to have a wide range of pharmacological effects, including anticancer, anti-inflammatory, antioxidant, antitumor, and analgesic properties (Wen *et al*., 2022). *Zanthoxylum chiloperon*, a plant commonly found in South America, has shown great potential due to its emmenagogue and antirheumatic properties, which have traditionally been used to treat malaria (Bhattarcharya, 2011). *Punica granatum* is another plant known for its antimalarial and anti-inflammatory effects (Dell'Agli *et al*., 2010).

Botanical screening efforts in Cameroon, along with experimental studies on antimalarial plants, have identified 217 different species cited for their use in traditional medicine as antimalarials. From these, about 100 phytochemicals have been isolated from 26 species, some of which show promise as potential leads for

developing new antimalarial drugs. Crude extracts and essential oils from 54 other species have demonstrated a wide range of activity against *Plasmodium* spp. However, 137 plants from 48 families, which are commonly used by traditional healers in Cameroon, remain uninvestigated for their potential antimalarial properties (Titanji *et al*., 2008). In India, there is a growing preference for herbal drugs, with more than 50% of conventional drugs being synthesized from medicinal plants. Plant-based medicines are often used alone or in combination with other drugs and generally have fewer side effects compared to modern pharmaceuticals. Several traditional medicinal plants in India have been scientifically assessed for their antimalarial properties, including *Azadirachta indica*, *Ocimum sanctum*, *Andrographis paniculata*, *Lantana camara*, *Calotropis gigantea*, and *Rauwolfia serpentina*. These plants are found in various regions and continue to be an important part of traditional medicine in the country (Sharma and Kaur, 2019).

Traditional Mizo Plants used for Malaria treatment

The search for an alternative malaria treatment spurred significant efforts to develop new antimalarial drugs from indigenous plants. Information was gathered from various tribal communities in northeast India, as well as research papers, books, journals, and documents from universities and institutes in the region, focusing on botanical therapies and plant species used for malaria. The people of northeast India utilize 68 plant species from 33 families to treat malaria. Six species such as *Crotolaria occulta*, *Polygala persicariaefolia*, *A. scholaris*, *Vitex peduncularis*, *Ocimum sanctum*, and *Coptis teeta* were reported by multiple researchers from different parts of the region. These species were found either near inhabited areas or within the forests of northeast India. The most commonly used plant parts for treatment were leaves (33%), and roots (31%), followed by bark and the entire plant (12%) (Shankar *et al*., 2012).

Through extensive interviews with local healers who practice indigenous medicine, 52 plant species from 36 families have been documented for their therapeutic use in treating fever and malaria. Many of these species are cultivated, while others are found in the wild in Mizoram. The most frequently mentioned plant families include

Apocynaceae, *Euphorbiaceae*, *Verbenaceae*, and *Leguminosae*. Various parts of the plants, such as flowers, rhizomes, bark, roots, bulbs, leaves, and even the entire plant, are used as herbal remedies. These are prepared in the form of decoctions, infusions, pastes, and crushed juice. The survey also revealed that these remedies are applied multiple times daily until the symptoms of the disease subside (Laldinsanga *et al*.,2019).

Various plant species are traditionally utilized for treating malaria through different preparations. For instance, decoctions made from the whole plant of *Lantana camara* (Hling pangpar), as well as from the leaves of *Artemesia nilagirica* (Sai), *Citrus sinensis* (Serthlum), and *Azadirachta indica* (Nim thing), are commonly used. Leaf and bark decoctions of *Cinnamomum bejolghota* (Thakthing suak). Fresh leaf juices from *Clerodendron infortunatum* (Phuihnam) and *Mikania micrantha* (Japan hlo), are also employed in treatment practices. Additionally, decoctions prepared from the leaves and roots of *Dichroa febrifuga***,** and root decoctions of *Ocimum sanctum* (Tulsi) and *Dendrocnide sinuata* (Thakpui), are used against malaria. The bark of *Prunus cerasoides* (Tlaizawng) and *Betula alnoides* (Hriang) is processed into decoctions for similar purposes, while the fruit of *Piper nigrum* is also utilized in decoction form. Infusions play a significant role as well, with those made from the leaves of *Stereospermum colais* (Zihnghal), *Acacia concinna* (Khangthur), and *Andrographis paniculata* being commonly administered. Further, infusions from the leaves, roots, or young stems of *Vitex peduncularis Wall* (Thingkhawilu), and the roots and leaves of *Hydyotes scandens* (Kelhnamtur) and *Plantago major* (Kelbaan), are traditional remedies for curing malaria. The bark of *A. scholaris* (Thuamriat) and *Picrasma javanica* (Thingdamdawi) is also used to prepare therapeutic infusions. Other treatment methods include the boiled juices of roots and leaves from *Mimosa pudica* (Hlonuar), and the leaves of *Justicia zeylanica* (Kawldai). Additionally, the seeds of *Costus speciosus* (Sumbul sen) and *Allium cepa* (Purun sen) bulbs are incorporated into medicinal practices for combating malaria (Lalramnghinglova, 2003; Sawmliana, 2003; Neeti Mahanti, 1994; Thanga IFS Rtd,)

Phytochemicals

Plants are rich in various phytochemicals, which have been used for centuries in traditional medicines or ethnomedicines. Historical records of medicinal plant use date back to China around 5000 BC (Greathead, 2003) and to India, specifically in texts like the Rigveda and Atharvaveda, around 2000 BC (Ramawat *et al*., 2008). Phytochemicals, also known as phytonutrients, are bioactive compounds naturally found in foods such as vegetables, fruits, whole grains, nuts, seeds, legumes, tea, and dark chocolate. Although tens of thousands of these compounds exist, only a few have been identified and isolated from plants (Cao *et al*., 2017; Singh and Chaudhuri, 2018). Common phytochemicals in food include polyphenols, carotenoids, flavonoids, and many others. (Xiao, 2017; Zhao *et al*., 2018).

Phytochemicals can be categorized into major classes based on their chemical structure, botanical origin, biosynthetic pathways, or biological properties. The most common classification is based on their chemical structures, such as phenolics, alkaloids, saponins, terpenoids, and others (Patra, 2012). These compounds are naturally occurring, biologically active chemicals in plants that offer health benefits, both as medicinal ingredients and nutrients (Hasler and Blumberg, 1999). They protect plants from diseases, damage, and environmental hazards like pollution, stress, drought, UV exposure, and pathogens (Gibson *et al*., 1998; Mathai, 2000). Phytochemicals are crucial for plant survival and proper functioning, providing protection against herbivores, microorganisms, and competitors, regulating growth, and controlling pollination, fertilization, and the rhizosphere environment (Molyneux *et al*., 2007).

Recent studies have shown that phytochemicals also play a significant role in human health, particularly when consumed in sufficient quantities through diet (Samrot *et al*., 2009; Koche *et al*., 2010). These compounds have nutraceutical properties and are highly beneficial for human health, offering protection against a wide range of diseases and disorders, including cancers, coronary heart disease, diabetes, high blood pressure, inflammation, infections, psychotic diseases, spasms, ulcers, osteoporosis, and related conditions (Prakash *et al*.,2012).

Antioxidant

Plant antioxidants are a natural source of bioactive compounds (Gabriel *et al*., 2020). About two-thirds of the world's plant species are estimated to possess therapeutic properties, with nearly all exhibiting strong antioxidant potential (Krishnaiah *et al*., 2011). The discovery and isolation of ascorbic acid from plants sparked significant interest in studying exogenous plant antioxidants (Szent, 1963). Recent developments in understanding free radicals and reactive oxygen species (ROS) are driving a medical shift, opening new possibilities for managing health and diseases. External ROS sources include electromagnetic and cosmic radiation, UV light, ozone, cigarette smoke, and low-wavelength electromagnetic radiation. Meanwhile, oxidants can damage key macromolecules such as proteins, lipids, and DNA, leading to cellular and tissue damage (Krupanidhi *et al*., 2022).

Antioxidants safeguard cells from damage caused by free radicals. It has been demonstrated that antioxidants can stop or slow down the oxidation of other substances (Goodarzi *et al*., 2018). They can halt chain reactions and inhibit oxidation by neutralizing radical intermediates and undergoing oxidation themselves (Yasueda *et al*., 2016; Awuchi and Okpala, 2022). Antioxidants are categorized into "primary" and "secondary" types. Primary antioxidants directly prevent oxidation, while secondary antioxidants work indirectly by interacting with pro-oxidants or scavenging oxygen (Craft *et al*.,2012; Shahidi and Wanasundara, 1992). Phenolic compounds, which are primary antioxidants, function through two mechanisms: hydrogen-atom transfer (HAT) and single-electron transfer (SET). In the HAT mechanism, antioxidants neutralize free radicals by donating hydrogen atoms (Amarowicz and Ronald, 2019).

Elevated levels of oxidants or reactive oxygen species (ROS) lead to oxidative stress, which has been associated with the development of numerous chronic conditions, including cardiovascular and periodontal diseases (Pampani *et al*., 2021). Free radicals are believed to contribute to the onset of serious diseases such as diabetes, cancer, cirrhosis, obesity, and cardiovascular disorders (Wang *et al.,* 2013). Antioxidant compounds, which can neutralize free radicals, offer a potential solution

for alleviating these conditions (Speakman and Selman, 2011). Plants with high antioxidant potential contain a diverse range of free radical-scavenging components, such as phenols, vitamins, terpenoids, and flavonoids (Miguel, 2018). The antioxidant properties of plants have gained significant attention because oxidative stress has been recognized as a key factor in the onset and progression of life-threatening diseases, including neurodegenerative and cardiovascular conditions. Supplementation with external antioxidants or enhancing the body's internal antioxidant defenses is a promising strategy for combating the negative effects of oxidative stress (Kasote *et al*., 2013). Antioxidant-based drugs are used for preventing and treating complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer (Devasagayam *et al*., 2004).

To combat oxidative damage, plants have developed a comprehensive antioxidant defense system consisting of enzymes and metabolites. Key water-soluble antioxidants include ascorbate (vitamin C) and glutathione, along with secondary metabolites such as polyphenols, flavonoids, and terpenoids, which help detoxify ROS under various environmental stressors (Nadaraijah *et al*., 2004; Du *et al*., 2018; Hashim *et al*., 2020). Medicinal plants owe their antioxidant properties to phytonutrients like phenols, flavonoids, and terpenoids (D'souza *et al*., 2014; Li *et al*., 2019). Studies have shown that many medicinal plants possess anti-cancer, immunomodulatory (Madhuri *et al*., 2011), hepatoprotective, and hypolipidemic activities (Gopa and Hemavathi, 2012).

In traditional Indian medicine, herbal antioxidants have been used for centuries as rejuvenators. Research has confirmed that various herbal remedies contain compounds with antibacterial and free radical-scavenging properties, protecting the body against infections and oxidative damage (Parham *et al*., 2020).

Antimicrobial

The use of medicinal plants for alleviating illness dates back over five millennia, with early records from civilizations in China, India, and the northeast, though its origins are likely as ancient as humanity itself (Mahesh and Satish, 2008). Medicinal

plants are valuable bases of antimicrobial agents and are used in various countries as potential sources of influential drugs (Sivastavas, 1997). In recent decades, the search for new anti-infection agents has been a major focus in ethnopharmacology research (Recio *et al*., 1989). Microbes, which are significant human pathogens causing diseases and death, have been a major target for therapeutic discovery, with antimicrobial agents being one of the 20th century's key breakthroughs (Peterson and Dalhoff, 2004). Numerous plant species have been pharmacologically and clinically documented worldwide, revealing their phytochemicals with notable effects on human pathogenic bacteria (Jai Bahadur *et al*., 2012). Plants produce many important compounds, including phenolics and flavonoids, which have antioxidant and antimicrobial properties (Arulmozhi *et al*., 2010).

Infectious diseases remain a significant cause of illness and death, particularly in developing countries. As a result, pharmaceutical companies have been driven to create new antimicrobial drugs, especially in response to the growing resistance of microorganisms to traditional treatments (Nascimento *et al*., 2000). The misuse and overuse of antibiotics is a key factor contributing to the rise of resistant bacteria, posing a major challenge in controlling infectious diseases (Radji *et al*., 2013). Hospital-acquired infections are a primary source of spreading antibiotic-resistant bacteria (Talbot *et al*., 2006). In light of this, numerous studies have focused on the pharmacological potential of medicinal plants, as they may provide new drug candidates and enhance the effectiveness of existing antimicrobial treatments, potentially lowering costs and improving treatment outcomes (Silva and Fernandes, 2010). Bioactive compounds from plants have gained interest for their promising antimicrobial properties (Chakraborty *et al*., 2016). In particular, plant-derived chemicals that increase bacterial susceptibility to antibiotics are receiving increased attention. Several terpenoids, diterpenoids, and sesquiterpenoids have demonstrated the ability to work synergistically with antibiotics, suggesting that plant-based compounds could be used to combat multidrug-resistant pathogens (Kurck *et al*., 2012; Walencka *et al*., 2007; Brehm-Stecher and Johnson, 2003). Many plants contain antimicrobial compounds to protect against harmful microorganisms (Silva and Fernandes, 2010). The antibacterial activity of plants is a key area of interest in

the search for new antibiotics and antifungal agents (Zazharskyi *et al*., 2019). New antimicrobial substances are needed to treat diseases caused by drug-resistant pathogens (Zink, 1997). The rise of antibiotic-resistant microorganisms has prompted the search for new materials, with plants offering a promising source of potential antimicrobial agents (Prabha shetty *et al*., 2014).

Antiparasitic

Helminths, derived from the Greek word *vermes* for "worms," (Faust *et al*., 1970) have impacted human health for millennia. Evidence of intestinal helminths can be found in mummified human remains, and ancient texts such as those by Hippocrates, Egyptian medical papyri, and the Bible describe symptoms characteristic of helminth infections. These parasites also influenced modern history, particularly during the Cold War in China, where schistosomiasis, caused by blood flukes, played a significant role (referred to as "the blood-fluke that saved Formosa") (Cox, 2002; Hotex, 2008). Soil-transmitted helminths (STHs) are especially concerning due to their serious global health impact (WHO, 2005). The global burden from STHs is estimated at 39 million disability-adjusted life years (Utzinger and Keiser, 2004) with anemia and other morbidities like stunted physical and cognitive development being major consequences (King and Bertino, 2008).

STH infections affect around 1.5 billion people globally, or 24% of the world's population, predominantly in tropical and subtropical regions like sub-Saharan Africa, China, South America, and Asia. The infections are primarily transmitted through eggs in contaminated soil, resulting from poor sanitation practices. Vulnerable groups include over 260 million preschool-age children, 654 million school-age children, 108 million adolescent girls, and 138.8 million pregnant and lactating women, all of whom are at high risk of STH transmission and in need of treatment. The most common species of helminths infecting humans are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*). These helminths are typically addressed as a group because they require similar diagnostic approaches and respond to the same medications (WHO, 2023). The World Health Organization (WHO) leads efforts in

controlling STH infections through preventive chemotherapy (PC), which involves regular administration of anthelminthic drugs to at-risk populations without prior diagnosis (Keiser, 2023).

Several potent anthelmintic drugs are available for the treatment of helminth infections. These include benzimidazoles such as albendazole, flubendazole, thiabendazole, and mebendazole. Other classes of anthelmintic such as levamisole (butamisole, pyrantel, morantel, oxantel, bephenium, thenium), macrocyclic lactones and milbecymins (avermectin, ivermectin), amino acetonitrile derivatives and spiroindoles. other notable anthelmintics were niclosamide, diethylcarbamazine, oxamniquine, and praziquantel (Lahane *et al*., 2014). These drugs are part of the standard treatment regimens for various helminthiases. They target different stages of the helminth's life cycle and are often used in large-scale preventive chemotherapy campaigns to reduce transmission and morbidity.

Globally, worm control in livestock, including sheep, primarily relies on the use of anthelmintic drugs (Levecke *et al*., 2018). However, a major challenge in managing helminth infections in animals is the growing issue of treatment failure due to anthelmintic resistance (AR) (Seifu *et al*., 2019). Benzimidazoles, in particular, are at risk of losing effectiveness as drug resistance develops, alongside limitations in their therapeutic profile (Keiser, 2023). The situation is worsened by widespread resistance among major helminths to all available anthelmintic drugs, highlighting the urgent need for improvements and alternative treatments (Becker *et al*., 2018; Schulz *et al*., 2018). AR represents a significant global concern that requires careful monitoring and management. Although some studies have been conducted in Western Oromia (Khak *et al*., 1999), these challenges have driven the search for sustainable, effective, and safe alternatives to traditional anthelmintics (Tariq *et al*., 2009). Plant-based products offer potential solutions for treating various conditions, including parasitic infections. Numerous plant species have been utilized and documented worldwide for their effectiveness against nematode infections in both animals and humans (Akhter *et al*., 2000). Natural plant compounds provide a promising opportunity for the discovery of new, safe, and effective anthelmintics

(Waller *et al*., 2001). As a result, the development of new substances with anthelmintic properties, particularly from plants, is gaining momentum as they are considered a valuable source of bioactive compounds (Yadav and Singh, 2011).

Malaria resistence

Malaria parasite resistance develops due to various factors, including the overuse of antimalarial drugs for prevention, incomplete or inadequate treatments, the parasite's high genetic and metabolic adaptability, and its rapid reproduction rate, which allows resistant populations to emerge quickly (Talisuna *et al*., 2004). According to the WHO, antimalarial drug resistance occurs when a parasite strain can survive or multiply despite the administration and absorption of medication at doses equal to or exceeding the usual recommended levels, provided the drug reaches the site of action effectively. This resistance is driven by the selection of parasites with genetic mutations or gene amplifications that reduce their susceptibility to the drug (Reyburn, 2010). Quinine remains a valuable and effective treatment for malaria, although instances of resistance have been observed intermittently. Chloroquine-resistant *P. falciparum* likely emerged in four distinct regions, beginning at the Thai-Cambodian border in 1957, followed by Venezuela and parts of Colombia around 1960, Papua New Guinea in the mid-1970s, and Africa starting in 1978 in Kenya and Tanzania, spreading to Sudan, Uganda, Zambia, and Malawi by 1983. In an attempt to enhance effectiveness and delay resistance, sulfones and sulfonamides were combined with proguanil or pyrimethamine. However, by 1953, *P. falciparum* resistance was already noted in Tanzania. Sulfadoxine/pyrimethamine (SP) was introduced in Thailand in 1967, but resistance appeared the same year and quickly spread throughout Southeast Asia. In Africa, resistance to SP remained low until the late 1990s, after which it spread rapidly. Mefloquine resistance began emerging in Asia in 1985, coinciding with the drug's widespread availability (US Institute of Medicine Report, 2004).

The rise of *P. falciparum* strains resistant to all antimalarial drugs, including artemisinin-based therapies, has made the fight against malaria increasingly difficult

(WHO, 2017). This growing resistance has prompted researchers to continue the search for new, more effective antimalarial treatments (Akuodor *et al*., 2015).

Natural products have recently played a crucial role in discovering new chemical compounds (Mayo *et al*., 2020), with plant extracts offering diverse metabolites (Chen *et al*., 2020). These natural compounds have been key sources of lead treatments for various infectious diseases, including malaria. Notable examples include quinine, derived from the bark of *Cinchona* species, and artemisinin, extracted from the Chinese medicinal plant *Artemisia annua*, both of which have significantly contributed to reducing malaria deaths worldwide (Schwikkard and Heerden, 2002). The success of these plant-derived compounds highlights the potential of natural sources in yielding new antimalarial drugs (Ribeiro *et al*., 2023). Plants are a vast resource for novel molecular compounds, and efforts to discover new antimalarial agents should focus on evaluating the safety and efficacy of traditionally used medicinal plants (Feiz Haddad *et al*., 2017). Traditional medicine systems across various cultures have long utilized plants to treat malaria, making them a valuable source for new antimalarial discoveries (Willcox and Bodeker, 2004). By prioritizing plants with a history of traditional use for malaria treatment, researchers can save time, resources, and investment compared to a random selection approach. Further research into medicinal plants from different traditional systems could provide important leads for developing effective antimalarial drugs (Lemma *et al*., 2017).

The present study aims to screen potential antimalarial plants for future phytochemical investigations and to identify a single candidate for a comprehensive phytochemical analysis, in alignment with the objectives outlined below:

RESEARCH OBJECTIVES

1. Phytochemical screening of 10 antimalarial plants
2. Gas chromatography-mass spectrometry (GC-MS) analyses of phytoconstituents of three selected plants.

3. To study the *in vitro* antioxidant, antimicrobial, and antiparasitic activity of one selected plant.

CHAPTER 2

REVIEW OF LITERATURE

Herbal medicines are vital for both the prevention and treatment of various diseases and had served as a staple therapy of most diseases in human history. These plants synthesize a vast array of chemical compounds, known as phytochemicals, which fulfill diverse roles, such as defense mechanisms against bacteria, fungi, viruses, insects, and herbivores. This chemical diversity positions plants as promising candidates for the development of novel herbal medications (Parwiz *et al*., 2024). Addressing complex chronic diseases often requires targeting multiple biological pathways, and in conventional drug therapy, plant-derived products—characterized by their chemically complex mixtures—offer multiple potential targets and mechanisms through a range of primary and secondary constituents (Pelkonen *et al*., 2014). The application of natural products, including botanical amendments and extracts, for controlling fungal plant diseases is seen as an alternative to synthetic fungicides, with fewer adverse effects on both human health and the environment (Kumar and kumar, 2015).

The rising incidence of anthelmintic-resistant helminth strains, along with concerns over drug residues in animal products and the high costs associated with conventional anthelmintics, has intensified interest in medicinal plants as alternative anthelmintic sources (Tariq *et al*., 2009). In the search for potent antimalarial agents, research into medicinal plants within traditional medicine systems may provide critical insights and potential leads (Lemma *et al*., 2017). Strengthening the connection between traditional knowledge and scientific research is essential to identify and optimize natural compounds with pharmacological activities. Advanced analytical techniques are indispensable for accurately characterizing the structures and biological targets of these bioactive compounds (Rebeiro *et al*., 2023).

This review examines the systematic study of medicinal plants conducted through ethnobotanical research by various researchers, which has been instrumental in identifying plants with potential for phytochemical studies.

According to reports, some traditional antimalarial herbs were evaluated in vitro against *P. falciparum* malaria parasites. Experimental evidence shows that *Azadirachta indica* completely inhibited *P. falciparum* in both aqueous and ethanolic extracts at 100 µg/ml, with IC_{50} values of 7.39 µg/ml and 8.6 µg/ml (Abdulrazaq *et al*., 2020). *Vitex negundo* is one of the plants that tribal groups in the Amarkantak region utilize extensively to cure malaria and other ailments. Chloroform extracts of the leaf revealed substantial antimalarial activity, with IC_{50} values of 7.21 µg/ml and 7.43 µg/ml against 3D7 and K1 strains, respectively, with no indication of significant cytotoxicity against the mammalian cell line (VERO) and no toxicity as shown in the hemolysis experiment (Dwivedi *et al*., 2021). The root of *Goniothalamus lanceolatus* methanol extract reveals strong antiplasmodial activity against both *P. falciparum* 3D7 (IC_{50} = 2.7 µg/ml) and K1 strains (IC_{50} = 1.7 µg/ml) (Kaharudin *et al*., 2020). *Nauclea lanceolatus* hexane and methanol fractions showed potent antimalarial activity with IC_{50} values of 1.93 6 µg/ml and 3.91 6 µg/ml respectively and hence were classified as 'highly active' and tended to be able to match with chloroquine phosphate activity. The perspective for antimalarial activity in both fractions was affected by the presence of flavonoids, alkaloids, terpenoids, and steroids which had evolved as bioactive compounds for the malarial drug (Budiarti *et al*., 2020). Ethanol extract of different parts of *Helianthus annuus* displays strong in vitro antimalarial activity, roots exhibit the highest activity followed by leaf and flower extracts with an IC_{50} value of 2.3 ± 1.4, 4.3 ± 2.2, and 4.8 ± 0.0 µg/mL, respectively. The results of the 4-day suppressive test show that the ethanol extract of *H. annuus* root has an ED50 value of 10.6 ± 0.2 mg/kg (Ekasar *et al*., 2019). At a dose of 100 µg/ml, the dichloromethane/methanol (1:1) extract from the roots of *Pleiocarpa bicarpellata* showed no cytotoxicity towards mammalian L6 cells, however, it did exhibit action against the NF54 strain of *P. falciparum* with an IC_{50} value of 3.5 µg/ml. The solid antiplasmodial action of the extract is caused by alkaloids that were extracted from *Pleiocarpa mutica* roots which demonstrated considerable activity against *P. falciparum* (Sevik *et al*., 2022). The ethanolic extracts of three plants, *Mammea siamensis* flowers (IC_{50} = 1.50 µg/ml), *Garcina malaccensis* rhizomes (IC_{50} = 5.33 µg/ml), and *Mesua ferrea* flowers (IC_{50} = 7.39 µg/m), showed promising antiplasmodial action against the *P. falciparum* K1 strain. Furthermore, Vero cells

were toxically affected by the ethanolic extract of *M. siamensis*, which exhibited the strongest antimalarial activity (CC_{50} < 5 µg/ml) (Chaniad *et al*., 2022). The n-hexane, ethyl acetate, and ethanolic extracts of *Soncgus arvensis* leaf showed good antiplasmodial action in vitro, with IC_{50} values of 5.119±3.27, 2.916±2.34, and 8.026±1.23, respectively. Each extract also had a high antioxidant content and low cytotoxic effects. The ethyl acetate extract demonstrated antiplasmodial action (ED_{50} = 46.31±9.36 mg/kg body weight) (Wahyuni *et al*., 2023). The IC_{50} values for *Syzygium aromaticum* aqueous flower extract, *Piper chaba* ethanolic flower extract, *Zingiber officinale* ethanolic rhizome extract, *Terminalia arjuna* aqueous fruit extract, *Piper nigrum* ethanolic fruit extract, and *T. arjun* ethanolic fruit extract were 1.96, 2.06, 3.42, 4.05, 4.38, and 4.72 µg/ml, respectively. *S. aromaticum* has the most activity against *P. falciparum*, with the lowest IC_{50} value (1.96 µg/ml). The ethanolic extract of *Prabchompoothaweep* (a mixture of Thai herbs) showed considerable antimalarial activity against the K1 strain of *P. falciparum* (IC_{50} = 14.13 µg/ml). *Artesunate,* the positive control, displayed antimalarial activity with an IC_{50} of 1.25 µg/ml (Chaniad *et al*., 2023). In Madagascar, *Hernandia voyroni* (*Hernandiaceae*) has traditionally been used as a chloroquine replacement. The crude alkaloid extracts of stem barks revealed inherent in vitro antimalarial activity and chloroquine resistant against *P. falciparum* strain, FCM-29, with an IC_{50} value of 3.53 µg/ml (Ratsimamanga *et al*., 1994). *Bridelia atroviridis* showed excellent antiplasmodial activity (IC_{50} = 8.08 µg/mL) and low cytotoxicity (CC_{50} > 100 µg/mL). *Bridelia atroviridis* extract significantly decreased parasitemia ($p < 0.05$) with an effective dose 50 (ED_{50}) of 89 mg/kg (Djouwoug *et al*., 2020). The Indigenous people of Nsukka utilize *cucurbita pepo* to treat malaria. *Cucurbita pepo* ethanol extract demonstrated strong antiplasmodial action in vitro, with an IC_{50} of 3.05 µg/ml. Mice receiving an oral dosage of 500 mg/kg showed a significant ($p < 0.01$) 51% reduction in parasitemia. The extract had no appreciable hemolytic action up to a concentration of 1 mg/ml. A dose of 300 mg/kg significantly ($p < 0.01$) rescued mice from low hematocrit-induced anemia after infection (Chinelo Ezeani *et al*., 2022). The parasite levels significantly decreased in a dose-dependent manner after *Acacia nilotica* extract was administered. At 100, 200, and 400 mg extract/kg body weight, the average parasite percentage of chemo suppression was found to be 68.8, 78.5, and

79.5%, respectively. Complete chemo suppression was achieved with the administration of 5 mg chloroquine/kg body weight (Alli *et al*., 2011)

Root extracts of *Sarcococca hookeriana* have been used as a gout remedy by rural communities in Nepal (Rajbhandari, 2002). The steroidal alkaloids, hookerianamide and hookerianamide, as well as the three known alkaloids Na-emethylepipachysamine, sarcovagine, and dictyophlebine, have all been identified. Except for the compound dictyophlebine, which had the highest potency (IC_{50} 2.4 mM), all of these compounds demonstrated good inhibitory activity (IC_{50} 2.4—10.3 mM) against the *P. falciparum* strain that is resistant to chloroquine. (Devkota *et al*., 2007). The isolated compounds such as the flavonoids 7-dimethyl artonol, artonin and cyclo-artobiloxanthone from the root bark of *Artocarpus rigidus* shows significant antiplasmodial activity against *P. falciparum* with IC_{50} values of 7.9 µg/ml, 2.4 9 µg/ml and 3.7 9 µg/ml respectively (Namdaung *et al*., 2006). With an IC_{50} value of 2.21 µM, the isolated chemical isoxanthochymol from the roots of *Garcinia polyantha* showed strong chemo-suppressor parasite growth against *P. falciparum* (Lannang *et al*., 2008) *Diospyros quaesita* leaves, twigs, and branches had strong antimalarial activity. With IC_{50} values of 1.40 and 0.98 mm, respectively, one isolate, betulinic acid 3-caffeate, showed antimalarial activity in vitro against clones of *P. falciparum* resistant to and sensitive to chloroquine. At an ED_{50} of 4.0 mm, the isolated compound showed cytotoxicity in the human oral epidermoid (KB) cancer cell line (Cui-Ying *et al*., 2008). From genus *Erythrina* four compounds 1, 2, 4, eryvarin, hydroxycristacarpone, abyssinone, lespedezaflavanone, and vogelin were tested for, antimycobacterial, cytotoxic activities, and antiplasmodial activities. Vogelin displayed the strongest antiplasmodial activity against *P. falciparum* with an IC_{50} value of 2.8 µg/ml, whereas lespedezaflavanone and abyssinone displayed lower activity, with IC_{50} values of 3.7 and 7.0 µg/ml, respectively (Rukachaisirikul *et al*., 2008)

Tests for antimalarial activity have been conducted on around 54 species (21 families) of Cameroonian medicinal plants, crude extracts, and essential oils; some of them have demonstrated strong antiplasmodial properties, with IC_{50} values ranging from less than 5 µg/ml to less than 100 ng/ml on a variety of *Plasmodium* species

and strains. 26 Cameroonian medicinal plants (from 18 families) have produced almost a hundred active compounds that show a broad spectrum of efficacy against *Plasmodium* species. The separated products belong to several different chemical classes such as various terpenes, anthraquinones, saponins, flavonoids, labdanes, various alkaloids, xanthones, quassinoids, sugar, essential oils, amino acids, sungucine derivatives, xanthenes (Titanji *et al*., 2008). Some of the experimental findings revealed that the essential oils extracted by hydro distillation from fresh leaves of *Cymbopogon citratus* and *Ocimum gratissimum* booming in Cameroon were investigated, and the principal constituents of *O. gratissimum* oil were y-terpinene, ß-phellandrene, limonene, and thymol. The oil of *Cymbopogon citratus* contained geranial, myrcene, and ß-pinene. The effects of these oils on the growth of *Plasmodium berghii* were examined. Both oils demonstrated considerable antimalarial activity in a four-day suppressive in vivo test. The essential oil of *Citrus citratus* produced the maximum action at dosages of 200, 300, and 500 mg/kg of mouse per day, as measured by parasitemia suppression percentages 62.1%, 81.7%, and 86.6%. The equivalent values for the oil of *O.gratissimum* at the same concentrations were 55.0%, 75.2%, and 77.8%. respectively (Choumbougnang *et al*., 2005). The Baka pygmies of the Dja Division have used *Holarrhena floribunda* to cure malaria for decades. Extracts of the stem bark were tested against *P. falciparum* strains. The aqueous extract had the most activity against Indochina (W-2) strain, with an IC_{50} of 1 µg/mL, while the ethanol extract had the best activity against *Sierra Leone P. falciparum* (D-6) strain, with an IC_{50} of 4.3 µg/m. Lupeol and its derivatives have been isolated namely -3-O-(3'hydroxyeicosanoyl) lupeol, 3-O-(2'tetracosyloxy) acetyl lupeol and 3-O[(1''-hydroxyoctadecyloxy) 2-hydroxypropanoyl] lupeol were isolated from this plant and showed considerable antimalarial activity in vitro (Fotie *et al*., 2006). *Tithonia diversifolia*, an Asteraceae plant historically used to treat malaria, was tested in vitro against three strains of *P. falciparum*. The ether extract from aerial portions of the plant obtained in São Tomé e Príncipe showed strong antiplasmodial action (IC_{50} on FCA strain: 0.75 µg/ml). A bioassay-guided fractionation of this extract resulted in the discovery of the known sesquiterpene lactone tagitinin as an active component against *Plasmodium* (IC_{50} on FCA strain: 0.33 µg/ml) and bearing cytotoxic effects (IC_{50} on HTC-116 cells: 0.706

µg/ml) (Goffin *et al*., 2002). In Southwest Cameroon, a plant called *Turreanthus africanus* (Meliaceae) is used in traditional medicine to cure malaria. Seven chemicals were extracted from this plant's seeds. Four of them, which were oils, were tested in vitro using the chloroquine-sensitive strain of *P. falciparum* F32. The compound with the strongest antiplasmodial activity was compound 1 (16-oxolabda-8, 12(E)-dien-15-oic acid). Two additional compounds, methyl-14, 15-epoxylabda-8, 12(E)-diene-16-oate, and turreanin, had moderate action, while one compound was inert. These results are in line with the usual treatment of *P. falciparum* malaria with *T. africanus* (Ngemenya *et al*., 2006). The five isolated compounds from *Aframomum Zambesiacum* seeds were examined against antiplasmodial efficacy the result demonstrated that compound Zambisiacolactone exhibited the highest level of activity with a $1C_{50}$ value of 4.97 µM in vitro among the compounds tested (Kenmogne *et al*., 2006). Ajoene (4,5,9-trithiadodeca-1,6,11-triene 9-oxide), a substance first identified from *Alium sativum* extracts, was examined for antimalarial properties in vivo using a well-established mouse model. On the day of infection, a single 50 mg/kg dosage of ajoene inhibited the growth of *parasitemia* the tested amount had no discernible acute adverse effects. When administered as a single dosage on the day of infection, ajoene (50 mg/kg) and chloroquine (4.5 mg/kg) stopped treated mice's future development of *parasitemia* (Perez *et al*., 1994).

Ethnobotanical research in Nigeria has documented numerous plants local communities use to treat various illnesses, including malaria. Among 51 plant species studied, extracts from 45 showed antimalarial and antiplasmodial activity, while 24 species demonstrated antiplasmodial properties in vitro specifically against *P. falciparum*. Sixteen plants yielded active antimalarial compounds (IC_{50} values < 5 µg/ml), predominantly alkaloids and limonoids. Due to limited quantities obtained, many of these compounds have only been studied in vitro (Adebayoa, 2011).

The nitro-tetrazolium blue-based parasite lactate dehydrogenase test was used to assess the antimalarial activity of four traditional East and Central African medicinal herbs—*Emilia discifolia*, *Senecio stuhlmannii*, *Indigofera emarginella*, and *Aspilia africana*. Among them, the ethyl acetate extract of *A. africana* showed significant antiplasmodial activity, effectively inhibiting both chloroquine-sensitive (D10, IC_{50}:

9.3 µg/ml) and chloroquine-resistant (K1, IC_{50}: 11.5 µg/ml) strains of *P. falciparum*. The strong correlation between extract efficacy against the two strains (Pearson's r = 0.9691, P = 0.05) suggests that antimalarial sesquiterpenes in *A. africana* may contribute to its high antiplasmodial activity (Waako *et al*., 2007). From the root bark of *Harungana madagascariensis*, a novel anthrone derivative called bazouanthrone has been identified together with other well-known chemicals including betulinic acid, feruginin A, harunganin, harunganol A, harunganol B, and friedelan-3-one. The obtained compounds' antiplasmodial activity was assessed in culture against the *P. falciparum* W2 strain. It was discovered that all of the substances were effective against the *Plasmodium* parasites, with bazouanthrone exhibiting the highest potency (IC_{50} = 1.80 mM) (Lenta *et al*., 2007). Thus, with an IC_{50} value of 0.052 and 0.517 µg/ml, the stem bark extract of *Harungana madagascariensis* showed strong anti-protozoan activities against *Plasmodium* and *Trichomonas* both in vitro and in vivo. The extract's effects on *Plasmodium yoelii nigeriensis* were investigated in vivo. Results indicated that only 80 mg/kg of the extract decreased the level of *parasitemia*. At the same time, the percentages of chemo-suppressive samples in both the prophylactic and suppressive tests ranged from 28.6–44.8% and 30.2–78.2%, respectively (Iwalewa *et al*., 2008). In northern Nigeria, *Cassia singueana* is used to treat acute malaria attacks. This plant's root bark methanol extract was tested in mice and rats to see how it affected inflammation, nociception, pyrexia, and rodent plasmodia infection. The outcomes demonstrated that in every model employed, the extract had notable antinociceptive, antipyretic, and antiplasmodial properties. The extract's phytochemical screening identified phenols, tannins, saponins, and trace amounts of anthraquinones. In mice, the extract's LD_{50} was determined to be 847 ± 30 mg/kg (Adzu *et al*., 2003). To assess the antimalarial efficacy of traditional medicines used in French Guiana, 35 traditional remedies were produced and tested in vitro for blood schizonticidal activity against a chloroquine-resistant strain of *P. falciparum* (W2). A few of these extracts underwent in vivo screening for the presence of *Plasmodium yoelii rodent* malaria. An inhibition test for ferriprotoporphyrin was also conducted. *Plasmodium* liver stage testing was utilized to evaluate four common preventative treatments used by the populace. All told, five treatments such as *Irlbachia alata* (Gentiananceae), *Picrolemma pseudocoffea*

(Simaroubaceae), *Quassia amara* (Simaroubaceae), *Tinospora crispa* (Menispermaceae), and *Zanthoxylum rhoifolium* (Rutaceae) were able to block over 50% of the parasite development in vivo at around 100 mg/kg (Okokon *et al*., 2007). The aerial portions of *Abrus precatorius* were used to isolate abruquinone and isoflavanquinone, a novel derivative. By using spectrum analysis, the chemical structures of these substances were clarified isoflavanquinone had weak antiviral and cytotoxic properties, whereas abruquinone has shown antitubercular, antiplasmodial, and cytotoxic properties (Limmatvapirat *et al*., 2004). Ethnobotanical studies in southwest Nigeria identified *Cassia siamea* as a treatment for fever. Researchers fractionated its stem bark extract, using bioassays to test its antimalarial activity against the multi-resistant *P. falciparum* strain (K1) via the parasite lactate dehydrogenase test. Chromatographic techniques led to isolating emodin and lupeol from the ethyl acetate fraction. Spectroscopic analysis confirmed their structures, revealing that both compounds exhibited antiplasmodial activity with IC_{50} values of 5 µg/ml (Ajaiyeoba *et al*., 2008).

Recent research has discovered aqueous, butanol, and ethyl acetate extracts of *A. scholaris* leaf and bark. Compared to the ethyl acetate extract, the aqueous and butanol extracts showed significantly higher antioxidant activity in the DPPH, ABTS, and FRAP experiments. The IC_{50} values for aqueous bark and leaf extracts were 1.21 mg/ml and 2.83 mg/ml in the DPPH assay, and 210 µg/mL and 523 µg/ml in the ABTS assay. In the FRAP experiment, the aqueous bark and leaf extract demonstrated a ferric reducing power of 130.70 µm/mg and 109.52 µm/mg at 2 mg/ml (Antony *et al*., 2011). Furthermore, the antioxidant capacity of both aqueous and methanolic extracts from *A. scholaris* bark was investigated in vitro, and the results revealed a high total antioxidant activity level. At each concentration studied, aqueous extracts scavenged more superoxide radicals than conventional gallic acid at the same concentrations. Similarly, the findings of the DPPH free radical scavenging experiment demonstrated that the aqueous extracts better scavenged free radicals than the methanolic extract and standard ascorbic acid tested at corresponding doses (Mistry *et al*., 2016). Compared to the standard butylated hydroxytoluene (BHT) and L-ascorbic acid, the methanol extracts of the flower of *A. scholaris* demonstrated

stronger antioxidant activity than the fruit (Joel *et al*., 2011). Methanolic extracts of leaves, follicles, and latex from the Indian devil tree, *A. scholaris* (L.) R. Br). All of the extracts had robust DPPH free radical and superoxide anion scavenging activities, except the leaf extract, which demonstrated potent reducing and ferrous ion chelating activities (Ganjewala and Gupta, 2013). For the first time, the methanol extract of *A. scholaris* stem bark has been shown to have a potential anti-inflammatory effect. The extract's antioxidant properties were quantitatively validated using β-carotene-linoleate oxidation using thin-layer chromatography (Subraya *et al*., 2012). According to reports, the dichloromethane and ethyl acetate fractions of *A. scholaris* have antioxidant effects, which are equivalent to those of BHA and L-ascorbic acid (Arulmozhi *et al*., 2010). The results demonstrated the antioxidant activity of all *A. scholaris* extracts, whether they were bark or leaves. The IC50 values for the hexane, methanol, ethyl acetate, and leaf extracts were 295.40, 84.48, and 237.29 µg/ml, respectively, and the IC50 values for the stem bark extracts, bark, ethyl acetate, methanol, and hexane extracts were 156.59, 89.36, and 189.27 µg/ml, respectively. The total phenolic content of the stem bark, methanol, ethyl acetate, and hexane extract was 3.32 µg of gallic acid equivalents (GAE)/10 mg dried extract, while the total phenolic content of the leaves, stem bark, and ethyl acetate extracts was 18.80, 5.33, and 2.63 µg, all expressed in mg of dried extract (Itam *et al*., 2018). *A. scholaris* methanol leaf extract had IC_{50} values of 45.88 µg/ml for crude extract, 42.87 µg/ml for column purified samples, and 38.5 µg/ml for ascorbic acid. The results show that the DPPH leaf extract increased with increasing concentration, implying that the purified methanolic extract of *A. scholaris* could be a potential source of antioxidants and phytochemicals in the future, making it useful as a therapeutic agent against oxidative stress-related degenerative diseases and disorders (Shanmugapriya and Jayanti, 2019). The EC_{50} value for the hexane extract of *A. scholaris* stem bark was 68.75 µg/ml, whereas the n-hexane extract of the leaves was higher at 118.75 µg/ml. *Alstonia venenata* had considerable in vitro superoxide scavenging efficacy, surpassing that of quercetin. *A. venenata* showed reduced superoxide scavenging efficacy. The IC_{50} values for hexane extract *A. scholaris,* isopropanol extract *A. venenata*, and quercetin were 90.5 ± 6.2, 7.5 ± 1.2, and 31.5 ± 2.5 µg/ml, respectively. The hexane extract of stem bark from *A.*

scholaris and the isopropanol extract of leaves from *A. venenata* are potential candidates for the development of a new generation of anticancer medications to treat illnesses such as lymphoma and leukemia. These can also be utilized as antioxidants in nutritional supplements (Bagheri *et al*., 2016). The methanol extract of *A. Scholaris* bark showed scavenging action with an IC_{50} value of 39 g/ml, indicating mild to moderate antioxidant activity. In the brine shrimp lethality test, the methanol extract had an average LC_{50} of 0.91 µg/ml. This revealed that the methanolic extract had substantial cytotoxicity (Bellah *et al*., 2017).

Picralima nitida (Apocynaceae*)* bark extract in methanolic, hydro-methanolic, and aqueous forms is high in phenol and flavonoid components. The DPPH anti-radical scavenging capabilities demonstrated that the different extracts of *P. nitida* have a moderated decrease (2, 867 ± 0,002 mg/ml, 3,161 ± 0,016 mg/ml, and 2,693 ± 0,004 mg/ml), but comparatively low compared to the molecule of reference, ascorbic acid (0,064 ± 0,000 mg/ml) (Ngaisonna *et al*., 2016). *Carissa edulis* (Apocynaceae) is a plant used to treat oxidative stress and inflammatory conditions such as malaria, rheumatoid arthritis, and cardiovascular disease. The hydroethanolic and methanolic extracts had a high concentration of flavonoids, with 14.84 mg RE/g extract and 12.02 mg RE/g extract, respectively. Tannin levels were likewise high in the two extracts, with 26.76 mg TAE/g extract and 34.67 mg TEE/g extract. The aqueous extract displayed free radical scavenging activity ranging from 58.63% to 94.67%, whereas the methanolic extract showed ABTS ranging from 51.39% to 94.12%. The methanolic extract exhibited the greatest FRAP (6.73 g AAE/100 g extract). HPLC examination confirmed the presence of quercetin, rutin, and gallic acid in the various extracts. *C. edulis* is a possible source of bioactive components with antioxidant properties (Fanta *et al*., 2019). *Hoodia gordonii* extract effectively inhibited HIV RT, with IC_{50} values of 73.55 ± 0.04 and 69.81 ± 9.45 µg/ml for ethanol and ethyl acetate extracts, respectively. Both extracts showed inhibitory efficacy against HIV PR, with IC_{50} values of 97.29 ± 0.01 and 63.76 ± 9.01 µg/ml for ethanol and ethyl acetate respectively. *H. gordonii* demonstrated strong antioxidant activity, with IC_{50} values of 124.6 ± 11.3 and 126.2 ± 3.15 µg/ml for ethanol and ethyl acetate extracts, respectively. The reducing power of *H. gordonii* extracts increased with

concentration, confirming the presence of antioxidants (reductants) in the extracts (Kapewangolo *et al*., 2016).

The results of the investigation showed that 13 bacterial species and strains were tested in vitro using the agar diffusion method to determine the antibacterial activities of the acetone, chloroform, alcohol, and ethyl acetate extracts of *Glycyrrhiza glabra*, *Juniperus oxycedrus*, *Pimpinella anisum*, *Cinnamomum cassia*, and *Coriandrum sativum*. They were shown to effective against *Listeria monocytogenes*, *Yersinia enterocolitica* P 41797, *Enterococcus faecalis*, *Mycobacterium smegmatus* RUT, *Bacillus brevis* FMC 3, *Bacillus cereus* EU, *Bacillus megaterium* DSM 32, *Pseudomonas aeruginosa*, *Bacillus subtilis* IMG 22, *Staphylococcus aureus* ATCC 25923. *Micrococcus luteus* LA 2971, *Bacillus subtilis* var. *niger* ATCC 10, and *Klebsiella pneumoniae* FMC 5. According to the study, the above microorganisms were resistant to the various antimicrobial agents tested, alcohol extracts from *Pimpinella anisum* seeds, ethyl acetate, acetone, and chloroform extracts from *Glycyrrhiza glabra* roots, bark from *Cinnamomum cassia*, *Glycyrrhiza glabra* roots, and *Juniperus oxycedrus* seeds. However, *Coriandrum sativum* extracts did not affect the same organisms (Ates *et al*., 2003). Using the agar disc diffusion technique, ethanolic extracts of *Zingiber officinales*, *Punica granatum, Cuminum cyminum*, *Thymus vulgaris*, and *Syzygium aromaticum* were tested against *Salmonella typhi*, *B. cereus, P. aeruginosa*, and *Escherichia coli*. At a dose of 10 mg/ml, the findings showed varying efficacy against the investigated bacterial strains, with the extract of *Cuminum cyminum* showing very little effectiveness against *S. aureus.* The two most effective plant extracts were the ethanolic extracts of *P. granatum* and *S. aromaticum*, which demonstrated both bactericidal and bacteriostatic properties against the highly sensitive strains of food-borne pathogenic bacteria (*S. aureus* and *P. aeruginosa*). These potentially beneficial plant extracts can be utilized as natural alternatives to prevent food poisoning and preserve food while avoiding the health risks associated with chemically antibacterial agent applications (Mostafa *et al*., 2018).

The antibacterial efficacy of aqueous, ethanolic, methanolic, and phenolic extracts from three traditional Palestinian medicinal plants and their commercial oils was

investigated against pathogenic microorganisms. The results suggest that aqueous extracts of sage and thyme were effective against most of the bacteria tested. The phenolic extracts of sage and thyme were bactericidal against *S. aureus* and *Enterococcus* sp., respectively. In contrast, the ethanolic extract of parsley had a greater effect on *E. coli*. However, the extract had no discernible effect on the tested Gram-positive organisms. Commercial sage, thyme, and parsley oils showed negligible antibacterial efficacy against *E. coli*, *Proteus mirabilis*, and *S. typhi* (Elastay *et al*., 2005). An entire antimicrobial spectrum and an exceptional inhibitory effect were demonstrated by the combined extract (8:1:1, v/v/v) of *Corni fructus*, *Cinnamon*, and Chinese chive, which was used to assess the antimicrobial activity against common foodborne microorganisms, such as bacteria, yeasts, and molds (Hsieh *et al*., 2001). The alcoholic extract of curry leaves demonstrated the greatest zone of inhibition on *Candida albicans*, followed by aqueous tea leaves. Other plant extracts such as alcoholic onion leaves, tea leaves, onion bulbs, *Aloe vera*, and mint leaves all suppressed *C. albicans* development, albeit to varying degrees. A recent study identifies a few medicinal plants as alternative medicines in the field of dentistry that can be used in conjunction with conventional therapy for oral candidiasis. (Doddanna *et al*., 2013). The study investigated the effects of *Vitex negundo, Terminalia bellerica, Andrographis paniculata, Terminalia chebula, and Phyllanthus niruri* on four Gram-negative and one Gram-positive bacteria. The findings indicated that *Phyllanthus niruri* leaf extract had a minimum inhibitory concentration (MIC) of 50 µg/ml against both *S. aureus* and *S. typhi*. In contrast, *Terminalia bellerica* fruit extract had MICs of 50 µg/ml against *Escherichia coli* and 200 µg/ml against *S. aureus*. No antimicrobial activity was observed for the leaf extracts of *Vitex negundo*, *Terminalia chebula*, and *Andrographis paniculata* at the tested concentrations (Sumathi and Parvathi, 2010). Methanol and aqueous extracts from *Catharanthus roseus*, *Wrightia tinctoria*, *Sapindus emarginatus*, and *Annona squamosa* were analyzed, revealing that *Wrightia tinctoria* leaf extracts exhibited the strongest antibacterial effects against *S. aureus* and *P. aeruginosa*. Additionally, methanol extracts demonstrated 30% antifungal inhibition against *Curvularia* sp. (Shalini and Prema, 2012). The methanol extracts from the leaves, roots, or bark of *Acacia nilotica*, *Sida cordifolia*, *Tinospora cordifolia*, *Withania somnifera*, and

Ziziphus mauritiana demonstrated activity against various microbes, including *B. subtilis*, *E. coli*, *Pseudomonas fluorescens*, *S. aureus*, *Xanthomonas axonopodis* pathovar *malvacearum*, *Aspergillus flavus*, *Drechslera turcica*, and *Fusarium verticillioides*. Plant-derived products are effective sources of antimicrobial compounds (Mahesh and Satish, 2008). Ethanolic extracts of *Nigella sativa*, *Anthemis nobilis*, *Zingiber officinale*, *Cinnamomum verum*, *Syzygium aromaticum*, and *Piper nigrum*, sourced from a local market in Dammam, Saudi Arabia, were evaluated using the hole-plate diffusion method against various microorganisms. These included Gram-positive bacteria *B. subtilis* and *S. aureus*, Gram-negative bacteria *P. aeruginosa* and *E. coli*, and the yeast *C. albicans*. *Zingiber officinale* and *Anthemis nobilis* demonstrated stimulating effects on *B. subtilis* and *C. albicans*. *Syzygium aromaticum* effectively inhibited the growth of *C. albicans*, *B. subtilis*, and *P. aeruginosa*. *Cinnamomum verum* was notably effective against *B. subtilis* and *C. albicans* only. The remaining plants either showed no inhibition or exhibited stimulating effects on the test organisms. *S. aureus* and *E. coli* were resistant to all the plant extracts, while *P. aeruginosa* was resistant to nearly all extracts except for *Syzygium aromaticum* (Ababutian, 2011). Acetone and ethanol extracts of *M. longifolia*, *P. biglandulosa*, and *P. acerifolium* have higher antibacterial activity than water extracts and might be used to generate novel antimicrobial agents (Khond *et al*., 2009).

A. scholaris extracts in hexane, benzene, isopropanol, ethyl alcohol, methanol, acetonitrile, and water were tested for antimicrobial activity against Gram-positive and Gram-negative bacteria such as *S. aureus*, *B. cereus*, *Lactobacillus* sp., *E. coli*, *P. aeruginosa*, and *S. typhi*. The benzene, methanol, and water extracts all showed considerable antibacterial activity, which was more prominent against Gram-positive (20-25mm) than Gram-negative bacteria (18-21mm). The ethyl acetate extract also showed greater efficacy against Gram-positive (20-23mm) than Gram-negative (14-18mm). Though all of the extracts showed significant suppression of all of the organisms tested, the hexane extract exhibited no cidal action against *Lactobacillus* or *S. typhi*. The aqueous extract has no cidal action against *P. aeruginosa* or *S. typhi* at the tested doses (200-240 μg) (Thankamani *et al*., 2011). Crude extracts of

petroleum ether, carbon tetrachloride, and chloroform were tested for antibacterial activity. The petroleum ether extracts have modest inhibitory activity against *Shigella boydii* and *V. parahemolyticus*. However, carbon tetrachloride and chloroform extracts inhibited *V. mimicus* and *V. parahemolyticus*. The chloroform extracts also had an inhibiting effect on *E. coli*. In the presence of other bacteria and fungi, the inhibition was determined to be weak to moderate (Bellah *et al*., 2017).

The antibacterial activity of various chemicals extracted from the *Alstonia* species suggests that long-chain hydrocarbons are active against both Gram-positive and Gram-negative bacteria. Although esters and alcohols are ineffective against all bacteria, α-amyrin and lupeol are more effective than *B. subtilis* and *E. coli*. Sterols, as opposed to aliphatic long-chain hydrocarbons, are either more or equally effective against all Gram-negative bacteria. Adding acetyl groups to α-amyrin, lupeol, and sterols lowers their activity, similar to *Shigella aureus*. *S. albus*, *E. coli*, *Proteus vulgaris*, and *P. pyocyanea* are either ineffective or inactive, as are *Klebsiella* and *Shigella dysentriae* (Varshney *et al*., 1995). The compounds extracted from *A. scholaris* leaf extract show that pentacyclic triterpenoids are the antibacterial chemicals in *A. scholaris*, and ursolic acid's capacity to improve antibiotic activity may make it a valuable group of medicinal agents in the future (Wang *et al*.,2016). Silver nanoparticles derived from *A. scholaris* have high inhibitory activity against fungal species, Gram-positive and Gram-negative bacteria (Shetty, *et al*., 2014).

In vitro, antifungal assays against *Fusarium oxysporum, Fusarium lycopersici* were performed utilizing plant extracts of *Solanum indicum*, *Azadirachta indica*, and *Oxalis latifolia* using the poison food approach. The study suggests that botanical extracts might be a useful option in generating powerful plant-based fungicides that can be utilized in organic farming to control *F. oxysporum*, and *F. lycopersici*. (Ramaiah and Garampalli, 2015). Ethanolic leaf extracts of *Datura metel, Jatropha carcus*, and *Ruellia tuberosa* were evaluated against the isolated pathogen that causes sheath blight and rice blast disease. When compared to mancozeb, the three plant extracts inhibited mycelial growth much more. As a result, extracts from these three plants have the active capacity to limit fungal development and may be utilized as

bio fungicides to manage rice blasts and sheath blight infections. (Durgeshlal *et al*., 2019).

The cattle business in developing nations faces a significant danger from infections brought on by gastrointestinal nematodes. Due to the high cost of anthelmintic medications, they have direct repercussions in the form of production and indirect economic losses (Kassai, 1999). Many tactics combat parasitism, such as immunization, nutritional management, biological control, pasture management, and anthelmintic medications (FAO, 2002). Chemical anthelmintics such as levamisole, flubendazole, and thiabendazole were used to treat gastrointestinal nematodiasis (roundworm infection) in sheep and goats but were not always effective. This made people want to learn more about medicinal plant extracts as possible alternative sources of anthelmintics. (Kamaraj *et al*., 2011).

A common method for determining anthelmintic effectiveness is a direct examination of helminthic worm viability after exposure to the anthelmintic agent. Following this fundamental method, various therapeutic plants, such as *Cocus nucifera* (Palmae), *Hedera helix* (Araliaceae), *Myrsine Africana* (myrisinaceae), *Rapanea melanophloeos* (myrisinaceae), *Abizia schimperiana oliv,* (Fabaceae), *Leucas martinicensis* (Lamiaceae), *Rumex abyssinisus* (Polygonaceae), *Leonotis ocymfolia* (Lamiaceae), *Combretum mole* (Combretaceae), *Brucea javanica* (Simaroubaceae), *Carica papaya* (Caricaceae), *Coriandrum sativum* (Apiaceae), *Artemesia brevifolia* (Asteraceae), *Pycnanthus angolensis (*Myristicaceae*)*, *Sphenocentrum jollyanum* (Menispermaceae), *Nauclea talifolia* (rubiaceae), and *Zingiber officinale* (Zingiberaceae) have already been recognized to display anthelmintic activity against different nematodes of veterinary reputation (Olivera *et al*., 2009; Equale *et al*., 2007a; Githiori *et al*., 2002; Ademola and Eloff, 2010; Wang *et al*., 2011; Eguale *et al*., 2007b; Ademola *et al*., 2004; Tariq *et al*., 2009; Wang *et al*., 2010; Asha *et al*.,2001; De Amorin *et al*., 1999; Preshant *et al*., 2001; Iqbal *et al*., 2004; Gbolade and Adeyemi, 2008; Onyeyili *et al*., 2007; Igbal *et al*., 2006).

Crude aqueous and methanolic extracts of *Chenopodium album, Caesalpinia crista, Trianthema portulacastrum, Musa paradiscaia, Ziziphus mummularia, Calatropis procera,* and *Azadirachta indica* exhibited dose and time-dependent anthelmintic effects by causing mortality of worms and inhibition of egg hatching when tested against trichostrongylids nematodes of sheep (Jabbar *et al*., 2007; Hussain *et al*., 2011; Bachaya *et al*., 2009; Iqbal *et al*., 2002; Iqbal *et al*., 2010).

According to reports, hydroacetonic and hydroalcoholic extracts from the seeds and leaves of *Parkia platycephala, Dimorphandra gardneriana,* and *Turnera ulmifolia* barks and leaves were chosen naturally by goats in the Brazilian savanna (cerrado), were tested in vitro against *Haemonchus contortus*, a common roundworm of sheep and goat, and have been referenced in ethnoveterinary investigations, were used to assess larval development, larval exsheathment inhibition, and egg hatching assays. The outcomes demonstrated distinct in vitro anthelmintic activity against *H. contortus* at various phases, suggesting that these species might be used as a viable substitute strategy to manage helminthic infections in small ruminants (Oliveira *et al*., 2017). Plants of the genus *Artemisia* are recognized to have medicinal and anthelmintic properties. A recent study examined the in vitro and in vivo effects of methanol extracts of *Artemisia sieversiana* and *A. parviflora* on *H. contortus*. The bioassays employed a variety of doses, and adult worms and infective larvae died after 8 hours of exposure. Both plant extracts demonstrated a 100% inhibition of egg hatching. It was determined that certain plant extracts were beneficial in lowering the worm load in animals (Irum *et al*., 2015). The effects of crude aqueous and crude ethanolic extracts of *Artemisia absinthium* aerial parts on sheep gastrointestinal (GI) nematodes were compared to albendazole and displayed considerable ($P < 0.005$) anthelmintic action against adult *H. contortus* worms, as evidenced by the paralysis or death of the worms after exposure to various treatments (Tariq *et al*., 2009). According to investigations, *Picra fel-terrae* leaf and whole plant extracts exhibit encouraging anthelmintic activity. In general, plant extracts are a potentially abundant source of anthelmintics to treat helminthic disorders (Kumarasingha *et al*., 2016). Several species have already been found to have anthelmintic effects, including *Artemisia annua* extracts (Cala *et al*., 2014).

The rhizome of *Stephania glabra* and the aerial root of *Trichosanthes multilobe* were tested against the different helminth parasites including nematodes like *Heterakis gallinarum, Ascaridia galli, Ancylostoma ceylanicum*, and *Ascaris suum*; cestodes like *Raillietina echinobothrida* and trematodes like *Fasciolopsis buski*. These plants were used in traditional medicinal practices in Meghalaya (Northeast India) as an anthelmintic agent against intestinal worms. The alcoholic crude extracts had a noticeable impact on the trematode and cestode, with *R. echinobothrida* and *F. buski* exhibiting a dose-dependent progressive reduction in physical motility. The phytochemical of *S. glabra* appears to be useful against cuticle-covered nematodes, but not so much against platyhelminth parasites with a tegumental interface (Tandon *et al*., 2004). The study examined the in vitro anthelmintic efficacy of *Cassia alata, C. angustifolia,* and *C. occidentalis* and found that paralysis of worms occurred for a very short period after parasites were exposed to these plant extracts and that all tested plants exhibited broad-spectrum vermifugal activity (Kundu *et al*., 2014). The aerial portions of *Hygrophila difformis (*Acanthaceae) were extracted using petroleum ether, benzene, chloroform, and ethanol. The extracts efficiently paralyzed and killed the worm at varied dosage levels ($p < 0.001$). The most anthelmintic activity was reported with benzene extract, which may be used on ruminants. Phytochemical studies revealed positive results for cardiac glycosides, tannins, steroids, flavonoids, and saponins (Samanta *et al*., 2012). The leaf, bark, and seed extracts of *Anisomeles malabarica, Andrographis paniculata, Solanum torvum, Annona squamosa, Datura metel*, and *Solanum torvum* were tested against *H. contortus* using egg hatch assay (EHA) and larval development assay (LDA). All plant extracts inhibited parasite effects to a modest degree. The EHA results revealed that the ED_{50} of methanol extracts of *A. paniculata* and *D. metel* were 2.90 and 3.08 mg/ml, respectively, whereas, in the larval development experiment, the ED_{50} was 4.26 and 3.86 mg/ml. In vivo research is still needed to validate these benefits. (Nandi *et al*., 2017). At the quantities and dosage levels examined, extracts from *Coriandrum sativum* seeds demonstrated some in vitro and in vivo anthelmintic activity against *H. contortus*. Alkaloids and flavonoids, which are present in the current experiment, belong to a class of secondary metabolites that are thought to be the source of the chemical components underlying the various medicinal properties

of various plants (Debella, 2002). A tapeworm *Raillietina tetragona* was used as an in vitro test subject for the crude extract of *C. viscosum*. Significant structural changes in the tegument suggest that the plant-derived components modify the parasite's permeability, causing paralysis and eventual death. As a result, they may be used as an alternative chemotherapeutic drug (Kamaraj *et al*., 2011). The crude aqueous and methanolic extracts of *Butea frondosa* seeds extracts were tested against benzimidazole-resistant gastrointestinal nematodes in goats using in vitro and in vivo methods such as egg hatch assay (EHA), larval paralysis test (LPT), and adult mortality test (AMT) against various stages of gastrointestinal nematodes. Based on the outcomes of studies conducted in vitro and in vivo, *B. frondosa* appears to have anthelmintic activity and may be a viable substitute for pharmaceutical anthelmintics (Saiyam *et al*., 2021). Tribal groups in the Bodoland area of Assam employ *A. scholaris*, *Cardiospermum halicacabum*, *Hydrocotyle sibthorpioides*, and *Hypericum japonicum* as essential folk medicinal herbs to cure helminth infections. A research on anthelmintics was carried out by administering several dosages of plant extracts to the trematode, *Paramphistomum* sp. All plants exhibited a dose-dependent effectiveness in anthelmintic bioassays. The anthelmintic activity of *H. japonicum* was the highest (LC50 0.21 mg/ml), with *H. sibthorpioides* (5.36 mg/ml), *C. halicacabum* (13.40 mg/ml), and *A. scholaris* (18.40 mg/ml) following closely behind. The antiproliferative and anthelmintic effects of the plants were shown to positively correlate with their antioxidant characteristics (Ananta *et al*., 2021). When air-dried leaves of *Cassia alatas* ethanol extract were tested against the cestode *Hymenolepis diminuta*, the results showed tegument swelling, blebbing all over the tegumental surface, the destruction of microtriches, and alterations like shrinking in the scolex area. Parenchyma cell depletion, connective tissue breakdown, and sparsely cytoplasmic cytons were also noted; these characteristics are consistent with worms given the well-known medication praziquantel. These findings might point to the plant leaves as a potential treatment for helminth infection and be a positive development in the hunt for substitute anthelmintic medications (Kundu *et al*., 2012). An indigenous leguminous plant of Meghalaya, with putative anthelmintic use *Flemingia vestita* from crude root-tuber peel extract was evaluated against the chicken tapeworm *R. echinobothrida* and the active chemical principle, genistein.The

change in AChE activity observed following treatment with root-tuber peel extract or genistein implies that a component produced from *F. vestita* may be anthelmintic (Pal and Tandon, 1998). The anthelmintic efficacy of an ethanolic shoot extract of *Alpinia nigra*, a plant that the Tripuri tribes of northeast India have traditionally used as a medicinal remedy, was evaluated in vitro using changes in the activity of key tegumental enzymes, including acid phosphatase (AcPase), alkaline phosphatase (AlkPase), and adenosine triphosphatase (ATPase). The findings imply that the active component(s) of *A. nigra*, which appears to function transtegumentally, may target the parasite's tegumental enzymes as a major site of action (Roy and Swargiary, 2009).

Various ethnobotanists have conducted studies on medicinal plants in Mizoram through ethnobotanical investigations. Over the years, multiple studies have reported the use of a vast number of plant species for treating a wide range of diseases. The investigation reported on plants used to treat 97 different diseases (Darlianthanga, 1989), while 58 species were identified (Saptawna, 1990) and 128 species were documented (Lallianthanga, 1990), Recognized the medicinal use of 434 plant species by the people of Mizoram to treat various diseases (Mahanti, 1994) noted 85 ethnomedicinal plants (Chawngkunga, 1996).
Some significant contributions, grounded on ethnobotanical surveys have been made by Lalramnghinglova (1996); Lalnundanga *et al*., (1997).

Enriched significantly to this field, documenting 966 plant species used for medicinal purposes across Mizoram (Sawmliana, 2003).17 plant species utilized by Mizoram's tribes to treat cuts and wounds (Bhardwaj and Gakhar, 2004) while reported 204 medicinal plant species in the region (Rozika, 2005).In western Mizoram, 89 plant species serve as herbal medicines (Lalfakzuala *et al*., 2007), and 39 species are employed in various districts (Rama Shankar, 2009). 159 ethnomedicinal plants from 134 genera and 56 families, providing detailed descriptions of plant parts used, modes of application, and their roles in treating diseases prevalent in local communities. (Rai and Lalramnghinglova, 2010)

Further exploration was made in the Kolasib, Aizawl, Champhai, Serchhip, and Lunglei districts highlighting the role of folk medicinal plants in treating conditions such as diarrhea, dysentery, respiratory issues, and skin ailments. Some traditional healers have even cultivated their herbal gardens, incorporating endangered species like *Oroxylum indicum*, *Cissampelos pareira*, and *Stephania japonica* (Rama Shankar *et al*., 2012). 82 medicinal plant species across 42 families and 76 genera, documenting their use in treating 34 ailments, with malaria, stomachaches, diarrhea, dysentery, jaundice, and kidney issues being the most common (Lalmuanpuii *et al*., 2013). Further contributions include research on 207 plant species, with 29 species used in combination, and his findings on 36 species used in ethnoveterinary medicine (Lalramnghinglova, 2016) and 53 for diabetes treatment (Laha *et al*., 2016). while documenting 56 traditionally used species in Aizawl (Lalzarzovi *et al*., 2016). Ten plants commonly used in Mualcheng village were identified (Tlau and Lalawmpuii, 2020), and 24 species have been scientifically evaluated for antidiarrheal properties, while 44 are yet to be studied (Zothantluanga *et al*., 2020). Recent research highlights 93 plant species for treating muscle, bone, gastrointestinal, and skin disorders (Laldingliani *et al*., 2022). Garcinia sopsopia contains terpenoids and polyphenols, while Mallotus roxburghianus is rich in flavonoids, and Vitex penduncularis has sitosterol and glucosides (Lalnundanga, 2000). The Hiptage benghalensis root bark's methanol extract reveals alkaloids, tannins, and reducing sugars. Alkaloids, known for their significant medicinal properties, are likely the key components behind the plant's traditional therapeutic effects (Lalnundanga *et al*., 2012).

The following plant extracts were tested against several parasites, including *R. echinobothrida*, *R. tetragona*, and *Ascaridia galli*, and the findings demonstrated substantial anthelmintic activity among the Mizo traditional anthelmintic plants.

It has been shown that a crude alcoholic extract of the stem bark of *Acacia oxyphylla*, an anthelmintic plant native to the Mizo tribes of northeastern India has been proven to have substantial anthelmintic effects against the avian gastrointestinal cestode, *R. echinobothrida* (Lalchhandama *et al*., 2007). *Ascaridia galli* (Nematoda) treated with

20 mg/ml of the plant *Acasia oxyphylla* exhibited substantial structural changes including cuticle peeling, muscle layer disintegration, ovarian rupture, and egg membrane deformation. The findings indicate that *A. oxyphylla* is an effective nematocide, supporting its use as a traditional anthelmintic (Lalchhandama, 2008). *Raillietina echinobothrida Megnin,* an avian gastrointestinal cestode, was used to investigate the plant extract of stem bark from *Acacia caesia Linnaeus* (Mimosaceae). The plant extract showed strong anthelmintic effects and worked transtegumentally to induce morphological impairments. It also revealed the cause of dose-dependent paralysis and death effects comparable to those of albendazole (Lalchhandama, 2009). The ethanol extract of *A. oxyphylla* (Mimosaceae) stem bark was tested against *Ascaridia galli,* the intestinal roundworm found in domestic poultry. Thus, structural changes detected on *A. galli* revealed changes in the fine topography of *A. galli* treated with 20 mg/mL of the plant extract. The worm treated with plant extract showed severe cuticle shrinking, lip loosening and collapse, and widespread irregular creases all over the body surface (Lalchhandama *et al*., 2009). The cestode *R. tetragona* and the nematode *A. galli* were exposed to Artesunate plant extracts at concentrations of 0.7, 1.5, 3, 6, and 12 mg/ml. While the cestodes were highly responsive to the different doses, the nematodes showed no reaction. These findings suggest that Artesunate holds the potential as an effective treatment for cestode infections (Lalchhandama *et al*., 2015). A survival test against *A. galli* was done using a methanolic extract of *Millettia pachycarpa's* root bark. The results show that the plant extract is efficient against parasitic roundworms (Lalchhandama, 2019). The chloroform extract of *Spilanthes acmella* induced shrinkage and folds in the main body but not in the scolex. The suckers show greater damage than cestodes treated with praziquantel. The spines were eliminated. The results show that *S. acmella* is an excellent source of chemicals with anthelmintic action. Regarding efficacy, praziquantel was more powerful, although the plant extract performed well at all doses tested. (Lalthanpuii *et al*., 2020). The hexane extract of *Acmella oleracea* contains significant bioactive compounds, including the alkylamide N-isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide and the triterpenoid lupeol. The extract's lethal concentration (LC_{50}) was found to be 5128.61 ppm for *T. tetragona* and 8921.50 ppm for *A. perspicillum*. Effects observed in the parasites included shrinkage of the

tegument, erosion of microtriches, and deformation of suckers in cestodes, along with lip collapse and cuticle shrinkage in nematodes. These compounds are key contributors to the extract's broad-spectrum anthelmintic activity (Lalthanpuii and Lalchhandama, 2020). The chloroform extract of the whole subterranean sections of *Imperata cylindrica* was considerably effective on both helminths and showed dose-dependent anthelmintic activity similar to albendazole, efficiently killing and inflicting adverse effects on parasitic tapeworm and roundworm. The analysis thus verifies the Mizo people's traditional usage (Lalthanpui and Lalchhandama, 2020). The methanol extract of *Schima wallichi* was tested against the chicken cestode *R. tetragona*.The plant extract suppressed the cestode parasite at varying concentrations. The plant extract's anthelmintic action was demonstrated by visible damage to the parasite's outer structure. *S. wallichii* bark contains phytosterols that are responsible for its anthelmintic effects (Lalthanpuii and Lalchhandama, 2024).

According to the information provided above, it is evident that medicinal plants in Mizoram have been the subject of considerable exploration and documentation by various ethnobotanists. There are several herbal medications available that claim to treat a variety of ailments. While some phytochemical analyses of plant extracts have been conducted by several researchers, comprehensive scientific validation of the antimalarial properties of many plants within Mizoram remains insufficiently and meticulously examined. There are ongoing efforts to validate these traditional uses through modern research and incorporate this knowledge into mainstream practices to benefit society. These medicinal plants, recognized by the Ayurvedic Pharmacopeia of India, hold the potential to improve the socioeconomic status of the people of Mizoram (Shankara and Rawatb, 2012).

CHAPTER 3

METHODOLOGY

REVIEW OF ETHNOBOTANICAL STUDIES TO SELECT PROSPECTIVE OF ANTIMALARIAL PLANTS FOR PHYTOCHEMICAL SCREENING

All relevant materials were reviewed to identify the set of ten plant species utilized as an antimalarial by the tribal population of Mizoram, India. For the selection, the ten candidate plants were subjected to phytochemical examination following the standard technique (Gokhale *et al*., 2017). It is vital to validate the prospective ethno-medicinal plant before handing out it for phytochemical analysis. Identification was carried out with the assistance of Taxonomists from Mizoram University, as well as flora such as *The Book of Mizoram Plants* (Sawmliana, 2003). The eleven groups of the chemical test was carried out to investigate the presence of secondary metabolites. The three plant samples that demonstrated good secondary metabolites was chosen based on the phytochemical analysis results. The three samples were then analyzed again using the gas chromatography-mass spectrometry (GCMS) technique to determine the presence of bioactive chemicals in the samples. The most appealing plant species was recognized for profundity based on the frequent occurrence of secondary metabolites and the presence of bioactive compounds.

COLLECTION AND EXTRACTION

All the selected 10 plants were collected from different parts of Mizoram, India. The samples were collected based on the plant parts used by the tribal people of Mizoram to cure malarial diseases. The collected specimens were washed thoroughly with distilled water to clean the dirt and dried in a closely ventilated area to avoid direct contact with the sunlight. After it completely dried, the leaves were crushed into small pieces or bark, on the other hand, they were crushed using a mechanical grinder to obtain coarse powder. Then, 30 gm of leaves and 20 gm of bark dried weight of the sample were packed into the Soxhlet apparatus for continuous extraction using solvents of varying polarities such as petroleum ether, chloroform,

and methanol. Successive extraction was done for the three solvents at 72 hours each. To get concentrated crude extracts, the solvents were evaporated in a Buchi Rotavapor® R-100 vacuum rotatory evaporator. The plant extracts were then kept in a refrigerator at 4°C, and subjected to preliminary screening for phytochemical analysis to investigate the presence of various secondary metabolites.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The qualitative phytochemical investigation was carried out according to the standard protocol proposed by Gokhale *et al*., 2017. Different chemical tests were performed to determine the presence of chemical constituents listed below:

i) Alkaloids
ii) Flavonoids
iii) Tannins
iv) Phytosterols
v) Saponin
vi) Carbohydrates
vii) Reducing sugars
viii) Glycosides
ix) Amino acids
x) Proteins
xi) Gums
xii) Anthraquinone

Reagents and chemical used for various group tests

i) Mayer's reagent: 5 gm potassium iodide dissolved in 20 ml of distilled water was combined with a solution containing 1.36 gm of mercuric iodide in 60 ml of distilled water.

ii) Liberman-Burchard reagent: 5 gm of acetic anhydride was carefully mixed with 5 ml of concentrated sulfuric acid while cooling, and added continually to 50 ml of 100% ethanol while cooling.

iii) Dragendorff's reagent: In 80 ml of water, 1.7 gm of bismuth nitrate and 20 gm of tartaric acids were melted. This solution was then combined with 16 gm potassium iodide and 40 ml of water.

iv) Fehling's solution A: 500 ml of solution was made by dissolving 34.64 g of copper sulphate in 0.5 ml of sulfuric acid by adding water.

v) Fehling's solution B: In 500 ml of water, 77 gm of NaOH and 176 gm of sodium potassium tartarates were added. At the time of usage, equal quantities of the aforesaid solutions were combined.

vi) Benedict's reagent: 10 gm anhydrous sodium carbonate, 1.73 gm solution citrate, and 1.73 gm cupric sulphates were dissolved in water and the volume was made up to 100 ml.

vii) Molish's reagent: 2.5 gm of untainted a-napthol was melted in 25 ml of ethanol.

PHYTOCHEMICAL GROUP TEST:

Test for Alkaloids:

a) **Mayer's test:** In a test tube, 2ml of each extract solution was placed. 0.1 ml of Mayer's reagent and 0.2 ml of dilute hydrochloric acids were added. The production of a yellowish-buff-colored precipitate indicated the presence of an alkaloid.

b) **Dragendorff's test:** In a test tube containing 2 ml of each extract solution, 0.1 ml of diluted hydrochloric acid and 0.1 ml of Dragendorff's reagent were added. An orange-brown colored precipitate that formed showed an alkaloid was present.

c) **Wagner's test:** Wagner's reagent and diluted hydrochloric acid were added to 2 ml of extract solution. The appearance of a reddish-brown precipitate demonstrates the positive test for an alkaloid.

d) **Hager's test:** Hager's reagent and diluted hydrochloric acid were added to 2 ml of each extract solution, and the mixture was allowed to react. Alkaloid was indicated by a yellowish precipitate.

Detection of flavonoids:

a) **Shinoda test (magnesium hydrochloride reduction test):**

A few bits of magnesium ribbon and strong hydrochloric acid are added to the extract solution dropwise, and after a few minutes, a pink scarlet, crimson red, or occasionally green to blue colour develops.

b) **Zinc-hydrochloride reduction test**: A combination of zinc dust and concentrated hydrochloric acid was added to the extract solution. After a few minutes, it becomes crimson

Test for tannins:

a) 1 ml of a 5% ferric chloride solution and 5 ml of the extract solution were given time to react. The presence of tannins was suggested by the greenish-black coloring.

b) 1 ml of 10% aqueous potassium dichromate solution was added to 5 ml of the extract solution. The presence of tannins was suggested by the production of a yellowish-brown precipitate.

c) 1 ml of 10% lead acetate solution was added to 5 ml of the extract solution. Precipitation with a yellow hue served as a tannin test.

Detection of phytosterols:

a) **Liebermann-Burchards's test:** After the extract had been dissolved in acetic anhydride, the mixture had been heated to boiling, and cooled, and 1 ml of Conc. H_2SO_4 had been poured along the test tube's side. The presence of steroids or triterpenoids and their glycosides are indicated by the red, pink, or violet color at the junction.

b) **Salkwoski reaction**: 2ml of the extract was combined with 2ml of chloroform. A little amount of Conc. H_2SO_4 2ml was progressively poured onto the test tube's side, shaken thoroughly, and then left to stand for a while. The creation of a yellow-colored bottom layer and the appearance of red color in the lower layer both suggest the existence of triterpenoids.

Detection of Saponins:

Foam test: A small amount of each extract was diluted to 20ml with distilled water. For 15 minutes, the suspension was shaken in a graded cylinder. Saponins are detected by a 2cm layer of foam or froth that remains stable for 10 minutes.

Detection of carbohydrates:

The following tests were performed on the filtrate after each extract's 100 mg was dissolved in 5ml of distilled water:

a) **Molisch's test**: To 2ml of the filtrate, 2 drops of an alcoholic solution of α-naphthol was added. Following a thorough shaking of the mixture, 1 ml of concentrated H_2SO_4 was poured along the test tube's side. The test tube was then chilled in cold water and left to stand; the presence of carbohydrates is indicated by a violet ring at the junction.

b) **Fehling's test**: 1 ml of the filtrate was heated in a water bath with 1 ml each of Fehling's solution A and Fehling's solution B. The formation of a red precipitate serves as a confirmation of the presence of reducing sugars.

c) **Barfoed's test**: To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and the mixture was heated in a boiling water bath for 2 minutes. The appearance of a red precipitate indicates the presence of reducing sugars.

d) **Benedict's test**: 0.5 ml of Benedict's reagent was added to the filtrate, and the mixture was heated in a water bath for 2 minutes. The presence of sugars is indicated by a distinctively colored precipitate.

Test for reducing sugars:

a) 5 ml of the extract solution was mixed with 5ml of Fehling's solution and heated for 5 minutes. The appearance of a brick-red precipitate suggests that the test for reducing sugars was successful.

b) In a test tube, 5 ml of the extract solution was mixed with 5ml of Benedict's solution and heated for a few minutes. The presence of reducing sugars was established by the formation of the brick-red precipitate.

Detection of proteins and amino acids:

The filtrate was tested for proteins and amino acids after 100 mg of each extract was diluted in 10ml of distilled water and filtered through Whatman filter paper No. 1.

a) **Biuret test:** A drop of 2% copper sulphate solution was added to an aliquot of 2 ml of filtrate. 1 ml of 95% ethanol was then added to the mixture, followed by an excessive amount of potassium hydroxide pellets. The ethanolic layer's pink tint denotes the presence of proteins.

b) **Ninhydrin test**: To 2 ml of aqueous filtrate, around 2 drops of ninhydrin solution were added. The presence of amino acids is indicated by a distinctive purple color.

Detection of glycosides:

About 50 mg of extract was hydrolyzed with concentrated HCl for two hours in a water bath to identify glycosides. After filtering, the sample was tested as follows:

a) **Legal's test**: The solvent was used to dissolve 50 mg of the extract. A 10% sodium hydroxide solution was used to make the sodium nitroprusside solution alkaline before being added. A distinctive pink tint indicates the presence of glycoside.

b) **Keller-Kiliani's test**: 1ml of glacial acetic acid containing traces of ferric chloride and 1 ml conc. H_2SO_4 was added to the extract solution. The development of the reddish-brown color at the intersection of two layers and the higher layers becoming bluish-green represents the positive test for glycosides.

Detection of gums:

Stirring continuously, dissolve 100 mg of extract in 10 ml of distilled water and 25 ml of pure alcohol. It produces a hazy or white precipitate

Detection of anthraquinone:

a) Borntrager's test: A few ml of plant extracts filtrate was mixed with 10 ml 10% Ammonia solution (shake vigorously for 30 secs). Pink, violet, or red colored solution indicates the presence of Anthraquinone.

b) Ammonium hydroxide test: 10 mg extract was dissolved in isopropyl alcohol and few drops of concentrated ammonium hydroxide solution was added. The formation of a red color after 2 minutes indicated the presence of anthraquinone.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC - MS)

The extracts were dissolved in acetonitrile at a concentration of 50 mg per 3 ml and analyzed using gas chromatography- ass spectrometry (GC-MS) (Thermo Scientific TRACE™ 1300 ISQ™) (Hussain, 2014). A sample volume of 1 µl was injected with 5 plunger strokes, with the sampling depth set at the center of the vial. A non-polar TR-5ms column (260 F 142 P) was used as the stationary phase, measuring 30 m x 0.25 mm x 0.25 µm film thickness of 0.26 µm. The MS transfer line temperature was 250°C, and the ion source temperature was 220°C. Helium, used as the carrier gas, flowed into the oven chamber at a constant rate of 1 ml/min. The mass spectrometer operated over a range of 10-1100 amu for a total run time of 54 minutes. The final chromatogram was generated using Thermo Scientific™ Xcalibur™ software, and compound identification was done based on retention time, chemical formula, and molecular weight using the Wiley Registry™ and National Institute of Standards and Technology (NIST) databases.

QUANTITATIVE TEST

TOTAL PHENOL CONTENT

A quantitative phytochemical assay of *A. scholaris* bark of three different extracts was done by estimating the total phenolic content of the plant using the (Singleton *et al*., 1965) technique with minimal modifications. Gallic acid was used as a standard to calculate and quantify phenols. In summary, 1 ml of the plant extract (concentration 100 µg/ml) and standard gallic acid concentrations (10, 20, 40, 60, 80, and 100 µg/ml) were prepared. Following that, 5 ml of Folin-Ciocalteu reagent (FCR) was added to each sample. For 3 minutes, the reaction was allowed to proceed at room temperature. The solution was then combined in a rotary mixer with 4 ml of 0.7 M sodium carbonate solution. The samples were kept at room temperature for one hour without being disturbed. 1 ml of methanol, 5 ml of FCR, and 4 ml of sodium carbonate were combined to make a blank concentration. The absorbances of

all samples were measured in a UV-Vis spectrophotometer at 765 nm. For the standard gallic acid, a calibration curve was created. The total phenolic content of the plant samples was determined as milligrams of gallic acid equivalent per gram (GAE mg/g). The experiment was conducted three times.

TOTAL FLAVONOID CONTENT

Using an aluminum chloride analysis based on the technique suggested by (Zhensen *et al*., 1999), the total flavonoid content of *A. scholaris* bark extracted by altering three polarity solvents such as petroleum ether, chloroform, and methanol was determined. As a reference flavonoid compound, quercetin was employed. 1 ml of the plant extract (100 µg/ml) and various concentrations of quercetin (10, 20, 40, 60, 80, and 100 µg/ml) were made, and 2 ml of distilled water was poured into each sample. After 5 minutes, 3 ml of 5% sodium nitrite and 0.3 ml of 10% aluminium chloride were added. After 6 minutes, 2 ml of 1 M sodium hydroxide was added. The ultimate amount was increased by adding distilled water to each sample to 10 ml. After one hour, the absorbances at 510 nm were measured. Using the quercetin standard curve as a reference, the total flavonoid concentration of the three plant extracts was calculated and represented as milligrams of quercetin equivalent per gram (QE mg/g) of the dried weight of the sample.

TOTAL ANTIOXIDANT ACTIVITY

The total antioxidant activity was determined by phosphomolybdate estimation using ascorbic acid, which was significantly modified from a standard approach developed by (Prieto *et al*., 1999). Ascorbic acid was utilized as a control to assess total antioxidant activity. To make a stock solution, 1 mg/ml of each plant extract was dissolved in distilled water. 3 ml of the reagent solution was added to 1 ml of plant extract (100 µg/ml). The Reagent solution comprised a combination of three compounds: 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After that, it was incubated at 95°C for 90 minutes. The mixture was allowed to cool to room temperature and absorbance wa taken at 695 nm. The total antioxidant activity was calculated using the ascorbic acid equivalent in milligrams

per gram (AAE mg/g) of dry weight of the sample. The experiment was repeated three times.

DPPH FREE RADICAL SCAVENGING ACTIVITY AASSAY

The antioxidant activity of *A. scholaris* bark extract was investigated using the (Blois, 1958) technique with some modifications. Butylated hydroxytoluene (BHT) was utilized as a control antioxidant. The antioxidation procedure utilized DPPH (2,2-diphenyl-1-picrylhydrazyl), a free radical-producing substrate. Both the plant extract and the standard BHT were prepared at various concentrations, namely 10, 20, 40, 60, 80, and 100 µg/ml. 3 ml of each sample was combined with 0.5 ml of 1 mM DPPH. The control sample was generated from 3 ml methanol and 1 ml DPPH. All of the samples were incubated and allowed to respond for 30 minutes at 37°C. The absorbances were measured at 517 nm. The absorbance data were then converted into a percentage of antioxidant activity using the following formula

$$\text{Scavenging (\%)} = \frac{\text{Absorbance control - Absorbance of extract}}{\text{Absorbance of Control}} \times 100$$

The IC_{50} values were computed using Prism GraphPad version 8.0.2. The log dosage was calculated using the extract concentrations of 1, 0.5, and 0.25 mg/ml.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

The reducing (antioxidant) capacity of *A. scholaris* bark extract was evaluated using the (Oyaizu, 1986) modified FRAP technique. Ascorbic acid, a compound antioxidant, and the plant extract were prepared in a variety of quantities, including 10, 20, 40, 60, 80, and 100 µg/ml. 2.5 ml of phosphate buffer (6.6 pH) and 2.5 ml of 10% potassium ferricyanide were added to 1 ml of the total sample. The solutions were centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant from each sample was taken and diluted with 2.5 ml of distilled water. After properly mixing, 0.5 ml of freshly prepared 0.1% ferric chloride was added. To create the blank concentration, 1 ml of distilled water, 2.5 ml of phosphate buffer, and 2.5 ml of potassium ferricyanide were mixed. After waiting for 10 minutes, the absorbance at 700 nm was measured against the blank concentration.

ANTIBACTERIAL ASSAY

The antibacterial efficacy of plant extracts such as petroleum ether, chloroform, methanol, and aqueous extract was investigated since they have traditionally been utilized to treat many types of microbial infections.

Microorganisms: The microorganisms employed in this investigation include *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus 1(*ATCC 11623), *Staphylococcus aureus 2* (ATCC 700698), *Bacillus cereus* (ATCC 13061), *Escherichia coli* (ATCC 10536), *Salmonella typhyii* (ATCC 51812), *Micrococcus luteus* (MTCC-106), *Pseudomonas aeruginosa* (MTCC-424), *Klebsiellla pneumonia* (MTCC-39), Before the experiment, the bacteria were subcultured in nutrient broth and incubated for 24 hours at 37°C.

Plant sample preparation and extraction. The petroleum ether, chloroform, methanol, and aqueous extract, which had previously been extracted for other experiments, were employed**.**

Well diffusion method: The crude extract's antibacterial activity was evaluated using the well diffusion technique on the various test microorganisms listed (Devillers *et al*., 1989).

Preparation of stock solution: The dried petroleum ether, chloroform, methanol, and aqueous extract of the plant sample were weighed and dissolved in 1% dimethyl sulfoxide (DMSO) at three concentrations: 200 mg/ml, 100 mg/ml, and 50 mg/ml.

Preparation of media: 200 ml of nutrient agar (Himedia) was prepared by dissolving premade nutrient agar powder in distilled water. After that, the dissolved nutrient agar was sterilized**.**

Evaluation of the antimicrobial activity of plant extract: The antimicrobial activity of four varying solvent plant extracts was assessed by the well diffusion method (Devillers *et al*., 1989). Agar plates are prepared which were inoculated with the test microorganisms by pour plate method and allowed to dry at room temperature. A common antibiotic, 2% Ciprofloxacin, was employed as a positive reference for antibacterial activity, and 1% DMSO was used as a negative control. To

compare the zone of inhibition with the experimental plant extracts, three concentrations of the plant extract (200, 100, and 50 mg/ml) were used. Using a spreader, 40 µl of various inoculum bacteria were uniformly distributed over the nutritional agar. A hole was drilled in the well using a 6 mm sterile cork borer. 50 µl of different concentrations were placed in each well, along with 0.5 µl ciprofloxacin and 10 µl DMSO in separate wells. Bacteria were allowed to grow at 37°C for 24 hours. The inhibitory zones were measured, and the results were reported as means standard error of the means. Tukey's multiple comparison test was applied in Prism Graphpad version 8.2.0 to compare the means, and *p* values less than 0.5 were used to determine the significance of the difference from the control.

ANTIFUNGAL ACTIVITY

The antifungal activity of different crude extracts of *A. scholaris* were carried out following the poison food method (Mounyr Balouiri *et al*., 2016) *Candida albicans, Fusarium keratoplasticum*, and *Aspergillus fumigatus* were used for the test organisms. Plant extracts at various concentrations, including 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml, were prepared and blended well with molten agar. The medium was then poured into petri dishes. To inoculate, 2-5 mm mycelial discs were placed in the center of the plate and incubated at 25°C. The diameter of the mycelial growth of control and plant extracts were measured and recorded for seven days. The antifungal effect was estimated using the formula below.

Antifungal activity (%) = (DC-DS/DC) × 100

DC is the diameter of growth in the control plate, and DS is the diameter of growth in the plate containing the tested antifungal agent.

ANTIMALARIAL ACTIVITY

The antimalarial activity was assessed against *Plasmodium falciparum* by a fluorescence-based SYBR Green I assay to determine half-maximal inhibitory concentration (IC_{50}) values, as described by Smilkstein *et al*. (2004) and Anas *et al*. (2017). The parasites were cultured at 6-8% parasitemia and 2% hematocrit in complete RPMI medium (RPMI 1640 supplemented with HEPES, 0.2% sodium

bicarbonate, 0.2% D-glucose, 0.5% albumax, 45 mg/L hypoxanthine, 0.25 mg/L fungizone, and 50 mg/L gentamycin) at 37°C in a humidified CO_2 incubator. The SYBR Green I assay was employed to quantify parasite inhibition, with tests performed at 0.8% parasitemia and 1% hematocrit. Ring-stage parasites were exposed to various chemical dilutions in 96-well plates and incubated at 37°C for 72 hours. Controls included untreated parasites (infected red blood cells without treatment), DMSO-treated cells, and non-infected RBCs. Chloroquine (CQ, Sigma C6628) was used as a reference drug, prepared in water at stock concentration, and SYBR Green values were measured as previously reported.

Vero cells (C1008, monkey kidney epithelial cells) were used in an MTT assay to evaluate cytotoxicity. The Vero cells were cultured in RPMI medium supplemented with HEPES, 0.2% sodium bicarbonate, 0.2% D-glucose, 10% FBS, fungizone (0.25 mg/L), and gentamycin (50 mg/L) at 37°C in a humidified CO_2 incubator. After seeding 10^4 cells per well in 96-well plates, they were treated with varying chemical dilutions 16-18 hours post-seeding. Podophyllotoxin (Sigma P4405) served as the positive control. Following 72 hours of incubation, 25 µL of MTT reagent (Sigma M2128, stock concentration 5 mg/ml) was added to each well and incubated for an additional 2 hours in a CO_2 incubator. The assay readout was conducted according to established protocols.

IN VITRO ANTHELMINTIC ACTIVITY

In vitro anthelmintic activity was carried out following the standard technique (Lalchhandama *et al*., 2007). At a nearby abattoir, parasites were recovered from the intestines of local fowls. The parasites were retrieved and promptly transferred into a lukewarm phosphate-buffered saline solution (pH 7.1- 7.4). Various plant extracts were prepared in 0.9% phosphate-buffered saline (PBS) with 1% DMSO. For each extract, concentrations of 5, 10, and 20 mg/ml were made in three replicates. Similar concentrations of the standard albendazole, which acted as a positive control, were also prepared. As a negative control, parasites were kept in 0.9% PBS supplemented with 1% DMSO. The treated parasites were subsequently maintained in an incubator at 37°C. Six worms were introduced to each plate. Motility, survival, and

histomorphological changes were used to estimate the effectiveness of the standard and extracts. The worms' motility was assessed by voluntary movement and physical stimuli. The worms' paralysis and death were evaluated by maintaining them in lukewarm PBS regularly. For each worm, the time of paralysis and death were recorded.

HISTOLOGICAL PROCESSING FOR LIGHT MICROSCOPY

The worms treated with 20 mg/ml were selected for histological studies and were immediately fixed to Bouin's solution for tissue fixation for 24 hours (Roy *et al*., 2007) After fixing, the worms were rinsed multiple times with distilled water while being constantly stirred. Dehydration begins with 70% and 80% ethanol for 1 hour each, however, 90 and 100% ethanol with two 1-hour changes. After dehydration, they were treated with a combination of xylene and clove oil until they acquired clear translucent specimens, then cleared in pure xylene. The specimens were subsequently treated with a succession of mixes of paraffin and xylene in corresponding ratios to pure paraffin. Following full penetration of the tissue with molten wax, the tissue was retained vertically embedded in the wax and allowed for solidification. Using a microtome (Medimeas MRM-ST) blocks are sliced into 5 mm thick rectangular portions. The ribbons were stretched on an automated hot plate, glued on albumin-coated glass slides, and deparaffinized with pure xylene. The wax has now been entirely clear with pure xylene after it was deparaffinized for 10 minutes with xylene for two consecutive changes. Finally, they were thoroughly dehydrated with various ethanol grades and stained with hematoxylin and eosin. Then they were cleared in xylene for 10 minutes with two consecutive changes before mounting with DPX on glass slides. After covering with cover slips, they were oberved under an electrical compound microscope (Olympus CX41).

SCANNING ELECTRON MICROSCOPY (SEM)

Cestodes recovered after treatments with 20 mg/ml concentrations of plant extracts were rinsed with 0.8% normal saline and transferred to 10% neutral-buffered formalin (NBF) for fixation for a minimum of 24 hours (Roy *et al*., 2007). For post-fixation, the specimens were washed with phosphate-buffered saline (PBS) for 30

minutes, with two consecutive changes. Dehydration was subsequently performed using a graded acetone series, beginning with 30%, 50%, and 70% acetone for 15 minutes, followed by 80%, 90%, 95%, and 100% acetone for 30 minutes each, with two changes at each concentration. Specimens were then treated with trimethylsilane (TMS) and allowed to air-dry at 25°C for 15 minutes to ensure complete evaporation. The cestodes were mounted onto metal stubs and coated with gold using a fine-coat ion sputter (Hitachi MC1000) for 15 minutes. Examination of the specimens was conducted using a scanning electron microscope (HITACHI TM4000Plus) at magnifications of 100X, 250X, and 1000X to assess morphological changes induced by the standard and plant extract treatments.

STATISTICAL ANALYSES

Microsoft Excel (Microsoft Office 2016) was employed for the quantitative estimation of total phenolic content, antioxidant activity, and flavonoid concentration. Statistical analyses of differences in DPPH scavenging activity and ferric reducing antioxidant power (FRAP) between various plant extracts were performed using GraphPad Prism 8.0.2, applying one-way ANOVA followed by Tukey's post-hoc test. Similarly, one-way ANOVA with Tukey's multiple comparison tests in Prism GraphPad 8.0.2 was utilized to compare antibacterial and antifungal activities among the different plant extracts. To evaluate the relationship between dose and time to death at varying extract concentrations, one-way ANOVA with Tukey's multiple comparison test in Prism GraphPad 8.0.2 was conducted. All statistical results are presented as means ± standard error of the mean (SEM), with a significance level set at $p < 0.05$.

CHAPTER 4

RESULT

YIELD OF EXTRACTION:

The extractive weights and yields for ten collected samples of dried material (Table 4.2), including bark (20 g), root (20 g), leaves (30 g), and whole plant (30 g), were analyzed. Specifically, for *A. scholaris* (bark), extraction was performed using petroleum ether, chloroform, and methanol. The respective extractive weights and extraction yields were 1.909 g (6.36%), 2.026 g (6.75%), and 3.67 g (12.23%), with methanol yielding the highest extractive value. A similar trend was observed for *Betula alnoides* (bark), where the methanol extract yielded the highest values at 3.059 g (10.19%), followed by chloroform (0.432 g, 1.44%) and petroleum ether (0.022 g, 0.07%). For *Cinnamomum bejolghota* (leaves), the respective extractive weights and yields were 0.032 g (0.16%) for petroleum ether, 0.251 g (1.25%) for chloroform and 0.308 g (1.54%) for methanol. In the case of *Costus speciosus* (root), the extracts showed weights and yields of 0.519 g (1.73%) for petroleum ether, 1.853 g (6.17%) for chloroform, and 2.419 g (8.06%) for methanol. For *Dendrocnide sinuate* (bark), the extractive yields were 0.023 g (0.76%), 1.578 g (5.26%), and 1.362 g (4.54%) for petroleum ether, chloroform, and methanol, respectively. Similarly, *Hedyotes scandens* (leaves) exhibited weights of 0.049 g (0.245%), 0.513 g (2.56%), and 0.890 g (4.45%) for the three solvents. The whole plant of *Mimosa pudica* yielded extractive values ranging from 0.215 g (1.075%) for petroleum ether, 0.368 g (1.84%) for chloroform, and 0.783 g (3.915%) for methanol. *Prunus cerasoides* (bark) showed a significant difference in yields, with methanol extraction giving the highest yield of 3.238 g (10.79%), compared to 0.273 g (0.91%) for petroleum ether and 1.236 g (4.12%) for chloroform. For *Stereospermum colais* (bark), extractive yields were 0.812 g (2.70%) for petroleum ether, 1.054 g (3.51%) for chloroform and 3.738 g (12.46%) for methanol. Finally, *Vitex peduncularis* (bark) showed extractive yields of 0.881 g (2.93%) for petroleum ether, 1.735 g (5.78%) for chloroform, and 3.616 g (12.05%) for methanol.

PHYTOCHEMICAL SCREENING:

The most common and important phytocompounds of *A. scholaris* bark of different extracts were tested using 11 group tests (Table 4.4). The chemical analysis showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phytosterols, saponins, tannins, and reducing sugars in methanol extract. The petroleum ether extract showed the presence of flavonoids and phytosterols in both Liebermann Burchard's and Salkowki's tests. Phytosterols, saponins, carbohydrates, reducing sugars, protein and amino acids were spotted in chloroform extracts. The aqueous extract detected alkaloids, flavonoids, tannins, phytosterols, saponins, carbohydrates, reducing sugars, glycosides, and anthraquinones. Aqueous extract showed the presence of alkaloids, flavonoids, tannins, phytosterols, saponins, carbohydrates, reducing sugars, glycosides, and anthraquinones. However, two other important phytochemicals compounds such as protein and amino acids, and gums could not be detected by the specific test employed. Detection of chemicals by standard test indicated the presence of key phytochemicals in *B. alnoides* (Table 4.5) such as phytosterols in petroleum ether extract. The chloroform extract exposed the presence of flavonoids, phytosterols, carbohydrates, and reducing sugars. Whereas methanol extract showed the presence of flavonoids, tannins, saponins, carbohydrates, and reducing sugars However, important phytochemicals such as alkaloids, protein and amino acids, glycosides, gums, and anthraquinones could not be detected in any of the standard test being used. *C. bejolghota* (Table 4.6) detected the presence of phytosterols in petroleum ether. Chloroform extract revealed the presence of phytosterols, saponins, carbohydrates, and reducing sugars. Methanol extract showed the presence of flavonoids, tannins, phytosterols, saponins, carbohydrates, and reducing sugars. However, important phytochemical compounds like alkaloids, protein and amino acids, glycosides, gums, and anthraquinones could not be detected by the standard tests. *C. speciosus* (Table 4.7) showed the presence of phytosterols in petroleum ether extract. Phytosterols, carbohydrates, reducing sugars, and gums were detected in chloroform extract. Methanol extract showed the presence of tannins, phytosterols, saponins, carbohydrates, and reducing sugars. Important compounds such as alkaloids, flavonoids, protein and amino acids, and anthraquinones were not

detected. *D. sinuate* (Table 4.8) revealed the presence of phytosterols in petroleum ether. Carbohydrates and reducing sugars were detected in chloroform extract. In methanol extract, tannins, saponins, carbohydrates, and reducing sugars were present but important phytochemical compounds like alkaloids, flavonoids, protein and amino acids, glycosides, gums, and anthraquinones could not be detected. *H. scandens* (Table 4.9) revealed the presence of only glycosides in petroleum ether extract. Chloroform revealed only phytosterols while methanol showed the presence of tannins, saponins, carbohydrates, and reducing sugars. Key phytochemicals like alkaloids, flavonoids, saponins, proteins and amino acids, gums, and anthraquinones could not be detected by the tests. *M. pudica* (Table 4.10) exposed the presence of phytosterols and glycosides. Chloroform extract showed the presence of saponins, carbohydrates, and glycosides. The methanol extract showed the presence of flavonoids, tannins, carbohydrates, and glycosides. Important phytochemical compounds like alkaloids, protein and amino acids, gums, and anthraquinone could not be detected by the test. *P. cerosoides* (table 4.11) showed the presence of phytosterols in petroleum ether. phytosterols, carbohydrates, and reducing sugars were found in chloroform extract. In methanol extract, flavonoids, tannins, saponins, carbohydrates, and reducing sugars were present. Protein and amino acids, gums, and anthraquinones failed to be detected by the tests. *S. colais* (Table 4.12) discovered the important phytochemical compounds of phytosterols in petroleum ether extract. Chloroform extract showed the presence of phytosterols, carbohydrates, and reducing sugars. The methanol extract showed the presence of flavonoids, tannins, saponins, carbohydrates, and reducing sugars, alkaloids, protein and amino acids, glycosides, gums, and anthraquinones were absent. *V. peduncularis* (Table 4.13) wall showed the presence of phytosterols in petroleum ether extract. Chloroform revealed the presence of phytosterols, carbohydrates, and reducing sugars. While methanol showed the presence of flavonoids, tannins, saponins, carbohydrates, reducing sugars, and anthraquinones.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS):

The chromatogram of the different extracts of *A. scholaris* is shown in Fig: 4.11, 4.12, 4.13, and 4.14, and the consistent list of detected compounds identified from

the library of NIST is written down in Table 4.14, 4.15, 4.16, and 4.17. Petroleum ether extract (ASP) (Table 4.14) detected thirty-two bioactive compounds. The most abundant compound is 9,9-Dimethoxybicyclo [3.3.1] detected at 23.63 retention time with an abundance of 52.7%. An Octadecanoic acid was detected at 17.15 retention time with an abundance of 52. 67%. Another important compound was squalene detected at 27.46 retention time and 46.58% abundance.

Thirty-five different compounds were identified from the chloroform extract (ASC) (Table 4.15). Out of which the most abundant is benzene propanoic acid, 3,5-bis (1,1- dimethyl)-4 hydroxy-, methyl ester at 13.17 retention time with an abundance of 80.9%. Akuammilan-17-oic acid methyl ester at 25.06 retention time with an abundance of 72.11%. Another important compound lupeol and R-limonene 29.17 and 3.74 retention time with 38.91 and 14.2 abundance. 1-Hepatriacotanol detected three peaks with retention time at 26.46, 29.38, 30.89 with relative abundance of 49.33%, 13.72%, and 3.07%. Two peaks of 24-noroleana-3,12-diene with a retention time of 31.43 and 31.58 relative abundance of 18.52% and 18.18% respectively. Other major compound detected are octadecanoid acid, 9,19-cycloergost-24(28)-en-3-ol, 4, 14-dimethyl, acetate (3a,4a,5a).

Methanol extract (ASM) (Table 4.16) spotted 32 bioactive compounds. Phenol, 4-(1, 1-dimethyl ethyl)-2-(1, 1-dimethyl propyl) with retention time 5.42 and 73.17 abundance. 4 peaks of urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+) - were detected with 7.79, 8.3, 10.57, 11.8, 20.3 retention time and 28.51 %, 32.99%, 22.61% and 26.86% abundance. 8 peaks of lup-20 (29) - en- 3-ol, acetate, (32) - were distinguished from the extract.

Aqueous extract (ASAq) (Table 4.17) recognized 28 bioactive compounds. Major important compounds identified were 7, 9 di-tert-butyl,-1 oxaspiro (4, 5) deca-6, 9-diene-2, 8-dione with 12.76 retention time and 90.35% abundance. Akuammilan -17-oic acid methyl ester with 25.02 retention time and 73.73% of abundance. Two peaks detected are bicyclo (3.1.1) heptane-2,-diol, 2, 6, 6- trimethyl-.

The gas chromatogram result of different extracts of *C. speciosus* was shown in Figures 4.15 and 4.16. Thirty-four bioactive compounds were detected from

petroleum ether extract of *C. speciosus* (CSP) (Table: 4.18). The most abundant compound was Eicosanoic acid with 20.50 retention time and 70.10% abundance. Other major compounds present were octadecanoic acid, phenyl 4-(1, 1-dimethyl propyl), 8- pentadecanone and androstane-11, 17- dione, 3-[(trimethylsilyl) oxy]-.

Chloroform extract (CSC) (Table: 4.19) identified thirty-seven bioactive compounds on which the most abundant compound is 4H-1- benzopyran-4-one.2.3-dihydro5- hydroxy-2-(4-hydroxyphenyl)-7- methoxy-,(S)- with 26.83 retention time and 78.17% abundance. Another major abundant compounds are diosgenin with 73.80% abundance, Eicosanoic acid, octadecanoic acid, ethyl ester. 2, 4-di-tert-butyl phenol and vanillin lactoside.

The gas chromatogram result of different extracts of *V. peduncularis* wall were shown in Figures 4.17 and 4.18. Petroleum ether extract of *V. Peduncularis* (VPP) (Table 4.20) detected twenty-seven different bioactive compounds. Phenol, 4-(1, 1-dimethyl ethyl)-2- (1, 1-dimethyl propyl) with 5.47 retention time and 84.98 % abundance. Major compounds include 1,6:3,4-dianhydro-2-deoxy-a-d-lyxo-hexopyranose, 4- fluro-1-methyl-5- carboxylic acid, ethyl ester, octadecanoic acid, 2-(- hydroxyethoxy) ethyl ester and n-hexadecanoic acid with 7.12, 15.70, 17.09 retention time and 60.43%, 70.30 % and 55.99 % abundance.

Thirty-three bioactive compounds were detected in the chloroform extract VPC (Table: 4.21). Major important compounds found in the extract are n-hexadecanoic acid, octadecanoic acid, 2 (2-hydroxy ethoxy) ethyl ester, and C-sitosterol with 13.43, 17.06 and 26.38 retention time and 74.15%, 66.11% and 43.62 %. two peaks detected from phenol 4- (1,1-dimethyl ethyl)-2-(1,1-dimethyl propyl) and 2-propenoic acid, tridecyl ester.

SELECTION OF CANDIDATE PLANT:

A comprehensive review of relevant literature, including books and journals, was conducted to gather information on antimalarial plants. Based on this review, ten plants traditionally used for the treatment of malaria were selected for further study (Table 4.1). These plants were collected from various regions of Mizoram, with the collection sites chosen based on the optimal growth conditions of each species.

Specifically, six species—*A. scholaris*, *Costus speciosus*, *Cinnamomum bejolghota*, *Dendrocnide sinuata*, *Stereospermum colais*, and *Vitex peduncularis*—were gathered from the forests near Lungdai village in the Kolasib District. Two species, *Betula alnoides* and *Prunus cerasoides*, were collected from Champhai in the Champhai District, while the remaining two species, *Hedyotes scandens* and *Mimosa pudica*, were obtained from Aizawl. Phytochemical screening of these plants revealed significant findings. Alkaloids were detected in *A. scholaris*, gums in *C. speciosus*, and anthraquinones in *V. peduncularis*. These phytochemical constituents were absent in the other species collected. Notably, these compounds demonstrated a broad range of activity against *Plasmodium* species, as noted by Vincent (2008). Based on these findings, the three species—*A. scholaris*, *C. speciosus*, and *V. peduncularis*—were selected for further analysis through GC-MS. The GC-MS results revealed the presence of notable bioactive compounds, including lupeol, akuammine, and limonene, which exhibited significant activity against *P. falciparum*, as reported by Sharma *et al*. (2020), Hanne *et al*. (2002), Kapadia *et al*. (1993), and Moura *et al*. (2001). Of particular interest, these compounds were detected in the bark extract of *A. scholaris*, collected from Lungdai village, Kolasib district of Mizoram, India, located at 23°52' N 92° 44' E.

As a result, *A. scholaris* has emerged as the most promising candidate for further in-depth study.

The voucher specimen was identified and authenticated by the Botanical Survey of India, Shillong (no. BSI/ERC/ Tech/2023-24/102-17-05-23). A reference specimen with accession number PUC-A-23-01 (reference No. PUC-A-01) was deposited in the herbarium section of the Department of Botany, Pachhunga University College.

Candidate ethnomedicinal plants: *A. scholaris* (L.) R.Br

Systematic classification

Kingdom: Plantae

Phylum: Tracheophyta

Class : Magnoliopsida

Order : Gentianales

Family : Apocynaceae

Genus : *Alstonia*

Species : *scholaris*

Binomial name : *A. scholaris*

In various Indian traditional medicine practices, the *A. scholaris* tree's bark, latex, and leaves are shown to possess antibacterial, antifungal (shafique *et al*., 2014; Jabeen *et al*., 2008), herbicidal (Javaid *et al*., 2010), and antitussive, expectorant, and anti-asthmatic properties (Shang *et al*., 2010). The bark also treats chronic illnesses like leprosy, jaundice, asthma, malaria, and cancer (Baliga, 2012; Khayde *et al*., 2014). Also, latex is particularly applied to heal wounds, treat ulcers, and relieve pain in rheumatoid arthritis (Singh *et al*., 2017). The plant is recognized for its anthelmintic, analgesic, anticancer, anti-inflammatory, laxative effects, and anti-tumor property (Pandey *et al*., 2020). In traditional Mizo medicine, different parts of this tree are utilized for the treatment of wounds (Bhardwaj *et al*., 2005) and as therapeutic agents for a variety of ailments, including Malaria (Bora *et al*., 2007) typhoid fever, cardiovascular diseases dysentery, hypertension, diarrhea, and ear infections (Sharma *et al*., 2001).

The species is distinguished by its oblong leaves, arranged in three to ten whorls. In India, it commonly has whorls of seven leaves, giving rise to its name "*Saptaparna*," meaning seven leaves. It is also referred to as the Devil's tree because its pollen often triggers severe allergic reactions during its flowering period. Unlike the typical seven-whorled leaves found in other parts of India, the *A. scholaris* variety in Mizoram is distinct, having exactly eight leaves in each whorl. This unique characteristic has led the Mizo people to call it "*Ṭhuamriat*", meaning "eight-whorled."

Distribution: Native to Africa, China, Australia, Papua New Guinea, Vietnam, Bhutan, Cambodia, Thailand, India, Malaysia, Sri Lanka, Myanmar, Bangladesh, Nepal, and Malaysia. It is found throughout Mizoram.

YIELD OF EXTRACTION OF *A. SCHOLARIS*:

The extractive weight and values from 600 gm of the dried *A. scholaris* bark (Table 4.3) sample were determined using the extraction yield, as indicated in the table. In that order, petroleum ether, chloroform, methanol, and aqueous (290 gm) extracts had extractive weights of 57.28 g, 60.80 g, 110.10 g, and 15.43 g. The extraction value of aqueous extract is (5.19 %), petroleum ether extract is 9.54%, and methanol extract has the greatest yield (18.35%), followed by chloroform extract (10.13%).

IN VITRO ANTIOXIDANT ACTIVITY OF *A. SCHOLARIS* BARK:

The antioxidant activity of various *A. scholaris* bark extracts exhibited a clear concentration-dependent antioxidant reaction, as evidenced by the graph presented in Figure 4.19, which illustrates the percentage inhibition of free radicals. Across all tested concentrations, the plant extracts demonstrated notable free radical scavenging capacity against DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals. Detailed analysis of the dose-response relationship revealed that the half-maximal inhibitory concentration (IC_{50}) for the standard antioxidant, butylated hydroxytoluene (BHT), was 5.73 µg/ml. In contrast, the IC_{50} values for the different *A. scholaris* bark extracts were as follows: methanol extract (11.27 µg/ml), chloroform extract (28.96 µg/ml), petroleum ether extract (133.8 µg/ml), and aqueous extract (6.09 µg/ml). These findings suggest that both the aqueous and methanol extracts possess substantial antioxidant activity, though slightly lower than that of BHT.

A comparative analysis of the IC_{50} values (as depicted in Figure 4.20) for the *A. scholaris* bark extracts namely, ASM (methanol extract), ASC (chloroform extract), ASP (petroleum ether extract), and ASO (aqueous extract) alongside the standard BHT, revealed statistically significant differences in their free radical scavenging activities against DPPH. The statistical significance was confirmed at the $p < 0.05$ level, underscoring the efficacy of the aqueous and methanol extracts in exhibiting potent antioxidant properties in comparison to the standard antioxidant BHT.

TOTAL PHENOLIC CONTENT OF *A. SCHOLARIS* BARK:

The estimation of the total phenolic content in various extracts of *A. scholaris* bark, as determined using the Folin-Ciocalteu assay, is illustrated in Figure 4.21. Quantification of phenolics was performed by comparison with a gallic acid standard curve. Among the tested extracts, the ASM extract exhibited the highest phenolic content, with a value of 13.268 ± 0.088 mg of gallic acid equivalents (GAE) per gram of dry weight. This was followed by the ASAq extract, which contained 12.306± 0.121 GAE mg/g, the ASC extract with 11.197±0.068 GAE mg/g, and finally, the ASP extract, which recorded a phenolic content of 10.865±0.129 GAE mg/g of dry sample weight. These results indicate a notable variation in the phenolic content across the different extracts, with ASM demonstrating the most substantial phenolic activity.

TOTAL FLAVONOID CONTENT OF *A. SCHOLARIS* BARK:

The quantification of total flavonoid content in the various plant extracts, as determined from the aluminium chloride reaction method, is presented in Figure 4.22. Using quercetin as a standard reference compound, the flavonoid concentrations were calculated based on the linear calibration curve. Among the plant extracts analyzed, ASP exhibited the highest total flavonoid content, containing 117.54 ± 5.965 QE mg/ml of dried sample weight. This was followed by ASC, which contained 57.868 ± 3.583 QE mg/ml, ASAq with 43.114 ± 2.503 QE mg/ml, and ASM with 31.639 ± 2.504 QE mg/ml. These findings suggest that ASP possesses significantly higher flavonoid concentrations compared to the other extracts, indicating its potential as a rich source of these bioactive compounds.

TOTAL ANTIOXIDANT CONTENT OF *A. SCHOLARIS* BARK:

The total antioxidant content of the plant extracts was determined using a standard curve generated from the absorbance readings of ascorbic acid at varying concentrations, measured at 695 nm. The standard curve is depicted in Figure 4.23. Based on this standard curve, the antioxidant capacity of each plant extract was quantified, revealing significant differences among the extracts. Among the tested

extracts, the ASM extract exhibited the highest total antioxidant activity, with a value of 13.563 ± 0.088 mg of gallic acid equivalent (GAE) per gram of dry weight. The ASAq extract followed, with a total antioxidant content of 12.306 ± 0.121 GAE mg/g, while the ASC extract showed a slightly lower activity, measured at 11.42 ± 0.068 GAE mg/g. Finally, the ASP extract displayed the least activity, with a total antioxidant content of 10.865 ± 0.120 GAE mg/g of dried sample weight.

FERRIC REDUCING ANTIOXIDANT POWER ESSAY OF *A. SCHOLARIS* BARK:

The potassium ferricyanide-reducing activity of *A. scholaris* bark extract was assessed, with the results presented in Figure 4.24. The antioxidant capacity of different plant extracts was measured and compared against a standard antioxidant, ascorbic acid, across a range of concentrations. The findings revealed that both the plant extracts and ascorbic acid exhibited a concentration-dependent increase in antioxidant activity, with higher concentrations corresponding to greater reducing power. However, it was consistently observed that the reducing power of the plant extracts, while notable, was less potent than that of ascorbic acid at all tested concentrations. Among the *A. scholaris* extracts evaluated, the ASM extract demonstrated the highest reducing power, surpassing the other extracts. This was followed by the ASC, ASAq, and ASP extracts, in descending order of effectiveness. Despite their effectiveness, none of the plant extracts reached the antioxidant capacity of the standard ascorbic acid.

ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF *A. SCHOLARIS*:

The antibacterial activity of methanolic extracts derived from the bark of *A. scholaris* was evaluated at concentrations of 50, 100, and 200 mg/ml, alongside the antibiotic ciprofloxacin. The results, detailed in Table 4.25 and Figure 4.25, indicate that the four bacterial strains tested generally exhibited a positive response to ciprofloxacin. Though, *Staphylococcus aureus 2* did not show sensitivity to the antibiotic ciprofloxacin. However, *S. aureus* 2 demonstrated measurable inhibition

zones of 2.763 mm and 1.407 mm at concentrations of 200 mg/ml and 100 mg/ml, respectively.

For *Enterococcus faecalis*, the inhibition zones observed were 1.837 mm, 2.550 mm, and 3.707 mm at concentrations of 200 mg/ml, 100 mg/ml, and 50 mg/ml of the plant extract, with a standard inhibition zone of 15.273 mm produced by ciprofloxacin. *Escherichia coli*, in contrast, exhibited an inhibition zone only at the 50 mg/ml concentration of the extract, measuring 0.893 mm, which coincided with the inhibition zone of the standard ciprofloxacin. *Bacillus cereus* displayed inhibition zones of 0.893 mm, 2.597 mm, and 1.020 mm at 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively, whereas ciprofloxacin showed a significantly higher zone of inhibition at 31.900 mm. Similarly, *Salmonella typhi* exhibited inhibition zones of 7.197 mm and 4.403 mm at 100 mg/ml and 50 mg/ml of the extract, with the standard ciprofloxacin producing a robust inhibition zone of 25.427 mm.

A noteworthy finding from this study is the broad-spectrum antibacterial activity of the *A. scholaris* bark extract, which was effective against both Gram-positive and Gram-negative bacterial strains. The lowest efficacy was observed against *E. coli*, where inhibition (0.89 mm) occurred only at the 50 mg/ml concentration of the extract. In contrast, the extract demonstrated the greatest antibacterial effect against *S. typhi*, with significant inhibition zones recorded. A statistical comparison of the antibacterial efficacy of different concentrations of the plant extract and ciprofloxacin is depicted in Figure 4.26, highlighting the varying degrees of antibacterial activity across the tested concentrations and bacterial strains.

ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACT OF *A. SCHOLARIS*:

The antibacterial activity of the chloroform extracts of *A. scholaris* bark at concentrations of 50, 100, and 200 mg/ml, alongside ciprofloxacin as a standard antibiotic, is presented in Table 4.23 and Figure 4.27. The response of the four tested bacterial strains to the treatments varied. Notably, *S. aureus 2* exhibited no significant response to the 2% ciprofloxacin solution. In contrast, *S. aureus 1*

demonstrated distinct inhibition zones, measuring 8.646 mm, 7.486 mm, 5.343 mm, and 22.336 mm at concentrations of 200, 100, and 50 mg/ml of the extract, and ciprofloxacin, respectively. For *S. aureus 2*, the inhibition zones were smaller, measuring 2.253 mm and 1.473 mm at 200 mg/ml and 100 mg/ml, respectively.

E. coli exhibited inhibition zones of 2.720 mm, 3.076 mm, and 4.046 mm at extract concentrations of 200 mg/ml, 100 mg/ml, and 50 mg/ml, while the inhibition zone for ciprofloxacin was significantly larger, at 32.606 mm. Similarly, *B. cereus* displayed inhibition zones of 3.766 mm, 3.803 mm, and 4.046 mm at the same extract concentrations, with a ciprofloxacin-induced inhibition zone of 31.793 mm. For *S. typhi*, inhibition zones of 5.036 mm, 4.866 mm, and 4.473 mm were observed at the respective extract concentrations, while ciprofloxacin produced a zone of 26.003 mm.

One of the most notable findings was that the chloroform extract of *A. scholaris* bark demonstrated antibacterial activity against both Gram-positive and Gram-negative bacteria. Among the bacterial strains tested, *S. aureus 2* showed the least susceptibility to the extract, with inhibition zones of 2.253 ± 0.202 mm and 1.473 ± 0.333 mm at 200 mg/mL and 100 mg/ml, respectively. However, in terms of overall inhibition zones, *S. aureus 1* exhibited the greatest sensitivity to the plant extract.

A comprehensive statistical comparison between the antibacterial effects of the different concentrations of the plant extract and ciprofloxacin is provided in Figure 4.28. This analysis highlights the dose-dependent nature of the extract's antibacterial efficacy, with significant variations observed across the tested bacterial species.

ANTIFUNGAL ACTIVITY OF *A. SCHOLARIS* BARK:

The antifungal activity of different plant extracts of *A. scholaris* bark was performed following the poison food method. The result of petroleum ether extract showed that the highest percentage of inhibition was observed at 200 mg/ml concentration against *Candida albicans* followed by *Fusarium keratoplasticum* and *Aspergillus fumigatus*. The petroleum ether extract displayed a concentration-dependent antifungal activity against the three antifungals tested (Figure 4.29, 4.30, 4.31 and 4.32).

Chloroform extract of *A. scholaris* bark exhibited significant against *C. albicans* at 200 mg/ml as the highest percentage of inhibition was noted at this concentration followed by *F. keratoplasticum* and *A. fumigatus* and it showed a dose-dependent activity against the fungal strain tested. The least effectivity was observed at the lowest concentration at 25 mg/ml. A similar result was obtained regarding concentration-dependent activity against the two fungi tested (Figure 4.33, 4.34, 4.35 and 4.36).

The antifungal activity of methanol extract was observed and the fungal activity was up to seven days. The percentage of inhibition was calculated using the formula. The result obtained significantly exhibited the fungal growth at 200 mg/ml concentration. However, it showed less effectivity at the lowest concentration, i.e. at 25 mg/ml it caused a dose-dependent activity against the fungal *C. albicans* at a higher rate of percentage inhibition followed by against *F. keroplasticum* and finally against *A. fumigatus* (Figure 4.37, 4.38, 4.39 and 4.40).

ANTIMALARIAL ACTIVITY OF *A. SCHOLARIS* BARK:

The findings of our investigation into the antimalarial activity and cytotoxicity of various extracts from the bark of *A. scholaris* are presented in Table 4.25. The study utilized two strains of *P. falciparum* for comparative analysis: PF3D7, which is sensitive to chloroquine (CQ), and K1, which is resistant to chloroquine. For the CQ-sensitive PF3D7 strain, the IC_{50} value of chloroquine was established at 6.4 nM. In contrast, for the CQ-resistant K1 strain, the IC_{50} value of chloroquine was significantly higher at 500 nM, consistent with the known resistance profile of this strain.

Regarding the bark extracts, the IC_{50} values (µg/ml) for PF3D7 were as follows: petroleum ether extract, 22.0 ± 4 µg/ml; chloroform extract, 14.0 ± 4 µg/ml; methanol extract, 43.4 µg/ml; and aqueous extract, >50 µg/ml. A similar trend was observed for the K1 strain, with the IC_{50} values being 26.7 ± 4 µg/ml for petroleum ether, 10.0 µg/ml for chloroform, 45.6 ± 4 µg/ml for methanol, and >50 µg/ml for the aqueous extract.

The cytotoxicity assessment, measured by CC_{50} on Vero cells, revealed that all extracts exhibited a CC_{50} value exceeding 100 µg/ml, indicating a favorable safety profile in terms of cellular toxicity. Among the extracts, the chloroform extract exhibited the most promising antimalarial activity, with an IC_{50} value of 10.0 µg/ml against the K1 strain. This result is particularly noteworthy, as it suggests that the chloroform extract holds potential as a lead candidate for the development of novel antimalarial agents, corroborating findings by Lima (2015). The petroleum ether extract demonstrated moderate antimalarial activity with an IC_{50} value of 22.0 ± 4 µg/ml. However, the methanol and aqueous extracts displayed limited or no significant antimalarial activity, emphasizing their lack of potential for further development in this experiment.

INVITRO ANTHELMINTIC ACTIVITY OF *A. SCHOLARIS* BARK:

The present study investigates the anthelmintic effects of various concentrations of albendazole and *A. scholaris* bark of different extracts on the cestode *R. echinobothrida*. The study evaluates the survival rates of the worms under different treatments and compares the efficacy of the control group, Albendazole, and plant extracts, including methanol, chloroform, petroleum ether, and aqueous extracts. The results demonstrate a concentration-dependent effect across all test mediums, highlighting the superior efficacy of albendazole and the differential potency of the plant extracts. As shown in Table 4.26 anf Figure 4.41, the control group exhibited a mean survival time of 55.673 ± 4.253 hrs when incubated in phosphate-buffered saline (PBS) containing 1% DMSO at 37 ± 1°C. No significant anthelmintic activity was observed in the control group, stressing the role of the tested compounds in inducing mortality. Albendazole, a well-known synthetic anthelmintic agent, exhibited a rapid and potent effect on *R. echinobothrida*, with survival times of 9.400 ± 2.133 hours, 3.737 ± 0.970 hrs, and 1.190 ± 0.213 hrs at concentrations of 5 mg/ml, 10 mg/ml, and 20 mg/ml, respectively. These results indicate a strong concentration-dependent anthelmintic effect of albendazole. Similarly, the methanol extract of the tested plant demonstrated significant anthelmintic activity, with the cestodes succumbing after 23.682 ± 0.661 hrs, 10.019 ± 0.509 hrs, and 3.420 ± 0.428

hrs at the same concentrations (5 mg/ml, 10 mg/ml, and 20 mg/ml). This suggests a relatively high efficacy, albeit slightly lower than Albendazole, at equivalent concentrations. The chloroform extract also exhibited a potent effect, killing the worms within 18.025 ± 0.930 hrs, 10.979 ± 0.402 hrs, and 4.016 ± 0.742 hrs at 5 mg/ml, 10 mg/ml, and 20 mg/ml, respectively. These results closely paralleled the performance of the methanol extract, with a slight delay in the onset of lethality. The petroleum ether extract, while effective, demonstrated a comparatively weaker effect on *R. echinobothrida*, as the survival times were extended to 36.902 ± 4.223 hrs, 33.572 ± 0.562 hrs, and 14.039 ± 0.649 hrs at 5 mg/ml, 10 mg/ml, and 20 mg/ml, respectively. Despite its efficacy, this extract was notably less potent than both Albendazole and the methanol and chloroform extracts. Finally, the aqueous extract displayed the least anthelmintic activity among the tested plant extracts, with worms surviving for 39.525 ± 2.080 hrs, 31.691 ± 4.297 hrs, and 16.409 ± 4.980 hrs at the respective concentrations. Although it did induce mortality, the time required for the worm's death was significantly longer than for the other tested extracts.

HISTOLOGICAL STUDY OF UNTREATED AND TREATED PARASITES OF *A. SCHOLARIS* BARK:

Our histological examination of transverse sections of untreated *R. echinobothrida* revealed unique structural characteristics as shown in the Figure 4.42. The outermost layer, known as the tegument, forms the primary interface of the parasite. Immediately beneath the tegument lies a thicker layer, termed the sub-tegument, which is closely opposed to the tegument itself. The sub-tegument is considerably more robust compared to the tegument. Within the internal matrix, referred to as parenchymatous tissue, multiple eggs or oospheres are observed, surrounded by oncospheres**.** This parenchymatous tissue has a spongy appearance and is embedded within the cells. At the peripheral extremities of the organism, an excretory vacuole can be identified.

Upon treatment with albendazole at a concentration of 20 mg/ml (Fig. 4.43), significant histological alterations were observed. The tegument and sub-tegument exhibited notable structural changes, including partial disintegration. The

parenchymatous tissue underwent substantial degradation, leading to the irregular arrangement of oncospheres within the tissue matrix. Additionally, two large vacuoles emerged at the distal ends of the cestode, signifying pronounced vacuolar formation in response to the drug. Cestodes treated with methanol extract at 20 mg/ml (Figure 4.44) displayed severe shrinkage of both the tegument and sub-tegument layers. This reduction in structural integrity was accompanied by a disorganization of the parenchymatous tissue**,** which resulted in the abnormal positioning of oncospheres. The presence of two prominent excretory vacuoles in the peripheral regions was also a significant finding, indicating profound cellular and tissue disruption. Histological sections of cestodes treated with chloroform extract (20 mg/ml, Figure 4.45) revealed partial peeling of the tegument and sub-tegument, where portions of these outer layers were observed to be sloughing off. The internal eggs appeared compressed and displaced towards the central region of the cestode. Moreover, there was considerable disintegration of the parenchymatous tissue, and, consistent with previous treatments, two large vacuoles were observed at the organism's extremities. Treatment with petroleum ether extract at 20 mg/ml (Figure 4.46) induced significant alterations in the tegument, sub-tegument, and parenchymatous tissues. The structural integrity of these layers was compromised, leading to the irregular displacement of eggs within the tissue. Two large vacuoles, consistent with those observed in other treatments, were prominent at the distal ends of the organism. Finally, cestodes exposed to aqueous extract at a concentration of 20 mg/ml (Figure 4.47) exhibited extensive shrinkage of both the tegument and sub-tegument, affecting the internal architecture of the parasite. The eggs were disorganized and clustered in the central region of the tissue. As with other treatments, the formation of two large excretory vacuoles at the extremities was a recurrent finding, reflecting consistent vacuolar responses to the aqueous extracts.

SCANNING ELECTRON MICROSCOPY (SEM) OF *A. SCHOLARIS* BARK:

R. echinobothrida are soft-bodied cestodes lacking a digestive system. Consequently, nutrient and drug absorption occurs directly through the body surface, referred to as the tegument (Taman and Azab, 2014). The morphology of untreated *R.*

echinobothrida consists of several distinctive regions, beginning with an anterior knob-like structure known as the scolex. Following this is a flattened neck, which transitions into the elongated body proper termed the strobila. The scolex is equipped with four oval-shaped suckers that surround the apical region, known as the rostellum. These suckers serve as vital attachment organs and are lined with multiple rows of sharp spines. Additionally, the scolex possesses a ribbon-like chain of conjoined segments termed proglottids. Covering the entire body of the organism is the tegument, which features hair-like filaments called microtriches, imparting a velvety texture to the surface (Figure 4.48 A, B and C).

When *R. echinobothrida* was exposed to 20 mg/ml of albendazole, drastic structural changes were noted. The scolex exhibited profound shrinkage, with deep depressions forming between the suckers, causing the suckers to bulge outward. Furthermore, the characteristic spines or hooks were dislocated. The proglottids displayed extensive damage, including pitting, cracking, and peeling across the surface of the integument (Figure 4.49 A, B andC).

Treatment with a Methanol extract at 20 mg/ml led to comparable damage, with the scolex showing severe shrinkage and deep depressions between the suckers. Degeneration of the rostellum was also apparent, and the entire proglottid structure exhibited folding (Figure 4.50 A, B andC). Similarly, exposure to the chloroform extract at the same concentration induced extensive shrinkage of the scolex, accompanied by wrinkle formation on the tegument. Most of the spines were dislocated because of the treatment (Fig. 4.51 A, B andC).

When treated with petroleum ether extract at 20 mg/ml, *R. echinobothrida* displayed significant structural distortion. The proglottids were cracked and folded, and the scolex showed severe damage, characterized by shrinkage and the sharp crooking of the spines (Figure 4.52 A, B andC).

The least pronounced effects were observed in treatmets with aqueous extract at 20 mg/ml. While scolex shrinkage was evident, only some of the spines were dislocated, and the tegumentary surface exhibited general folding (Figure 4.53 A, B andC).

Sl. No	Botanical name	Local name	Family	Uses
1.	*A. scholaris* Common name- Devil Tree, Saptaparni	Thuamriat	Apocynaceae	Intestinal worms, antimalarial treatment, skin disorders, typhoid fever, cardiovascular conditions, dysentery, hypertension, diarrhea, and ear infections are among the ailments addressed. The infusion of bark is administered once daily.
2.	*Betula alnoides* Common name- Himalayan birch	Hriang	Betulaceae	Arthritis, rheumatism, gout, intestinal parasitic infections, scurvy, renal disorders, and malaria are among the conditions treated. A decoction is taken specifically to alleviate malarial fever.
3.	*Cinnamonum bejolghota* Common name- Chinese cinnamon	Thakthing suak	Lauraceae	Dyspepsia, hepatomegaly, indigestion, cold, fever, malarial fever, cough, headache, abdominal pain, and diarrhea are among the conditions addressed. The decoction is administered internally for malarial fever, with a recommended dosage of two tablespoonfuls (20 ml) twice daily.
4.	*Costus speciosus* Common name- Grepe ginger, spiral flag, cane reed	Sumbul	Costaeae	Conditions such as kidney disorders, leprosy, stomatitis, catarrhal fever, reduced breast milk production, and malaria. The rhizome, combined with local liquor, is administered to combat *Plasmodium falciparum* malaria at a dosage of two teaspoonfuls (20 ml) once daily.
5.	*Dendrocnide sinuate* Common name- Devil or fever nettle	Thakpui	Urticaceae	Liver ailments, jaundice, fever, chickenpox, skin itching, and malaria are among the conditions treated using this remedy. Additionally, a decoction prepared from the roots is commonly administered to address malaria specifically.
6.	*Hedyotes Scandens* Common name- Climbing diamond fever	Kelhnamtur	Araceae	Malarial fever, general fever, jaundice, kidney disorders, and the expulsion of kidney or gallbladder stones are effectively treated. An infusion made from the roots and leaves is particularly effective for managing malarial fever, with a recommended dosage of one

				teaspoon (10 ml) taken twice daily.
7.	*Mimosa pudica* Common name- Touch me not/ sensitive plant	Hlonuar/ hlo zak	Mimosaceae	malaria, leprosy, dysentery, gynecological and uterine issues, inflammations, burning sensations, fatigue, asthma, leukoderma, and blood disorders. A preparation from boiled roots or juice extracted from the leaves is specifically administered for the treatment of malaria.
8.	*Prunus cerosoides* Common name- Himalayan wild cherry	Tlaizawng	Rosaceae	This remedy addresses conditions such as fever, malaria, pain relief, and is known for its antipyretic, carminative, expectorant, febrifuge, spasmolytic, and astringent properties. A bark decoction is specifically utilized as a treatment for malaria
9.	*Stereospermum colais* Common name- Yellow snake tree/Trumpet flower	Zihnghal	Bigoniaceae	Anthelmintic, antifebrile, cardiotonic, antiasthmatic, antitussive, aphrodisiac, and anti-inflammatory properties. An infusion of the leaves is taken specifically for its antifebrile effects and to treat malarial fever, with a recommended dosage of one tablespoon (10 ml) administered twice daily.
10.	*Vitex Penduncularis wall* Common name- Charaiygoda (Hindi)	Thingkhawi lu	Verbenaceae	The plant is applied externally for relief from chest pain, malaria, blackwater fever, joint pain, typhoid, jaundice, and cough, as well as general aches and pains associated with fever.

Table 4.2: Yield of extraction from 10 dried samples collected

1. ***A. scholaris***

(g)	**Solvent**	**Extractive weight (g)**	**Extractive value (%)**
30	Petroleum ether	1.909	6.36 %
	Chloroform	2.026	6.75 %
	Methanol	3.67	12.23 %

2. ***Betula alnoides***

(g)	**Solvent**	**Extractive weight (g)**	**Extractive value (%)**
30	Petroleum ether	0.022	0.07 %
	Chloroform	0.432	1.44 %
	Methanol	3. 059	10.19 %

3***. Cinnamomum obtusofolium***

(g)	**Solvent**	**Extractive weight (g)**	**Extractive value (%)**
20	Petroleum ether	0.032	0.16 %
	Chloroform	0.251	1.25 %
	Methanol	0.308	1.54 %

4. ***Costus speciosus***

(g)	**Solvent**	**Extractive weight (g)**	**Extractive value (%)**
30	Petroleum ether	0.519	1.73 %
	Chloroform	1.853	6.17 %
	Methanol	2.419	8.06 %

5. ***Dendrocnide sinuate***

(g)	**Solvent**	**Extractive weight (g)**	**Extractive value (%)**
30	Petroleum ether	0.023	0.76 %
	Chloroform	1.578	5.26 %
	Methanol	1.362	4.54 %

6. ***Hedyotes scandens***

(g)	Solvent	Extractive weight (g)	Extractive value (%)
20	Petroleum ether	0.049	0.245 %
	Chloroform	0.513	2.56 %
	Methanol	0.890	4.45 %

7. ***Mimosa pudica***

(g)	Solvent	Extractive weight (g)	Extractive value (%)
20	Petroleum ether	0.215	1.075 %
	Chloroform	0.368	1.84 %
	Methanol	0.783	3.915 %

8. ***Prunus cerosoides***

(g)	Solvent	Extractive weight (g)	Extractive value (%)
30	Petroleum ether	0.273	0.91 %
	Chloroform	1.236	4.12 %
	Methanol	3.238	10.79 %

9. ***Stereospermum colais***

(g)	Solvent	Extractive weight (g)	Extractive value (%)
30	Petroleum ether	0.812	2.70 %
	Chloroform	1.054	3.51 %
	Methanol	3.738	12.46 %

10. ***Vitex penducularis wall***

(g)	Solvent	Extractive weight (g)	Extractive value (%)
30	Petroleum ether	0.881	2.93 %
	Chloroform	1.735	5.78 %
	Methanol	3.616	12.05 %

Table 4.3: Yield of extraction from dried sample of *A. scholaris*

(g)	Solvent	Extractive Weight (g)	Extractive value (%)
600	Petroleum ether	57.284	9.55
600	Chloroform	60.8	10.13
600	Methanol	110.1	18.35
297	Aqueous	15.428	5.19

Table 4.4: Phytochemical detection of different extracts of *A. scholaris* using various biochemical tests.

Phytochemical	**Name of test**	**ASP**	**ASC**	**ASM**	**ASA**
Alkaloids	Mayer's test	-	-	-	-
	Dragendorff's test	-	-	-	+
	Wagner's test	-	-	-	+
	Hager's test	-	-	+	-
Flavonoid	Shinoda test	+	-	+	+
	Zinc hydrochloride reduction test	-	-	-	+
Tannins	$FeCl_3$ test	-	-	-	+
	$K_2Cr_3O_7$ test	-	-	+	-
	Lead acetate test	-	-	+	-
Phytosterols	Libermann-Burchard's test	+	+	+	+
	Salkowski test	+	+	+	+
Saponins	Foam test	-	+	+	+
Carbohydrates	Molisch's test	-	+	+	+
	Fehling's test	-	+	+	-
	Barfoed's test	-	-	-	-
	Benedict's test	-	+	-	-
Reducing sugars	Fehling's test	-	+	-	-
	Benedict's test	-	+	+	+
Protein and Amino acids	Biuret test	-	-	-	-
	Ninhydrin test	-	-	-	-
Glycosides	Legal's test	-	-	-	-
	Keller-Kiliani test	-	-	+	+
Gums	Alcohol test	-	-	-	-
Anthraquinone	Borntrager's test	-	-	-	-
	Ammonium hydroxide test	-	-	-	+

ASP= *A. scholaris* petroleum ether extract

ASC = *A. scholaris* chloroform extract

ASM = *A. scholaris* methanol extract

ASA = *A. scholaris* aqueous extract

+ sign indicates the presence

- sign indicates the absence

Table 4.5: Phytochemical detection of different extracts of *Betula alnoides* using various biochemical tests.

Phytochemical	Name of test	BAP	BAC	BAM
Alkaloids	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoid	Shinoda test	-	+	+
	Zinc hydrochloride reduction test	-	-	+
Tannins	$FeCl_3$ test	-	-	+
	$K_2Cr_3O_7$ test	-	-	+
	Lead acetate test	-	-	+
Phytosterols	Libermann-Burchard's test	+	+	-
	Salkowski test	+	+	-
Saponins	Foam test	-	-	+
Carbohydrates	Molisch's test	-	-	+
	Fehling's test	-	+	+
	Barfoed's test	-	-	+
	Benedict's test	-	-	-
Reducing sugars	Fehling's test	-	+	+
	Benedict's test	-	-	+
Protein and Amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Glycosides	Legal's test	-	-	-
	Keller-Kiliani test	-	-	-
Gums	Alcohol test	-	-	-
Anthraquinone	Borntrager's test	-	-	-
	Ammonium hydroxide test	-	-	-

BAP = *Betula alnoides* petroleum ether extract

BAC = *Betula alnoides* chloroform extract

BAM = *Betula alnoides* methanol extract

+ sign indicates the presence

- sign indicates the absence

Table 4.6: Phytochemical detection of different extracts of *Cinnamomum obtusofolium* using various biochemical tests.

Phytochemical	Name of test	COP	COC	COM
Alkaloids	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoid	Shinoda test	-	-	-
	Zinc hydrochloride reduction test	-	-	+
Tannins	$FeCl_3$ test	-	-	-
	$K_2Cr_3O_7$ test	-	-	+
	Lead acetate test	-	-	+
Phytosterols	Libermann-Burchard's test	+	+	+
	Salkowski test	+	+	+
Saponins	Foam test	-	+	+
Carbohydrates	Molisch's test	-	+	+
	Fehling's test	-	+	-
	Barfoed's test	-	+	+
	Benedict's test	-	+	-
Reducing sugars	Fehling's test	-	+	-
	Benedict's test	-	+	+
Protein and Amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Glycosides	Legal's test	-	-	-
	Keller-Kiliani test	-	-	-
Gums	Alcohol test	-	-	-
Anthraquinone	Borntrager's test	-	-	-
	Ammonium hydroxide test	-	-	-

COP = *Cinnamomum obtusofolium* petroleum ether extract

COC = *Cinnamomum obtusofolium* chloroform extract

COM = *Cinnamomum obtusofolium* methanol extract

+ sign indicates the presence

- sign indicates the absence

Table 4.7: Phytochemical detection of different extracts of *Costus speciosus* using various biochemical tests.

Phytochemical	Name of test	CSP	CSC	CSM
Alkaloids	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoid	Shinoda test	-	-	-
	Zinc hydrochloride reduction test	-	-	-
Tannins	$FeCl_3$ test	-	-	+
	$K_2Cr_3O_7$ test	-	-	-
	Lead acetate test	-	-	+
Phytosterols	Libermann-Burchard's test	+	+	+
	Salkowski test	+	+	-
Saponins	Foam test	-	-	+
Carbohydrates	Molisch's test	-	-	+
	Fehling's test	-	+	-
	Barfoed's test	-	-	+
	Benedict's test	-	-	-
Reducing sugars	Fehling's test	-	+	-
	Benedict's test	-	-	+
Protein and Amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Glycosides	Legal's test	-	-	-
	Keller-Kiliani test	-	-	-
Gums	Alcohol test	-	+	-
Anthraquinone	Borntrager's test	-	-	-
	Ammonium hydroxide test	-	-	-

CSP = *Costus speciosus* petroleum ether extract

CSC = *Costus speciosus* chloroform extract

CSM = *Costus speciosus* methanol extract

+ sign indicates the presence

- sign indicates the absence

Table 4.8: Phytochemical detection of different extracts of *Dendrocnide sinuate* using various biochemical tests.

Phytochemical	Name of test	DSP	DSC	DSM
Alkaloids	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoid	Shinoda test	-	-	-
	Zinc hydrochloride reduction test	-	-	-
Tannins	$FeCl_3$ test	-	-	-
	$K_2Cr_3O_7$ test	-	-	+
	Lead acetate test	-	-	+
Phytosterols	Libermann-Burchard's test	+	-	-
	Salkowski test	+	-	-
Saponins	Foam test	-	-	+
Carbohydrates	Molisch's test	-	-	+
	Fehling's test	-	+	-
	Barfoed's test	-	-	+
	Benedict's test	-	-	-
Reducing sugars	Fehling's test	-	+	-
	Benedict's test	-	-	+
Protein and Amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Glycosides	Legal's test	-	-	-
	Keller-Kiliani test	-	-	-
Gums	Alcohol test	-	-	-
Anthraquinone	Borntrager's test	-	-	-
	Ammonium hydroxide test	-	-	-

DSP= *Dendrocnide sinuate* petroleum ether extract

DSC = *Dendrocnide sinuate* chloroform extract

DSM = *Dendrocnide sinuate* methanol extract

+ sign indicates the presence

- sign indicates the absence

Table 4.9: Phytochemical detection of different extracts of *Hedyotes scandens* using various biochemical tests.

Phytochemical	Name of test	HSP	HSC	HSM
Alkaloids	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoid	Shinoda test	-	-	-
	Zinc hydrochloride reduction test	-	-	-
Tannins	$FeCl_3$ test	-	-	-
	$K_2Cr_3O_7$ test	-	-	+
	Lead acetate test	-	-	+
Phytosterols	Libermann-Burchard's test	-	+	-
	Salkowski test	-	-	-
Saponins	Foam test	-	-	+
Carbohydrates	Molisch's test	-	-	+
	Fehling's test	-	-	-
	Barfoed's test	-	-	-
	Benedict's test	-	-	-
Reducing sugars	Fehling's test	-	-	+
	Benedict's test	-	-	-
Protein and Amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Glycosides	Legal's test	+	-	-
	Keller-Kiliani test	+	-	-
Gums	Alcohol test	-	-	-
Anthraquinone	Borntrager's test	-	-	-
	Ammonium hydroxide test	-	-	-

HSP = *Hedyotes scandens* petroleum ether extract

HSC = *Hedyotes scandens* chloroform extract

HSM = *Hedyotes scandens* methanol extract

+ sign indicates the presence

Table 4.10: Phytochemical detection of different extracts of *Mimosa pudica* using various biochemical tests.

Phytochemical	Name of test	MPP	MPC	MPM
Alkaloids	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoid	Shinoda test	-	-	+
	Zinc hydrochloride reduction test	-	-	+
Tannins	$FeCl_3$ test	-	-	+
	$K_2Cr_3O_7$ test	-	-	+
	Lead acetate test	-	-	-
Phytosterols	Libermann-Burchard's test	+	-	-
	Salkowski test	-	-	-
Saponins	Foam test	-	-	+
Carbohydrates	Molisch's test	-	+	-
	Fehling's test	-	+	+
	Barfoed's test	-	-	-
	Benedict's test	-	-	-
Reducing sugars	Fehling's test	-	+	+
	Benedict's test	-	+	-
Protein and Amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Glycosides	Legal's test	+	+	+
	Keller-Kiliani test	-	-	-
Gums	Alcohol test	-	-	-
Anthraquinone	Borntrager's test	-	-	-
	Ammonium hydroxide test	-	-	-

MPP = *Mimosa pudica* petroleum ether extract

MPC = *Mimosa pudica* chloroform extract

MPM = *Mimosa pudica* methanol extract

+ sign indicates the presence

- sign indicates the absence

Table 4.11: Phytochemical detection of different extracts of *Prunus cerosoides* using various biochemical tests.

Phytochemical	Name of test	PCP	PCC	PCM
Alkaloids	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoid	Shinoda test	-	-	+
	Zinc hydrochloride reduction test	-	-	+
Tannins	$FeCl_3$ test	-	-	+
	$K_2Cr_3O_7$ test	-	-	+
	Lead acetate test	-	-	-
Phytosterols	Libermann-Burchard's test	+	+	-
	Salkowski test	-	-	-
Saponins	Foam test	-	-	+
Carbohydrates	Molisch's test	-	+	+
	Fehling's test	-	+	+
	Barfoed's test	-	-	-
	Benedict's test	-	-	-
Reducing sugars	Fehling's test	-	+	+
	Benedict's test	-	+	+
Protein and Amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Glycosides	Legal's test	+	-	-
	Keller-Kiliani test	-	-	-
Gums	Alcohol test	-	-	-
Anthraquinone	Borntrager's test	-	-	-
	Ammonium hydroxide test	-	-	-

PCP = *Prunus cerosoides* petroleum ether extract

PCC = *Prunus cerosoides* chloroform extract

PCM = *Prunus cerosoides* methanol extract

+ sign indicates the presence

- sign indicates the absence

Table 4.12: Phytochemical detection of different extracts of *Stereospermum colais* using various biochemical tests.

Phytochemical	Name of test	SCP	SCC	SCM
Alkaloids	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoid	Shinoda test	-	-	-
	Zinc hydrochloride reduction test	-	-	+
Tannins	$FeCl_3$ test	-	-	+
	$K_2Cr_3O_7$ test	-	-	+
	Lead acetate test	-	-	+
Phytosterols	Libermann-Burchard's test	+	+	-
	Salkowski test	+	-	+
Saponins	Foam test	-	-	-
Carbohydrates	Molisch's test	-	-	+
	Fehling's test	-	+	+
	Barfoed's test	-	-	+
	Benedict's test	-	-	-
Reducing sugars	Fehling's test	-	+	+
	Benedict's test	-	-	+
Protein and Amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Glycosides	Legal's test	-	-	-
	Keller-Kiliani test	-	-	-
Gums	Alcohol test	-	-	-
Anthraquinone	Borntrager's test	-	-	-
	Ammonium hydroxide test	-	-	-

SCP = *Stereospermum colais* petroleum ether extract

SCC = *Stereospermum colais* chloroform extract

SCM = *Stereospermum colais* methanol extract

+ sign indicates the presence

- sign indicates the absence

Table 4.13: Phytochemical detection of different extracts of *Vitex penducularis wall* using various biochemical tests.

Phytochemical	Name of test	VPP	VPC	VPM
Alkaloids	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoid	Shinoda test	-	-	+
	Zinc hydrochloride reduction test	-	-	+
Tannins	$FeCl_3$ test	-	-	+
	$K_2Cr_3O_7$ test	-	-	+
	Lead acetate test	-	-	-
Phytosterols	Libermann-Burchard's test	+	+	-
	Salkowski test	-	+	-
Saponins	Foam test	-	-	+
Carbohydrates	Molisch's test	-	+	+
	Fehling's test	-	+	+
	Barfoed's test	-	-	+
	Benedict's test	-	-	-
Reducing sugars	Fehling's test	-	+	+
	Benedict's test	-	+	+
Protein and Amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Glycosides	Legal's test	-	-	-
	Keller-Kiliani test	-	-	-
Gums	Alcohol test	-	-	-
Anthraquinone	Borntrager's test	-	-	+
	Ammonium hydroxide test	-	-	-

VPP = *Vitex penducularis* petroleum ether extract

VPC = *Vitex penducularis* chloroform extract

VPM = *Vitex penducularis* methanol extract

+ sign indicates the presence

- sign indicates the absence

Table 4.14: Compounds identified from *A. scholaris* petroleum ether extract by GC-MS.

Sl.No.	RT	RA	Compound	Formula	MW
1.	3.08	14.2	3- Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324
2.	3.76	9.96	Hexadecen-1-ol,trans-9-	$C_{16}H_{32}O$	240
3.	4.15	11.18	3-Trifluoroacetoxytetra decane	$C_{16}H_{29}F_3O_2$	310
4.	5.46	24.57	2H-Indeno[1,2-b] furan-2-one,3,3a,4,5,6,7, 8,8b-octahydro-8,8-dimethyl	$C_{16}H_{18}O_2$	206
5.	6.63	4.9	1,9-Nonadecane	$C_{19}H_{38}$	266
6.	6.76	11.5	1-Decanol,2-hexyl	$C1_6H_{34}O$	242
7.	8.07	20.82	8-Pentadecanone	$C_{15}H_{30}O$	226
8.	10.14	5.33	4-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	310
9.	10.25	5.13	3-Eicosene,(E)-	$C_{20}H_{4O}$	280
10.	10.37	9.9	1-Decanol,2-hexyl	$C_{16}H_{34}O$	242
11.	11.81	10.21	8-Pentadecanone	$C_{15}H_{30}O$	226
12.	12.76	33.71	Pentadecanoic acid,14-methyl-methyl ester	$C_{17}H_{34}O_2$	270
13.	13.55	14.73	phthallic acid,butyl undecyl ester	$C_{23}H_{36}O_4$	376
14.	14	5.98	heptacos-1-ene	$C_{27}H_{54}$	378
15.	14.57	21.93	Isoshyobunone	$C_{15}H_{24}O$	220
16.	15.88	12.3	9,12-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
17.	16.78	17.69	Linoelaidic acid	$C_{18}H_{32}O_2$	280
18.	17.15	52.67	Otadecanoic acid	$C_{18}H_{36}O_2$	284
19.	17.59	5.86	Nonacos-1-ene	$C_{29}H_{58}$	406
20.	20.07	15.22	Z-8-methyl-9- tetradecenoic acid	$C_{15}H_{28}O_2$	240
21.	20.47	35.27	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256
22.	20.93	6.99	Nonacos-1-ene	$C_{29}H_{58}$	406
23.	23.07	11.86	cyclopentaeundecanoic acid methyl ester	$C_{17}H_{32}O_2$	268
24.	23.42	48.17	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390
25.	23.63	52.7	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212
26.	25.59	6.72	4-Fluoro-1-methyl-5-Carboxylic acid, ethyl ester	$C_7H_9FN_2O_2$	172
27.	26.31		Water	H_2O	18
28.	27.46	46.58	Squalene	$C_{30}H_{50}$	410
29.	28	33.64	Cedron-diol (8s,14)-	$C_{15}H_{26}O_2$	238
30.	29.15	8.54	1-Heptatriacotanol	$C_{37}H_{76}O$	536
31.	31.09	6.76	Cedron-diol (8s,14)-	$C_{15}H_{26}O_2$	238
32.	31.61	30.01	Methylene cyclo hexyl) butanol	$C_{13}H_{22}O$	194

RT= Retention time, RA= Relative abundance in percentage, MW= Molecular weight

Table 4.15: Compounds identified from *A. scholaris* chloroform extract by GC-MS

Sl.No	RT	RA	Compound	Formula	MW
1.	3.07	24.73	8-methylenecyclooctene-3-4-diol	$C_9H_{14}O_2$	154
2.	3.47	9.35	2-Pentadecyn-1-ol	$C_{15}H_{28}O$	224
3.	3.74	14.2	R-Limonene	$C_{10}H_{16}O_3$	184
4.	4.88	7	9-Oxabicyclo[4.2.1]nonan-2-ol	$C_8H_{14}O_2$	142
5.	5.36	6.69	3-Decyn-2-ol	$C_{10}H_{18}O$	154
6.	5.47	6.39	10-Heptadecen-8-ynoic acid, methyl ester, €	$C_{18}H_{30}O_2$	278
7.	7.06	25.06	6-Nonenal,3,7-dimethyl-	$C_{11}H_{20}O$	168
8.	7.34	9.04	Bicyclo[3.1.0] hexane-6-methanol,2-hydroxy-1,4,4-trimethyl-	$C_{10}H_{18}O_2$	170
9.	7.43	8.82	Cyclohexanene,2-(1-methyl-2-nitroethyl)-	$C_9H_{15}NO_3$	185
10.	8.41		Dodecyl acrylate	$C_{15}H_{28}O_2$	240
11.	9.09	14.05	11-Oxatetracyclo[5.3.2.0(2,7).oc2,8] dodecan-9-one	$C_{11}H_{14}O_2$	178
12.	9.2	7.04	Z,Z,Z-4,6,9-Nona decatriene	$C_{19}H_{34}$	262
13.	10.83	11.67	Bicyclo[2.2.1] heptan-2-one, 4,7,7-tri methyl-semicarbazone	$C_{11}H_{19}N_{30}$	209
14.	11.27	12.62	Bicyclo[2.2.1] heptan-2-one, 4,7,7-tri methyl-semicarbazone	$C_{11}H_{19}N_{30}$	209
15.	13.17	80.9	Benzenepropanoic acid,3,5-bis (1,-di methyl)-4-hydroxy-,methyl ester	$C_{18}H_{28}O_3$	292
16.	13.56	52.93	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
17.	13.99	8.39	1-Hexadecanol,2-methyl-	$C_{17}H_{36}O$	256
18.	15.25	12.78	[1,1-Bicyclopropyl]2-octanoid acid, 2-hexyl methyl ester	$C_{17}H_{36}O$	322
19.	16.72	13.19	Trans-13-octadecenoic acid	$C_{18}H_{36}O_2$	282
20.	17.11	67.54	Octadecanoic acid	$C_{18}H_{36}O_2$	284
21.	17.59	5.03	Trichloroacetic acid, Pentadecyl ester	$C_{17}H_{31}CL_3O_2$	372
22.	18.32	11.56	Cis-Z-a-Bisabolene epoxide	C15H24O	220
23.	20.94	10.02	3-trifluoroacetoxypenta decane	C17H31F3O2	324

24.	21.16	15.59	Campesterol	$C_{15}H_{24}O$	400
25.	23.43	21.58	9,10-Secocholesta -5,7,10(19)-triene-3,25, 26-triol, (3a,5Z,7E)	$C_{27}H_{44}O_3$	416
26.	23.56	20.11	Cholesta-22,24-dien-5-ol,4,4-dimethyl	$C_{29}H_{48}O$	412
27.	25.06	72.11	Akuammilan-17-oic acid methyl ester	$C_{20}H_{22}N_2O_2$	322
28.	26.46	13.72	1-Heptatriacotanol	$C_{37}H_{76}O$	536
29.	27.45	38.08	a- Amyrin	$C_{30}H_{50}O$	426
30.	27.84	54.18	9,19-cycloergost-24(28)-en-3-ol,4,14-dimethyl, acetate(3a,4a,5a)	$C_{32}H_{52}O_2$	468
31.	29.17	38.91	Lupeol	$C_{30}H_{50}O$	426
32.	29.38	49.33	1-Heptatriacotanol	$C_{37}H_{76}O$	536
33.	30.89	3.07	1-Heptatriacotanol	$C_{37}H_{76}O$	536
34.	31.43	18.52	24-Noroleana-3,12-diene	$C_{29}H_{46}$	394
35.	31.58	18.18	24-Noroleana-3,12-diene	$C_{29}H_{46}$	394

RT= Retention time, RA= Relative abundance in percentage, MW= Molecular weight

Table 4.16: Compounds identified from *A. scholaris* methanol extract by GC-MS

Sl. No	RT	RA	Compound	Formula	MW
1.	3.62		water	H_2O	
2.	3.92	50.42	2,6,10-Dodecatrien-1-ol,3,7,11- trimethyl	$C_{21}H_{30}O_3S$	362
3.	4.94		water	H_2O	18
4.	5.42	73.17	Phenol,4-(1,1-dimethylethyl)-2-(1,1-Dimethylpropyl	$C_{15}H_{24}O$	220
5.	5.86		water	H_2O	18
6.	7.97	28.51	Urs-12-en-24-oic acid,3-oxo-, methyl 24-oic,3-oxo-, methyl ester, (+) -	$C_{31}H_{48}O_3$	468
7.	8.3	32.99	Urs-12-en-24-oic acid,3-oxo-, methyl ester, (+) -	$C_{31}H_{48}O_3$	468
8.	10.57	22.61	Urs-12-en-24-oic acid,3-oxo-, methyl ester, (+) -	$C_{31}H_{48}O_3$	468
9.	11.83	26.86	Urs-12-en-24-oic acid,3-oxo-, methyl ester, (+) -	$C_{31}H_{48}O_3$	468
10.	13.37	7.62	1- Heptatriacotanol	$C_{37}H_{76}O$	536

11.	14.8	10.07	Lup-20(29)-en-3-ol, acetate, (3a)-	$C_{32}H_{52}O_2$	468
12.	15.72	9.54	Lup-20(29)-en-3-ol, acetate, (3a)-	$C_{32}H_{52}O_2$	468
13.	15.87	19.34	Lup-20(29)-en-3-ol, acetate, (3a)-	$C_{32}H_{52}O_2$	468
14.	16.53	18.05	Lup-20(29)-en-3-ol, acetate, (3a)-	$C_{32}H_{52}O_2$	468
15.	16.7	19.71	Lup-20(29)-en-3-ol, acetate, (3a)-	$C_{32}H_{52}O_2$	468
16.	17.41	22.42	Lup-20(29)-en-3-ol, acetate, (3a)-	$C_{32}H_{52}O_2$	468
17.	17.53	22.28	Lup-20(29)-en-3-ol, acetate, (3a)-	$C_{32}H_{52}O_2$	468
18.	18.04	26.56	1-Heptatriacotanol	$C_{37}H_{76}O$	536
19.	19.96	68.43	Water	H_2O	18
20.	20.93	69.37	Water	H_2O	338
21.	21.15	48.88	Diazoprogesterone	$C_{21}H_{30}N_4$	338
22.	23.22	92.37	Water	H_2O	18
23.	23.4	34.1	Bisnorallocholanic acid	$C_{21}H_{30}N_4$	332
24.	24.95	87.54	water	H_2O	18
25.	26.39	11.21	Kolavelool	$C_{20}H_{34}O$	290
26.	26.54	16.45	Kolavelool	$C_{20}H_{34}O$	290
27.	27.39	39.45	a-Amyrin	$C_{30}H_{50}O$	426
28.	28.91	11.65	Cedran-diol(8s,14)	$C_{15}H_{26}O_2$	238
29.	28.91	11.65	Cedran-diol(8s,14)	$C_{15}H_{26}O_2$	238
30.	30.19	37.9	Pregnan-18-oic acid,20-hydroxy-, C-lactone (5a)-	$C_{21}H_{32}O_2$	316
31.	31.16	9.41	a-Amyrin	$C_{30}H_{50}O$	426
32.	33.05	21.27	Lup-20(29)-en-3-ol, acetate, (3a)-	$C_{32}H_{52}O_2$	468

RT= Retention time, RA= Relative abundance in percentage, MW= Molecular weight

Table 4.17: Compounds identified from *A. scholaris* aqueous extract by GC-MS

Sl.No	RT	RA	Compounds	Formula	MW
1.	3.09	8.81	Benzoxazol,2,3-dihydro-2-thioxo-3 diallyamino methyl	$C_{14}H_{16}N_{20}S$	260
2.	4.16	35.17	Formamide, N-(2,4-dimethylphenyl)	$C_9H_{11}NO$	149
3.	5.36	9.08	2,4-Pentadien-1-ol, 3-pentyl-, (2Z)-	$C_{10}H_{18}O$	154
4.	5.46	21.05	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	$C_{19}H_{30}O_3$	306
5.	6.26	8.86	2(1H)-Naphthalenone, octahydro-8a-methyl-, cis-	$C_{11}H_{18}O$	166
6.	7.07	18.5	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl-	$C_{10}H_{18}O_2$	170
7.	7.37	29.23	Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-	$C_{10}H_{18}O_2$	170
8.	7.84	11.77	1-(Cyclopropyl-nitro-methyl)-cyclopentanol	$C_9H_{15}NO_3$	185
9.	8.16	22.4	6-Ethoxy-6-methyl-2-cyclohexenone	$C_9H_{14}O_2$	154
10.	9.96	66.31	water	H_2O	18
11.	10.09	57.19	water	H_2O	18
12.	12.76	90.35	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276
13.	13.46	34.07	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212
14.	15.27	42.16	water	H_2O	18
15.	15.47	76.86	water	H_2O	18
16.	17.11	96.72	water	H_2O	18
17.	18.44	94.91	water	H_2O	18
18.	20.77	98.29	water	H_2O	18
19.	21.46	97.43	water	H_2O	18
20.	22.78	63.31	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330
21.	18.44	68.57	water	H_2O	18
22.	25.02	73.73	Akuammilan-17-oic acid, methyl ester	$C_{20}H_{22}N_2O_2$	322
23.	25.86	14.73	Octadecanal, 2-bromo-	$C_{16}H_{35}B_rO$	346
24.	26.82	38.93	8H_Azecino[5,4-b]indol-8-one, 5-ethylidene- 1,2,3,4,5,6,7,9-octahydro-6-(2-hydroxyethyl-3-methyl-[S-(E)]-	$C_{20}H_{26}N_2O_2$	326

25.	27.75	35.83	6-Amino-5-cyano-4-(5-cyano-2,4-dimethyl-1H-pyrrol-3-yl) -2-methyl 4-pyran-3-carboxylic acid ethyl ester	$C_{17}H_{18}N_4O_3$	326
26.	28.63	28.96	6-Amino-5-cyano-4-(5-cyano-2,4-dimethyl-1H-pyrrol-3-yl) -2-methyl 4-pyran-3-carboxylic acid ethyl ester	$C_{17}H_{18}N_4O_3$	326
27.	30.05	15.05	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296
28.	30.05	27.81	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296

RT= Retention time, RA= Relative abundance in percentage, MW= Molecular weight

Table 4.18: Compounds identified from *Costus speciosus* petroleum ether extract by GC-MS.

Sl.No	RT	RA	Compounds	Formula	MW
1.	3.06	21.74	3- Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324
2.	3.87	23.5	2- Hexyl-1-octanol	$C_{14}H_{30}O$	214
3.	4.2	8.46	2- trifluoro-acetoxypentadecane	$C_{17}H_{31}F_3O_2$	324
4.	5.46	54.22	Phenol 4-dimethylethyl)-2-(1,1-dimethylpropyl)-	$C_{15}H_{24}O$	220
5.	6.65	9.56	E-15- Heptadecenal	$C_{17}H_{32}O$	252
6.	6.76	9.13	Silane, trichlorodocosyl-	$C_{22}H_{45}Cl_3Si$	442
7.	8.08	51.93	8- Pentadecanone	$C_{15}H_{30}O$	226
8.	9.65	37.97	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212
9.	10.26	6.55	E-15- Heptadecenal	$C_{17}H_{32}O$	252
10.	10.38	7.67	1- Decanol, 2-octyl-	$C_{18}H_{38}O$	270
11.	11.54	12.26	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212
12.	11.92	7.17	Ethanol,2-(octadecycloxyl)-	$C_{20}H_{42}O_2$	314
13.	13.12	17.82	Hexadecenoic acid, Z-11-	$C_{16}H_{30}O_2$	254
14.	13.12	17.82	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376
15.	13.65	49.26	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	256
16.	14.02	5.79	heptacos-1-ene	$C_{27}H_{54}$	378
17.	15.65	14.68	Z-8-methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240
18.	16.75	14.68	Linoelaidic acid	$C_{18}H_{32}O_2$	280
19.	16.84	6.72	2-methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O_2$	280
20.	17.19	59.03	Octadecanoic acid	$C_{18}H_{36}O_2$	284

21.	17.61	9.23	heptacos-1-ene	$C_{27}H_{54}$	378
22.	18.63	12.33	Cis- 13- Eicosenoic acid	$C_{20}H_{38}O_2$	310
23.	19.5	6.48	Trichloroacetic acid, hexadecyl ester	$C_{18}H_{33}Cl_3O_2$	386
24.	20.5	70.1	Eicosanoic acid	$C_{20}H_{40}O_2$	312
25.	21.07	51.97	Hexanedioic acid, bis (2-ethyl hexyl) ester	$C_{22}H_{42}O_4$	370
26.	22.67	6.31	Trichloroacetic acid, hexadecyl ester	$C_{18}H_{33}Cl_3O_2$	386
27.	23.43	9.75	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390
28.	23.66	13.77	heptadecanoic acid, heptadecyl ester	$C_{34}H_{68}O_2$	508
29.	25.79	30.04	Boldenone	$C_{19}H_{26}O_2$	286
30.	28.03	73.07	Water	H_2O	18
31.	28.4	7.93	Oxalic acid, cyclobutyl heptadecyl ester	$C_{23}H_{42}O_4$	382
32.	29.58	42.45	water	H_2O	18
33.	31.08	47.91	Androstane-11,17-dione,3-[(trimethylsilyloxy]-17-[0-phenylmethyl) oxime](3a,5a)-	$C_{29}H_{43}NO_3Si$	382
34.	32.29	13.34	26-Nor-5-cholesten-3a-ol-25-one	$C_{26}H_{42}O_2$	386

RT= Retention time, RA= Relative abundance in percentage, MW= Molecular weight

Table 4.19: Compounds identified from *Costus speciosus* chloroform extract by GC-MS.

Sl.No	RT	RA	Compounds	Formula	MW
1.	3.09	11.56	3-(prop-2 enoyloxy) tetradecane	$C_{17}H_{32}O_2$	268
2.	3.76	12.07	4- Trifluoroacetoxy tetradecane	$C_{16}H_{29}F_3O_2$	310
3.	3.84	8.31	Octadecane, 6- methyl-	$C_{19}H_{40}$	268
4.	4.11	56.94	Vanillin lactoside	$C_{20}H_{28}O_{13}$	476
5.	5.44	61.6	2,4-Di-tert-butyl phenol	$C_{14}H_{22}O$	206
6.	5.6	21.74	2H-indeno[1,2-b]furan-one,3,3a,4,5,6,7,8, 8b-octahydro-8,8-dimethyl	$C_{13}H_{18}O_2$	206
7.	6.73	4.52	10-methyl nonadecane	$C_{20}H_{42}$	282
8.	6.85	7.97	1-octanol,2-butyl-	$C_{12}H_{26}O$	186
9.	7.85	14.95	2- Methyl-Z,Z-3,13-Octadecadienol	$C_{19}H_{36}O$	280
10.	8.41	19.32	Dodecyl acrylate	$C_{15}H_{28}O_2$	240
11.	8.49	13.76	Dodecyl acrylate	$C_{15}H_{28}O_2$	240
12.	10.24	6.07	5- eicosene (E)-	$C_{20}H_{40}$	280
13.	10.37	7.25	Carbonic acid, eicosyl Vinyl ester	$C_{23}H_{44}O_3$	368

14.	11.56	31.67	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256
15.	11.63	15.36	Eicosanoic acid	$C_{20}H_{40}O_2$	312
16.	13.16	22.67	Cis-13- eicosenoic acid	$C_{20}H_{38}O_2$	310
17.	13.85	55.03	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256
18.	14.06	36.75	hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
19.	15.34	37.19	n- Hexadecanoic acid	$C_{20}H_{38}O_2$	256
20.	16.95	4.67	cis- Vaccenic acid	$C_{18}H_{36}O_2$	282
21.	17.31	62.64	Octadecanoic acid	$C_{18}H_{36}O_2$	284
22.	17.64	7.46	Pentadecenoic acid,2,6,10,14-tetramethyl-methyl ester	$C_{20}H_{40}O_2$	312
23.	18.66	12.31	Cis-13- eicosenoic acid	$C_{20}H_{38}O_2$	310
24.	18.83	35.18	Estra-1,3,5 (10)-trien-17 a-ol	$C_{18}H_{24}O$	256
25.	20.54	61.46	Eicosanoic acid	$C_{20}H_{40}O_2$	312
26.	20.96	6.11	Heptacos-1-ene	$C_{27}H_{54}$	378
27.	21.21	34.37	Aspidospermidin-17-ol,1-acetyl-19.21-epoxy-15,16-dimethoxy	$C_{23}H_{30}N_2O_5$	414
28.	21.41	19.77	9,12,15-octadecatrienoic acid, 2,3-bis (acetyloxy) propyl ester, (Z,Z,Z)-	$C_{25}H_{40}O_6$	436
29.	23.67	48.73	Estra-1,3,5 (10)-trien-17 a-ol	$C_{18}H_{24}O$	256
30.	24.85	73.8	Diosgenin	$C_{29}H_{50}O$	414
31.	26.43	35.79	C-Sitosterol	$C_{29}H_{50}O$	414
32.	26.83	78.17	4H-1-Benzopyran-4-one,2,3-dihydro-5-hydroxy-2-(4-hydroxyl phenol)-7-Methoxy-,S	$C_{16}H_{14}O_5$	286
33.	27.64	29.44	1- Heptatriacotanol	$C_{37}H_{76}O$	536
34.	28.98	28.28	1- Heptatriacotanol	$C_{37}H_{76}O$	536
35.	30.56	11.8	9,10-Secocholesta-5,7,10(19)-triene-3,24, 25-triol (3a,5Z,7E)	$C_{27}H_{44}O_3$	416
36.	31.19	31.98	1- Heptatriacotanol	$C_{37}H_{76}O$	536
37.	32.84	12.31	Urs-12-en-24-oic,3-oxo- methyl ester, (+)-	$C_{31}H_{48}O_3$	468

RT= Retention time, RA= Relative abundance in percentage, MW= Molecular weight

Table 4.20: Compounds identified from *Vitex peduncularis wall* petroleum ether extract by GC-MS.

Sl.No	RT	RA	Compounds	Formula	MW
1.	3	48.68	water	H_2O	18
2.	3.6	36.68	water	H_2O	18
3.	5.5	84.98	Phenol, 4-(1,1- dimethylethyl)-2-(1,1- dimethylpropyl)- tridecane	$C_{15}H_{24}O$	220
4.	6.68	5.47	3- Trifluoroacetoxy	$C_{15}H_{27}F_3O_2$	296
5.	7.12	60.43	1,6:3,4-Dianhydro-2-deoxy-a-d-lyxo-hexopyranose	$C_6H_8O_3$	128
6.	9.91	69.4	Water	H_2O	18
7.	10.26	15.13	E-15- Heptadecenal	$C_{17}H_{32}O$	252
8.	11.84	32.47	Cyclohexananone,4-hydroxy-3,3,5,5-tetramethyl	$C_{10}H_{18}O_2$	170
9.	13.51	53.7	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256
10.	14.02	6.18	4- trifluoroacetoxyhexadecane	$C_{18}H_{33}F_3O_2$	338
11.	14.19	17.4	2- Piperidinone,N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233
12.	15.7	70.3	4-Fluro1-methyl-5- carboxylic acid, ethyl ester	$C_7H_9FN_2O_2$	172
13.	16.61	17.37	Linoelaidic acid	$C_{18}H_{33}O_2$	280
14.	17.09	55.99	Octadecanoic acid,2-92-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	372
15.	17.61	7.46	1- Heneicosyl formate	$C_{22}H_{44}O_2$	340
16.	17.71	22.78	4-Fluro1-methyl-5- carboxylic acid, ethyl ester	$C_7H_9FN_2O_2$	172
17.	20.96	8.05	Hexadecen-1 ol, trans-9-	$C_{16}H_{32}O$	240
18.	21.08	51.06	Hexanedioic acid,bis (2- ethylhexyl) ester	$C_{22}H_{42}O_4$	370
19.	22.66	16.42	Silane, trichloro docosyl-	$C_{22}H_{45}Cl_3Si$	442
20.	23.43	28.45	Diisocotyl phthalate	$C_{24}H_{38}O_4$	390
21.	24.07	7.99	4- trifluoroacetoxyhexadecane	$C_{18}H_{33}F_3O_2$	338
22.	25.68	14.82	3-(prop-2-enoyloxy) tetradecane	$C_{17}H_{32}O_2$	268
23.	26.33	70.88	water	H_2O	18
24.	28.41	40.48	water	H_2O	18
25.	29.22	57	water	H_2O	18
26.	30.72	56.43	water	H_2O	18
27.	32.58	65.47	water	H_2O	18

RT= Retention time, RA= Relative abundance in percentage, MW= Molecular weight

Table 4.21: Compounds identified from *Vitex peduncularis wall* chloroform extract by GC-MS.

Sl.No	RT	RA	Compounds	Formula	MW
1.	3.04	54	water	H_2O	18
2.	3.26	58	water	H_2O	18
3.	4.7	49	water	H_2O	18
4.	5.45	39	phenol,4-(1,1-dimethylethyl)-2-(1,1-dimethylpropyl)	$C_{15}H_{24}O$	220
5.	5.58	70	phenol,4-(1,1-dimethylethyl)-2-(1,1-dimethylpropyl)	$C_{15}H_{24}O$	220
6.	5.91	49	water	H_2O	18
7.	7.99	84	water	H_2O	18
8.	8.44	23	2-Propenoic acid, tridecyl ester	$C_{16}H_{30}O_2$	254
9.	8.55	10	2-Propenoic acid, tridecyl ester	$C_{16}H_{30}O_2$	254
10.	10.3	8	4- Trifluoroacetoxyhexadecane	$C_{18}H_{33}F_3O_2$	338
11.	10.4	22	Silane, Trichlorodocosyl	$C_{22}H_{45}Cl_3Si$	442
12.	12.8	93	water	H_2O	18
13.	13.4	74	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256
14.	14	6.7	hexadecen-1-ol, trans-9-	$C_{16}H_{32}O$	240
15.	15.6	75	water	H_2O	18
16.	16.7	37	water	H_2O	18
17.	17.1	66	Octadecanoic acid,2(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	372
18.	17.6	6.3	hexadecen-1-ol, trans-9-	$C_{16}H_{32}O$	240
19.	17.7	15	decane,2,4,6- trimethyl-	$C_{13}H_{28}$	184
20.	20.6	96	water	H_2O	18
21.	21	8.3	4- Trifluoroacetoxyhexadecane	$C_{18}H_{33}F_3O_2$	338
22.	21.1	47	water	H_2O	18
23.	23.2	96	water	H_2O	18
24.	24.1	20	silane, Trichlorodocosyl-	$C_{22}H_{45}Cl_3Si$	442
25.	25.3	94	water	H_2O	18
26.	26.4	44	C-Sitosterol	$C_{29}H_{50}O$	414
27.	26.6	17	a- acorenol	$C_{15}H_{26}O$	222
28.	26.8	56	water	H_2O	18
29.	28.2	14	Cedron-diol(8s,14)-	$C_{15}H_{26}O_2$	238
30.	28.9	19	Cedron-diol(8s,14)-	$C_{15}H_{26}O_2$	238
31.	29.2	26	water	H_2O	18
32.	29.9	65	water	H_2O	18
33.	32.5	73	water	H_2O	18

RT= Retention time, RA= Relative abundance in percentage, MW= Molecular weight

Table 4.22: Antimalarial activity and cytotoxicity of different extracts of *A. scholaris*

Sl.No	Sample Name	IC_{50} (3D7)	IC_{50} (KI)	CC_{50} (Vero cells)
1.	Petroleum ether	22.0 µg/ml	26.7 µg/ml	>100 µg/ml
2.	Chloroform	14.0 µg/ml	10.0 µg/ml	>100 µg/ml
3.	Methanol	43.4 µg/ml	45.6 µg/ml	>100 µg/ml
4.	Aqueous	>50 µg/ml	>50 µg/ml	>100 µg/ml

3D7 is chloroquine-sensitive strain. IC_{50} value of CQ is 6.4 nM. K1 is CQ-resistant strain. IC_{50} value of CQ is 500 nM.

Table 4.23: *In vitro* survival test of the cestode *R. echonobothrida* upon treatment with albendazole and different extracts of *A. scholaris.*

Incubation medium	Dose (mg/ml)	Time of death (hr)
Control	0	55.673 ± 4.253
Albendazole	5	9.400 ± 2.133*
	10	3.737 ± 0.970*
	20	1.190 ± 0.213*
A. scholaris petroleum ether	5	36.902 ± 4.223*
	10	33.572 ± 0.562*
	20	14.039 ± 0.649*
A. scholaris chloroform	5	18.025 ± 0.930*
	10	10.979 ± 0.402*
	20	4.016 ± 0.742*
A. scholaris methanol	5	23.682 ± 0.661*
	10	10.019 ± 0.509*
	20	3.420 ± 0.428*
A. scholaris aqueous	5	39.525 ± 2.080*
	10	31.691 ± 4.297*
	20	16.409 ± 4.980*

Fig 4.1: Photograph of *A. scholaris* leaves and bark.

Fig 4.2: Photograph of *Betula alnoides* and a magnified view of its leaves *Betula alnoides.*

Fig 4.3: *Cinnamonum bejolghota* and magnified view of its leaves.

Fig 4.4: *Costus speciosus* and magnified view of its leaves.

Fig 4.5: *Dendrocnide sinuate* and magnified view of its leaves.

Fig 4.6: *Hydotes scandens* and magnified view of its leaves.

Fig 4.7: *Mimosa pudica* and magnified view of its leaves.

Fig 4.8: *Prunus cerosoides* and magnified view of its leaves.

Fig 4.9: *Stereospermum colais* and magnified view of its leaves.

Fig 4.10: *Vitex penducularis wall* and magnified view of its leaves.

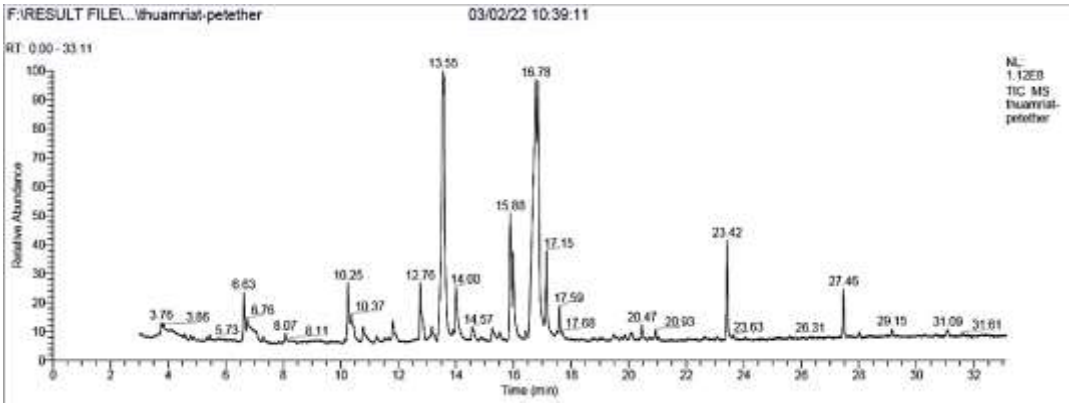

Fig 4.1: Chromatogram of petroleum ether extract of *A. scholaris* detected by gas chromatography-mass chromatography (GCMS).

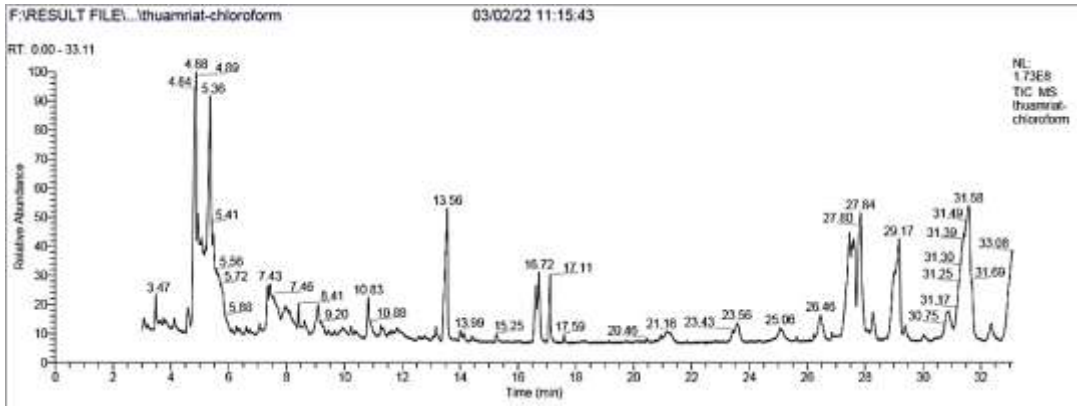

Fig 4.12: Chromatogram of chloroform extract of *A. scholaris* detected by gas chromatography-mass chromatography (GCMS).

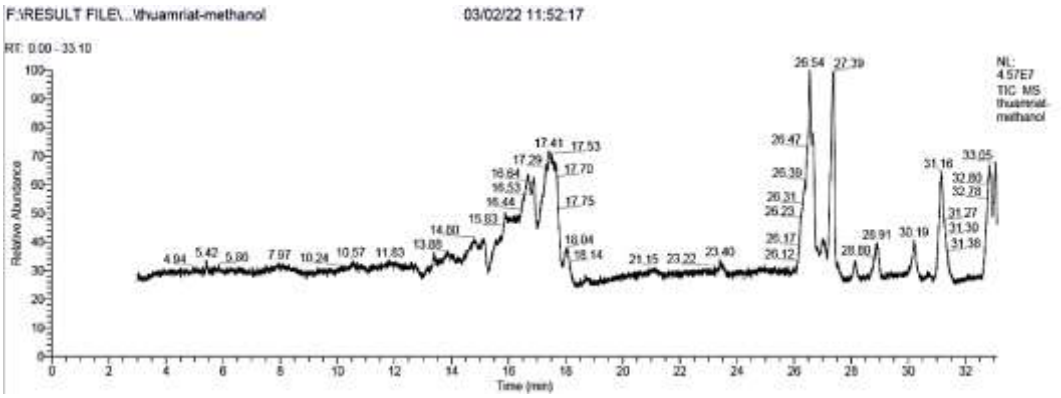

Fig 4.13: Chromatogram of methanol extract of *A. scholaris* detected by gas chromatography-mass chromatography (GCMS).

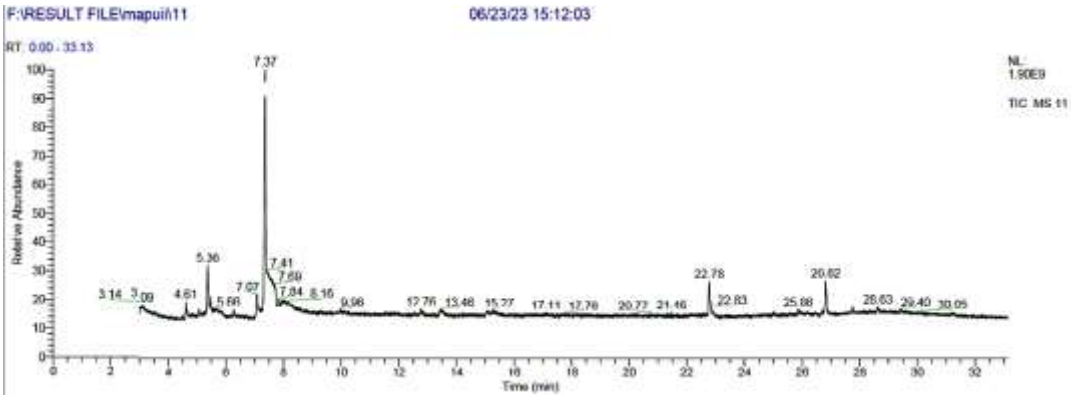

Fig 4.14: Chromatogram of aqueous extract of *A. scholaris* detected by gas chromatography-mass chromatography (GCMS).

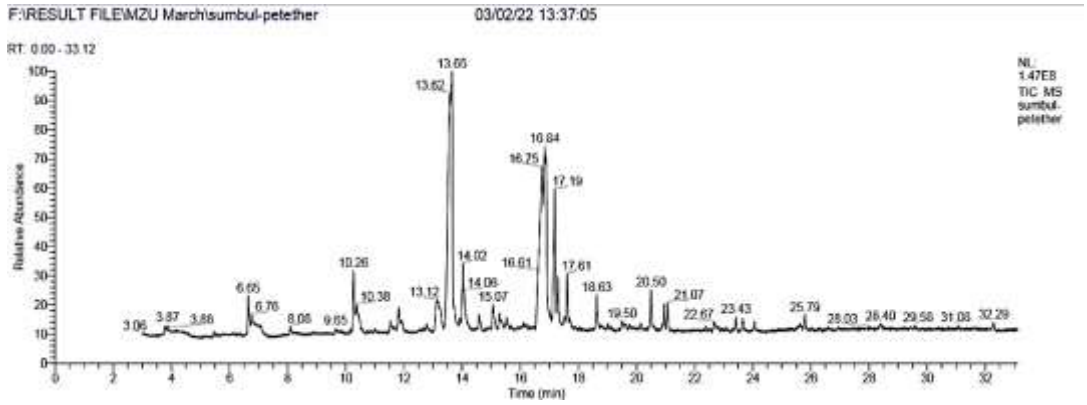

Fig 4.15: Chromatogram of petroleum ether extract of *Costus speciosus* detected by gas chromatography-mass chromatography (GCMS).

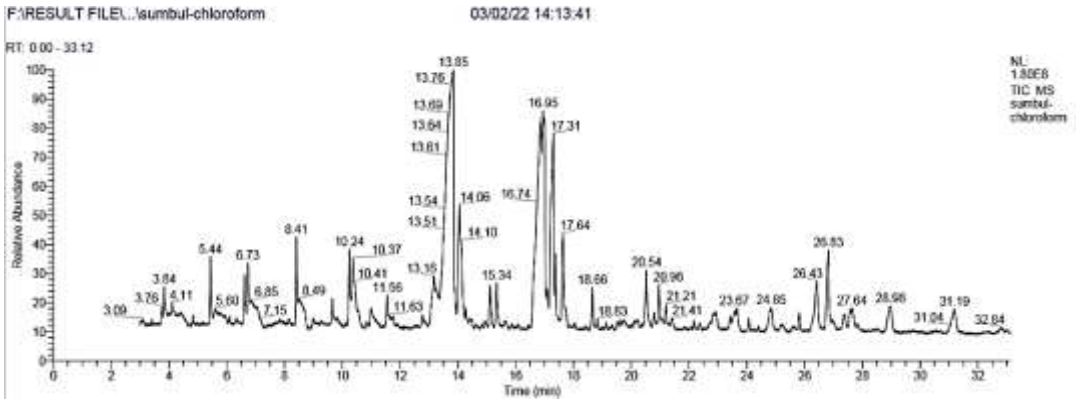

Fig 4.16: Chromatogram of chloroform extract of *Costus speciosus* detected by gas chromatography-mass chromatography (GCMS).

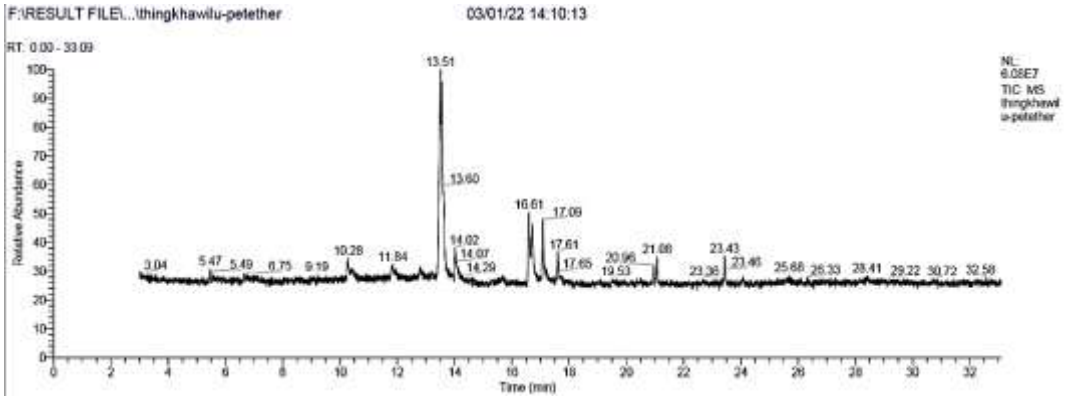

Fig 4.17: Chromatogram of petroleum ether extract of *Vitex peduncularis* detected by gas chromatography-mass chromatography (GCMS).

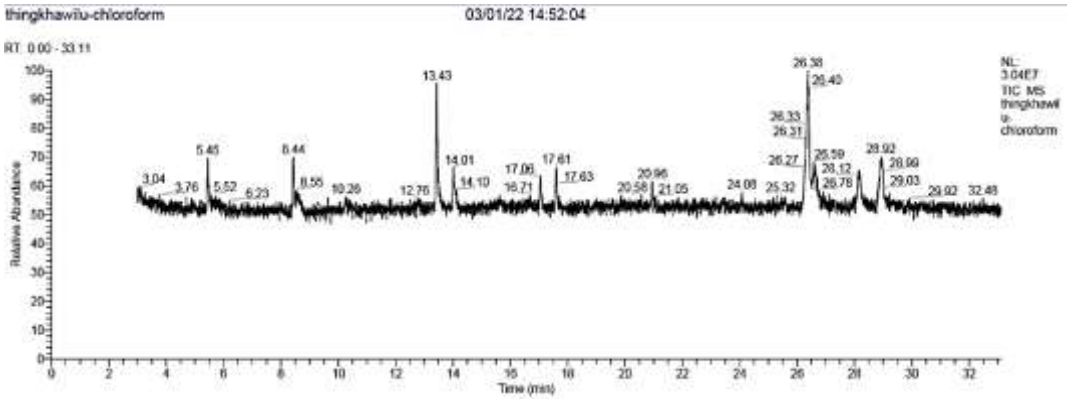

Fig 4.19: Chromatogram of chloroform extract of *Vitex peduncularis wall* detected by gas chromatography-mass chromatography (GCMS).

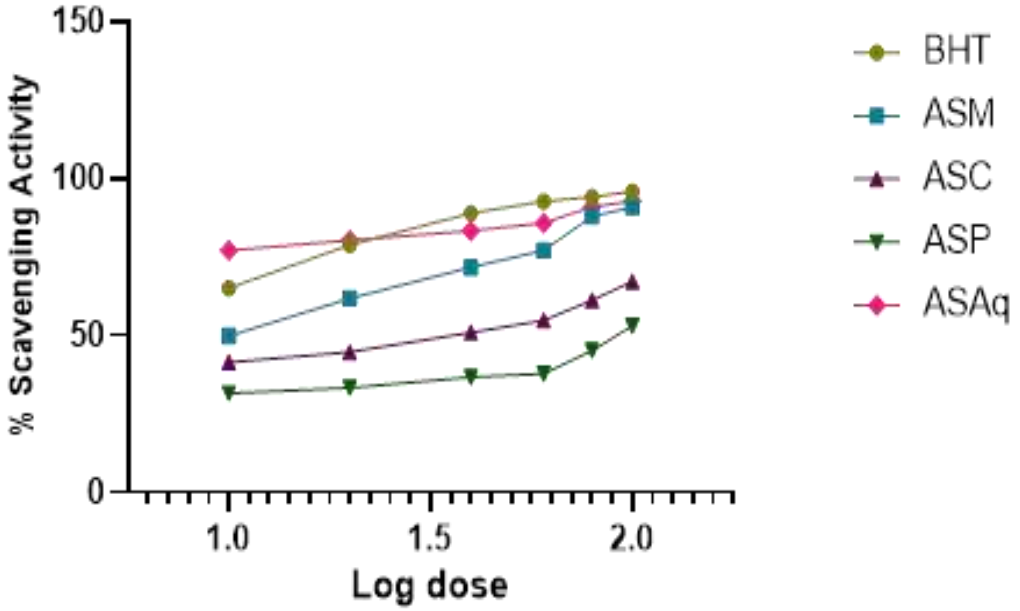

Fig 4.19: Free radical scavenging activities of BHT and different extracts of *A. scholaris* extracts on 2,2-diphenyl-1-picrylhydrazyl (DPPH). Values are expressed as means ± SEM (n=3).

(BHT= Butylated hydroxytoloune, ASM= *A. scholaris* methanol extract, ASC= *A. scholaris* choloform extract, ASP= *A. scholaris* extract, ASAq= *A. scholaris* aqueous extract)

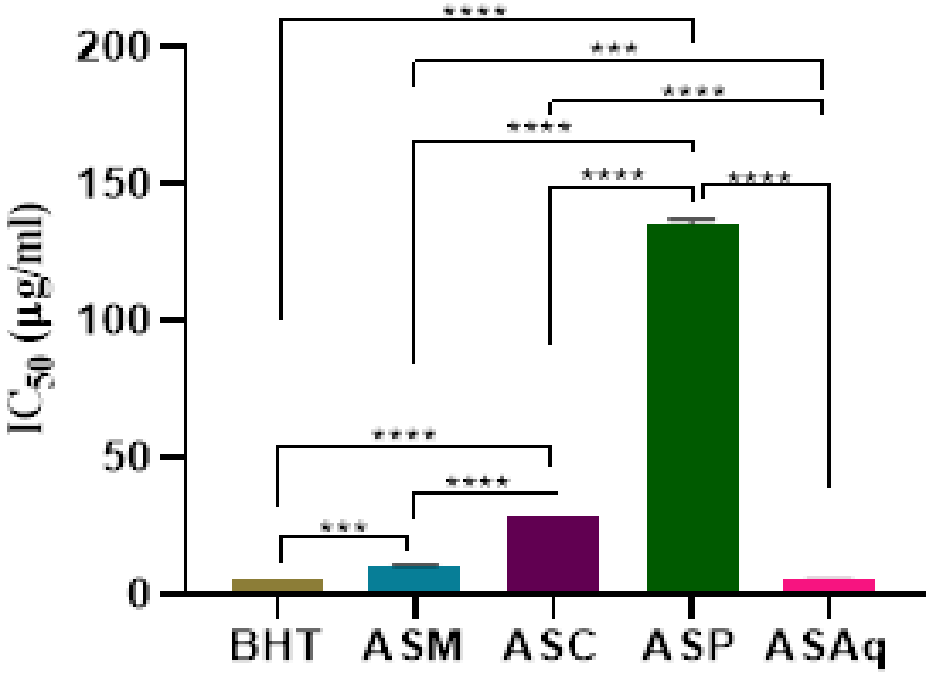

Fig 4.20: IC_{50} of butylated hydroxytoloune and different extracts of *A. scholaris* in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH).

(BHT= Butylated hydroxytoloune, ASM= *A. scholaris* methanol extract, ASC= *A. scholaris* choloform extract, ASP= *A. scholaris* extract, ASAq= *A. scholaris* aqueous extract, **** $P \leq 0.0001$, *** $P \leq 0.002$)

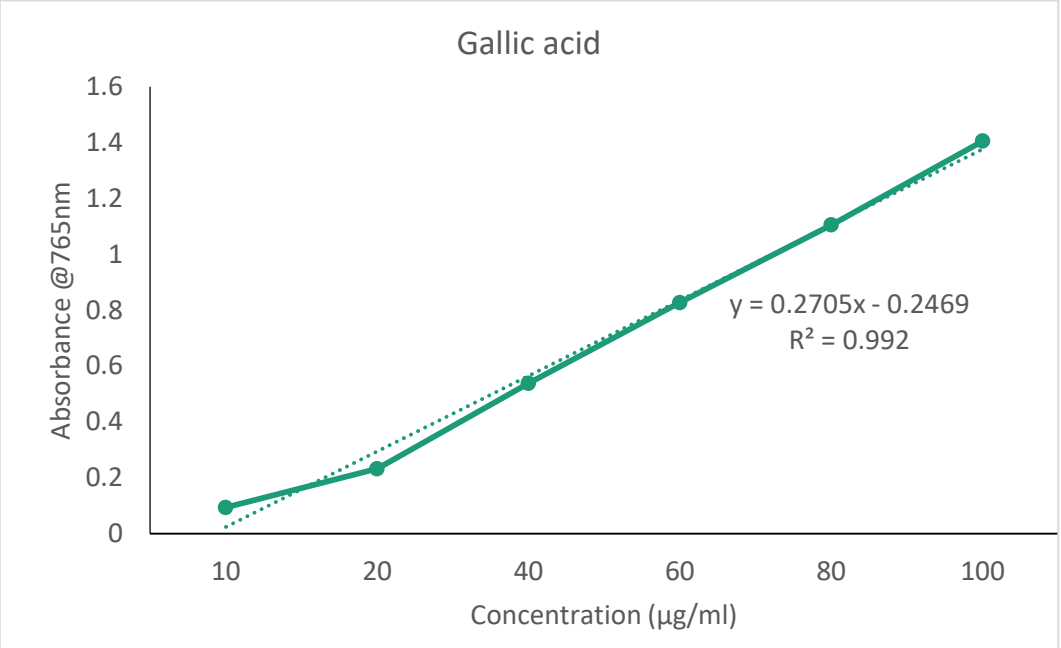

Fig 4.21: Standard graph of gallic acid for total phenol assay. The dotted line represents the linear graph.

GAE = Gallic acid equivalent

ASP = 10.865±0.129 GAE mg/g

ASC = 11.197±0.068 GAE mg/g

ASM = 13.268±0.088 GAE mg/g

ASAq = 12.306 ± 0.121GAE mg/g

(ASP = *A. scholaris* petroleum ether extract, ASC = *A. scholaris* petroleum ether extract, ASM = *A. scholaris* petroleum ether extract, ASAq = *A. scholaris* petroleum ether extract)

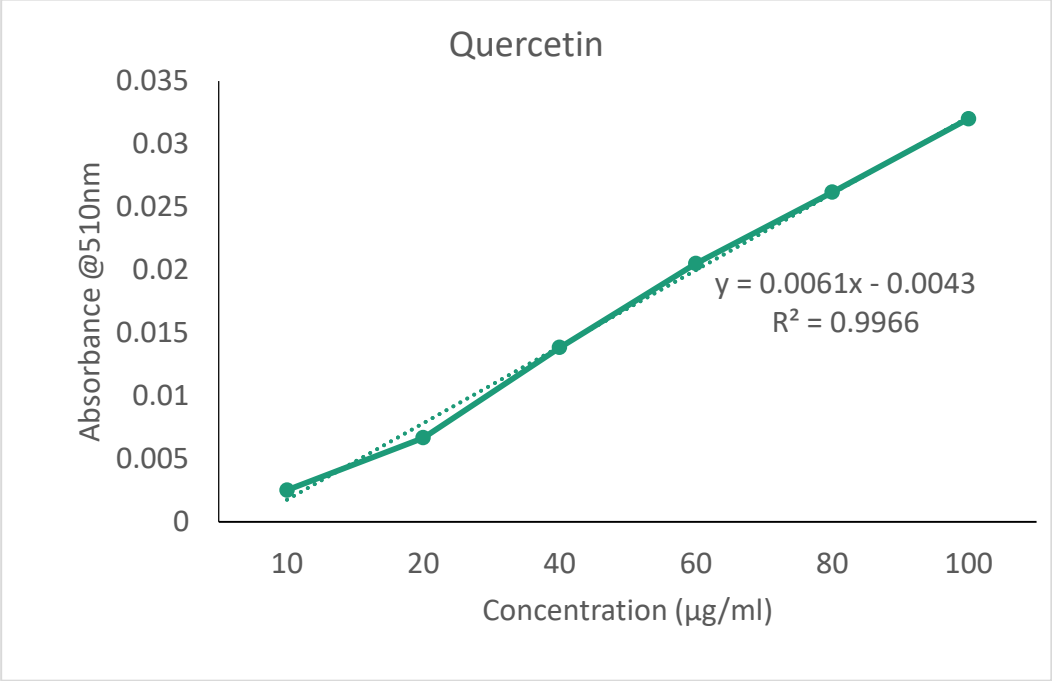

Fig 4.22: Standard graph of quercetin for total flavonoid assay. The dotted line represents the linear graph.

QE = Quercetin equivalent
ASP = 117.54 ± 5.965 QE mg/g
ASC = 57.868 ± 3.583 QE mg/g
ASM = 31.639 ± 2.504 QE mg/g
ASAq = 43.114 ± 2.503 QE mg/g

(ASP = *A. scholaris* petroleum ether extract, ASC = *A. scholaris* petroleum ether extract, ASM = *A. scholaris* petroleum ether extract, ASAq = *A. scholaris* petroleum ether extract)

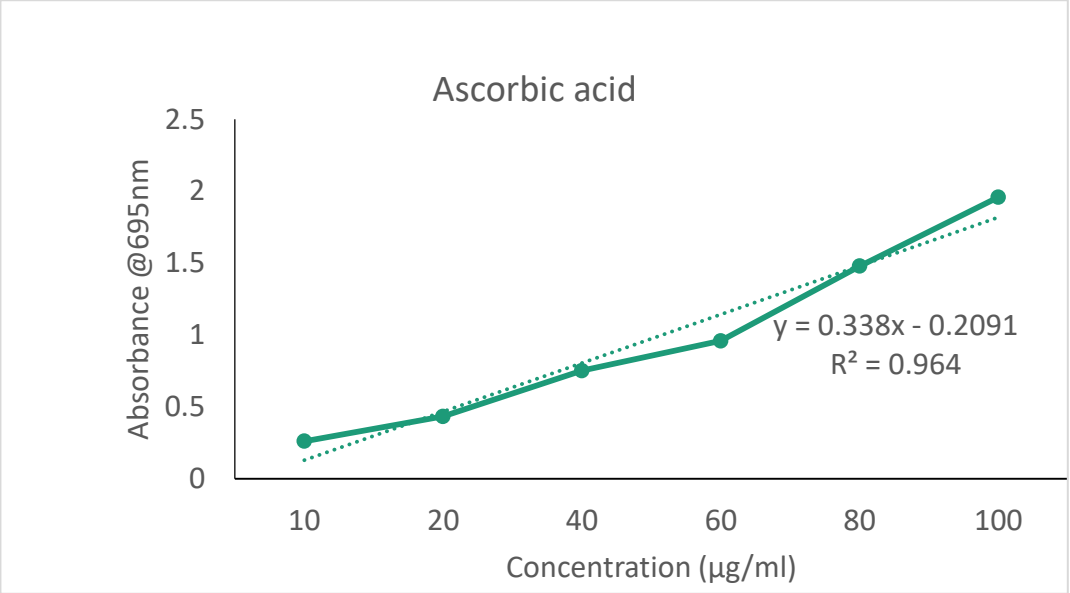

Fig 4.23: Standard graph of ascorbic acid for total antioxidant assay. The dotted line represents the linear graph

AAE = Gallic acid equivalent

ASP = 6.511±0.052 AAE mg/g

ASC = 7.221±0.009AAE mg/g

ASM = 10.476±0.843 AAE mg/g

ASAq= 7.281 ± 0.035 AAE mg/g

(ASP = *A. scholaris* petroleum ether extract, ASC = *A. scholaris* petroleum ether extract, ASM = *A. scholaris* petroleum ether extract, ASAq = *A. scholaris* petroleum ether extract)

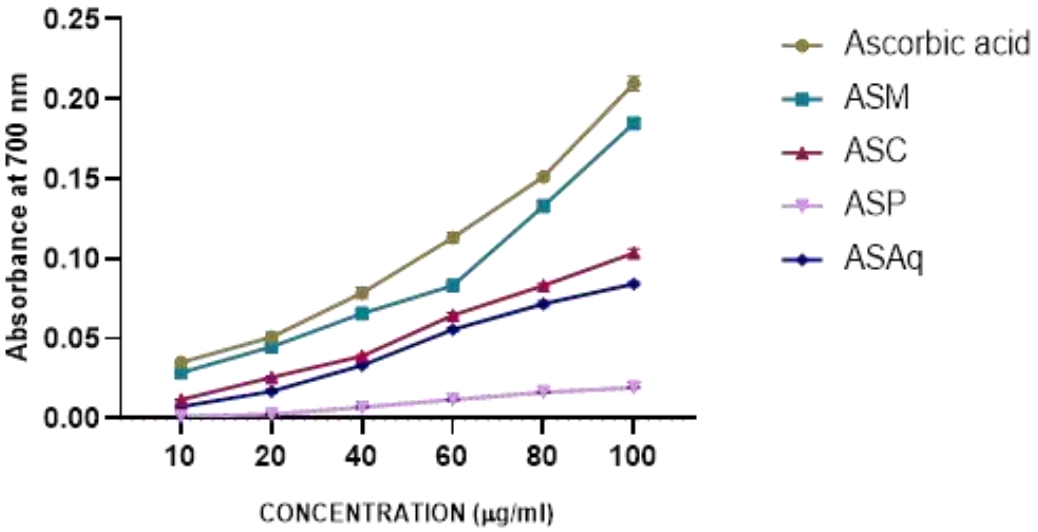

Fig 4.24: Potassium ferri-cyanide-reducing activity of ascorbic acid and different extracts of *A. scholaris*

(ASP = *A. scholaris* petroleum ether extract, ASC = *A. scholaris* petroleum ether extract, ASM = *A. scholaris* petroleum ether extract, ASAq = *A. scholaris* petroleum ether extract)

Table: Antimicrobial activity of the methanol extract of *A. scholaris* on different test microorganisms

Sl. No	Test microorganisms	Zone of Inhibition (in mm)			
		200 mg/ml	100 mg/ml	50 mg/ml	Standard (Ciprofloxacin)
1.	*Staphylococcus aureus 2* (ATCC 700698)	2.763	1.407	-	-
2.	*Enterococcus faecalis* (ATCC 29212)	1.837	2.550	3.707	15.273
3.	*Escherichia coli* (ATCC 10536)	-	-	0.893	33.163
4.	*Bacillus cereus* (ATCC 13061)	2.103	2.597	1.020	31.900
5.	*Salmonella typhii* (ATCC 51812)	-	7.197	4.403	25.427

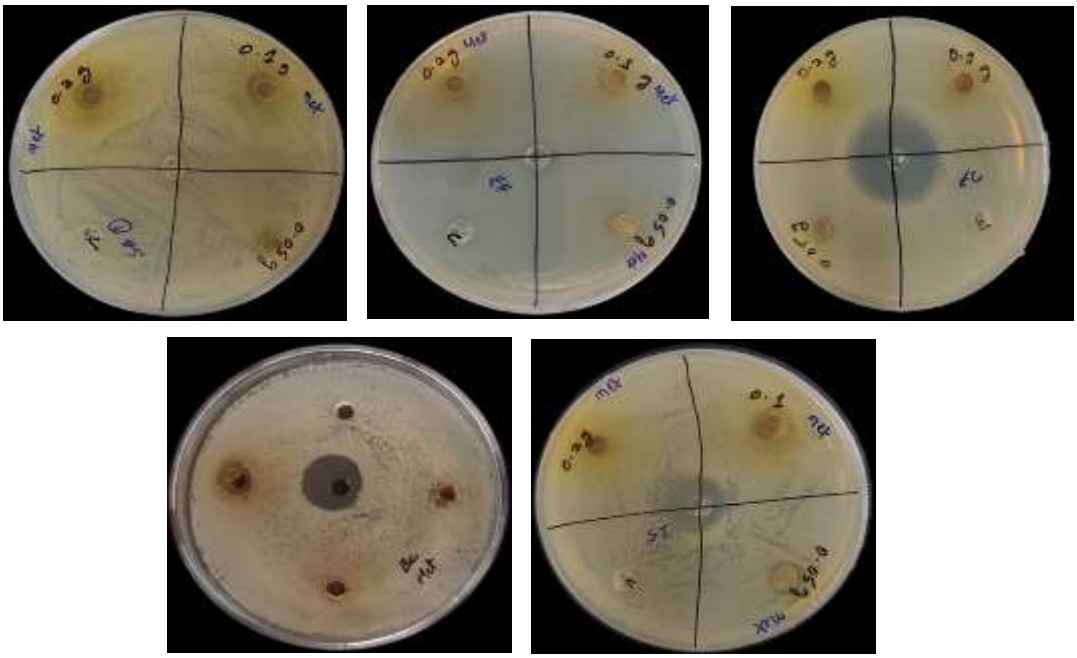

Fig:4.25: Antibacterial activity of *A. scholaris* methanol extract (ASM) at different concentrations and ciprofloxacin (Standard) against *Staphylococcus aureus* (SA2), *Enterococcus faecalis* (EF), *Escherichia coli* (EC), *Bacillus cereus* (BC), and *Salmonella typhi* (ST).

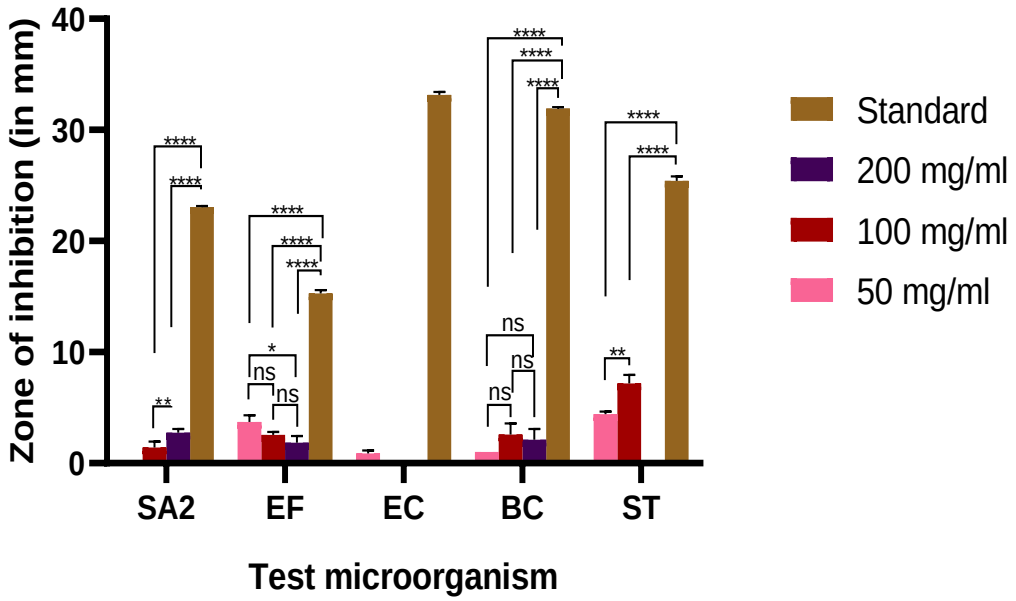

Fig 4.26: Comparison of antibacterial activity of *A. scholaris* methanol extract (ASM) at different concentrations and ciprofloxacin (standard) against *Staphylococcus aureus* (SA2), *Enterococcus faecalis* (EF), *Escherichia coli* (EC), *Bacillus cereus* (BC), and *Salmonella typhi* (ST). Values are in means ± Standard error of means (n=3); **** P≤ 0.0001, *** P≤ 0.0002,** P≤ 0.0021 *P≤ 0.0332 ns ≥ 0.05.

Table: Antimicrobial activity of the chloroform extract of *A. scholaris* on different test microorganisms

Sl. No	Test microorganisms	Zone of Inhibition (in mm)			
		200 mg/ml	100 mg/ml	50 mg/ml	Standard (Ciprofloxacin)
1.	*Staphylococcus aureus* 1 (ATCC 700698)	8.646	7.486	5.343	22.336
2.	*Staphylococcus aureus* 2 (ATCC 700698)	2.253	1.473	-	-
3.	*Escherichia coli* (ATCC 10536)	2.720	3.076	4.046	32.606
4.	*Bacillus cereus* (ATCC 13061)	3.766	3.803	4.460	31.793
5.	*Salmonella typhii* (ATCC 51812)	5.036	4.866	4.473	26.003

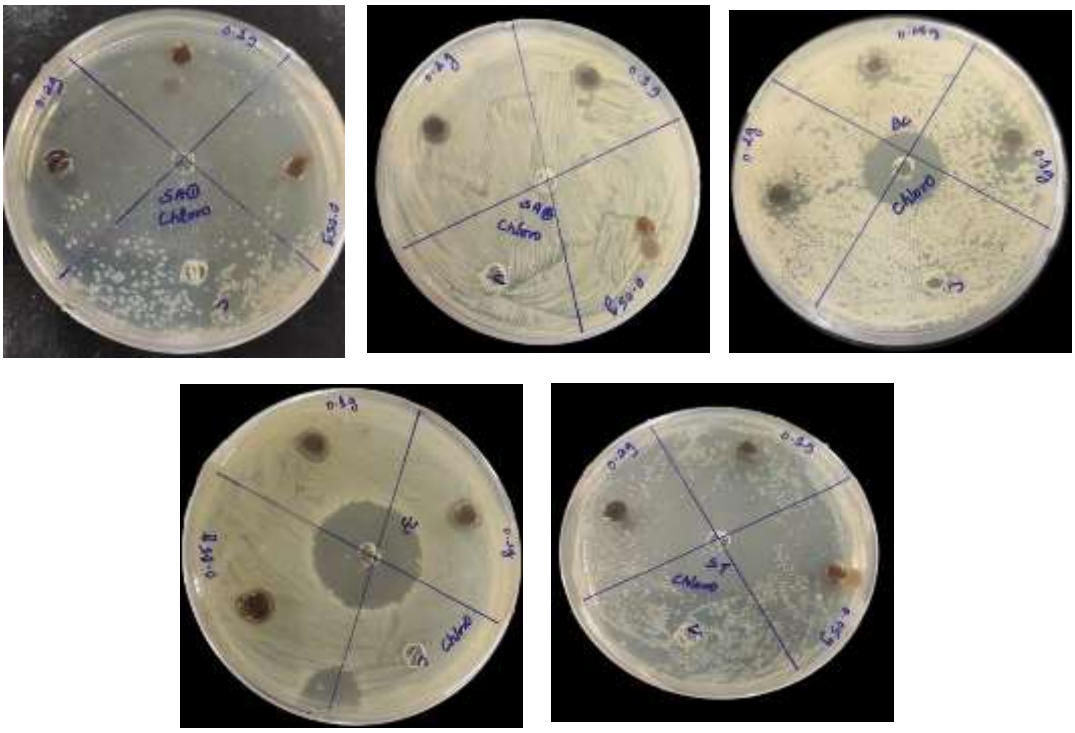

Fig 4.27: Antibacterial activity of *A. scholaris* chloroform extract (ASC) at different concentrations and ciprofloxacin (standard) against *Staphylococcus aureus* (SA1), *Staphylococcus aureus* (SA2), *Escherichia coli* (EC), *Bacillus cereus* (BC), and *Salmonella typhi* (ST).

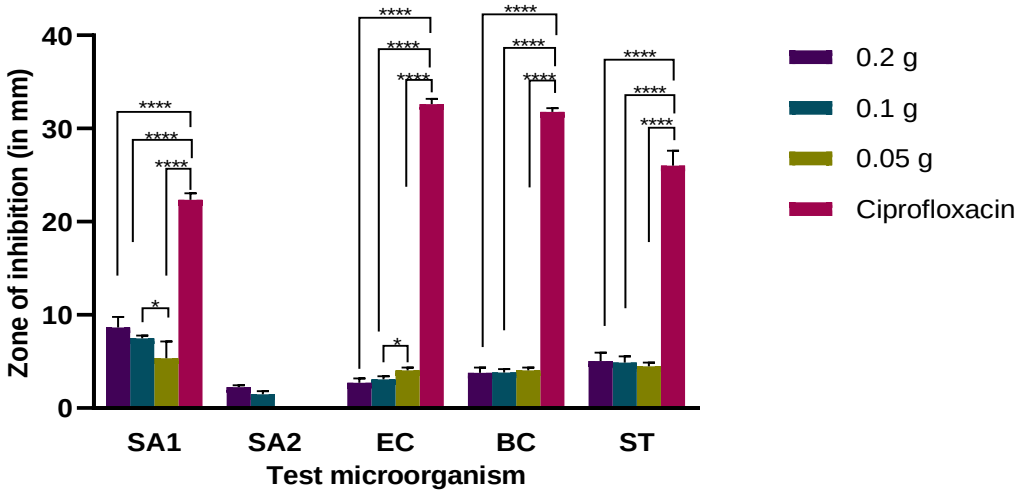

Fig 4.28: Comparison of antibacterial activity of *A. scholaris* chloroform extract (ASC) at different concentrations and ciprofloxacin (standard) against *Staphylococcus aureus* (SA1), *Staphylococcus aureus* (SA2), *Escherichia coli* (EC), *Bacillus cereus* (BC), and *Salmonella typhi* (ST). Values are in means ± Standard error of means (n=3); **** P≤ 0.0001, *** P≤ 0.002,** P ≤ 0.0021 *P≤ 0.0332.

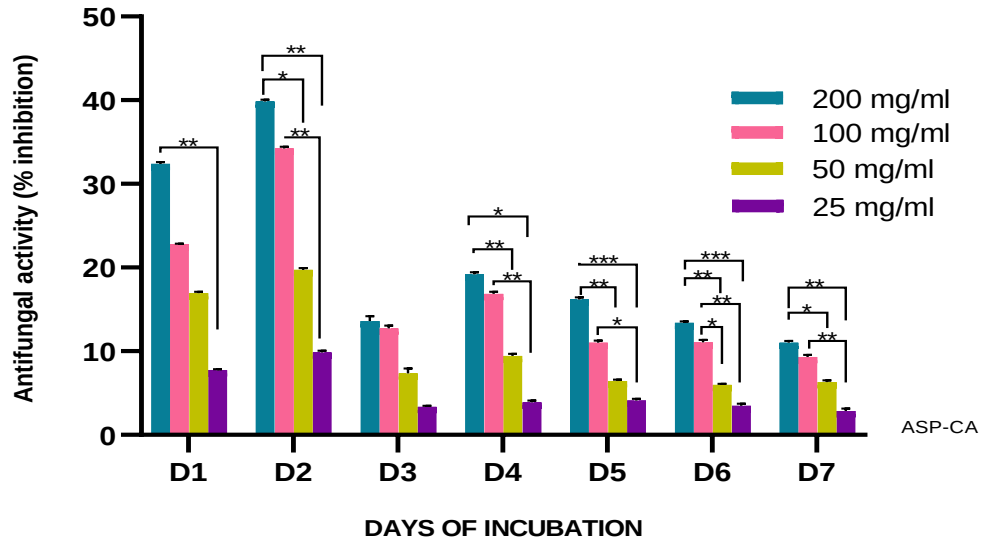

Fig 4.29: Comparison of the fungal activity of *A. scholaris* petroleum ether extract (ASP) at different concentrations against *Candida albicans* (CA).

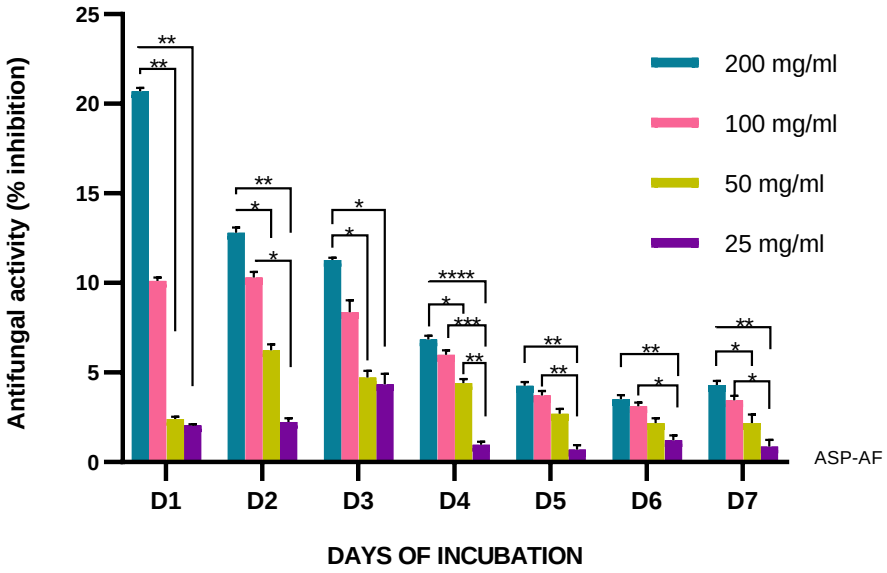

Fig 4.30: Comparison of the fungal activity of *A. scholaris* petroleum ether extract (ASP) at different concentrations against *Aspergillus fumigatus* (AF).

(Values are in means ± Standard error of means (n=3), ****P< 0.0001, ***P≤ 0.002, **P≤ 0.0021 *P≤ 0.0332).

(ASP= *A. scholaris* of petroleum ether extract, D1= Day1, D2= Day2, D3= Day3, D4= Day4, D5= Day5, D6= Day6, D7= Day7)

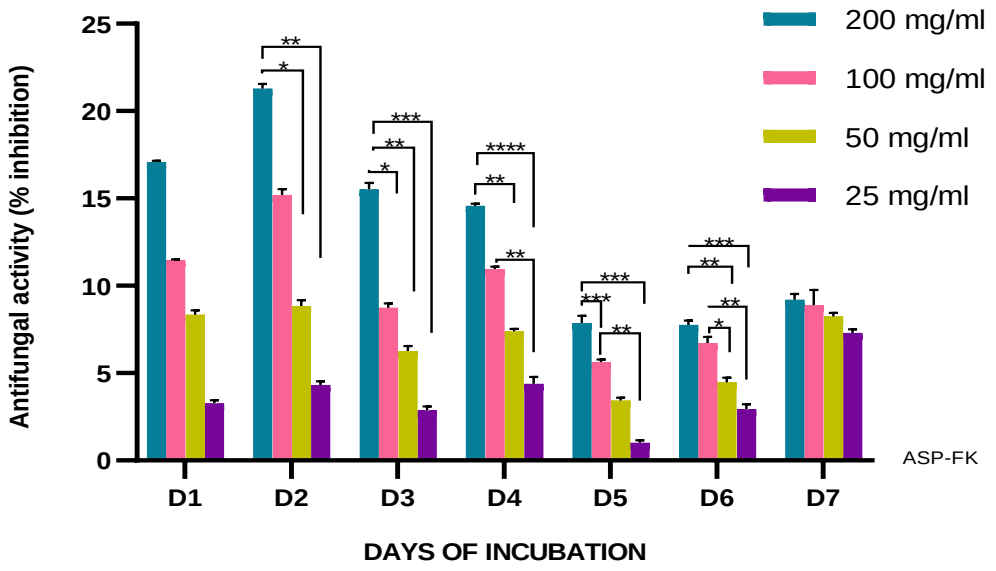

Fig 4.31: Comparison of the fungal activity of *A. scholaris* petroleum ether extract (ASP) at different concentrations against *Fusarium keratoplasticum* (FK). Values are in means ± Standard error of means (n=3), ****P< 0.0001, ***P≤ 0.002, **P≤ 0.0021 *P≤ 0.0332.

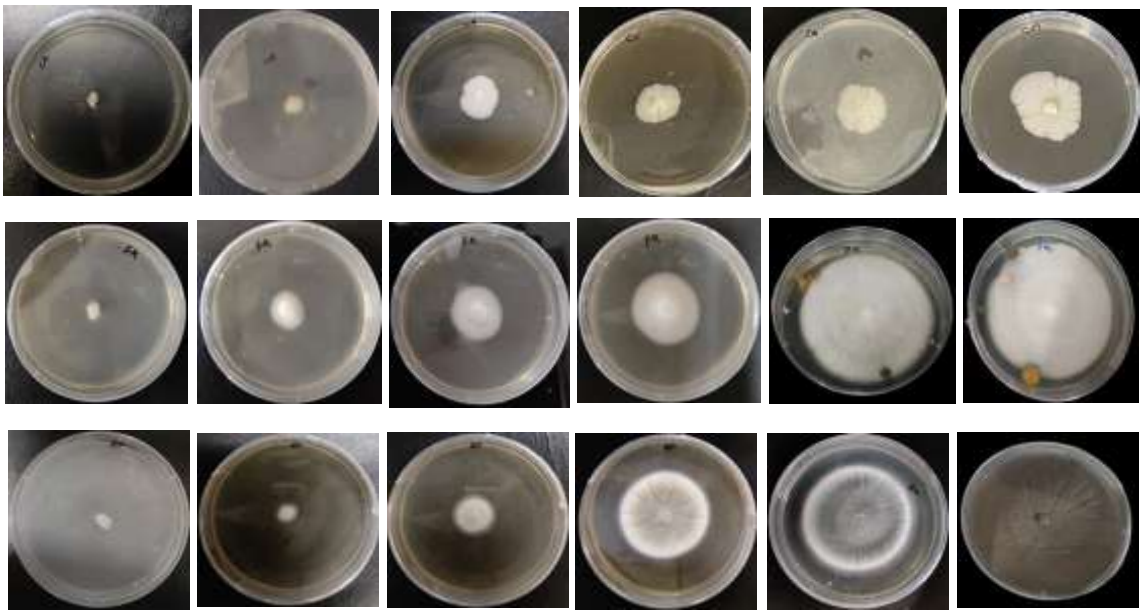

Fig 4.32: Antifungal activity of *A. scholaris* petroleum ether extract (ASP) at different concentrations against *Candida albicans* (CA), *Fusarium keratoplasticum* (FK), and *Aspergillus fumigatus* (AF).

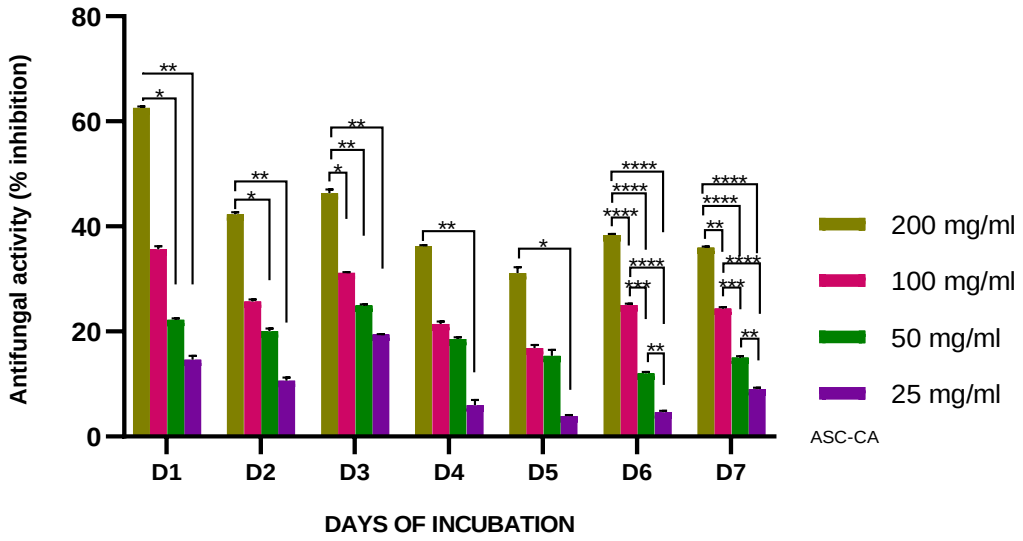

Fig 4.33: Comparison of the fungal activity of *A. scholaris* chloroform extract (ASC) at different concentrations against *Candida albicans* (CA).

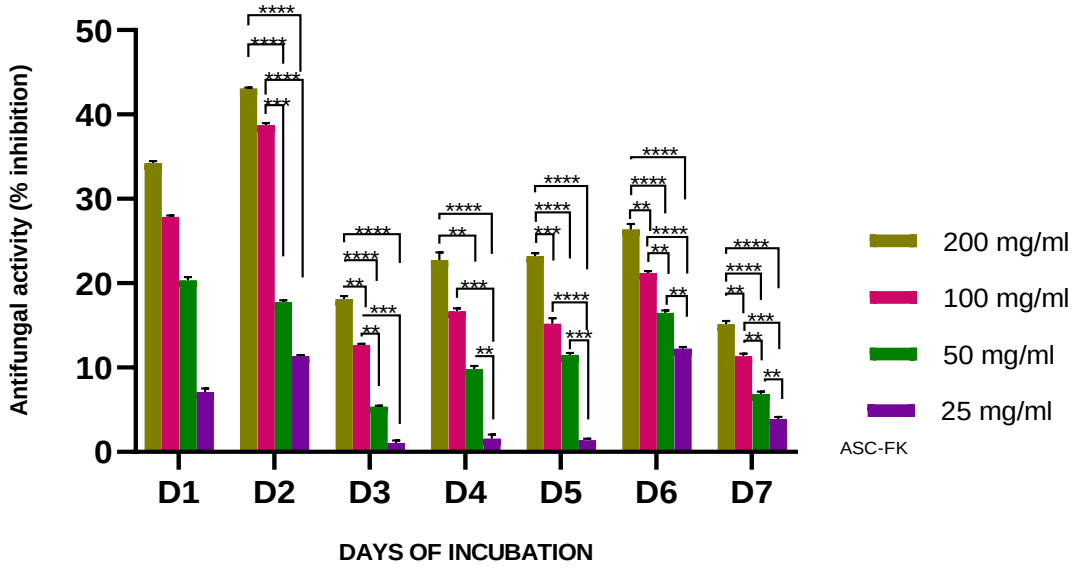

Fig 4.34: Comparison of the fungal activity of *A. scholaris* chloroform extract (ASC) at different concentrations against *Fusarium Keratoplasticum* (FK).

(Values are in means ± Standard error of means (n=3), ****P< 0.0001, ***P≤ 0.002, **P≤0.0021 *P≤ 0.0332).

(ASC= *A. scholaris* of chloroform extract, D1= Day1, D2= Day2, D3= Day3, D4= Day4, D5= Day5, D6= Day6, D7= Day7).

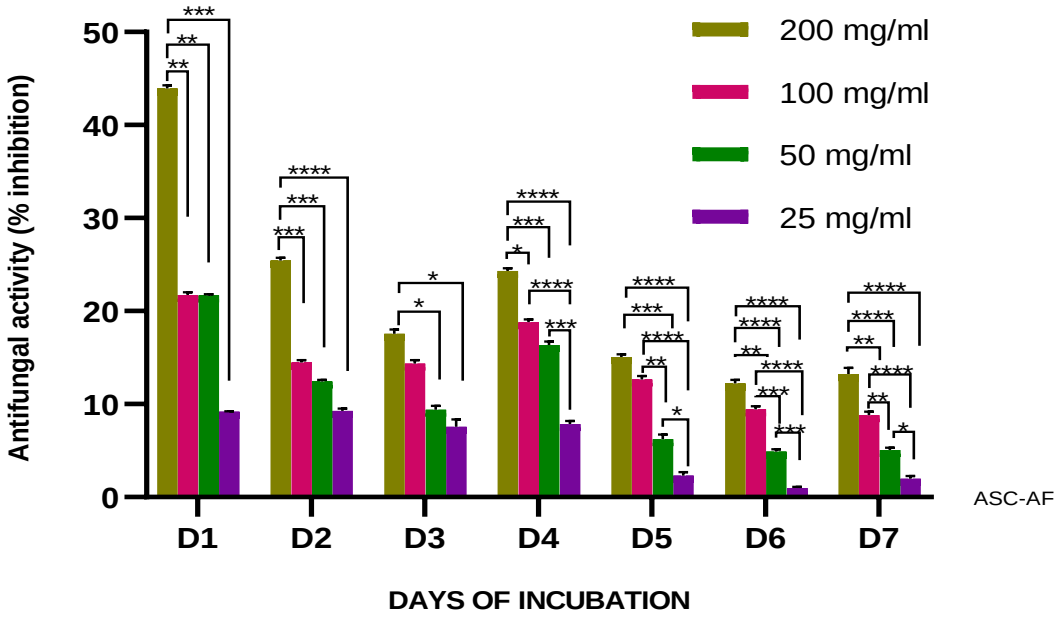

Fig 4.35: Comparison of the fungal activity of *A. scholaris* chloroform extract (ASC) at different concentrations against *Aspergillus fumigatus (*AF). Values are in means ± Standard error of means (n=3), ****P< 0.0001, ***P≤ 0.002, **P≤0.0021 *P≤ 0.0332.

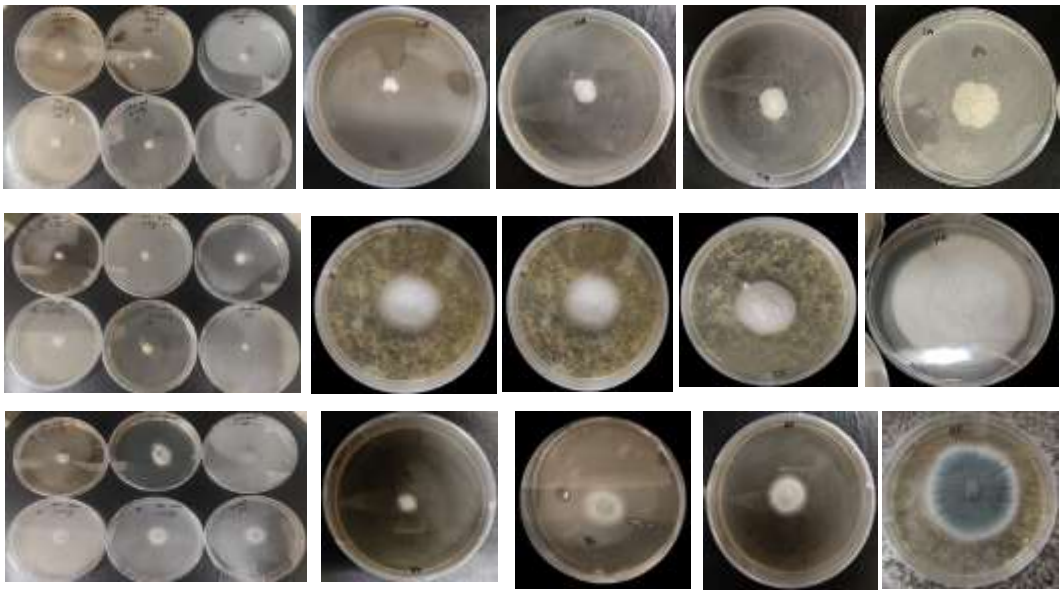

Fig 4.36: Antifungal activity of *A. scholaris* bark of Chloroform extract (ASC) at different concentrations against *Candida albicans* (CA), *Fusarium Keratoplasticum* (FK), *Aspergillus fumigatus* (AF).

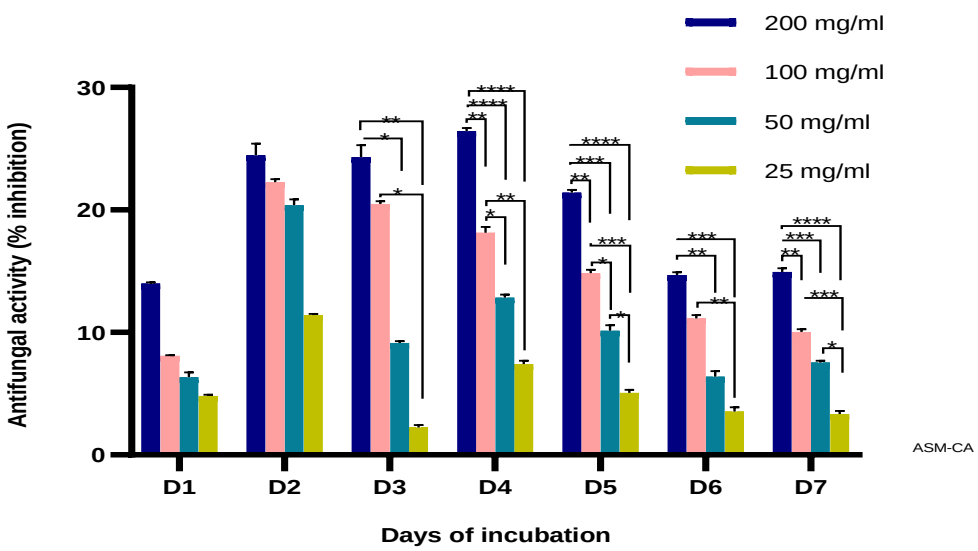

Fig 4.37: Comparison of the fungal activity of *A. scholaris* methanol extract (ASC) at different concentrations against *Candida albicans* (CA).

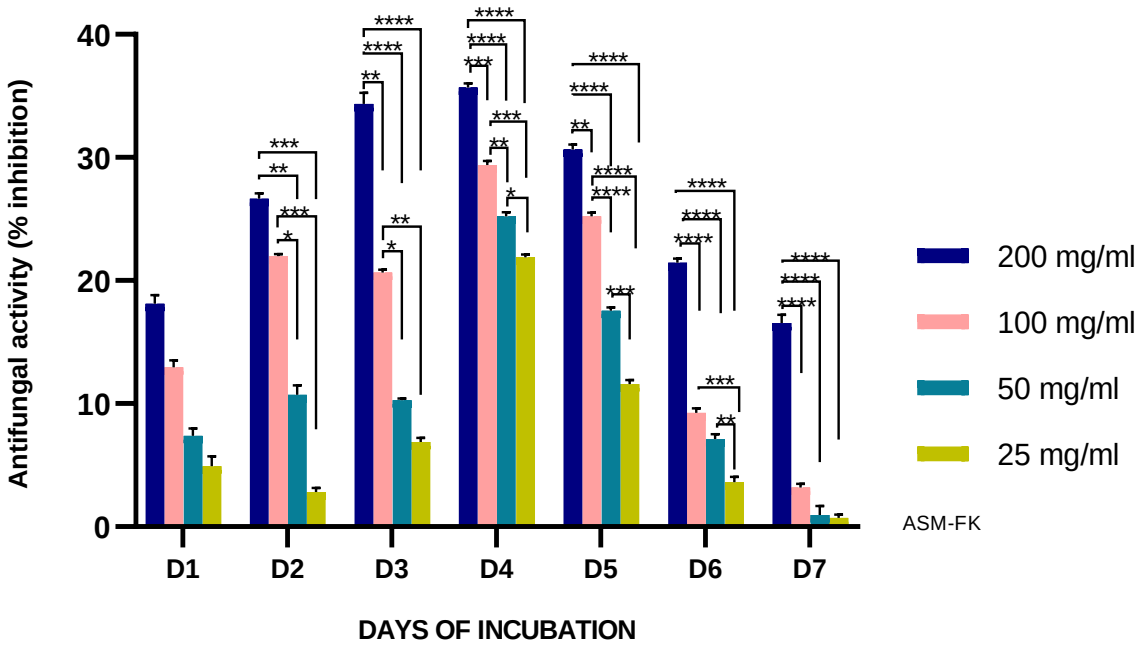

Fig 4.38: Comparison of the fungal activity of *A. scholaris* methanol extract (ASC) at different concentrations against *Fusarium Keratoplasticum* (FK).
(Values are in means ± Standard error of means (n=3), ****P< 0.0001, ***P≤ 0.002, **P≤ 0.0021 *P≤ 0.0332).
(ASM= *A. scholaris* of methanol extract, D1= Day1, D2= Day2, D3= Day3, D4= Day4, D5= Day5, D6= Day6, D7= Day7)

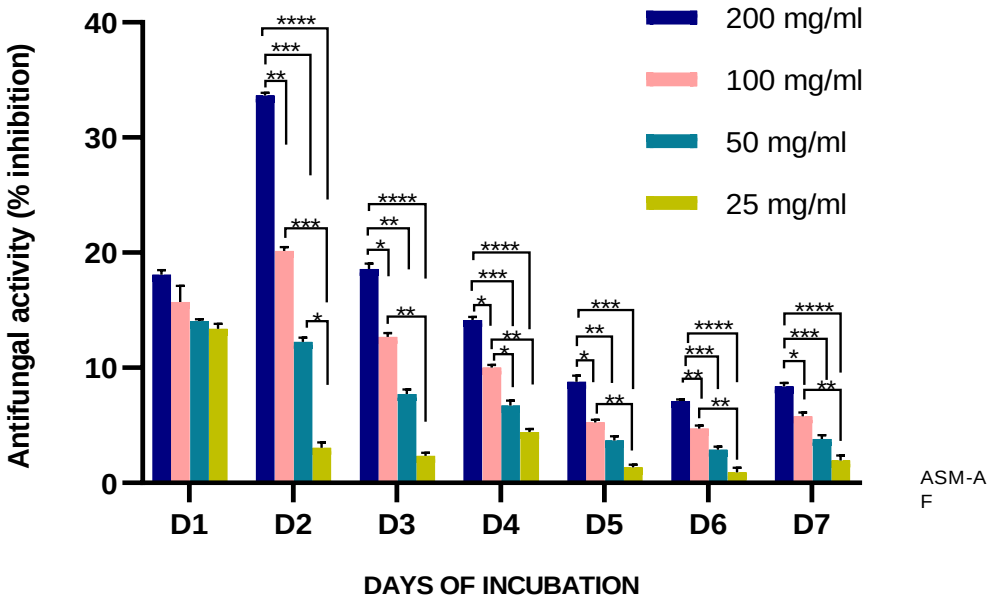

Fig 4.39: Comparison of the fungal activity of *A. scholaris* methanol extract (ASC) at different concentrations against *Aspergillus fumigatus* (AF). Values are in means ± Standard error of means (n=3), ****P< 0.0001, ***P≤ 0.002, **P≤ 0.0021 *P≤ 0.0332.

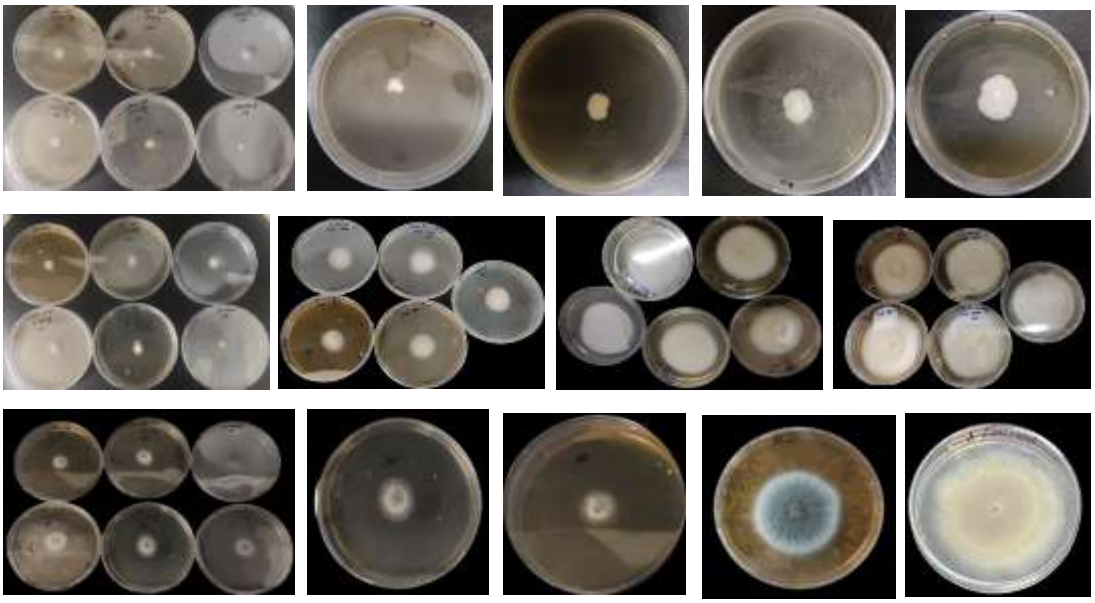

Fig 4.40: Antifungal activity of *A. scholaris* methanol extract (ASM) at different concentrations against *Candida albicans* (CA), *Fusarium keratoplasticum* (FK), and *Aspergillus fumigatus* (AF).

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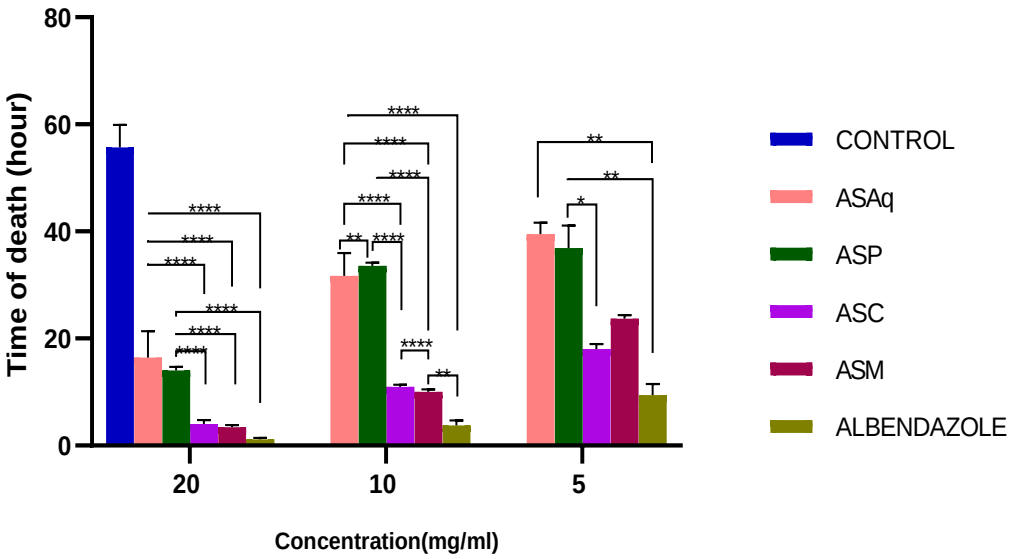


Fig 4.41: Effect of different concentrations of incubation medium on the survival of the cestode, *R. echinobothrida*. Values are in means ± Standard error of means (n=3); **** P≤ 0.0001, **P≤ 0.0021 *P≤ 0.0332.

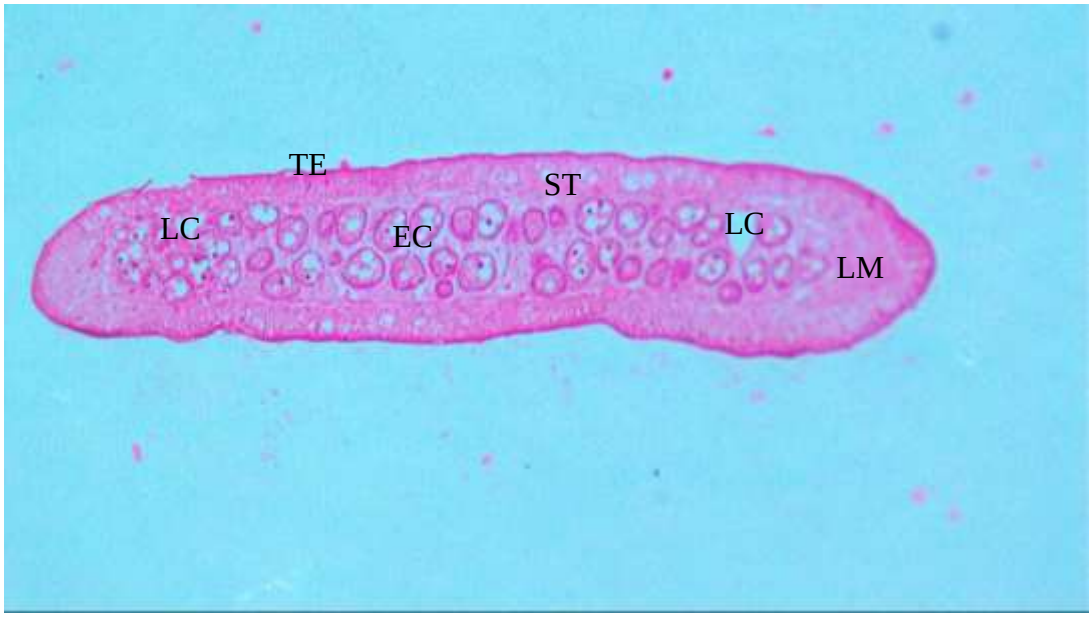


Fig4.42: Light micrographs of histological section of untreated control of *R. echinobothrida*.
Mature proglottid showing the tegument (TE) , the underlying subtegument (ST) and longitudinal muscle layer (LM), numerous eggs or oncospheres inside egg capsule (EC), and a lateral excretory canal (LC) on either side.

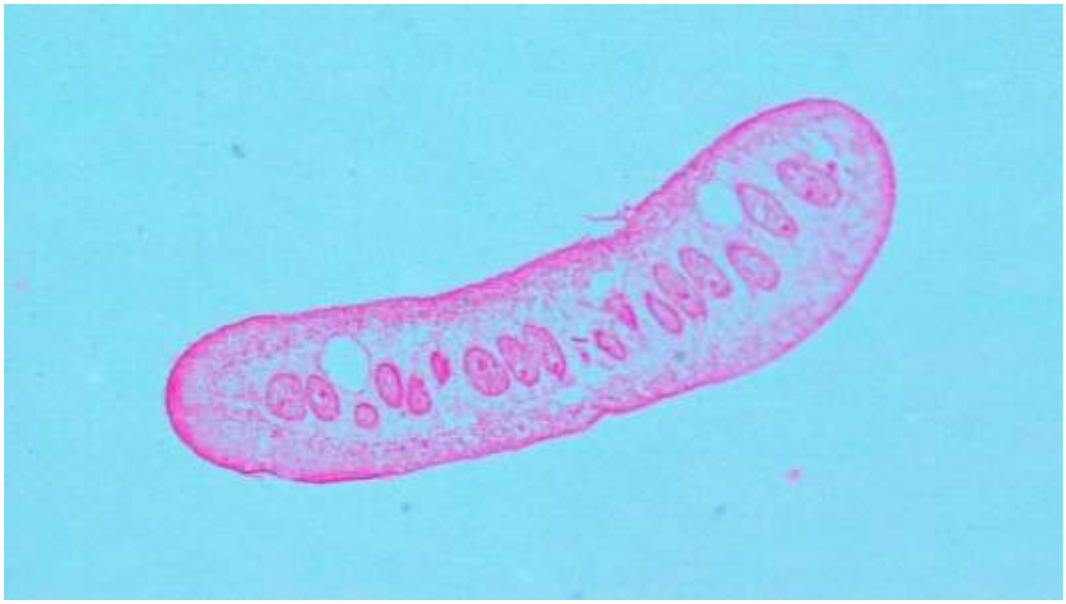


Fig 4.43: Light micrographs of histological section of *R. echinobothrida* treated with 20 mg/ml albendazole.
Alterations of tegument and sub-teguments are noticed. The disappearance of parenchymatous tissue leads to the irregular arrangement of oncospheres—two large vacuoles towards the extreme ends.

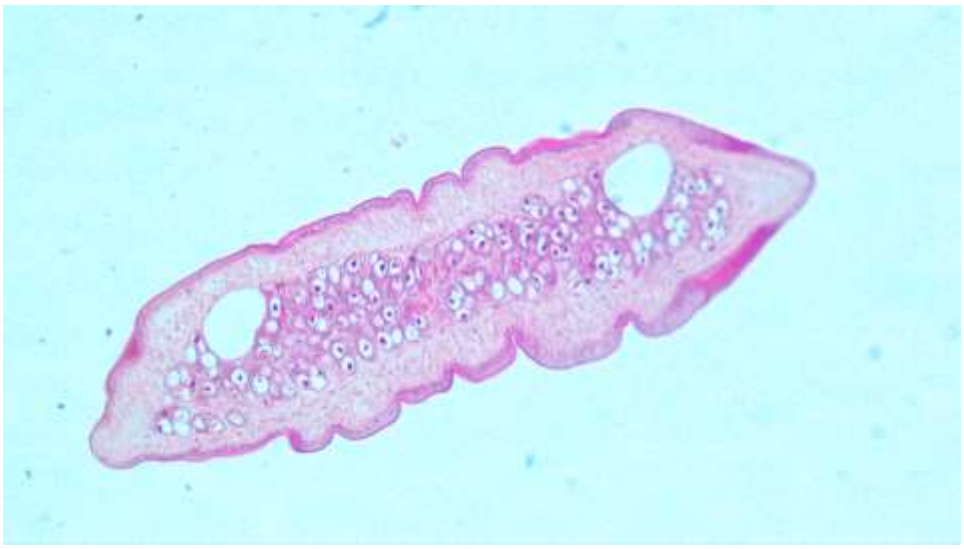


Fig 4.44: Light micrographs of histological section of *R. echinobothrida* treated with 20 mg/ml of *A. scholaris* methanol extract.
Massive shrinkage of tegument and sub-tegument. The disorganization of the parenchymatous tissue leading an irregular arrangement of the oncosphere and the presence of two large excretory vacuoles at the extreme ends.

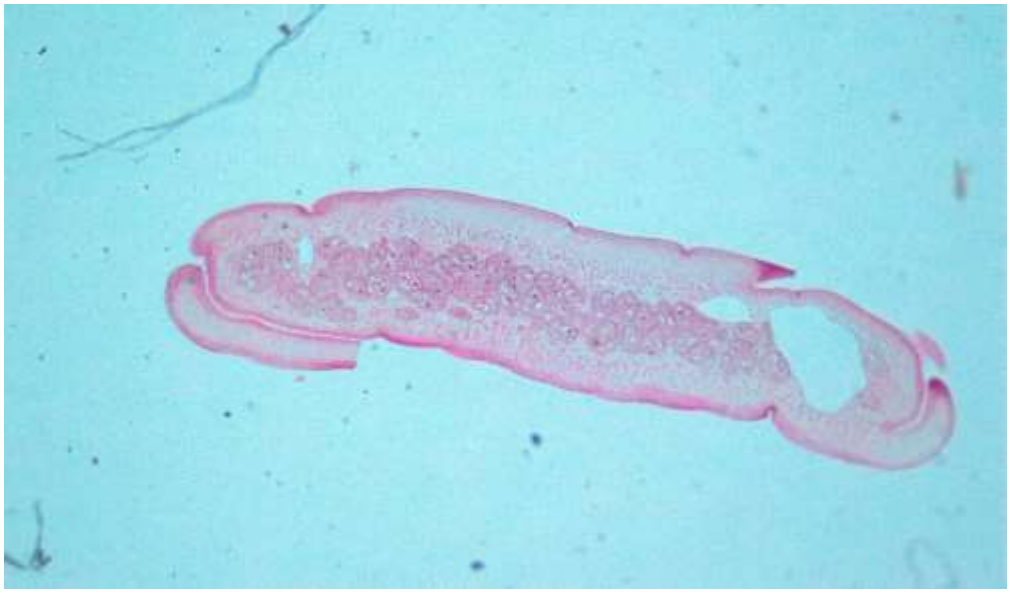


Fig 4.45: Light micrographs of histological section of *R. echinobothrida* treated with 20 mg/ml of *A. scholaris* chloroform extract.
Some portions of the tegument and sub-tegument are peeled off and the eggs are stomped up into the center. Disintegration of parenchymatous tissue and two large vacuoles appearing towards the extreme ends was noted.

Fig4.46: Light micrographs of histological section of *R. echinobothrida* treated with 20 mg/ml of *A. scholaris* petroleum ether extract.

Alterations of tegument, sub-tegument, and parenchymatous tissue which cause irregular displacement of eggs with the presence of two large vacuoles at the extreme ends.

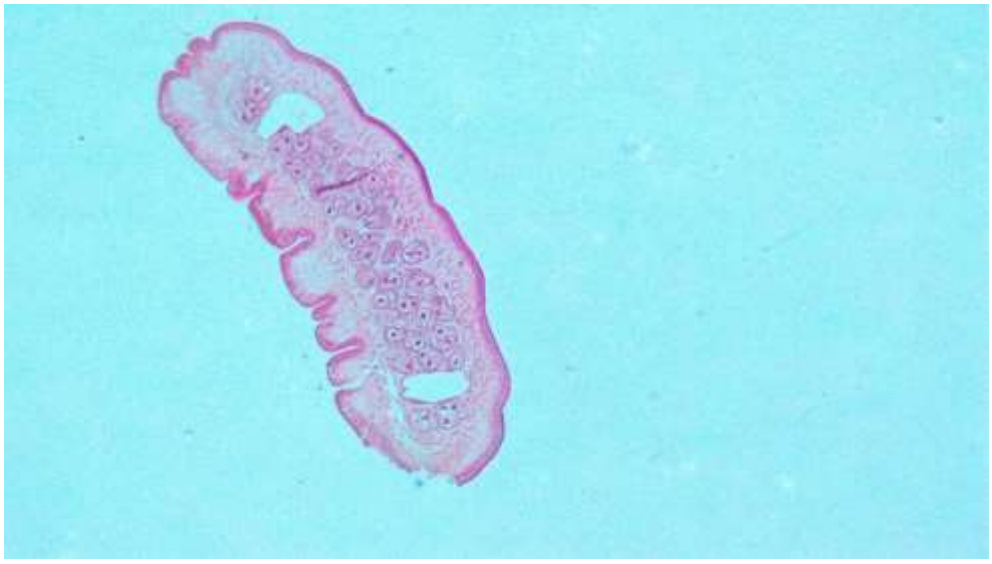
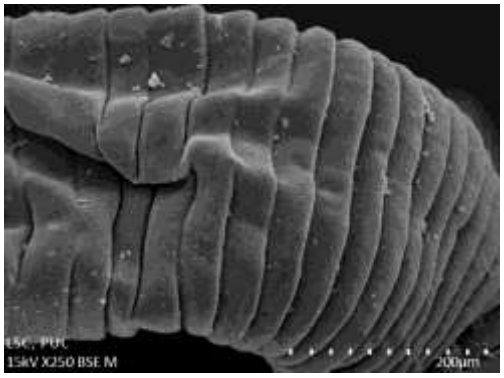


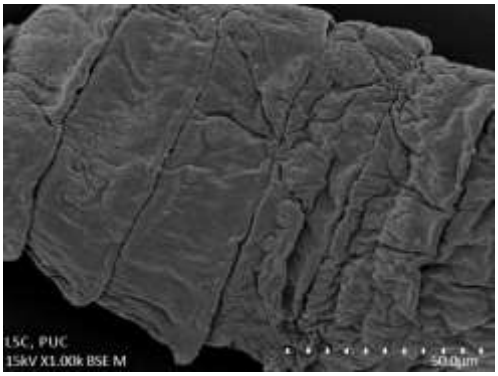
Fig 4.47: Light micrographs of histological section of *R. echinobothrida* treated with 20 mg/ml of *A. scholaris* aqueous extract.

Massive shrinkage on both tegument and sub-tegument on the internal organs of the cestodes with the disorganized eggs in the center thereby forming two large excretory vacuoles towards the extreme ends.

A



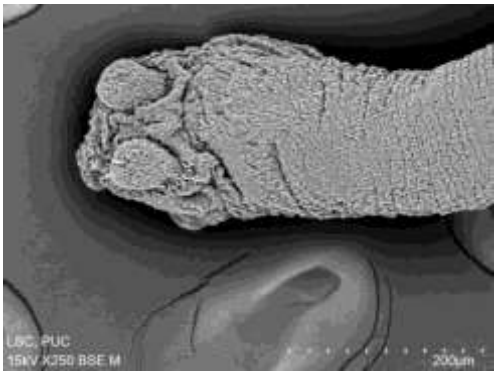
B



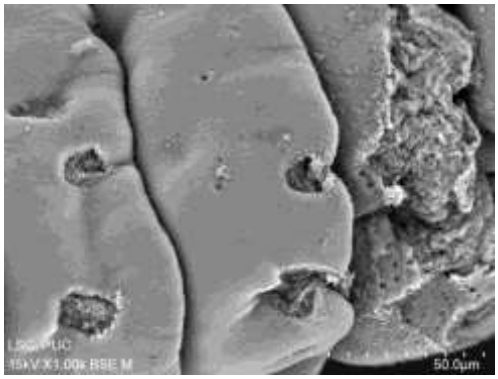
C

Fig 4.48: Scanning electron micrographs of untreated (control) cestode *R. echinobothrida*

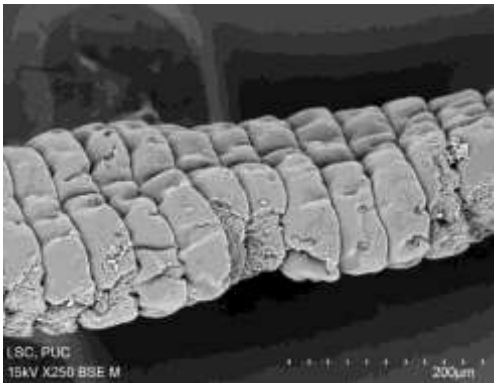
A. The anterior end of the body showing a bulbous scolex that bears mouth like rostellum at the tip, and oval eye-like suckers (two are visible).
B. The body segment (proglotids) towards the scolex/neck.
C. The tegument of mature proglottids.



A



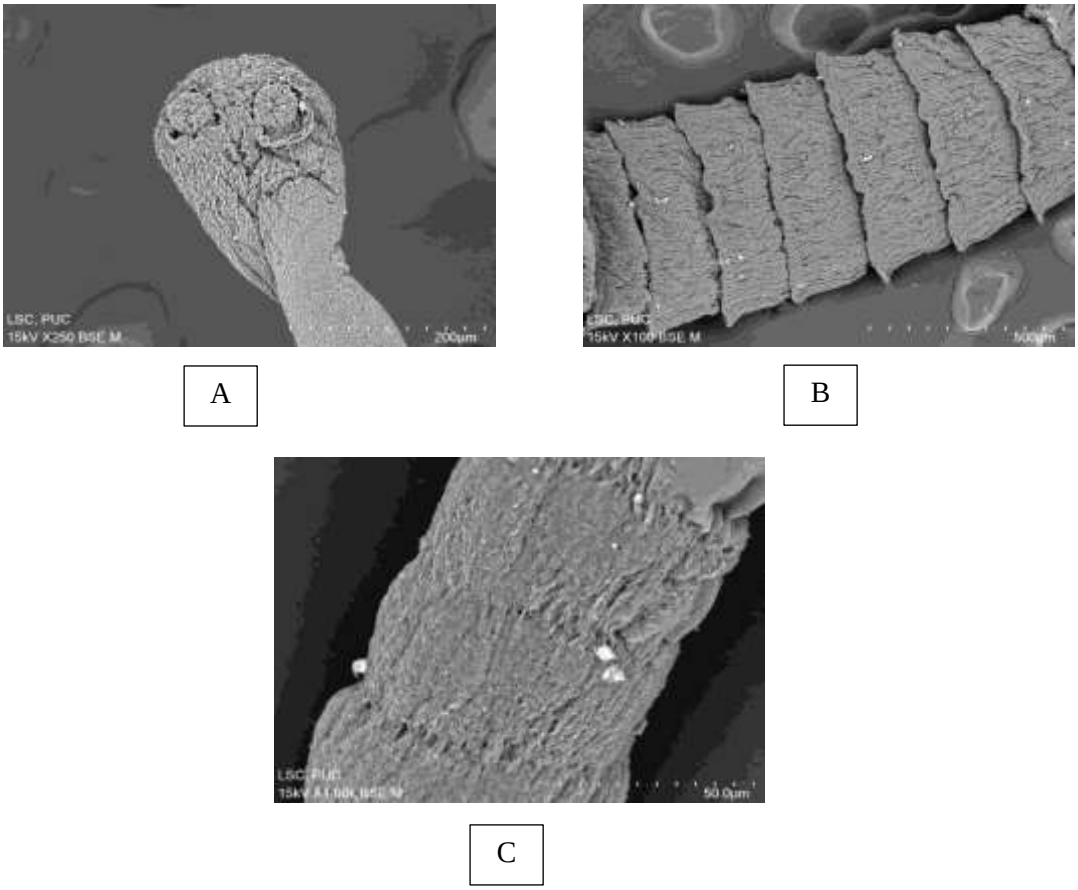
B



C

Fig 4.49: Scanning electron micrographs of a cestode *R. echinobothrida* treated with 20 mg/ml of albendazole

A. Severe shrinkage of the scolex forming deep depression. Spines or hook dislocated
B. Magnified surface of proglottids with pith formation, cracking and peeling of teguments.
C. Teguments with pith formation and cracking all over the topography.



A

B

C

Fig 4.50: Scanning electron micrographs of a cestode *R. echinobothrida* treated with 20 mg/ml of *A. scholaris* methanol extract.

A. The scolex showing shrinkage with deep depression between the suckers
B. Proglottids with the formation of wrinkles and folded all over the surface
C. Magnified portion of tegumental folds.

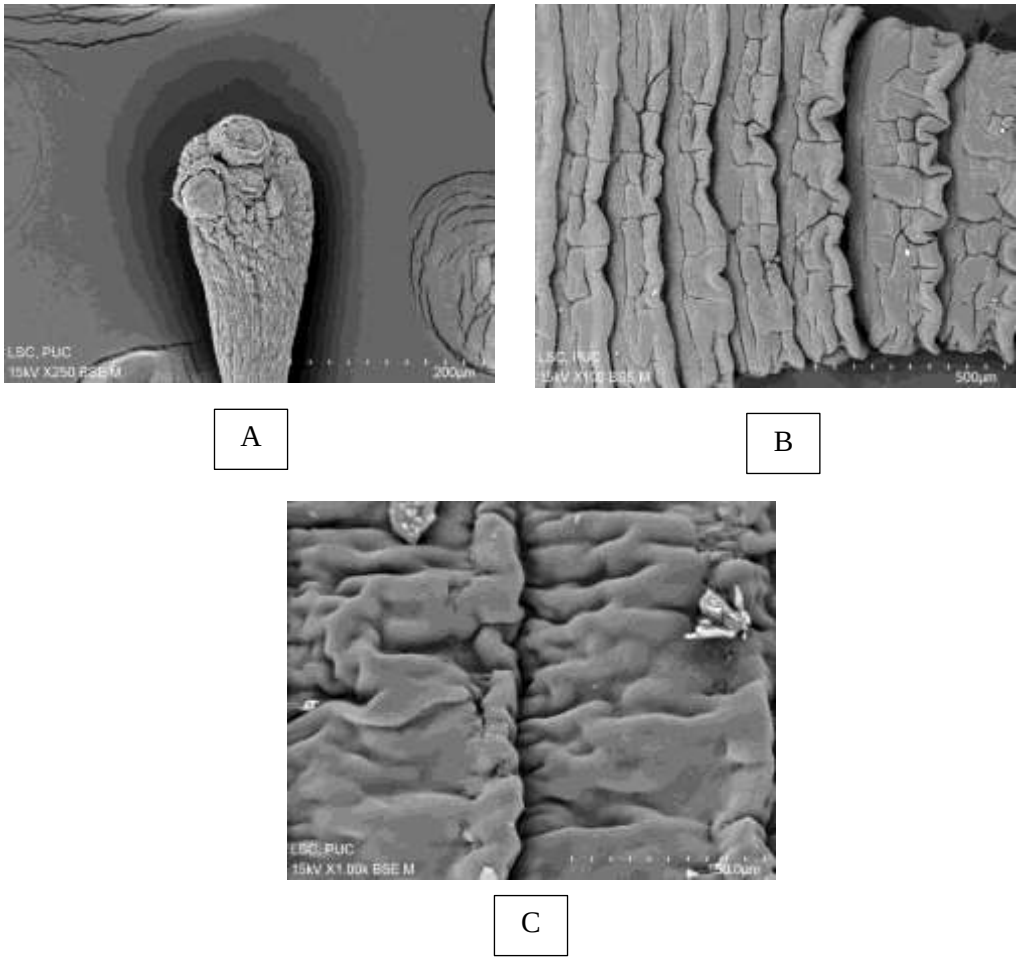


Fig 4.51: Scanning electron micrographs of a cestode *R. echinobothrida* treated with 20 mg/ml of *A. scholaris* chloroform extract.

A. The scolex with destruction of spines and dysfunction of restellum.
B. Immature body segment with formation of furrow and extensive cripple.
C. Magnified portion of proglotidds with shrinkage.

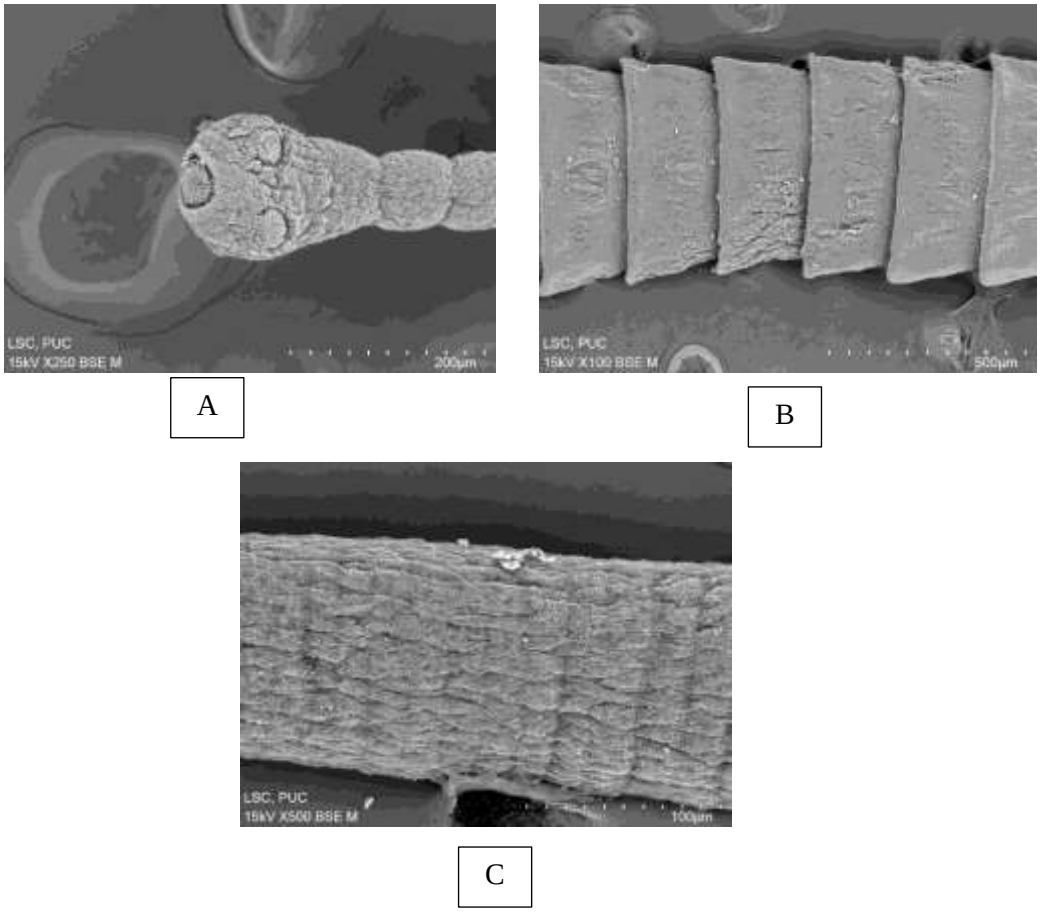


Fig 4.52: Scanning electron micrographs of a cestode *R. echinobothrida* treated with 20 mg/ml of *A. scholaris* petroleum ether extract.

A. scolex had severe damage with shrinkage and the spines are sharply crooked.
B. Proglotidds with cracking and peeling of all over topography
C. Magnified portion of tegumental folds and shrinkage.

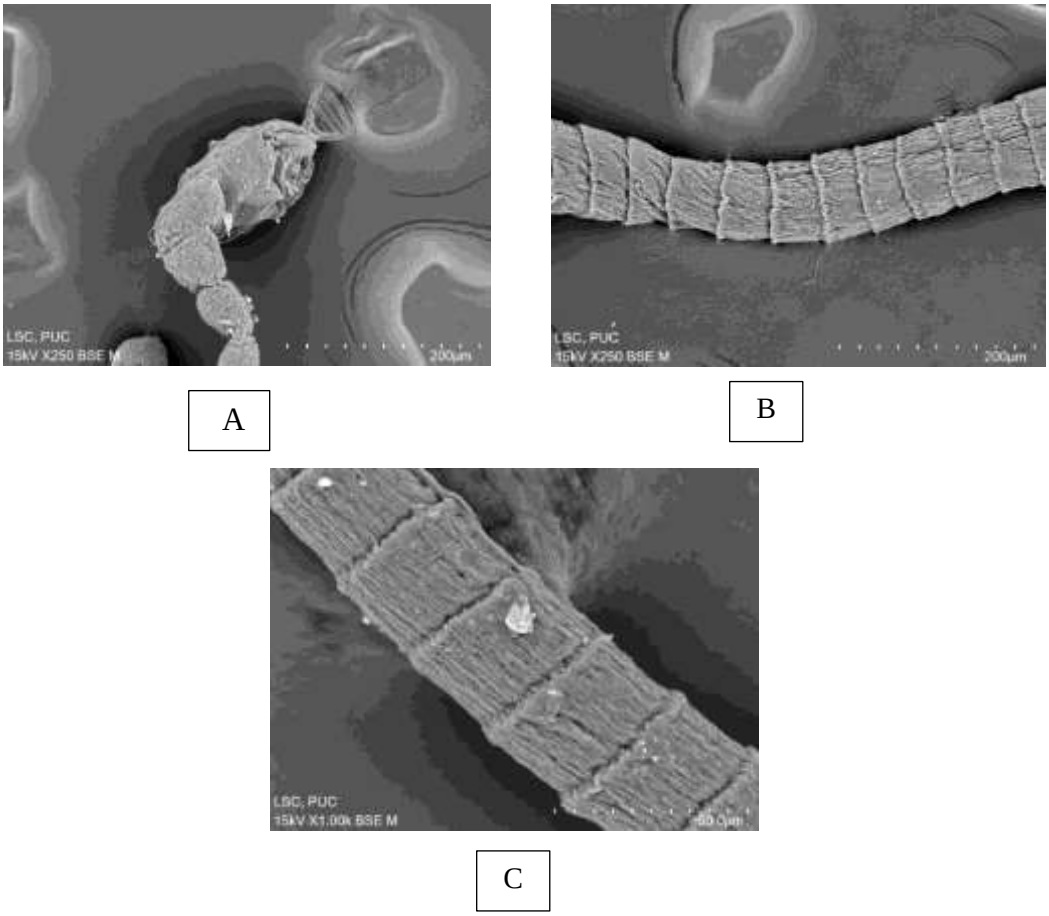


Fig 4.53: Scanning electron micrographs of a cestode *R. echinobothrida* treated with 20 mg/ml of *A. scholaris* aqueous extract.

A. The scolex with contort spines and dislocated. Anterior integuments with massive shrinkage and folds
B. The body segment with tegumental folds
C. Magnified portion of integuments with severe foldings.

CHAPTER 5

DISCUSSION

The antimalarial, antioxidant, antibacterial, and anthelmintic actions are solely dependent on the phytochemical substances found in plants. The presence of various phytocomponents varies depending on the kind of plant. However, phytochemicals found in plants have a wide spectrum of actions and effects in medical research. In addition, closely related species and varieties of plants can possess varying levels of their bioactive components as a result of their adaptation to a particular region. Therefore it is essential to identify the exact compounds present that are associated with the pharmacological properties of the plants.

Plants, constituting our primary diet, serve as the primary sources of nutraceuticals, fulfilling essential nutritional and therapeutic requirements. Their chemical constituents encompass molecules vital for normal cellular metabolisms, offering external sources to sustain cellular activities, especially those crucial in various cellular disorders (Makkar *et al*., 2020; Taroncher *et al*., 2021). Our study revealed the presence of biologically important phytocompounds, including alkaloids, carbohydrates, flavonoids, glycosides, phytosterols, saponins, tannins, and reducing sugars and Anthraquinone present in the three different extracts of *A. scholaris* bark. These diverse compounds, notably phenols, alkaloids, flavonoids, saponins, steroids, and tannins, are plant-derived elements from which various medicinal molecules, including pharmaceutical drugs and nutritional supplements, have been isolated and produced (Suntar, 2020; Huang *et al*., 2022). Similar findings on phytochemical identification in *A. scholaris* of Aqueous extract revealed the presence of Alkaloids, Tannin, saponin, glycosides, protein, phenols, steroids, and carbohydrates (Antony *et al*., 2011). Likewise, Alkaloids, glycosides, Flavonoids, Saponins, oilsand fats, Gumsand mucilage, and terpenoids were reported earlier (Mistry *et al*., 2016) Consequently, our data adds *A. scholaris* to the list of medicinal plants, establishing it as a source of important bioactive molecules and nutraceutical compounds.

Free radicals or reactive oxygen species (ROS) are normal biochemical products from cellular metabolic reactions. However, they become problematic when they

accumulate in the cells, transforming into highly toxic substances that cause damage to fundamental cellular components such as DNA, RNA, proteins, and lipids (Aliss *et al*., 2020). These cumulative damages can lead to the development of chronic diseases such as cancer, diabetes, and heart disease (Hajam *et al*., 2022). Balancing these effects is crucial, and external antioxidants play a significant role. Plants, forming a major part of our diet, serve as the primary source of these antioxidants (Aja and Awuchi, 2023). Our study observed appreciable amounts of phenols, flavonoids, and total antioxidants in *A. scholaris* indicating the plant's overall antioxidant value. Total phenolic and total flavonoids of 20.16±1.11 and 46.11±0.85 contributing to the antioxidant value were earlier reported (Ramachandra *et al*., 2012). A similar observation was also revealed from aqueous and methanol extracts of bark with total phenolic content of 46.87±0.10 and 49±0.08, flavonoid content of 37.51±0.04 and 26.38±0.10 respectively (Mistry *et al*., 2016). Reliable tests for evaluating antioxidant activity include the DPPH and FRAP assays (Moon and Shibamota, 2009; Mohammadnezhad *et al*., 2023). The present study results show a clear concentration-dependent activity and their ability to scavenge free radicals at all concentrations tested. The IC_{50} value of aqueous and methanol (6.09 and 11.23) strongly indicates good antioxidant activity, albeit slightly less potent than the standard BHT. FRAP essays validated that the plant extracts show increasing activity corresponding to the increase in concentration. However, at all the concentrations tested plant extract displayed lower effectiveness than the standard ascorbic acid. Our finding demonstrated that the bark extract of *A. scholaris* exhibited strong antioxidant activity in both DPPH and FRAP assays. A similar concentration-dependent DPPH scavenging activity of extracts of *A. scholaris* of methanolic extract of bark was earlier reported (Ramachandra *et al*., 2012). The aqueous extract of the bark was also found to have the most potent antioxidant property among all the other extracts with an IC_{50} value of 1.21 mg/ml (Antony *et al*., 2011). Compared to methanol and aqueous extracts from the bark of A. scholaris revealed that aqueous extracts had better potency for antioxidant activity particularly at higher concentrations than methanol extract (Mistry *et al*., 2016).

The development and spread of drug resistance in microbes have spurred research into new and alternative medications, with plants emerging as one of the most promising sources of leads (Vaoun *et al*., 2021). While many plants and their secondary metabolites have been investigated as potential antibacterial agents, the translation of this research into medical application has not yet reached a significant level (Mulat *et al*., 2019). Most plants, even if they exhibit antibacterial activity, often show limited prospects due to their poor and narrow effectiveness against the most important bacteria (Chen *et al*., 2021). Our data indicate that *A. scholaris* possesses broad-spectrum antibacterial activity against both Gram-negative and Gram-positive species. The lack of activity or diminished effectiveness of plant extracts or any drug at higher concentrations, particularly at 200 mg/ml in our study, can be attributed to the phenomenon of hormesis or the ceiling effect commonly encountered in microbes, where they become unresponsiveness beyond certain threshold doses (Sanders *et al*., 2019; Agathokleous *et al*., 2021). Therefore, the general susceptibility trend observed in different bacteria suggests that *A. scholaris* holds promise as a source of antibacterial molecules. Chemical fungicides are not readily biodegradable, can remain in the environment for years, and few fungi have acquired resistance to them (Bajwa *et al*., 2008). The use of natural items, such as botanical amendments or plant extracts, for the treatment of fungal infections in plants is regarded as a replacement technique for synthetic fungicides, due to their fewer negative effects on human and environmental health hazards or consequences (Anil Kumar and Raj Kumar, 2015). Since plant-derived antifungal chemicals are naturally less harmful and more ecologically friendly, they hold the most potential (Lee *et al*., 2007). Many plants have been isolated and tested for their antifungal properties throughout the past few decades. Among the natural compounds obtained from plants, essential oils have been shown to exhibit favorable bioactivities, such as fungicidal properties (Soliman *et al*., 2002). The current study found that three extracts of *A. scholaris* bark, namely petroleum ether, chloroform, and methanol, significantly inhibited antifungal activity against *C. albicans*, followed by *F. keratoplasticum* and *A. fumigatus*, with dose-dependent activity. The highest percentage of inhibition was recorded at 200 mg/ml. The earlier report of *A. scholaris* extract revealed the maximum activity against *B. subtilis*, followed by

Staphylococcus species, and the lowest activity against *Streptococcus* species and *Pseudomonas* species (Chakraborty *et al*., 2016). A comparable investigation on methanol extract indicated a broader spectrum of antibacterial activity, being active in both Gram-positive and Gram-negative bacteria (Singh *et al*., 2012), which is consistent with our current findings. The extracted methanol leaves had broad-spectrum antibacterial action against both Gram-positive and Gram-negative bacteria, with the highest activity against *B. subtilis*, followed by *E. coli* and *S. aureus*. Chloroform and acetone extracts had lower activity, but petroleum ether extract showed no inhibition (Kyde and Vaikos, 2008). In terms of inhibitory zones, chloroform and petroleum ether extracts exhibited similar poor antibacterial activity against *Klebsiella* and *E. coli*. However, the chloroform extract's antifungal efficacy against *Aspergillus niger* and *A. flavus* seemed promising (Virsha Parcha *et al*., 2003). Methanol extracts of *A. scholaris* plant components, including leaves, follicles, and latex, showed considerable antioxidant and antibacterial action (Ganjewala and Gupta, 2013). An interesting fact regarding the bark extracted with isopropanol: methanol displayed a rather significant action against the majority of Gram-positive and multiple drug resistance (MDR) bacteria. The antifungal properties of several leaf extracts, including hexane, benzene, isopropanol, methanol, and water extracts, were shown to strongly suppress the development of fungus, including human pathogenic strains (Gholamreza Bagheri *et al*., 2020). Six pentacyclic triterpenoids were extracted from the leaves of *A. scholaris*. Both oleanolic acid and ursolic acid demonstrated antibacterial action, although only against Gram-positive bacteria. (Wang *et al*., 2015). *A. scholaris* bark extract is a promising bio-source for the synthesis of silver nanoparticles. The produced nanoparticle is very stable and polydispersed, with an average size of 50 nm. Significant antimicrobial activity of silver nanoparticles has been recorded at higher concentrations (170 ppm) against fungal species and Gram-positive and Gram-negative bacteria, pointing to the commercial use of these photo-medicine-coated silver nanoparticles in biomedical applications and agriculture as fungicides for effective disease-causing pathogen control (Prabha Shetty *et al*., 2014). Our present data supports the conclusion that *A. scholaris* bark extract possesses notable

antibacterial properties, with varying degrees of effectiveness depending on bacterial strain and extract concentration.

The fight against malaria is becoming increasingly difficult due to the rise of *P. falciparum* strains resistant to all antimalarial medicines, including artemisinin-based combinations (WHO, 2017). Consequently, the search for novel chemical compounds possessing anti-malarial properties must continue. Medicinal herbs have been a significant part of the treatment of malaria for many years, leading to the development of two widely used drugs: quinine and artemisinin. Therefore, there is a considerable chance that medicinal plants will yield novel anti-malarial compounds. Additionally, it is believed that 80% of people in poor nations get their primary healthcare from medicinal plants (WHO, 2010). Identifying plant extracts with IC_{50} values less than 15 µg/ml is a crucial initial step in discovering novel anti-malarial medicines. Several techniques are available for this purpose (Kweyamba *et al*., 2019)

The result from our study shows that *A. scholaris* bark of chloroform extract shows significant inhibition against *P. falciparum* and 3D7 with an IC_{50} value of 10 µg/ml and 14 µg/ml petroleum ether extract shows moderately active against the *P. falciparum* with an IC_{50} value of 22.0 µg/ml and 26.7 µg/ml which can be considered as a promising plant for the discovery of new antimalarial drugs. However, methanol and Aqueous extracts show inactivity results. Parallel investigations of antimalarial activity were reported against *P. falciparum* upon treatment with chloroform extracts of the *Vitex* leaf. *Negundo* showed activity with IC_{50} values of 7.21 µg/ml and 7.43 µg/ml against 3D7 and K1 strains, respectively, with no indication of significant cytotoxicity against the mammalian cell line (Vero) and no toxicity as shown in the hemolysis experiment (Dwivedi *et al*., 2021) Comparable reports *in vitro* antimalarial efficacies against *Plasmodium* sp. were observed for the extracts of *P. angulata, J. curcas,* and *A. spectabilis* extracts show strong antiplasmodial activity with IC_{50} values of 0.22, 0.22, and 1.23 µg/ml; *N. podagrica, A. scholaris, F. pilosa,* and *P. alba* were moderate antiplasmodials with IC_{50} values of 11.60, 15.46, 24.92, and 36.39 µg/ml, respectively; and *C. rutidosperma, M. azedarach, S. ligustrina,* and *C. gigantea* were weak antiplasmodial with IC_{50} values of 54.25, 63.52, 63.91, and 66.49 µg/ml, respectively

(Taek *et al*., 2019). According to earlier research, *A. scholaris* bark and leaf extracts exhibit a dose-dependent strong inhibitory effect against *P. falciparum* (Ajana Singh *et al*., 2023). Stem, bark, leaf, and oil extracts of *Allanbackia foribunda* showed *in vitro* antiplasmodial effectiveness and cytotoxicity. Compared to leaf extract (IC_{50} on 3D7 was 8.0±0.28 µg/ml) and oil (IC_{50} on 3D7 was >100 µg/ml), stem bark extract showed superior antiplasmodial action (IC_{50} on 3D7 was 4.3±0.17 µg/ml). When contrasted with the cytotoxic medication doxorubicin, the leaf and stem bark extracts showed no cytotoxicity. The study of *A. foribunda* stem bark showed a harmless for normal cells and has strong anti-plasmodial action (Irabor *et al*., 2021). Upon screening, *in vitro* investigations revealed the plant's antimalarial usage in conventional pharmaceutical systems and showed strong antiplasmodial activity against *P. falciparum* 3D7 and K1, respectively, for the extracts of *Azadirachta indica, Homalolepsis suffruticosa, Goniothalamus lanceolatus, Vitex negundo, Petasites japonicus, Senna occidentalis, Nauclea orientalis, Alchornea cardifolia, Helianthus annus, Harungana madagascariensis, Pericopsis laxiflora, Psychotria apoda, Andrographis paniculate, Globba malaccensis, Sanchus arvensis, Terminalia arjuna, Pleiocarpa bicarpellate, Terminalia bentzoe,* and *Bridelia atrovirdi* (Abdulrazak, 2020; Boena *et al*., 2022; Kaharun *et al*., 2020; Dwivedi *et al*., 2021; Yun *et al*., 2022; Pratama *et al*., 2022; Budiarti *et al*., 2020; Cudjoe *et al*., 2020; Eksari *et al*., 2019; Koffi *et al*., 2020; Sevik *et al*., 2022; Rammun *et al*., 2023; Djouwoung *et al*., 2021; Erhunse *et al*., 2023). So, our present data on the chloroform extract's potent activity, coupled with its low cytotoxicity, highlights its promise in the search for new antimalarial therapies, while the other extracts, particularly methanol and aqueous, may require further investigation or modification to enhance their efficacy.

Plant compounds can treat a variety of disorders, including parasitism. Several plant species have been utilized and reported in various world regions to treat nematode infections in animals and humans (Akhtar *et al*., 2000). Many studies have documented the use of different plant species to manage parasite infestations in sheep, which are effective alternatives to traditional anthelmintics (Githiori *et al*., 2006). Previous studies revealed that extracts of *Turnera ulmifolia, Parkia*

platycephala, and *Dimorphandra gardneriana* were evaluated *in vitro* against *H. contortus*. The results demonstrated strong in vitro anthelmintic activity against *H. contortus* at different stages, indicating the possible use of these species as a feasible alternative way to manage helminthic infections in small ruminants (Oliveira *et al*., 2016). *Artemesia absinthium* exhibits strong in vitro and in vivo anthelmintic efficacy at concentrations and dosages evaluated against ovine nematodes as assessed by worm motility inhibition of *H. contortus* (Tariq *et al*., 2008). Consequently, the following plant extracts are a few therapeutic herbs capable of lowering helmintic infections, such as as *Chenopodium album, Caesalpinia crista, Trianthema portulacastrum, Musa paradisiaca, Cocos nucifera, Hedera helix, Myrsine Africana, Rapanea melanophloeos, Albizia schimperiana oliv, Combretum mole, Agave sisalana, Paris polyphylla, Leucas marttinicensis, Rumex abyssinicus, Leonotis ocymifolia, Senna occidentalis, Brucea javanica, Carica papaya, Coriandrum sativum, Khaya senegalensis, Ocimum sanctum, Ficus species*, and *Butea monosperma* (Jabbar *et al*., 2007; Hussain *et al*., 2011; Olivera *et al*., 2009; Equale *et al*., 2007a; Githiori *et al*., 2002; Equale *et al*., 2011; Ademola and Eloff, 2010; Botura *et al*., 2011; Wang *et al*., 2010, Wang *et al*., 2011; Satriji *et al*., 1995; Equale *et al*., 2007b; Ademola *et al*., 2004; Asha *et al*., 2001; De Amorin *et al*., 1999; Prasanth *et al*., 2001).

The current investigation supports the anthelmintic activity of *A. scholaris* bark extract against the cestode *R. echinobothrida*. The plant extract's effectiveness against *R. echinobothrida* was dose-dependent and comparable to albendazole. The various extracts of *A. scholaris* in methanol and chloroform extracts displayed substantial anthelmintic action against gastrointestinal worms *R. echinobothrida*, as evidenced by the worms' paralysis and death after exposure to varied doses (5, 10, and 20mg/ml). Both extracts studied resulted in a substantial amount of cestode death shortly after treatment. Methanol and chloroform had larger and faster anthelmintic action than Petroleum ether and Aqueous extracts, which took significantly longer to paralyze and kill the worms. However, all of the extracts had a dose-dependent action on cestodes, but albendazole is efficacious at all dosages tested. A similar effect was also shown by *Acasia caesia* stem bark extract against *R. echinobothrida*

in terms of dose-dependent efficacy (Lalchhandama, 2009). The aerial plant extract of *Acmella oleracea*, derived from the medicinal plants of a toothache plant cultivar from Mizoram, was similarly demonstrated to have comparable concentration-dependent cestocidal activity on *R. echinobothrida* (Lalthanpuii and Lalchhandama, 2020). At all tested doses, the in vitro anthelmintic activity of *Imperata cylindrica* chloroform extract against *R. tetragona* demonstrates strong concentration-dependent effects (Lalthanpuii and Lalchhandama, 2020) The findings for *Artemesia siversiana* and *A. parviflora*'s anthelmintic activity showed encouraging effects on nematode adult stages at various concentrations in a dose-dependent manner, and they can be seen as potential strategies to lessen worm burdens in animals (Irum *et al*., 2017). *Picria fel-terrae* extracts are a promising substitute for the commercially available anthelmintics for the treatment of gastrointestinal nematodes in sheep (Kumarasingha *et al*., 2016), and more generally, plant extracts of *Artemesia absinthium* are a potentially rich source of anthelmintics to fight against helminthic diseases (Tariq *et al.,* 2009). The present study highlights the potential of plant-derived extracts as alternative anthelmintic agents. While Albendazole remains the most effective treatment, the methanol and chloroform extracts demonstrate significant promise in combating parasitic infections caused by cestodes such as *R. echinobothrida*. Further studies exploring the specific phytochemicals responsible for this activity could pave the way for the development of novel plant-based anthelmintic therapies.

Scanning electron microscopic examinations revealed the tapeworm's characteristic form as well as structural damage that indicates antiparasitic actions. Scanning electron microscopy enhanced the data by showing unique micro-topographical aspects of the host-parasite interface, as well as giving useful information in helminth taxonomy and measuring the efficiency of test compounds in medication screening (Roy *et al*., 2008). The most effective method for examining the structures of a wide variety of species is microscopy. However, because various species have varying structures and compositions, processing specimens using typical microscopic methods can have serious limitations. *R. echinobothrida* is a helminth parasite that is often used in the pharmacology of anthelmintic medications. Its widespread distribution, ease of handling, and most importantly, exquisite form, have made it

one of the most researched helminth parasites. The tapeworm's exquisite morphological subtleties and fairly uncomplicated anatomy, owing to the absence of sophisticated digestive and respiratory systems, make it a good topic of study when treated with anthelmintics, because anthelmintics can inflict considerable structural damage (Lalchhandama, 2009). Our study of untreated which acts as a control of cestodes *R. echinobothrida* revealed that the body consists of a knob–like structure called scolex at the anterior end, followed by a flattened neck and an elongated body proper called strobila. On the scolex are four oval-shaped suckers that surround an apical opening called the rostellum. Each sucker is an organ of attachment and possesses several rows of sharply pointed spines and a ribbon-like structure composed of a chain of conjoined segments called proglottids. The entire body is covered with the tegument with hair-like filaments called microthrides throughout the surface giving the overall surface a velvety appearance. A comparable finding of untreated worms was reported (Roy *et al*. 2007; Lalchhandama, 2009; Giri and Roy, 2014; Lalthanpuii and Lalchhandama, 2020). Our study treated with 20 mg/ml of albendazole caused severe shrinkage of the scolex forming deep depression between the suckers thereby bulging out the suckers, and the spines or hooks are dislocated. Formation of pith, cracking, and peeling on the integument all over the surface of proglottids. Similar deformities of bulging of the suckers and rostellum were noticed in the praziquantel-treated parasite (Chandrani Mondal *et al*., 2023). There was a general contraction of the tegument on the suckers, rostellum, and scolex. The scolex was distorted when treated with 20 mg/ml of albendazole (Lalthanpuii and Lalchhandama, 2020; Lalthanpuii *et al*., 2019). Our study of methanol of plant extract showed severe shrinkage and distortion of the scolex forming deep depressions between the suckers wrinkle and folded teguments were observed all over the body surface. Similar observations were also made on *R. echinobothrida*, where the body segments were all wrinkled and completely devoid of microtriches, and the scolex, with its suckers and rostellum, was completely malformed along with the loss of rostellar hooks and sucker spines were reported on the methanol extract of *Acmella oleracea* (Lalthanpuii *et al*., 2020). Significant tegumental damage was produced by the methanol extract of *Ilex khasiana* leaves, including shrinkage, rostellum and spine deformation, and microtriche erosion. Concentration-dependent

fatal action on *R. tetragona* that was comparable to albendazole's supported the antiparasitic activity (Lalnunfela *et al*., 2020). Similarly, our investigation of aqueous extract, petroleum ether, and chloroform revealed a significant displacement of spines and scolex shrinkage and deformation. The teguments were crumpled and wrinkly throughout the whole body. significant scolex and neck area shrinking and tegumental folds. Constricted to asymmetrical forms, suckers. The chloroform extract of *Spilanthes acmella* revealed a complete loss of hairy absorptive filaments (microtriches), with a single sucker signifying enormous tegument erosion. At the same time, the spines are dislodged, shrinkage and folding are all over the segments, and widespread tegument collapse (Lalthanpuii *et al*., 2019). The overall topography of the cestode treated with resveratrol was irreversibly destroyed; the scolex was severely damaged, with the suckers substantially reduced and the spines surrounding the suckers sharply curved (Roy *et al*., 2008).

Drugs like albendazole and its related chemicals penetrate the parasite body by simple diffusion, disrupting the muscle and tegumental layers (Mottier *et al*., 2003; Markoski *et al*., 2006). Anthelmintic agent's effects on tegumental deformities have been widely established in several helminths (Kinstry *et al*., 2003; Xiao *et al*., 2003). Our histological observations on a transverse segment of untreated *R. echinobothrida* reveal a different pattern. The tegument is the outermost layer, and the layer underneath it is called the sub-tegument and is substantially thicker than the tegument. Several eggs or oospheres surrounded by oncospheres are lodged in the spongy tissue known as parenchymatous tissues within the cell. One excretory vacuole is located at the end. Comparable examinations of the cestodes were noticed before (Lalchhandama, 2009). In catechin-treated parasites, fissures and folds in the exterior tegument were visible, and the sub-tegument dissolved and disappeared (Chandrani Mondal *et al*., 2023). Treatment with albendazole had comparable outcomes, including outer integument folds and parenchymatous cell degeneration and the chloroform extract treatment induces cracking and peeling of various parts of the integument and sub integument. The cestode treated with plant extract displayed distinct degeneration in the muscle layer, resulting in the formation of vacuoles on many occasions. Additionally, the tegumental integrity was negotiated to the point

that the tissue began to fracture. There was also significant disruption to the interior parenchymatous cell's meshy network (Roy *et al*., 2007). In our current study, comparable morphological changes were observed following treatment with both methanol and aqueous extracts. These results are consistent with prior research mentioned above, which reported the formation of large vacuoles at the terminal regions, pronounced shrinkage of the tegument, the disintegration of sub-tegumental parenchymatous cells, and resulting in an irregular arrangement of egg cells.

CHAPTER 6

SUMMARY

CHAPTER 1

Chapter 1 provides an overview of malaria and traditional medicine, emphasizing the historical context, treatment evolution, and the role of herbal remedies. Malaria, an ancient disease likely originating in Africa, is caused by *Plasmodium* parasites transmitted by *Anopheles* mosquitoes (Tropical Medical Bureau Reports, 2023) with *Plasmodium falciparum* being the most lethal species (Medicine for Malaria Venture, 2023). Treatments have progressed from the use of cinchona bark in the 1600s to modern medications targeting the parasite's lifecycle (Institute of Medicine Report, 2004). However, malaria remains a significant global health threat, particularly in sub-Saharan Africa, with high infection rates and fatalities in 2022, despite modern medical advances. Challenges include drug resistance and climate change (WHO, 2023).

The chapter also highlights traditional medicine's rich history across cultures, with Indian systems like Ayurveda and Siddha utilizing plant-based remedies (Mukherjee, 2008) Approximately 80% of the global population relies on herbal medicine for primary care (Fabricant *et al*., 2001), It is hardly surprising that China and India control the majority of the global herbal trade business (Briskin, 2000; Khan, 2014). The use of herbal formulations as home remedies is common in many Asian and African countries (Gilani and Rahman, 2005). Many plants have therapeutic properties due to bioactive compounds (phytochemicals) offering antioxidant, antiviral, and antibacterial benefits (Chopra *et al*., 2002). 1,277 plant species from 160 families can be used to treat malaria (Ballou, 2009).In Mozambique, scientific studies have investigated the antimalarial potential of plants used in traditional medicine, particularly against malaria and fever. The most notable species include *Trichilia emetica*, *Tabernaemontana elegans*, *Schefflera actinophylla*, *Senna didymobotrya*, *Plumbago auriculata*, *Parkinsonia aculeata*, *Acacia karroo*, *Aloe parvibracteata*, *Bridelia cathartica*, *Cassia abbreviata*, *Cassia occidentalis*,

Crossopteryx febrifuga, *Leonotis leonurus*, and *Momordica balsamina* (Ramalhete *et al*., 2008).

As antibiotic resistance rises, interest grows in plant-based antimicrobials (Prabha shetty *et al*., 2014). Traditional medicine systems across various cultures have long utilized plants to treat malaria, making them a valuable source for new antimalarial discoveries (Willcox and Bodeker, 2004) and plants are also investigated as alternatives for treating helminth infections (Tariq *et al*., 2009), which affect billions globally (WHO, 2023). Overall, the chapter emphasizes the continued potential of medicinal plants in addressing global health issues, particularly malaria and related infections.

CHAPTER 2

Chapter 2 explores various traditional herbs and plant extracts with antimalarial, antioxidant, antibacterial, antifungal, and anthelmintic properties, highlighting their potential for new therapeutic developments. Key plants with promising antimalarial effects against *P. falciparum* include *Azadirachta indica*, *Vitex negundo*, and *Nauclea lanceolatus* (Abdulrazaq *et al*., 2020; Dwivedi *et al*., 2021and Budiarti *et al*., 2020), while compounds flavonoids 7-dimethyl artonol, artonin and cyclo artobiloxanthone from the root bark of *Artocarpus rigidus* (Namdaung *et al*., 2006) betulinic acid 3-caffeate (Cui-Ying *et al*., 2008). Additionally, studies on *Picralima nitida*, *Carissa edulis* (Apocynaceae), and *Hoodia gordonii* (Ngaisonna *et al*., 2016; Fanta *et al*., 2019 and Kapewangolo *et al*., 2016) reveal antioxidant effects, *Mimosa pudica* extract was evaluated against three potentially harmful microorganisms such as *Aspergillus fumigatus*, *Citrobacter diversus*, and *Klebsiella pneumoniae*. The results suggest that these traditional plants could offer new antimicrobial sources with stable, biologically active compounds, potentially providing a scientific foundation for modern medicine (Gandhiraja *et al*., 2009). Notable plants like *Picra fel-terrae* (Kumarasingha *et al*., 2016) and *Artemisia annua* (Cala *et al*., 2014) have demonstrated anthelmintic properties. *Imperata cylindrica* (Lalthanpuii and Lalchhandama, 2020) with evidence supporting the efficacy traditionally used by the Mizo people for helminth infections.

CHAPTER 3

This chapter outlines the comprehensive methodologies employed in a study investigating the phytochemical properties of traditional medicinal plants, with a focus on their potential antimalarial effects. Ten plant species, traditionally used by the Mizoram tribal community for treating malaria, were selected. These selections were substantiated through ethnobotanical references and taxonomic consultation. The plant materials were dried, ground into fine powders, and subjected to extraction using solvents of varying polarity—petroleum ether, chloroform, and methanol for 72 hours. Extracts were then analyzed through eleven chemical tests to detect the presence of secondary metabolites (Gokhale *et al*., 2017). Based on the outcomes of these results, three extracts showing high potential were selected for further analysis. Gas chromatography-mass spectrometry (GC-MS) was employed to identify bioactive compounds within these promising extracts (Hussain, 2014). The leading candidate extract underwent quantitative assessment for total phenolics, flavonoids, and antioxidant content (Zhensen *et al*.,1999; Singleton *et al*.,1965; Prieto *et al*.,1999). Antioxidant activity was evaluated using the DPPH and FRAP assays (Blois,1958; Oyaizu, 1986), while antimicrobial properties were investigated through well-diffusion and poisoned food techniques (Devillers *et al*.,1989; Mounyr Balouiri *et al*., 2016). The antimalarial potential of each extract was quantified using SYBR Green I fluorescence to determine IC_{50} values (Smilkstein *et al*., 2004; Anas *et al*., 2017), and anthelmintic efficacy was evaluated on parasites from local fowl, i.e. *Raillietina echinobothrida*. To assess morphological alterations in cestodes following treatment, histomorphological analyses and scanning electron microscopy were performed, providing insights into structural changes induced by the extracts (Lalchhandama *et al*., 2007; Roy and Lalchhandama, 2007; Roy and Tandon, 1991).

CHAPTER 4

This chapter explored the phytochemical composition of ten medicinal plants—*Alstonia scholaris*, *Betula alnoides*, *Cinnamomum bejolghota*, *Costus speciosus*, *Dendrocnide sinuata*, *Hedyotis scandens*, *Mimosa pudica*, *Prunus cerasoides*, *Stereospermum colais*, and *Vitex penduncularis*—using petroleum ether, chloroform,

methanol, and aqueous extractions. Screening showed alkaloids, flavonoids, phytosterols, saponins, and carbohydrates, while proteins, amino acids, gums, and anthraquinones were absent in some extracts. Alkaloids, gums, and anthraquinones were present in *A. scholaris*, *C. speciosus*, and *V. penduncularis*, respectively. *A. scholaris*, *C. speciosus*, and *V. penduncularis* were further analyzed with GC-MS revealing bioactive compounds such as diosgenin in *C. speciosus*, β-sitosterol in *V. penduncularis*, and *A. scholaris* contains bioactive compounds like lupeol, akuammine, and limonene, which exhibit potent antimalarial activity, particularly against *P. falciparum* (Sharma *et al*., 2020; Hanne *et al*., 2002; Kapadia *et al*., 1993; Moura *et al*., 2001). This highlights *A. scholaris* as a promising candidate for continued antimalarial research.

A. scholaris extracts showed significant antioxidant activity, with aqueous and methanol extracts displaying low IC_{50} values, though less effective than BHT. The methanol extract had the highest phenolic content, while petroleum ether showed the highest flavonoid levels. Methanol and chloroform extracts inhibited both Gram-positive and Gram-negative bacteria, especially *Salmonella typhi*. Petroleum ether and chloroform extracts also exhibited antifungal activity against *Candida albicans*, while the chloroform extract had notable antimalarial effects (IC_{50} = 10.0 µg/ml). The various extracts were effective against *R. echinobothrida*, causing paralysis and death in a dose-dependent manner and significant morphological damage was observed in the tegument and sub-tegument structures, with disorganized parenchymatous tissues and the formation of large excretory vacuoles. Treated parasites also exhibited severe scolex shrinkage, proglottid folding, and tegument surface disruption, especially with methanol and albendazole treatments.

CHAPTER 5

This chapter reviews the rationale behind studying *A. scholaris* bark and its significant bioactive phytochemicals with potential medicinal applications. Known for antimalarial, antioxidant, antibacterial, and anthelmintic properties, *A. scholaris* bark contains beneficial compounds like alkaloids, flavonoids, tannins, and saponins, contributing to health benefits and pharmaceutical uses. Similar findings were earlier

reported (Mistry *et al*., 2016). The bark exhibits strong antioxidant activity, neutralizing free radicals that can lead to chronic diseases, due to its phenol and flavonoid content measured through DPPH and FRAP assays. Given rising antibiotic resistance, *A. scholaris* is also promising for its antibacterial activity. A comparable effect was earlier reported (Jai Bahadur Singh *et al*., 2012) and antifungal properties, effectively inhibiting pathogens like *Candida albicans* and *Aspergillus fumigatus*.

With drug-resistant malaria strains on the rise, medicinal plants are essential for new antimalarial compounds. *A. scholaris* chloroform extract showed potent antimalarial activity (IC_{50} values of 10 µg/mL and 14 µg/mL) against *Plasmodium falciparum*, while other extracts had varying efficacy. A similar antimalarial activity was reported against *P. falciparum* from the extracts of *N. podagrica* with IC_{50} values of 11.60 µg/ml (Taek *et al*., 2019). In anthelmintic applications, all of the extracts had a dose-dependent action on cestodes, but albendazole is efficacious at all dosages tested. A similar effect was also shown by *Acasia caesia* stem bark extract against *R. echinobothrida* in terms of dose-dependent efficacy (Lalchhandama, 2009). Methanol and chloroform extracts of *A. scholaris* caused structural damage to gastrointestinal worms. Microscopy studies confirmed these effects, showing significant parasite damage.

Overall, the findings support *A. scholaris* as a valuable source of bioactive compounds with antioxidant, antimicrobial, antimalarial, and anthelmintic potential.

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PUBLICATIONS

1. **Lalngaihmanawmi**, Pawi Bawitlung Lalthanpuii, Lalbiakngheti Tlau, Lucy Lalawmpuii, Lalnundanga & Khawlhring Lalchhandama. Phytochemical analysis and antioxidant and antibacterial activities of *Alstonia scholaris* from Mizoram, India. *Plant Science Today*, Vol 11, No.1 (2024)

2. **Lalngaihmanawmi**, Pawi Bawitlung Lalthanpuii, Lalnundanga, Khawlhring Lalchhandama, Anticestodal evaluation of the Devil's tree (Echites scholaris) against an intestinal parasite and assessment of the anthelmintic effects using scanning electron microscopy. International Journal of Research in Pharmaceutical Sciences. 2025, 16(2). 1-8.

PAPER PRESENTED IN SEMINAR/CONFERENCE

1. **Lalngaihmanawmi**, Lalnundanga, PB lalthanpuii and K. lalchhandama (2023). Investigating the therapeutic potential of *Alstonia scholaris* by phytochemical analysis, GC-MS, and free radical scavenging. National Conference on Recent Advances in Plant Biology with special references to North-East India, 20-21 April 2023, Department of Botany, School of Life Sciences, Mizoram University.

2. **Lalngaihmanawmi**. Phytochemical study and Antioxidant activity of *Alstonia scholaris* (L) R.Br. International Conference on Biodiversity, Biogeochemistry and Ecosystem Sustainability in a changing environment. 14-16 June. 2023. Department of Forestry, Mizoram University, and Indian Ecological Society.

TRAINING/ WORKSHOP ATTENDED

1. Refresher Course on 'Research Methodology: Tools and Techniques' on 07th July- 20th July 2020 sponsored by UGC STRIDE Scheme, HRDC, Mizoram University.
2. One Week International Webinar on Natural Disaster on 20th July- 24th July 2020 sponsored by IQAC Govt. Champhai College.
3. Faculty Development Programme on Moodle Learning Management System on 24th Aug 2020-28th Aug 2020 organised by Government Zirtiri Residential Science College.
4. 5-Day Online International Workshop on Sustainability Post COVID-19 on 27th July 2020- 31st July 2020 organised by Planning & Programme Implementation Dept. Govt Of Mizoram.
5. Online Short Term Course (one Week) on Leadership Development Programme on 12th March 2021- 18th March 2021sponsored by HRDC. Mizoram University.
6. National Level Online Faculty Development Programme on Intellectual Property Rights on 21st Feb. 2022- 25th Feb 2022, organised by the Govt.Aizawl West College.
7. Participated in a Two-day International Webinar on Recent Advances in Science on 6th and 7th July.2020 organised by the Govt. Serchhip College.
8. Participated in the webinar on "Biomedical Sciences: A Touch of Medicinal Plants" on 17th June 2020, organized by the Department of HAMP. Mizoram University.
9. Participated in a Webinar on Tree improvement at a glance and Wildlife Conservation Why and How? On 30th June 2020 Dept. of Forestry. Mizoram University.
10. Participated in the International Webinar on Ecosystem Restoration-Reimagine, Recreate, and Restore on 5th June 2021 organized by Govt. Zirtiri Residential Science College.

11. Participated in an International webinar on Understanding Changes in Indigenous Farming Systems and Livelihood- 14th June 2021 organized by MZU and Minnesota.
12. Participated in the training entitled ‘Hands-on Training on Techniques in DNA barcoding of some Orchid species of Mizoram’on 22nd -31st August, 2023 organized by DBT-NER Advance Level Biotech Hub.

PARTICULARS OF THE CANDIDATE

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DEGREE	: DOCTOR OF PHILOSOPHY
DEPARTMENT	: FORESTRY
TITLE OF THE THESIS	: PHYTOCHEMICAL INVESTIGATION OF ANTIMALARIAL PLANTS IN MIZORAM
DATE OF ADMISSION	: 26.07.2019

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BOARD OF SCHOOL	: 11.05.2020
SCHOOL BOARD	: 29.05.2020
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ABSTRACT

PHYTOCHEMICAL INVESTIGATION OF ANTIMALARIAL PLANTS IN MIZORAM

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

LALNGAIHMANAWMI

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DEPARTMENT OF FORESTRY

SCHOOL OF EARTH SCIENCES AND NATURAL RESOURCES MANAGEMENT

MAY 2025

PHYTOCHEMICAL INVESTIGATION OF ANTIMALARIAL PLANTS IN MIZORAM

BY

LALNGAIHMANAWMI

Department of Forestry

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Submitted

In partial fulfillment of the requirement of the degree of Doctor of Philosophy in Forestry of Mizoram University, Aizawl.

CHAPTER 1

INTRODUCTION

Chapter 1 deals with malaria, an ancient infectious disease that has been documented for over 4,000 years, and its management down the ages. Historically, it was thought to stem from "bad air" (*mala* aria in Italian) but was discovered in the late 19th century that mosquitoes transmitted it (Tropical Medical Bureau Reports, 2023), with significant historical figures like Alexander the Great affected (Mertens, 2024). The disease is caused by unicellular protozoan parasites of the *Plasmodium* genus, transmitted through the bites of infected female *Anopheles* mosquitoes, with *Plasmodium falciparum* being the deadliest species in humans (Medicine for Malaria Venture, 2023). Treatments began with cinchona bark in the 1600s and evolved into quinine, chloroquine, artemisinin, and newer medications in the 20th century targeting different stages of the parasite's lifecycle (US Institute of Medicine Report, 2004). Despite these advancements, malaria remains a global health threat, particularly in tropical regions, with the WHO reporting 249 million cases and 608,000 deaths in 2022, predominantly in Sub-Saharan Africa. Drug resistance and factors like climate change challenge ongoing efforts against the disease (WHO 2023).

Traditional herbal remedies have been documented for around 5,000 years in various cultures, including Chinese, Indian, Egyptian, Greek, and Roman medicine. In India, extensive traditional medicinal knowledge exists, particularly in folk remedies, deeply rooted in systems like Ayurveda and Siddha. These systems utilize plant-based formulations to treat various ailments. Key ancient texts like the Rigveda and Charaka Samhita document these rich traditional woods (De *et al*., 2010, Kamboj 2000; Mukherjee, 2008). Herbal medicine is widely practiced globally, with about 80% of the world's population relying on it for primary health care (Fabricant *et al*., 2001). Many medicinal plants contain beneficial compounds with antiviral, antibacterial, and antioxidant properties (Chopra *et al*., 2002). The herbal medicine market is projected to reach $550 billion by 2030 (Saklani & Kutty, 2008; Willison & Andrews, 2004), Traditional medical knowledge, such as Ayurveda, is gaining

recognition globally (Modak, B *et al*., 2015). It is estimated that 250,000 to 350,000 plant species exist globally, but only around 35,000 are used for medicinal purposes (Kong *et al*., 2003). Various cultures utilize traditional plant-based treatments for malaria. In Kenya, alternative medicine, particularly herbal remedies, is commonly employed to treat malaria. In this region, 27 plant species from 24 genera across 20 families have been reported for malaria treatment (Nguta *et al*., 2010). In Nigeria, 59 medicinal plants are utilized as antimalarial remedies. The most commonly cited species include *Anacardium occidentale, Azadirachta indica, Carica papaya, Cassia fistula, Chromolaena odorata, Cymbopogon citratus, Enantia chlorantha, Helianthus annuus, Mangifera indica, Morinda lucida, Moringa oleifera, Psidium guajava,* and *Vernonia amygdalina* (Oladeiji *et al*., 2020).

Bioactive phytochemicals have a long history in traditional as well as mainstream medicine (Greathead, 2003). They are basically secondary metabolites that protect plants from environmental threats such as pollution, pathogens, UV exposure, and drought, thereby playing a crucial role in plant survival (Gibson *et al*., 1998; Mathai, 2000). For human health, phytochemicals are beneficial due to their nutraceutical properties, therapeutic benefits, and antioxidant properties (Prakash *et al*.,2012). Plant antioxidants are a vital source of bioactive compounds with pharmaceutical applications, with around two-thirds of plant species known for their strong antioxidant potential (Krishnaiah *et al*., 2011). The isolation of ascorbic acid sparked significant interest in plant antioxidants (Szent, 1963). The compound was identified to protect cells from free radicals and reactive oxygen species, linked to diseases like diabetes, cancer, and cardiovascular disorders (Devasagayam *et al*., 2004). Antioxidants work by neutralizing free radicals through mechanisms such as hydrogen-atom transfer and single-electron transfer (Amarowicz & Ronald, 2019).

All main antimalarial classes have acquired resistance during the last two decades, except for artemisinin derivatives, which are also showing reduced efficacy in some parts of the world (Cook & Zuma, 2009). Antiparasitic resistance, particularly antimalarial resistance, has been significantly under-explored, especially in the Indian setting (Parijia & Praharaj, 2011). The rise of drug-resistant pathogens has

prompted the search for new materials, with plants offering a promising source of potential antiparasitic agents (Shetty *et al*., 2014). The rise of helminth infections, affecting about 1.5 billion people (WHO, 2023), highlights the need for effective treatments, leading to the exploration of plant-based solutions as alternatives to conventional anthelmintics (Tariq *et al*., 2009). Overall, there is a growing interest in the therapeutic potential of medicinal plants across various health challenges, including malaria and helminth infections. In northeastern India, research on indigenous plants had identified 68 species used for malaria treatment, generally emphasizing the leaves and roots (Shankar *et al*., 2012). There is only limited study on the plants used by the Mizo people for antiparasitic applications.

The present study aims to screen potential antimalarial plants for future phytochemical investigations and to identify a single candidate for a comprehensive phytochemical analysis, in alignment with the objectives outlined below.

RESEARCH OBJECTIVES

1. Phytochemical screening of 10 antimalarial plants
2. Gas chromatography-mass spectrometry (GC-MS) analyses of phytoconstituents of three selected plants.
3. To study the invitro antioxidant, antimicrobial, and antiparasitic activity of one selected plant.

CHAPTER 2

REVIEW OF LITERATURE

Chapter 2 illustrates various traditional herbs and plant extracts evaluated for their antimalarial properties against *Plasmodium falciparum* and highlights their potential for developing new antimalarial treatments. Key finding includes *Acacia nilotica, Azadirachta indica, Goniothalamus lanceolatus, Cucurbita pepo, Garcinia malaccensis, Helianthus annuus, Nauclea lanceolatus, Pleiocarpa bicarpellata, Sonchus arvensis, S. Mammea siamensis, aromaticum,* and *Vitex negundo,* (Abdulrazaq *et al.*, 2020; Dwivedi *et al.*, 2021; Kaharudin *et al.*, 2020; Budiarti *et*

al., 2020; Ekasar *et al.*, 2019; Sevik *et al.*, 2022; Chaniad *et al.*, 2022;)**.** The text also discusses isolated compounds from various plants including *Sarcococca hookeriana*, which contains alkaloids with moderate to strong antimalarial (Devkota *et al.*, 2007), *Artocarpus rigidus* contains flavonoids with significant antiplasmodial activity, *Garcinia polyantha* that contains isoxanthochymol with strong activity, *Diospyros quaesita* having betulinic acid 3-caffeate showed potent effects. Various Cameroonian medicinal plants exhibited strong antiplasmodial activity (IC_{50} < 5 µg/mL) such as *Harungana madagascariensis*, iparticualrly a novel anthrone derivative called bazouanthrone demonstrating strong activity against *P. falciparium* (Namdaung *et al.*, 2006; Lenta *et al* 2007; Alain Meli Lannang *et al.*, 2008; Cui-Ying Ma *et al*. 2008; Titanji *et al* 2008). Additionally, the content outlines the therapeutic potential of various plant extracts, particularly focusing on their antioxidant, antibacterial, antifungal, and anthelmintic properties of *Picralima nitida* and *Carissa edulis* (Ngaisonna *et al*., 2016; Fanta Yadang *et al*., 2019) that showed antioxidant potential, and *Hoodia gordonii* extracts that inhibits HIV enzymes while exhibiting strong antioxidant activity (Kapewangolo *et al.*, 2016). Studies on *Cuminum cyminum*, *Punica granatum*, *Syzygium aromaticum*, *Thymus vulgaris* and *Zingiber officinale* demonstrated significant antibacterial effects, particularly against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, suggesting their potential for antibacterial medication and food preservation (Mostafa *et al*.,2 018). Alcoholic extracts of curry leaves and tea leaves were effective against *Candida albicans* (Doddanna *et al*., 2013), while extracts from *Phyllanthus niruri* and *Terminalia bellerica* showed activity against *S. aureus* and *Escherichia coli* (Sumathi & Parvathi, 2010). Gastrointestinal nematodes significantly threaten livestock, prompting interest in medicinal plant extracts as potential alternatives to traditional anthelmintics (Kassai, 1999). Moreover, extracts from *Artemisia absinthium, Azadirachta indica*, *Chenopodium album,* and *Parkia platycephala* are effective against parasites such as *Hymenolepis diminuta, Hypericum japonicum,* and *Trichosanthes multilobe* (Jabbar *et al*., 2007; Hussain *et al*., 2011; Irum *et al*., 2015; Kundu *et al*., 2012; Saiyam *et al*., 2021; Tandon *et al*., 2004). Traditional uses of Mizo plants for helminth infections supported by research indicating their effectiveness in bioassays were also emphasized.

CHAPTER 3

METHODOLOGY

This chapter copes with the methods used throughout the entire study. The study systematically identifies and analyses traditional medicinal plants for their phytochemical properties, emphasizing their potential as effective natural remedies against malaria, showcasing the convergence of ethnobotany and phytochemistry. Ten plant species used by the Mizoram tribal population for malaria treatment were selected for phytochemical examination. The selection involved validating their ethnomedicinal use with guidance from taxonomists and relevant botanical literature. The selected 10 plants underwent eleven chemical tests to identify secondary metabolites. Three promising samples were chosen for further analysis based on their secondary metabolites. Gas chromatography-mass spectrometry (GC-MS) was used to identify bioactive compounds in the selected plant species. The most promising species were identified based on the abundance of secondary metabolites (Hussain, 2014). Samples were cleaned, dried, and ground into powder. Dried samples were extracted using the Soxhlet apparatus with solvents (petroleum ether, chloroform, methanol) for about 72 hours. Crude extracts were concentrated and stored for preliminary screening. Qualitative Analysis was conducted using standard protocols to identify constituents such as alkaloids, flavonoids, and tannins, among others (Gokhale *et al*., 2017). Quantitative tests of the total flavonoid, total phenolic contents, and total antioxidant were measured using established methods, reporting results in equivalents of quercetin, gallic acid, and ascorbic acid (Zhensen *et al*., 1999; Singleton *et al*., 1965; Prieto *et al*., 1999). DPPH free radical scavenging activity and ferric-reducing antioxidant power (FRAP) assay were used to assess the antioxidant properties (Blois, 1958; Oyaizu, 1986). Antimicrobial assay was performed using a well diffusion method (Devillers *et al*., 1989) against various microorganisms. Antifungal Activity was assessed using the poisoned food method (Balouiri *et al*., 2016), measuring fungi growth inhibition such as *Aspergillus fumigatus*, *Candida albicans,* and *Fusarium keratoplasticum*. The antimalarial activity was evaluated on *Plasmodium* parasites using a SYBR Green I-based fluorescence assay to determine IC_{50} values (Stein, *et al*., 2004; Anas *et al*., 2017). *In*

vitro anthelmintic activity was conducted using extracts against intestinal parasites from local fowls, evaluating motility and survival. Fixed worms were processed for histological studies, involving dehydration, embedding, and staining. For the studies of scanning electron microscopy, cestodes were examined for morphological changes post-treatment with plant extracts, observed at varying magnifications (Roy & Tandon, 1991; Lalchhandama *et al*., 2007; Roy & Lalchhandama, 2007).

CHAPTER 4

RESULT

The study investigated the phytochemical profiles of *Alstonia scholaris*, *Betula alnoides*, *Cinnamomum bejolghota*, *Costus speciosus*, *Dendrocnide sinuata*, *Hedyotis scandens*, *Mimosa pudica*, *Prunus cerasoides*, *Stereospermum colais*, and *Vitex penduncularis* using various solvent extractions, including petroleum ether, chloroform, methanol, and aqueous solutions. Phytochemical screening revealed the presence of alkaloids, flavonoids, phytosterols, saponins, and carbohydrates, although certain compounds such as proteins, amino acids, gums, and anthraquinones were notably absent in several extracts. Alkaloids, gums, and anthraquinones were found exclusively in *A. scholaris*, *C. speciosus*, and *V. peduncularis*, respectively, with other collected species lacking these constituents. Given their demonstrated activity against *Plasmodium* species (Vincent, 2008), these three species were selected for further analysis using GC-MS. Further analysis through GC-MS identified a range of bioactive compounds. In *C. speciosus*, compounds such as eicosanoic acid, diosgenin, and vanillin lactoside were predominant, while *V. penduncularis* yielded compounds like hexadecanoic acid, β-sitosterol, and 2-propenoic acid tridecyl ester. Additionally, various extracts of *A. scholaris* contain bioactive compounds such as lupeol, akuammine, and limonene, which show strong antimalarial effects, especially against *P. falciparum* reported (Sharma *et al*., 2020; Hanne *et al*., 2002; Kapadia *et al*., 1993; Moura *et al*., 2001). This suggests that *A. scholaris* is a promising candidate for further antimalarial research.

A. scholaris bark extracts showed concentration-dependent scavenging of DPPH free radicals. Aqueous and methanol extracts had lower IC_{50} values (6.09 and 11.27 µg/ml, respectively), indicating strong antioxidant properties, though less effective than the standard butylated hydroxytoluene (IC_{50} = 5.73 µg/ml). The methanol extract had the highest total phenolic content (13.56 GAE mg/g), while the petroleum ether extract had the highest total flavonoid content (117.54 QE mg/g). The methanolic extract showed the highest antioxidant activity at 13.56 GAE mg/g and the most effective reducing power, though all extracts showed lower activity compared to ascorbic acid. Methanol and chloroform extracts demonstrated inhibitory effects against both Gram-positive and Gram-negative bacteria. Methanol was most effective against *Salmonella typhi* and least effective against *E. coli*. Petroleum ether and chloroform extracts displayed concentration-dependent antifungal activity, particularly against *Candida albicans.* Chloroform extract showed potent antimalarial activity (IC_{50} = 10.0 µg/ml), outperforming methanol, petroleum ether, and aqueous extracts. Methanol extract exhibited significant activity against the cestode, *Raillietina echinobothrida*, inducing paralysis and death in a dose-dependent manner. Treated parasites showed alterations in tegument and sub-tegument, disorganized parenchymatous tissues, and formation of large excretory vacuole. Treated parasites showed severe shrinkage of the scolex, folding of proglottids, and disruption of the tegument surface, particularly with methanol and albendazole treatments.

CHAPTER 5

DISCUSSION

This chapter examines the justification for the current study and discusses relevant work in the field of significant biological activities of phytochemicals found in plants, particularly *A. scholaris* bark, which exhibits antimalarial, antioxidant, antibacterial, and anthelmintic properties. Plants are primary sources of nutraceuticals and contain vital compounds such as alkaloids, flavonoids, tannins, and steroids that contribute to medicinal and nutritional benefits (Suntar, 2020; Huang *et al.*, 2022). *A. scholaris* bark contains various important phytochemicals

used to produce pharmaceutical drugs and supplements. Free radicals, which result from cellular metabolism, can cause damage to DNA, proteins, and lipids, leading to chronic diseases (Aliss *et al*., 2020; Hajam *et al*., 2022). Antioxidants from plants help neutralize these radicals (Aja & Awuchi, 2023). *A. scholaris* bark shows strong antioxidant activity due to its high levels of phenols and flavonoids, which were measured through reliable assays like DPPH and FRAP. With growing antibiotic resistance, plant-based compounds are being explored as alternatives (Vaoun *et al*., 2021). *A. scholaris* bark shows broad-spectrum antibacterial and antifungal activity, effectively inhibiting various bacteria and fungi, including *C. albicans* and *A. fumigatus*. It has the potential as a source of antibacterial and antifungal molecules. Additionally, *A. scholaris* bark extract has shown promise in the synthesis of silver nanoparticles with strong antimicrobial properties (Shetty *et al*., 2014). The fight against malaria is becoming harder due to *P. falciparum* strains resistant to all antimalarial drugs, including artemisinin-based treatments (WHO 2017). Thus, the search for new antimalarial compounds continues, with medicinal plants being a key focus. Plants have historically provided effective malaria treatments, like quinine and artemisinin, and remain promising for finding novel compounds. Many in poorer nations rely on medicinal plants for healthcare (WHO, 2010), and plant extracts with IC_{50} values below 15 µg/mL are considered potent against malaria (Kweyamba *et al*., 2019). The study found that the chloroform extract of *A. scholaris* bark exhibited significant antimalarial activity against *P. falciparum* with IC_{50} values of 10 µg/mL against 3D7 strain and 14 µg/mL against drug-resistant K1 strain, making it a candidate for new antimalarial drug discovery. Other extracts, such as petroleum ether, showed moderate activity, while methanol and aqueous extracts were inactive. Several other plant species that showed varying degrees of antiplasmodial activity upon treatment with different extracts are also mentioned. For anthelmintic activity, plant extracts, such as *A. scholaris* bark, demonstrated dose-dependent efficacy against gastrointestinal worms, comparable to the standard anthelmintic, albendazole. Methanol and chloroform extracts had stronger effects, causing significant structural damage to the worms, and leading to paralysis or death. Microscopy studies revealed the structural effects of plant extracts on parasites, with treated worms showing severe damage to their body structures. Histological analysis showed extensive

damage to the parasitess teguments and muscle layers when treated with plant extracts, further supporting their potential use as anthelmintics.

Based on the information provided, *A. scholaris* is a potent source of bioactive compounds with medicinal applications in antioxidant, antimicrobial, antimalarial and anthelmintic applications. Among its many reputed benefits, the findings of this study may provide scientific support for its use as a composite antiparasitic agent.

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