SCIENTIFIC EXPLORATION OF SELECTED ETHNOMEDICINAL FORMULATIONS AGAINST SKIN AILMENTS IN MIZORAM AND THEIR ANTIFUNGAL EFFICACY

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
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OF PHILOSOPHY

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DECLARATION

I, **Laldingngheti Bawitlung**, hereby declare that the subject matter of this thesis entitled **"Scientific Exploration of Selected Ethnomedicinal Formulations against Skin Ailments in Mizoram and their Antifungal Efficacy"** is the record of the work done by me, that the contents of this thesis did not form basis of the award of any previous degree 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 thesis is being submitted to the Mizoram University for the degree of **Doctor of Philosophy** in the Department of Horticulture Aromatic and Medicinal Plants.

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CERTIFICATE

This is to certify that the thesis entitled **"Scientific Exploration of Selected Ethnomedicinal Formulations against Skin Ailments in Mizoram and their Antifungal Efficacy"** submitted by **Ms. Laldingngheti Bawitlung (**Ph.D. Regn. No. **MZU/ Ph.D/739 of 19.05.2015)**, in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Horticulture Aromatic and Medicinal Plants of Mizoram University, Aizawl embodies the record of her original investigation under my supervision. She has duly registered and the thesis presented is worthy of being considered for the award of the Doctor of Philosophy (Ph. D.) Degree. The work has not been submitted previously for any degree to this or any other university.

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Place: Aizawl (**Laldingngheti Bawitlung**)

Date:

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CHAPTER - 1

INTRODUCTION

INTRODUCTION

Mizoram (an Indo Burma hot spot region) is tucked away in the southern tip of the North East Region of India. It is a picturesque mountainous state of lush forests pulsating with wildlife and dotted with natural wonders and fabled landmarks. The total area of Mizoram is 21087 sq .km, the total forest area being 15825 sq. km, rural area covers 20,761.31 sq. km and urban area cover 325.37 sq. km. Mizoram is one of the 34 mega-biodiversity hotspots of the world, flanked on the west by Bangladesh and the east and south by Myanmar. Mainly inhabited by Mizos (Lusei, Hmar, Ralte, Lai, Mara, Kuki, Thado etc), their main staple is rice. Mizoram has a mild, sub-tropical climate, tolerably warm in summer, temperature ranges from 20–30 °C (68–86 °F) and not so cold in the winter 11–21 °C (52–70 °F) temperature; rich in flora and fauna.

Though there was a strong influence of modern medicine, a large number of people practice and search for remedies from traditional methods of treatment based on herbal drugs. Information is available on the use of traditionally used medicines and their distribution in the areas with the traditional use of commercially important medicinal plants suitable for developing traditional knowledge for socio-economic upliftment. Information on the medicinal wealth and various aspects of folklore medicines used by the natives of Mizoram has been reported previously (Shankar et al., 2012). As per the WHO, about 80% of the world population is dependent on traditional medicines for primary healthcare. The use of folk medicine, mainly based on plants has been increasing in the recent past and gaining a respectable position and is commonly prevalent in the developing countries due to cultural acceptability, low cost, easy drug chemistry and antibiotics. In many countries including India, it was traditionally available and faith in the system. Plants continue to be one of the major raw materials for drugs treating various ailments of humans. A good amount of modern drugs has been isolated from natural sources that take part in a key role in the treatment of diseases. More importantly, they are not known to cause any notable derogatory effects and are readily available at an affordable price and provide "Socioeconomic security" to millions of people against unemployment.

Biodiversity plays prime importance in human survival, economic well-being and provides the resources upon which families, communities, nations and future generations depend. India is a vast repository of medicinal plants that are used in traditional medicinal treatments (Chopra *et al*., 1956). Various indigenous systems such as Ayurveda, Siddha, Unani and Allopathy use several plant species to treat different ailments (Rabe and Van Staden., 1997). In Indian society, traditional medicine plays a dominant role. For primary health care, tribal people in different parts of India use their traditional ecological knowledge, received from their ancestors and contemporary society. More than 500 traditional communities use about 800 plant species for curing different diseases (Kamboj, 2000). Almost, 70% of modern medicines in India are derived from natural products.

Modern pharmacopoeia also enlists about 25% of drugs derived from plants. Herbal drugs are popular owing to their acceptability, safety and economical nature. This makes the indigenous remedies popular among the people of both the urban and rural areas, especially in South-East Asian countries (Katewa *et al*., 2004). By its population practising several different well recognized indigenous systems of medicine, India occupies a unique position in the world.

Plant-derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparations that are currently recommended by medical practitioners and they form an important part of the healthcare system in the western world (Phillipson and Anderson., 1989). Plants are important sources of medicines and presently about 25 % of pharmaceutical prescriptions in the United State contain at least one plant-derived ingredient. Roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources in the last century. The rich diversity of medicinal plants in India is distributed in different geographical and environmental conditions. India is the home of about 43000 plant species and is one of the 33 hotspots of the world.

Search for new medicines for the prevention and cure of deadly diseases provide prospects for developing medicinal plants. In the present time, pharmaceutical and chemical sciences have greatly contributed to the enhancement of the utility of medicinal plants. Several scientific investigations have been conducted to seek the potential of the extract from the plant or isolated compounds for the continued use of these products in the treatment and prevention of various kinds of human diseases.

Various plant materials are believed to have antifungal activity and many essential oils have been reported to have antifungal activities with no side effects on humans and animals (Sokmen et al., 1999). The essential oils and compounds isolated originating from natural products were enriched with a wide range of activities (Burt, 2004), they represent an antimicrobial activity or can improve the efficiency of antibiotics, improving their mechanism of action and hindering microorganism adaptation (Teixeira, 2009). These antimicrobial agents are obtained majorly from microorganisms, plants and some animal products that exhibit activity that is less or higher than synthesized antibiotics, in some cases, they are a safe and good source of pharmacological effect for a human being (Omoya and Akharaiyi, 2012). The medicinal value of a plant lies in the bioactive physiochemical constituents of the plant and which shows various physiological effects on the human body. The active substances of many species have been isolated and in some cases duplicated in the form of synthetic drugs. Various synthetic antimicrobial drugs have been successfully commercialized in recent years, however, the adverse side effects and the development of resistance by microorganisms encounter major problems (Tolouee et al., 2010). The natural remedies with ethnomedicinal plants have more advantages when compared to those of synthetic drugs but there is an important task to investigate these ethnomedicinal plants scientifically as potential sources of novel antimicrobial compounds.

Plants are an important source of traditional medicine and have been used for the treatment of various skin ailments and dermatological disorders especially cuts, wounds and burns. It has been estimated that skin diseases amount to as high as 34% of all occupational diseases (Spiewak and Szostak, 2000). The skin infections caused by dermatophytes account for 20–25% worldwide, with a prevalence approaching one billion as of 2010 (Havlickova et al., 2008) and become common disorders worldwide to humans and animals (Weitzman and Summerbell, 2001). These infections occur in both healthy and immune-compromised persons. They are not life-threatening but chronic fungal infections of the skin can carry considerable morbidity (Bell-Sayer et al., 1998). Millions of people are affected by superficial fungal infections, which are the most common skin diseases. However, the existing treatments for these infections are still limited to a few antifungal agents and the clinical values of these agents have been limited due to having high toxicity and the emergence of drug resistance in their

antifungal activities (Watanabe., 1999; De Pauw., 2000). On the other hand, Medicinal plants that occurred abundantly in extensive areas are disappearing fast due to the lack of human care, urbanization, developmental activities and population explosion. Thus, it is essential to have proper documentation of such plants and to know the potential and values of medicinal plants for the improvement of health and hygiene through eco-friendly systems. And it is needed medicinal plants have been subjected to rigorous chemical analysis and the bioactive compounds have been isolated and variously evaluated.

Several new topical synthetic anti-dermatophytes drugs have been introduced in the recent past. Important among those are fluconazole, ketoconazole, griseofulvin, terbinafine, miconazole and econazole. Treatment failure and relapses occur with all presently available drugs due to mycostatic nature of these drugs. The ever increasing rate of multiple drug resistance of the pathogens makes it all the more difficult to complete cure of the fungal infections. Even the systemic antimycotic drug griseofulvin, is by no means the perfect treatment of dermatophytosis. All these topical and oral drugs have been found to have various serious side effects, so there is a need for the development of safe, effective and cheap topical chemo-therapeutics for the control of dermatophytoses. Plants and their secondary metabolites are the ultimate choices for this purpose. The knowledge of traditional medicine provides the basis for indicating which biological activities of plant extract and essential oils may be useful for specific medical conditions, so scientific exploration is required to investigate those plants which have been used in traditional medicines. Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required.

Considering the facts and figures, the following objectives were framed.

1. Collection and categorization of some formulated products, using against skin ailments/ remedies and practices among the tribal communities/ traditional healers in Mizoram
2. Physicochemical analysis of the selected formulations
3. Phytochemical analysis of the selected formulations
4. Bioprospection against the pathogenic fungi/ Dermatophytes

CHAPTER -2

REVIEW OF LITERATURE

2.1 Dermal infections:

Skin or dermal infections are prevalent in populations residing in highly humid places and individuals with lower tolerance to commensal skin pathogens. Commensal skin pathogens comprise bacteria like *Staphylococcus*, yeasts like *Candida* and fungi like *Malasezzia*, *Trichophyton* and *Microsporum*. The fungi causing infections are also called dermatophytes and they thrive in keratin degradation while exclusively infecting the stratum corneum, hair, claws or nails. Depending on their host prevalence they are classified as zoophilic or anthropophilic fungi. Zoophilic species cause infections in both animals and humans and are responsible for acute infections while anthropophilic infect only humans and are associated with chronic infections (Hube et al., 2015). Dermatophytes comprise a class of primary pathogenic fungi, that have a high affinity to invade and parasitize the non-living keratinized layer of skin, nails and hair by spreading developing hyphae through the keratin layer. Secretion of a broad spectrum of lytic enzymes especially keratinases represents their most studied virulence factor allowing their colonization and maintenance in the host tissue (Monod; 2008; Peres et al., 2010). Sulphitolysis is likely to be an essential step in the digestion of compact keratinized tissues which precedes the action of all proteases. The infections caused by fungus are classified into "Superficial" or "Dermatomycoses" which affect the outer layer of the skin, hair, nails and mucous membrane, or "Systemic" affecting the body as a whole, and their lesions are characterized by irritation, scaling, local redness, swelling and inflammation (Kyle and Dahl, 2004; Gupta and Cooper, 2008; Patel and Schwartz, 2011). Besides dermatophytes, yeasts from the genera *Candida* and *Malassezia* serve as the most prevalent etiologic agents of fungal infections (Havlickova et al., 2008). Meanwhile, in association with dermatomycoses, the opportunistic yeast pathogens, the genus *Candida*, can quickly colonize damaged nails or skin, especially the mucous membranes (Eggimann et al., 2003), bacterial contaminants have also been found in cutaneous lesions (Chung and Bennet, 1992).

Trichophyton rubrum is considered the most frequently isolated agent of dermatophytosis worldwide and is known to account for as many as 69.5% of all dermatophyte infections (Chen et al., 2001). The conidia produced by asexual sporulation of *T. rubrum* are thought to be the primary cause of human infections (Leng et al., 2008). It is transmitted via direct

contact with infected skin or hair retained in clothing, combs, caps, socks, and towels. Among other dermatophytes, they are the major causative agent for superficial dermatomycoses like onychomycosis and Tinea pedis (Kemnaand Elewsk., 1996; Weitzman and Summerbell, 1995). In addition, they also cause superficial infections such as Tinea capitis, Tinea corporis, Tinea inguinalis, Tinea manus and Tinea unguium. *T. rubrum* is also responsible for deep dermal invasion in immune-compromised patients (Sentamilselvi, 1998).

Microsporum gypseum is filamentous keratinophilic fungi that colonise in the keratinic substrates such as feathers, hair and nails. They are geophilic dermatophytes. According to the new taxonomy, *M. gypseum* is now called *Nannizia gypsea* (de Hoog et al., 2017). *Microsporum gypseum* is an infrequent agent of mycotic infection in humans but are common in domestic animals; occurs in dogs, cats, rabbits and horses. However, the animals may also be healthy carriers (Romano et al., 1997). Most frequently *Microsporum gypseum* caused Tinea corporis and attack various parts of the body. It is also responsible for Tinea capitis, Kerion and Tinea barbae. Immuno depression may cause a greater extension or unusual features of mycosis. According to the World Health Organization (WHO), 30 to 70% of adults are asymptomatic hosts to these pathogens and that the incidence of the disease increases with age (Seebacher et al., 2008; Havlickovaet al., 2008).

Malassezia furfur is one of the major representative species of the *Malassezia* genus which are found mainly in the skin of humans and pets such as dogs and cats, respectively (Cabañes, 2014). The genus *Malassezia* currently comprises 17 species and has recently been assigned its class, Malasseziomycetes (Theelen et al., 2018). They represent a member of a monophyletic genus of fungi. Based on both culture-based and culture-independent methods that used ITS length polymorphisms assessed by polymerase chain reaction (PCR), they are a major component of the skin mycobiome, (Gemmer et al., 2002) representing 50%–80% of the total skin mycobiome using culture-independent methods. They are lipophilic and lipid dependent basidiomycetous yeasts, most often found in lipid-rich body areas, such as the scalp, face, and trunk (Gupta et al., 2004). They may also be found on other areas of the body including arms, legs and genitalia. Although they are commensals, under specific conditions, they become opportunistic cutaneous pathogen and are associated with multiple skin disorders, such as Pityriasis

Versicolor (PV), *Malassezia folliculitis* (MF), Seborrheic dermatitis/dandruff (D/SD), Atopic dermatitis (AD), and Psoriasis (Prohic et al., 2016).

Candida albicans is an opportunistic human fungal pathogen that causes candidiasis and is the most frequently recovered species from *Candida* infected patients. It has emerged as a major public health problem during the past two decades. It is the causative agent and most associated with a serious fungal infection, accounting for more than 90% of cases (Kauffman, 2006). It became the most common cause of deep mycoses and vulvovaginal candidiasis (70 to 90% of vulvovaginitis cases) (Sobel, 2007). Candida infections are regarded as a significant cause of patient morbidity and mortality (Sardi et al., 2013). The infections caused by *Candida albicans* reside mainly on the skin, mucosa and gastrointestinal tract of 30 to 50% of healthy adults at any given time (Brown and Netea, 2007).

Staphylococcus aureus is a potential pathogenic Gram-positive bacterium that was discovered in the 1880s. It is responsible for the vast majority of infections, ranging from minor infections of the skin to post-operative wound infections. It is a leading cause of various human bacterial infections, most notable for its ability to infect any tissue in the human host. The most common sites of *S. aureus* infection is the skin including superficial infections such as impetigo and infected abrasions as well as more invasive infections such as cellulitis, folliculitis, furuncles, carbuncles, subcutaneous abscesses, scalded skin syndrome and infected ulcers and wounds. The mortality rate of individuals with an *S. aureus* infection was about 80% (Skinner and Keefer, 1941) in the early 1940s, prior to the introduction of penicillin for the treatment. Approximately 30% of healthy people are asymptomatically colonized by *S. aureus* (Gorwitz et al., 2008), importantly this colonization is a known risk factor for infection.

2.2 *Litsea cubeba*:

Litsea cubeba called "mountain pepper" belongs to the family Lauraceae, is an evergreen, aromatic tree that reaches a height of 8 - 10 metres with dioecious flowers and small pepper-like fruits. Leaves, branches, fruits and flowers are aromatic. Leaves are 5.2 - 13.5 cm x 1.4 - 3.9 cm, simple, alternate, bright green, glabrous. Inflorescence in umbels is generally 10 - 12 mm x 6 - 7 mm, axillary and solitary. Flower size measures 3.7-5 mm x 4 - 5 mm, yellowish-white. Fruit berries are 4 - 6 mm in diameter, glabrous, green (black when ripe) fruit pedicel 2 - 4 mm long; fruit peduncle 4 - 8 mm long. It grows wild in

south-east Asian countries, including India, China, Bhutan, Nepal, Myanmar, Vietnam, Korea, Taiwan and Indonesia. The essential oil of the plant plays an important role in aromatherapy and treatment of many diseases from ancient times to the present day, widely in perfumery, soap industry, sanitary products and as a deodorizer. The oil is also a popular skincare ingredient for oily, mature and inflamed skin (in moderation). In northeastern states of India, the plant is cultivated for rearing Muga silkworms (Choudhury, 1988).

Zhang et al., (2010) reported that *Litsea cubeba* extract can efficiently and persistently promote hematoid, cholesterol and mucin secretion as well as inhibit gallstone growth. It possesses analgesic, anti-inflammatory and antibacterial effects and was traditionally used for the treatment of rheumatic diseases (Lin et al., 2016), cardiovascular and cerebrovascular diseases (Zhang et al., 2015) and also used for cold treatment in Chinese medicine (Zhang et al., 2014). The methanol extract (Hwang et al., 2005), ethyl acetate extract of barks (Dalimunthe et al., 2016), ethanol extract of heartwood (Dalimunthe et al., 2018), chloroform extract of bark (Yu et al., 1998), alkaloid fraction of fruits (Dalimunthe et al., 2018), essential oil from both the fruit and leaf (Gogoi et al., 2018) and female flower (Zhong et al., 2013) are reported to possess strong antioxidant potential. Citral also plays an important role in antioxidant activity and free radical scavenging (Wang et al., 2012) activity. It was reported that essential oil is slightly toxic at 4,000 mg/kg of body weight (Luo et al., 2005), but the essential oil is applicable for food preservatives, flavoring, antibacterial agents, medicine, health product, and cosmetics (Chen et al., 2018). Citral from fruit essential oil is the precursor for vitamin A synthesis and is potentially useful as a flavour in food, medicinal and other industrial applications (Punyarajun and Nandhasri, 1981). The methanol extract of bark and its fractions (0.01 mg/ml) (Choi and Hwang, 2004), essential oil from both the fruit and leaf (Gogoi et al., 2018), the compound 9-fluorenone, 1-ethoxy-3,7-dihydroxy- 4,6-dimethoxy-9-fluorenone (Lin et al., 2016), 9,9'-Odi-(E)-feruloyl-meso-5,5'-di-methoxy-secoisolarici-resinol (Zhang et al., 2015) and aporphine alkaloids from root and rhizome, boldine and reticuline (Yang et al., 2018) exhibited potent anti-inflammatory activity. Aporphine alkaloids also possess analgesic effects (Zhang et al., 2015). The compound boldine and root extract from ethanol and water possess therapeutic agents for the treatment of human arthritis (Zhao et al., 2017; Lin et al., 2013). Yokogawa et al., (1997) reported that *Litsea cubeba* extract showed melanin formation-inhibiting and tyrosinase

inhibitory activity. It has been reported that plant extract possesses anticancer properties (Zhang et al., 2012) and seed oil had anti-tumour activity (Chang-jiang et al., 2014). Fruit essential oil possesses anxiolytic activity and analgesic activity and has a potent effect on the central nervous system in mice (Chen et al., 2012) also fruit essential oil can reduce cardiac arrhythmia induced in mice by CHCl3 or in rats by BaCl2 (Zhang, 1985). It has been reported that the compound litebamine possess antithrombotic activity and also attained beneficial effects in preventing cardiovascular diseases (Huang et al., 2008). It was also reported that that the compound citral present in essential oil plays an important role in the treatment of coronary heart disease (Wang et al., 1985). Ho et al., (2010) reported that essential oil from leaves and fruit exhibited cytotoxic activity against human lungs, liver and oral cancer cells. Sesquiterpenoid ester glycoside and a monoterpenoid ester glycoside obtained from ethanol extracts of the twigs also exhibited cytotoxicity against A549 and HCT-8 cell lines (Wang et al., 2018). Lignans of *Litsea cubeba* 7',9-epoxylignans with feruloyl or cinnamoyl groups were selectively cytotoxic against NCI-H1650 cell line also dibenzylbutyrolactone lignans 17-19 exerted cytotoxicities against HCT-116 and A2780 cell lines (Li et al., 2019). Benzylisoquinoline alkaloid N-methoxycarbonyl-norjuziphine are cytotoxicity against HL-60 and MCF-7 cells (Tang et al., 2017). The compound Cubelin exhibited activity against HeLa cell viability and proliferation (Trisonthi et al., 2014). The compound isolated from the root and stem exhibited a significant neuroprotective effect against hydrogen peroxide-induced oxidative damage in the rat adrenal pheochromocytoma (PC12) cell line (Guo et al., 2016). The citral of essential oil exhibits an immunosuppressive effect on dendritic cells and mice and can potentially be applied in the treatment of contact hypersensitivity, inflammatory diseases, and autoimmune diseases (Chen et al., 2016). It has been reported that *Litsea* essential oil is applicable as a detergent (Lin et al., 2017) and the kernel oil and seeds oil is applicable for the production of biodiesel (Zhong et al., 2009). The essential oil has also a repellency effect against yellow fever mosquito, *Aedes aegypti* (Linnaeus), the malaria vector, *Anopheles stephensii* (Liston), and the filariasis and encephalitis vector, *Culex quinquefasciatus* (Amer et al., 2006). Chloroform extract of fruit possesses natural insecticide-like activities against *Zeamais motschulsky* (Coleoptera: Curculionidae) (Zhang et al., 2017). The compound chlorobutanol from fruit extract showed fumigant toxicity and contact toxicity and repellent activity against *Sitophilus zeamais* (Zhang et al., 2017). Jeon et al., (2016) has reported that fruit essential oil possesses eco-friendly acaricides against house dust mites *Dermatophagoides farinae*,

Dermatophagoides pteronyssinus and stored food mite, *Tyrophagus putrescentiae.* The mixture of *Litsea cubeba* and *Litsea salicifolia* essential oil showed activities for mosquito repellents (*Aedes aegypti*). 0.075% demonstrated the highest non-contact repellency (62.7%) (Noosidum et al., 2014). *Oleum Litsea* possess a parasiticide effect on mite, louse, and flea, it also attains antibacterial and antifungal effects (Wang et al., 2003).

Su et al., (2016) reported that the excellent antimicrobial properties of fruit oil were mainly due to the presence of the compound Citral. It was also reported the presence of Aldehydes and alcohol is attributed to the antimicrobial properties of *Litsea cubeba* oil (Li et al., 2013). The extract obtained by supercritical CO_2 extraction possesses antibacterial properties against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger* (Zhou et al., 2019). Essential oil possess antimicrobial properties against *Fusarium moniliforme*, *Fusarium solani*, *Alternaria alternata* and *Aspergillus niger* (Gogoi et al., 1997), *Bacillus subtilis*, *Escherichia coli*, Actinomyces 5406 and *Staphylococcus aureus* (Yu et al., 1999), *Candida albicans*, *Candida tropicalis*, *Candida glabr*ata, *Candida parapsilosis*, *Candida krusei* (Fang et al., 1994), *Aspergillus flavus* (Luo et al., 2004), *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Lactobacillus plantarum*, and *Hansenula anomala* (Liu and Yang, 2012), *Pseudomonas aeruginosa* (Wu et al., 2013), *Streptococcus mutans*, *Streptococcus sobrinus* and *Streptococcus sanguinis* (Yang et al., 2013), *Penicillium funiculosum*, (Li et al., 2013), *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton erinacei*, *Trichophyton* terrestre and *Microsporum gypseum* (Nardoni et al., 2015), *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum* and *Penicillium expansum* (Tripathi et al., 2016), *Saprolegnia parasitica* (Nardoni et al., 2019), *Salmonella* Typhimurium (She et al., 2020), Methicillin-Resistant *Staphylococcu aureus* (Hu et al., 2019), antimycotic activity against *Aspergillus flavus* and *Ascosphaera apis* (Nardoni et al., 2018). The fruit essential oil possesses antimicrobial activity against *Escherichia coli* (Li et al., 2014). Contact and fumigant toxicities against *Sitophilus zeamais* and *Tribolium castaneum* have also been reported (Ko et al., 2009). Acaricides against *Dermatophagoides farina*, *Dermatophagoide spteronyssinus* mites and *Tyrophagus putrescentiae* (Jeon et al., 2016). Candida, Sporothrix and Cryptococcus (Tu et al., 1985), *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* (Saikia et al., 2013). Fruit extracts possess

natural insecticide against *Sitophilus zeamais* (Zhang et al., 2017). The Leaf oil possesses bactericidal properties against *Aeromonas hydrophila, Edwarsiella tarda, Vibrio furnissii, Vibrio parahaemolyticus, Streptococcus garvieae, Escherichia coli, Salmonella* Typhimurium (Nguyen et al., 2016), *Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* (Saikia et al., 2013). The essential oil from the leaves also has great potential as botanical insecticides against *Tenebrio molitor* (Wang et al., 2015). The seed oil possesses antimicrobial properties against aflatoxin production of *Aspergillus flavus* and *Saccharomyces rouxii* (Yu et al., 1998). It has been reported that Citral plays the main antifungal active component against *Fusarium oxysporum, Helminthosporium*, and *Stemphylium* (Huargliang et al., 1994). The compounds 5, 8-dihydroxy-6, 7-dimethoxyflavone possess antimicrobial properties against *Trichothecium roseum* (Shuaiwen et al., 2011). (+)-N-(methoxylcarbonyl)-N-nordicentrin, (+)-N-(methoxylcarbonyl)-Nnorpredicentrine, and (+)-N-(methoxylcarbonyl)- N-norglaucine showed antimicrobial activity against the bacterium *Staphylococcus aureus* and two fungi *Alternaria alternata* and *Cercospora nicotianae* (Zhang et al., 2012). 2, 6-dimethyl-6- hydroxy-2E, 4E-hepta-2, 4-diene acid, (6R)-3,7-dimethyl-7-hydroxy-2-octen-6-olide showed good fungicidal activities against *Sclerotinia sclerotiorum, Thanatephorus cucumeris, Pseudocer-cospora musae* and *Colletotrichum gloeosporioides* (Yang et al., 2010). Linalool-typeleaf essential oil possesses antibacterial activities against *Escherichia coli* (Nguyen et al., 2018). It has been reported that 1, 8-cineole and linalool type *Litsea cubeba* essential oil showed bactericidal activities against *Escherichia coli,* the essential oil which is enriched with the compound 1, 8-cineole can affect cell membrane, leading to cell filamentation and perturbation of cell width, while the linalool-rich induced damages in the cell membrane and changes in the nucleoid morphology (Nguyen et al., 2018). Citral and linalool showed strong contact toxicity and fumigant toxicity against *Lasioderma serricorne* and *Liposcelis bostrychophila* (Yang et al., 2014). Citral acetal 1, 2-propanediol, β-ionone and iron also possess powerful anti-fungal properties against *Poria vaporaria, Trichoderma viride* and *Caribena versicolor*. Further, it has also been reported that the cell membranes of the fungi were damage by this compound (Guo et al., 2016).

Choudhury et al., (2009) reported that the *Litsea cubeba* plants which possess citral and sabinene are found up to the altitude of 1660m, whereas the availability of linalool and citronellal was between 125 and 1000m. It has also been reported that the compound

Neral and Geranial played an important role in the sweet-lemon fragrance of the flowers (Asakawa et al., 2017). It has been reported that the fruit diameter of *Litsea cubeba* was 0.55-0.62 cm, fruit longitudinal diameter was 0.57-0.65 cm and one thousand fruit weight of 103.89-141.48 g; (He et al., 2012). It was reported that *Litsea cubeba* contains palmitic acid; oleic acid and linoleic acid (Yu et al., 2008), rutin (Zhang et al., 2009). Nucleolus oil contains capric acid and hydroxyalkyl amide (Wang et al., 1993). The main components of essential oils are Ethyl acetate, α-pinene, β-pinene, camphene, limonene, p-cymene, β -methylheptеneketone, methylheptylketone, citronellal, linalool, α -a-isopulegol, citral b, α -terpinol, citral a, citronellol, geraiol, safrol, and eugenol (Zhan et al., 1985) and Citral 64.6% (Li et al., 1998), Methylheptenone, citronellal and citral (Yu et al., 2018). β -Ionone (Fan et al., 2002), 6-methyl-5-hepten-2-one, limonene (Yu et al., 2007), myrcene, 6-methyl-heptenone, (Zhang et al., 2010), 2,6-dimethyl-6- hydroxy-2E,4E-hepta-2,4-diene acid, (6R)-3,7-dimethyl-7-hydroxy-2-octen-6-olide, along with three known compounds - vanillic acid, trans-3,4,5-tri methoxyl cinnamyl alcohol, and oxonantenine (Yang et al., 2010), Geranial, neral and d-limonene (Chen et al., 2012), Methyl heptenone (Zhao et al., 2015). Total fruit essential oil content ranged from 3.04% to 4.56%, dominated by monoterpenes (94.4-98.4 %), represented mainly by neral and geranial (78.7-87.4 %) and Limonene (0.7-5.3 %), ocymene and eremophilene (Si et al., 2012) and Citronellal (Saikia et al., 2013). The other compound present in the fruit essential oil were methyl-heptenone, linalool, laurene, β-pinene, eucalyptus, terpinol (Chen et al., 2018), Methyl heptenone 30.9% (Gogoi et al., 2018), Citral (70%) and methyl heptenone (20%), dipentene (Kahuku and Kato,1938), citronellol (10.9-14.0%) (Choudhury et al., 1998), C9 monoterpenoid acid (2,6-dimethyl-6-hydroxy-2E,4E-hepta-2,4-diene acid), monoterpene lactone (6R)-3,7-dimethyl-7-hydroxy- 2-octen-6-olide, vanillic acid, trans-3,4,5-tri methoxyl cinnamyl alcohol, and oxonantenine (Yang et al., 2010), sabinene (Choudhury et al., 2009). Unripe fruits yield 3.04% of volatile oil and are constituted mainly of citral (49.6%) (Punyarajun and Nandhasri, 1981). Dried cubeb berries were characterized by a higher level of d-limonene ("fruit, citrus"), citral ("fruit, lemon") and dodecanoic acid (Cheng et al., 2018). Chloroform extract of fruit contains the components Laurine (21.15%) and 2,6-diisopropyl aniline (16.14%), followed by chlorobutanol (10.54%), 3-O-methyl-N-acetyl-d-glucosamine (10.03%), and 6-methyl-5-hepten-2-one (8.33%) (Zhang et al., 2017), Citral (Liu et al., 2015),E-citral (geranial) (27.49%), Z-citral (neral) (23.57%) and D-limonene (18.82%) followed by 3,7-Dimethyl-7-hydroxy-2-octen-1,6-olide and 3,7-dimethyl-2,6-octadien-1,6-olide (Dong et al., 2013),

citronellal, d-limonene and citronellol (Hammid and Ahmad, 2015), alpha-pinene, beta-phellandrene, sabinene, and 1,8-cineol (Vongsombath et al., 2014), Diterpene, identified as (+)-6-(4-hydroxy-4-methyl-2-pentenoyl)-4,6-dimethyl-5-(3-methyl-2-butenyl)-1,3 cyclo-hexa-diene-carbaldehyde (1, cubelin) were isolated from methanol fruit extract (Trisonthi et al., 2014). Fruit ethanol extracts contain d-limonene (8.52%), α-citral (26.15%), β-citral (33.16%), hydrocarbon monoterpenes, monoterpenes oxide and aliphatic acids and esters. P-Cymene, thujanol, dlimonene, linalool, caryophyllene, (Hu et al., 2011). Seed oil possesses fifty-two compounds which were 95.65% of the total essential oil (Chang-jiang et al., 2014). Ether extract of seeds gave 44% of fatty substance, solid below 30°. Steam distillation extraction gave 3.1% of almost colourless volatile oil, consisting mainly of citral and methyl heptenone. The fatty matter1.3% unsaponifiable matter was obtained, from which 3.5% sitosterol was isolated as digitonide. It was also reported that the constituents of the acid mixture are lauric 53.2%, capric 22.9%, myristic 4.5%, unsaturated acids 19.6%. Linderic, tetradecenic, oleic and linoleic acids and a minute amount of hexadecanoic acid (Hata, 1939). The main constituents in the kernel were reported ascAlkanolamide (Li et al., 1997), the other compound present in the kernel were 6-methyl-5-hepten-2-one, d-Limonene, 3, 7-Dimethyl-1, 6-octadien-3-ol, alpha, alpha-4-trimethyl-3-Cyclohexene-1-methanol, 3-Methylcrotonaldehyde, n- Decanoic, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-Cyclohexene, and 2, 6, 6-trimethyl-Bicycloc[3.1.1] heptan-3-one (Chang-jiang et al., 2013). The essential oil of fruit peel obtained 91.95% of the total oil and possess D-Limonene, 3,7-dimethyl-1,6-octadien-3-ol, (Z)-3,7-dimethyl-2,6-octadienal, (E)-3,7-dimethyl-2,6-octadienal, geranic acid, and caryophyllene oxide compounds (Chang-jiang et al., 2013), Citral 71% (Lin and Hua, 1987). Flower bud essential oil contains 40 compounds which accounted for 87.27% of the total contents. There were 14 monoterpenes, which accounted for 21.90%, 12 monoterpene oxides, accounted for 60.44%, and 12 sesquiterpenes, accounted for 4.85% (Luo et al., 2012). Flower essential oils were rich in sabinene (41.8-42.3%), citronellal (14.3-17.3%), β-phellandrene (7.7-9.0%), α-pinene (6.6-7.6%) and β-pinene (5.8-6.1%) (Choudhury et al., 1998). The major constituent of the Inflorescence part is α-terpineol and cis-β-terpineol (Wang et al., 2011). Stem bark essential oil contains citronellol (11.9- 20.4%) and 7.7-10.0% of citronellal (Choudhury et al., 1998), limonene (Su and Ho, 2016). 6-selinene-4-ol, (R)-4-terpineol and α-citral (Luo, 2011), 1,8-cineole, caryophyllene, neral and geranial (Cheng et al., 1983). Eucalyptol, d-limonene and α-terpineol (Hammid et al., 2015). The essential oil

from the trunk possesses d-Limonene, linalool, citronellal, and some pelargonic acid. Major compound in the leaf essential oils are Cineol (80%), camphene (4%), α-terpineol (7%), and sesquiterpene (1%) (Kato, 1951), 1,8-cineole, α-terpinyl acetate, sabinene, and α- and β-pinene (Cheng et al., 1983), Linalool 78.3% (Nath et al., 1996), Alpha-cis-ocimene (25.11%), 3,7-dimethyl-1,6-octadien-3-ol(16.85%) and n-transnerolidol (13.89%) (Wang et al., 1999), 1,8-cineol (Ho et al., 2010), eucalyptol (39.86%), 3-cyclohexene-1-methanol, α, α,4-trimethyl-(S)-(15.62%), α- phellandrene (4.79%) (Luo et al., 2012), Citral, Citronellol, 6-Octenal, 3,7-dimethyl- (Zhao et al., 2010), β-elemene, γ-elemene, caryophyllene and caryophyllene oxide (Wang et al., 2011), Flavonoids 2.14% (Chen et al., 2012), a-terpineol (Hammid et al., 2015) and (E)-3, 7-dimethyl-2, 6-octadienal (49.78%) (Wang et al., 2015). Essential oil from the root contains limonene, α-citral and β-citral linalool, citronellal, α-pinene, camphene, citronellol, α-terpineol, and 2-methyl-6-keto-2-heptene (Jian, et al., 1986), Lignoceric acid, β-sitosterol, β-daucosterol, atheroline, N-Me laurotetamine, laurotetamine, N-trans-feruloyl-3-methoxytyramine and N-cis-feruloyl-3-methoxytyramine (Zhu et al., 2007), Citral, 6-Octenal, 3,7-dimethyl-, 2-Octen-1-ol, 3,7-dimethyl (Zhao et al., 2010), 9-fluorenone, 1-ethoxy-3,7-dihydroxy- 4,6-dimethoxy-9-fluorenone, pinoresinol, syringaresinol, 9,9'-Odi-(E)-feruloyl-meso-5,5' di-methoxy-secoisolariciresinol and lyoniresinol (Lin et al., 2016), litsecols A and litsecols B (Guo et al., 2016). (+)-norboldine, (+)-boldine, (+)-reticuline, (+)-laurotetanine, (+)-isoboldine, (+)-N-methyl-laurotetanine, and berberine (Zhang et al., 2014). Water-soluble constituents of twenty-five known aromatic glycosides and three known sesquiterpene glycosides were obtained from the twigs which were (7S,8R)-dehydro-diconiferyl alcohol 4,9'-di-O-b-Dglucopyranoside, (7S,8R)-5 methoxy di-hydro-dehydro-diconiferyl alcohol 4-O-b-D-glucopyranoside, (7S,8R)- urolignoside, (7R,8S)-dihydrodehydrodiconiferyl alcohol 4-O-b-D glucopyranoside, saposide B, lanicepside A, matairesinol-4-O-b-D-glucopyranoside, tyraxjaponoside B, (+)-lyoniresinol-9'-O-b-D-glucopyranoside, alaschanisoside A, syringin, psoralenoside, isopsoralenoside, scopolin, 2,6-dimethoxy-4- hydroxyphenol-1-O-b-D-glucopyranoside, 3-hydroxy-4,5-dimethoxyphenyl-b-D-glucopyranoside, 2-(3,4- dihydroxyphenyl)ethyl-b-D-glucopyrnoside, 2-(4-dihydroxyphenyl)ethyl-b-D-glucopyranoside, (+)-catechin-7- O-b-D-glucopyranoside, 3'-O-methylepicatechin-7-O-b-D-glucopyranoside, kaempferitrin , quercetin-3-O- a-L-rhamnopyranside (22), kaempferol-3-O-b-D-glucopyranoside, kaempferol 3-O-b-D glucopyranosyl(1-2)-O-b- D-galactopyranoside-7-O-a-L-rhamnopyranoside, quercetin 3-O-a-L-rhamnopyranosyl(1®6)-O-b-

Dglucopyranosyl(1®3)-O-a-L-rhamnopyranosyl(1®2)-O-b-D-glucopyranoside, staphylionoside D, vomifoliol 9- O-b-D-glucopyranoside (27), dihydrovomifoliol-O-b-D-glucopyranoside (28). Compounds 1-21 and 24-28 were obtained from this genus for the first time (Wang et al., 2017). Two sesquiterpenoid ester glycoside monoterpenoid ester glycoside have been isolated from an ethanol extract of the twigs of *Litseacubeba* (Wang et al., 2018). It has also been reported that the reaction of acetone with citral from essential oil can produce pseudoionone (Huang et al., 1993).

2.3 *Cymbopogon flexuosus* (lemongrass):

Cymbopogon flexuosus of the Poaceae family belongs to the genus Cymbopogon and is a tall perennial grass. It comprises a genus of about 55 species of grasses, native to warm temperate and tropical regions of the Old World and Oceania. The floral arrangement of this aromatic grass gives it the name 'Cymbopogon'. It is widespread in the semi-temperate and tropical regions of Asian, American and African continents. The plant is erect, attains a height up to 1.8 meters and 1.2 meters in width and grows in clusters. It has long, slender, drooping bright green leaves that measure from 1.3-2.5cm in width and 0.9 meters in length. Leaves are simple with entire margins, sheaths are cylindrical, barren shoots widened at the base and tightly clasping at the bottom, others narrow and separating. Flowers grow on spikes. It has a lengthy inflorescence approximately one-meter long. A strong lemon fragrance is a predominant feature of this grass which is due to the high citral content in its oil (Ranade et al., 2015).

Somparn et al., (2014) reported that in Southeast Asia lemongrass is traditionally used as an analgesic, antipyretic and antiseptic, it also possesses therapeutic use in hypertension and diabetes treatment (Campos et al., 2014). It has been reported that plant extract is useful for cardioprotective and anti-lipid peroxidation by increasing various antioxidants at a dose of 200 mg/kg body weight (Gayathri et al., 2011). The report says that ethanol and aqueous extracts (200 mg/kg body wt.) were safer with no adverse effects and did not exert oxidative damage and is possible for use in weight gain (Ademuyiwa et al., 2015). Kanatt et al., (2014) reported that aqueous extracts of lemongrass possess the antioxidant potential and were able to protect against radiation-induced DNA damage in pBR322 plasmid. The essential oil was also enriched with anti-oxidant activity (Li et al., 2015), (Samusenko, 2008), (Shaker et al., 1999) and Anti-inflammatory activity (Boukhatem et al., 2014). The antioxidant properties of the plant can prevent endothelial dysfunction associated with an oxidative imbalance promoted by different oxidative stimuli (Campos

et al., 2014). Hot water extract showed significantly ($P<0.05$) higher DPPH radical scavenging ability, Fe2+ chelating ability and OH* scavenging ability (Oboh et al., 2010). Aqueous extracts of the plant exhibit antioxidant enzymes induction *in vivo* (Somparn et al., 2014), besides possessing a nephroprotective effect in Wistar albino (Ademuyiwa et al., 2015). Puatanachokchai et al., (2002) reported that the plant extract possesses inhibitory effects of the early phase of hepatocarcinogenesis in rats. It has also been reported that methanol extract of the leaf has anti-diarrheal activities and was also found to be non-toxic (Okere et al.,2015). The compound citral was effective in the prevention of influenza infection (Imayoshi et al., 2011). Lemongrass synthesized zinc oxide nanoparticles have a potential for cancer treatment, and they can enhance the reactive oxygen species levels, inhibit cell proliferation and prominent cytotoxicity against THP-1 leukaemia cells (Kumari et al., 2018). The compound Citral 3,7-dimethyl-2,6-octadien-1-al of essential oil possess anti-cancer potential (Dudai et al., 2005). The compound citral also exhibits a potential to control the locoregional spread due to any remaining cancerous cells (White et al., 2017), It can also potentiate the cytotoxicity of doxorubicin by increasing apoptotic effects, (Dangkong and Limpanasithikul,2015). 80%-Ethanol extracts showed chemoprevention in an animal carcinogenesis model (Suaeyun et al., 1997), it also possesses anti proliferative efficacy against human cancer cell lines (Halabi et al., 2014) and it can inhibit the formation of azoxymethane-induced DNA adducts and aberrant crypt foci formation in the rat colon (Suaeyun et al., 1997). Geraniol possesses a potential anti-inflammatory agent and a capability to obstruct 4NQO initiated NF-κB activation and modulated the expression of inflammatory mediators (Madankumar et al., 2017). Citral of essential can be used for the synthesis of vitamin A, ionones and play an important role in perfumery and flavoring (Rao et al., 1993). It has been reported that 2.5% essential oil was demonstrated to be the minimum concentration for the preparation of antifungal cream for subsequent clinical study (Wannissorn et al.,1996). It also has been reported that a single dermal application of the undiluted essential oil (a volume of 0.5 mL) can cause very slight to severe oedema, erythema, and eschar formation in rabbits but no adverse effects on clinical signs, body weight (Shin et al., 2005). The compound Myrcene, a monoterpene from essential oil was capable of inducing antinociception in mice (Rao et al., 1990). Lemongrass essential oil can also be used in biological control programs, as well as, it can play an effective role in the integrated management programs against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Heikal, 2015).

Ramroop et al., (2018) has reported that the antimicrobial properties of Lemongrass essential oil are due to the presence of citral. The essential oil can inhibit the effect of Candida biofilm formation and its major constituents can inhibit germ tube formation (Taweechaisupapong et al., 2012). Methanol extract showed antimicrobial activities against *Escherichia coli, Salmonella typhi, Shigella* spp. and *Staphylococcus aureus* (Shinde et al., 2015). Aqueous extract of lemongrass species was active against *Staphylococcus aureus, Escherichia coli, Salmonella choleraesuis* and *Proteus vulgaris* (Bajpai et al., 2018). Ethyl acetate leaf extract showed the inhibitory activity against *Microsporum canis* and *Microsporum gypseum* (Krishnaveni et al., 2016). The vapour phase of lemongrass oil is highly effective against *Escherichia coli* (Tyagi and Malik,2012). 80% of ethanol extracts possess antimutagenic properties towards chemical-induced mutation in *Salmonella* Typhimurium strains TA98 and TA100 (Vinitketkumnuen et al., 1994). The essential oil showed vibriocidal activity against *Vibrio comma* (Nayak et al., 1961), anti-microbial properties against *Trichophyton mentagrophytes, Trichophyton rubrum, Epidermophyton floccosum,* and *Microsporum gypseum* (Wannissorn et al.,1996), *Microsporum cookei*, *Microsporum mycetomi* (Kokate and Verma,1971), *Salmonella enterica* and *Escherichia coli* (Friedman et al., 2002), *Pseudomonas aeruginosa* (Oboh et al., 2004), *Staphylococcus aureus, Candida albicans* and *Aspergillus niger* (Okamoto et al., 2005), *Tribolium castaneum* (Chahal et al., 2007), *Alternaria alternata, Fusarium oxysporum*, and *Penicillium roquefortii* and yeasts *Candida oleophila, Hansenula anomala, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Saccharomyces uvarum*, and *Metschnikowia fructicola* (Irkin et al., 2009), *Aspergillus flavus* and *Aspergillus ochraceus* (Eissa et al.,2008), *Candida tropicalis* (Boukhatem et al., 2014), *Streptococcus mutans* (Banu and Geeta, 2015). *Staphylococcus epidermidis* (Utakod et al.,2017), *Microsporum canis, Microsporum fulvum* (Jain et al., 2017), *Listeria monocytogenes* (Ramroop and Neetoo,2018), *Aeromonas hydrophila, Aeromonas caviae, Citrobacter freundii, Salmonella enterica, Edwardsiella tarda* and *Proteus mirabilis* (De Silva et al., 2017), *Listeria monocytogenes* (Friedman,2002). It has been reported that major constituents of essential oils in the gaseous state contains antimicrobial properties against *Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes* and *Staphylococcus aureus* (Inouye et al., 2001) also vapour phase of essential oil is highly effective against *Candida albicans* (Tyagi and Malik, 2010). The essential oil in combination with phenoxyethanol has the potential to increase antibacterial activity against *Escherichia coli*

and *Staphylococcus aureus* (Onawunmi**,**1988). Combinations of essential oil and Amoxicillin possess antimicrobial activity against all methicillin-resistant *Staphylococcus aureus*. Besides, the essential oil also plays a key role in the elevation of susceptibility to Amoxicillin (Abd el-kalek and Mohamed,2012). Citral of essential oil can completely inhibit the mycelial growth of fungus *Colletotrichum acutatum* (Alzate et al., 2009).

Yang and Hwang (2000) reported that essential oil of lemongrass possesses germicidal power, Anti-mycotic activity (Lean and Mohamed, 1999), acaricidal activity on larvae and engorge cattle ticks (*Boophilus microplus*) (Chungsamarnyart et al., 1992), acaricidal properties against the cattle tick (*Rhipicephalus microplus*) (Pazinato et al., 2016), repellent activity against *Aedes aegypti* (Hsu et al., 2013). It has also been reported that the essential oil posse's anti-angiogenic with 99 ± 0.8% of inhibition at 100 µg/mL and potential cytotoxic effect on HCT-116 with the IC50 value of 27.41 ± 4.3 mg/mL (Piaru et al.,2012). Singh et al., (2019) reported that a combination of cinnamon essential oil and lemongrass essential oil showed acaricidal activity against *Rhipicephalus* (Boophilus) *microplus*. A combination of negative air ion essential oil vapours has a greater bactericidal effect (100% reduction in viability) (Tyagi and Malik, 2012). Essential oil microencapsulated polyester fabric possesses the highest mosquito repellent activity at the concentration of 92% (Anitha et al., 2011). It has also been reported that essential oil possesses mosquito repellent activity and the compound Citral 3,7-dimethyl-2,6-octadien-1-al of essential oil possess insecticidal properties (Abdalla, 2000). Whole-plant extracts also possess anti-malarial properties against *Plasmodium chabaudi* AS or *Plasmodium berghei* ANKA (Chukwuocha et al., 2016) and anti-repellent activity against adult sand flies, *Phlebotomus duboscqi* (Kimutai et al., 2017).

Joshua et al., (2012) reported that the moisture content of lemongrass leaves was 13.50%, ash content 11.17%, fat content 10.00%, crude fiber 19.54%, crude protein 17.50 % and carbohydrate 28.29 % and the stem moisture content was 7.98%, ash content 14.29%, fat content 13.50%, crude fiber 12.5%, crude protein 17.50 % and carbohydrate 34.23 %. It has been reported that the yield of fresh lemongrass essential oil ranged from 0.26% to 0.52% and the citral content of the oil ranges from 78 to 85.5% and the highest percentage of oil was obtained when the grass was harvested late in the season (9 months old). It has also been reported that dry steam distillation gives a higher yield of essential oil than water distillation also higher yield of essential and aldehyde content was obtained from distillates run at 40° than from those run at 85° (Squibbs, 1938). Oliveros-Belardo et

al., (1978) reported that lemongrass collected in the hot season gave the highest amount of oil (0.3-0.48%) and 31.05-45.21% citral, as compared to 0.1-2.8% and 25-39.14% obtained during the cool season. It has also been reported that hilly areas gave a higher yield of oil than that thrived in the lowlands. Kumar et al., (2015) reported that the maximum essential oil obtained in a sun-dried sample was (2.50 %) and the oil yield from the fresh sample was (2.52%), also it was reported that different drying treatments did not show any significant changes for the main components (Citral a and Citral b) of the essential oil when compared to the fresh sample. It was reported that the citral content in the aqueous ethanol solution of the oil of lemongrass (*Cymbopogoncitratus*) increase with the age of the plantation (Spoon,1954). Shaikh et al., (2019) reported that Rhizosphere fungi *Trichoderma viridae* have the potential to enhance the production of essential oil and the content of citral a, citral b and linalool compounds. Supercritical extraction of Lemongrass essential oil with CO_2 extracts more than 68% citral of the essential oil and the extraction yield was 0.65% while the yield obtained from hydrodistillation was 0.43% with 73% citral content (Marongiu et al., 2006). Lemongrass leaves contains appreciable levels of Sodium (18.65 ± 0.05), Magnesium (20.86 ± 0.01), Potassium (30.31 ± 0.01), Calcium (5.27 ± 0.02), Iron (0.99 ± 0.01) respectively, also the anti-nutrient compound for phytic acid (0.16 ± 0.01), cyanide (0.35 ± 0.05), tannin (14.47 ± 0.02) and oxalate (2.37 ± 0.01) in mg/100g (Oyeleke**,** 2009). Wariyar (1954) reported that Citral was the major compound of the essential oil. The other compound which are found in the essential oil are 20% myrcene, and trace of linalool (Fujita,1951), l-α-thujene, l-α-pinene, l-camphene, l-limonene, l-borneol, l-terpineol and a residue 4.8% of the total oil, sesquiterpene hydrocarbons and alcohols and some diterpenic derivatives (Chakravarti et al., 1954), Aldehydes and ketone (Stenlake and William, 1957), furfural; terpenesmyrcene (10-20%) and dipentene (trace); alcohols (1-2%)-geraniol, linalool, nerol, and methylheptenol; ketones methyl heptenone (0.2-0.3%); and sesquiterpenes (1-0%) (De Silva, 1959), 80% citral, 12% myrcene, 3-4% dipentene, and a trace amount of methylheptenone (Rovesti and Variati, 1960), Farnesol and nerolidol (Naves, 1960), Citronellol and tetrahydrogeraniol (Daniewski and Strojny et al.,1962), oxygenated monoterpenes (78.2%); α-citral or geranial (36.2%) and β-citral or neral (26.5%), monoterpene hydrocarbons (7.9%) and sesquiterpene hydrocarbons (3.8%) (Tyagi and Malik, 2010), nerolic acid, and geranic (Sargenti and Lancas,1997). Geranialdehyde, rhodinal, nerol (Zhao,2001), cis-pino carveol (20.2%) have also been reported by Ali and Singh, (2004). Further, Propyl amyl ketone (1.88%), (3E)-3,7-dimethylocta- 3,6- dienal

(1.43%) and caryophyllene oxide (1.07%) (Jain and Sharma, 2017). (E)-3,7-dimethyl-2,6-octadienal, neral (Z)-3,7-dimethyl-2,6-octadienal, geraniol (E)-3,7-dimethyl-2,6-octadien-1-ol, 1-butyl-3-methylimidazolium chloride ([C4mim] Cl) and1-ethyl-3-methylimidazolium methyl phosphonate ([C2mim][(MeO)(H)PO2]) have also been reported (Murata et al., 2017). Essential oil vapor contains -Myrcene (3.5%), Limonene (30.3%), Camphene (6.5%), α-Citral (17.6%), β-Citral (11.3%), 6-Me hepten-2-one (14.6%) and linalool (1.5%) (Tyagi and malik, 2012), 3,7-dimethyl-2,6-octadienal acetals (Citral acetals) also citral ethylene glycol acetal and citral propyleneglycol acetal are obtained by redistillation (Shahzadi et al., 2014). 80% acetone extract contains phenolic compounds (Sepahpour et al., 2018). Yeh (1973), reported that the roots part of the plant attains 0.2% essential oil and contains 3.1% terpene aldehydes, mainly citral a and a small amount of citral b. About 80.8 % of the root oil is sesquiterpenes with cadinene making up 30.2%, and Sesquiterpene alcohol consists mainly of a cadalene-type bicyclic sesquiterpene alcohol (13.6% of the root oil). Ethanol extracts of both the leaves and stems contain tannins, flavonoids, phlorotannins and cardiac glycosides but the absence of alkaloid and saponin. Chloroform extracts contain saponins, glycosides and volatile oils (Vellore et al., 2016). Trace elements, namely, Mg, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Mo, As, Cd, and Pb. Toxic metals like As, Cd, and Pb, analysed are within the tolerable daily diet limit and at low concentrations (Anal,2014).

2.4 *Zingiber officinale*:

Ginger has been cultivated for thousands of years as a spice. The most well-known member of Zingiber (ginger) is *Zingiber officinale* belongs to the family Zingiberaceae and is native to Southeast Asia, comprising of 22 genera and 178 species in India (Jain et al., 1995). It occurs chiefly in the tropics. The plant is an herbaceous, perennial flowering plant, inflorescences are solitary, carry pale yellow with purple flowers and the plant reaches up to 90 cm in height. The leaves are simple, distichously narrow, alternate, lanceolate and oblong with a sheathing base (Jyotsna et al., 2017). The flowering stalk or inflorescence arises directly from the root ending in a solitary, pedunculated oblong scallop spike. (Ali et al., 2008) The flowers are calyx superior, gamosepalous, open splitting on one side. The rhizome and leaves are widely used around the world in food as a spice and as folk medicine. The use of ginger in food preparation play important role in the maintenance of health as well as prevention of food spoilage. The volatile oil, gingerol and other pungent principles not only give ginger its pungent aroma but are also

therapeutically powerful. Ginger is also reported to prevent rancidity, thereby increasing the shelf life of lipid-containing foods. More than 400 accessions of ginger are maintained at the Indian Institute for Spice Research in Calicut, Kerala, India (Vasala, 2004).

Ginger and its compounds have antimicrobial, immunomodulatory, cytoprotective/regenerative actions and possess a potential source for the treatment and prevention of necrotizing enterocolitis (Cakir et al., 2018). Indeed, in traditional medicine, ginger is administered to cure movement inabilities, nausea and vomiting during pregnancy. More importantly, apart from sedation and drowsiness, there is no report of any side effects for ginger (White,2007: Riazipour, 2011). Khaliq et al., 2017 reported that an aqueous extract of Ginger can be used as a memory-enhancing drug in various memory disorders. The essential oil and oleoresin (Singh et al., 2008), Silver nanoparticles synthesized from the extract (Saraswathi et al., 2016), the Compound 6-Gingerol [5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) (Haris et al., 2018), shogaols (Ghasemzadeh et al., 2018), chloroform and diethyl ether extracts (Awan et al., 2017), ethanol extract (Munawar et al., 2017) and acetone extract possess antioxidant activity (Aziz et al., 2015). It has been reported that Phenolic and flavonoids play a key role in antioxidant activity (Munawar et al.,2017). The methanol extracts possess nitrite-scavenging abilities, antioxidative activities and electron-donating ability (Son,2010). 80% ethanol extract treated with ultrasound for 2 hours exhibited ·OH and O2 -·scavenging ability (Yu et al., 2009). It has been reported that the compound 6-shogaol, 6-gingerol, and 6-dehydrogingerdione have active agents for the treatment of fungal infections, dandruff and seborrheic dermatitis (Wu et al., 2002). Zerumbone a tropical ginger sesquiterpene of *Zingiber officinale* have an anti-melanogenic effect and can be used as active ingredients in skin-whitening cosmetics (Oh et al.,2018), besides possessing anti-inflammatory, anti-allergic, anti-microbial, and anti-cancer activity. Silver nanoparticles synthesized from the extract of *Zingiber officinale* also possess anti-inflammatory properties and can be used as a curative agent for targeted drug delivery to cure diseases (Saraswathi et al., 2016). It has been reported that the compound 6-Gingerol [5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one] have the potential of antiemetic, antiulcer, antimicrobial, analgesic, hypoglycemic, antihypertensive, antihyperlipidemic, immunostimulant, anti-inflammatory and cardiotonic. 6-Gingerol is a good candidate for the treatment of various prostate, pancreatic, breast, skin, gastrointestinal, pulmonary and renal cancer (Haris et al., 2018). Oleoresin [10]-gingerol

possess inhibitory properties against metastatic triple-negative breast cancer *in vitro* and *in vivo* (Martin et al., 2017). Hydroalcoholic extract of *Zingiber officinale* 10 mg/kg on mice, once daily for five consecutive days before exposure to 6-12 Gy of γ radiation can reduce the severity of radiation sickness and the mortality and protected mice from GI syndrome as well as bone marrow syndrome (Jagetia et al., 2003). Ultrafine active fibres encapsulated with 12% ginger essential oil possess a high potential to be applied in food packaging to reduce microbial contamination (da Silva et al., 2018).

Rawal et al., 2016 reported that Ginger (*Zingiber officinale*) has long been used for naturopathy due to its potential antimicrobial activity against different pathogens. The aqueous extract (Khaliq et al., 2017), ethanol extract (Munawar et al**.,** 2017) and the compound shogaols or Gingerol (Ghasemzadeh et al., 2018), Zerumbone (Oh et al., 2018), silver nanoparticles (Saraswathi et al., 2016) of *Zingiber officinale* has been reported to possess antimicrobial properties.Wang et al., (2010) reported that the antioxidant effects of ginger compound partially contribute to antimicrobial activity. It was also reported that phenolic and flavonoids play a key role in antibacterial activities. Oleoresin obtained by supercritical CO_2 fluid extraction had antimicrobial activity against *Penicillium citrinum*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* and *Escherichia coli* (Chen et al., 2001). 5% ginger nanofibers bio-nanocomposites showed antibacterial activity against *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* (Jacob et al., 2019). *Zingiber officinale* photoactivated cow urine extract posse's antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger* and *Aspergillus flavus* (Dhiman et al., 2017). Zincoxide nanoparticle produced by *Zingiber officinale* is reported to have antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus subtilus* (Raj et al., 2015). Copper nanoparticles (CuNPs) synthesized using the extract of *Zingiber officinale* and its bio-conjugate also possess antimicrobial activity against food spoilage microorganisms such as *Staphylococcus aureus, Pseudomonas fluorescens, Listeria monocytogenes, Fusarium moniliforme* and *Aspergillus niger* (Pandit et al., 2017). Pad-dry-cure method ginger 25% clove 75% shows antibacterial activity against *Moraxella* sp, *Staphylococcus* spp, *Escherichia* spp, *Pseudomonas* spp, *Enterobacter cloacae* and *Klebsiella pneumoniae* (Saravanan et al.,2017). Chakotiya et al., (2017) reported that Ginger is effective for killing multi-drug resistant *P. aeruginosa*. It has been reported that the essential oil of

rhizome possesses antimicrobial activities against *Pseudomonas aeruginosa*, *Trichoderma* spp, *Pencillium* spp. and *Saccharomyces cerevisiae* (Sasidharan et al., 2010). *Klebsiella pneumoniae* and *Shigella dysenteriae*, (Zhong et al.,2011). *Aspergillus niger* (Obad et al., 2016), *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* (Sharma et al., 2016), *Staphylococcus aureus*, *Staphylococcus epidemidis*, *Enterococcus faecalis*, *Candida tropicalis* and *Trichophyton mentagrophytes* (Lopez et al., 2017). It has also been reported that the essential oil was active against food borne bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhymurium and *Vibrio cholerae* (Hamad et al., 2016), It was also active against oral-dental microorganisms such as *Micrococcus luteus* and *Pseudomonas aeruginosa* (Bonou et al., 2016). Antibiofilm effects of essential oils could be used against biofilm *Klebsiella* acquired infections (Avcioglu et al., 2016). The essential oil and CCl (4) oleoresin showed 100% zone inhibition against *Fusarium moniliforme* (Singh et al., 2008). The essential oil of Ginger dissolved in methanol (1:10), possesses antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Candida albicans* (Hassan et al., 2017). Chitosan film with ginger essential oil was also effective against *Staphylococcus aureus* and *Escherichia coli* at 0.3 % concentrations (Remya et al., 2016). Aqueous extract showed inhibitory effects against *Salmonella typhi* and *Salmonella paratyphi* (Jayanthi 2010). *Vibrio parahaemolyticus* (Shukla et al.,2016), *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* (Okigbo et al., 2009). Hydroalcoholic extract possesses antimicrobial activity against *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, *Escherichia coli* and *Candida albicans* (Jagetia et al., 2003). Methanol extract of therhizomepossesses significant antibacterial activity against *Bacillus cereus* (Son, 2010), *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aerogenosa*, *Proteus mirabilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae* (Shareef et al., 2016). Ethanol extracts showed antimicrobial activities against *Porphyromonas gingivalis*, *Porphyromonas endodontalis* (Park et al., 2008), *Staphylococcus aureus*, *Klebsiella*, *Escherichia coli* and *Streptococcus* (Aziz et al., 2015). *Candida albicans* (Sukandar et al., 2016), *Fusarium oxysporum* f. sp. Lycopersici (Rawal et al., 2016), *Staphylococcus aureus* and *Enterococcus feacalis* (Santo et al., 2017). 80% ethanol extract treated with ultrasound for 2 hours also exhibited antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Saccharomyces cerevisiae* (Yu et al., 2009). The chloroform extract possesses antibacterial activities against

Fusarium oxysporum f. sp. Lycopersici (Rawal et al., 2016), *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis* and *Serratia marcesnces* (Awan et al., 2017), Acetone extract had antimicrobial activity against *Bacillus subtilis* and *Escherichia coli* K-12 (Yamada et al.,1992), *Staphylococcus aureus*, *Klebsiella*, *Escherichia coli* and *Streptococcus* (Aziz et al., 2015), *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio vulnificus* (Shukla et al.,2016). Glycolic extracts possess antimicrobial activity against *Enterococcus faecalis* (Maekawa et al., 2015). It has also been reported that Glycolic extract combined with calcium hydroxide and chlorhexidine gel as an auxiliary chemical substance used for intra-canal medication was effective on *Candida albicans* and *Escherichia coli* (Valera et al., 2015). N-hexane extracts exhibited antibacterial activities against *Porphyromonas gingivalis*, *Porphyromonas endodontalis* oral pathogens (Park et al., 2008). Diethyl ether extract possesses antibacterial activities against *Klebsiella pneumonia* , *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis* and *Serratia marcesnces* (Awan et al., 2017). Petroleum ether solvent extract of ginger powder shows antimicrobial activity against *Fusarium oxysporum* f. sp. Lycopersici (Rawal et al., 2016). Organic acid solvent 1, 4-Dioxan extracts and turmeric possess activity against *Escherichia coli* and *Staphylococcus aureus* (Chandarana et al., 2005). The compound monoterpenoid glycosides, trans-1,8-cineole-3,6-dihydroxy-3-O-β-D-glucopyranoside and 5,9-dihydroxy borneol 2-O-β-D-glucopyranoside possess antibacterial activities against *Staphylococcus aureus* and *Staphylococcus epidermidis* Compound Trans-1, 8-cineole-3,6-dihydroxy-3-O-β-D-glucopyranoside also possess significant activity against Staphylococcus *aureus* and *Staphylococcus epidermidis* (Guo et al., 2018). The two highly alkylated gingerols, [10]-gingerol and [12]-gingerol effectively inhibited the growth of oral pathogens *Porphyromonas gingivalis* and *Porphyromonas endodontalis* (Park et al., 2008). Ginger paste and fresh garlic paste have activity for complete inactivation of *Escherichia coli* O157:H7 in the paste at 3 days at 4°C and 8°C (Gupta et al., 2005). A combination of nisin and red ginger essential oil has a fungicidal effect against *Aspergillus niger*. (Nissa et al.,2014). Photoactivated cow urine extracts possess the larvicidal activity and antihelmintic activity (Dhiman et al., 2017). The compound 1,7-bis(4-hydroxy-3-methoxyphenyl) hept-4-en-3-one (gingerenone A) had anti coccidial activity *in vitro* and a strong antifungal effect on *Pyricularia oryzae* (Endo et al., 1990). Nagoshi et al., 2006 reported that [10]-gingerol reduced the minimum inhibitory concentrations of aminoglycosides in vancomycin-resistant *Enterococci* and it reduced the minimum

inhibitory concentration of other aminoglycosides, also bacitracin and polymyxin B. of ethanol extract showed anti-enterococcal activity (Revati et al., 2015).

Phytochemical studies have shown that ginger rhizome contains 3-6% fatty oil, 9% protein, 60-70% carbohydrates, 3-8% crude fibre, about 8% ash, 9-12% water and 2-3% volatile oil (Govindarajan, 1982 a, b; Ali et al., 2008), It also contains proteolytic enzyme zingibain, extractable oleoresins, vitamins and minerals (Govindarajan, 1982a, b; Vasala 2004). The non-volatile pungent phytochemicals such as gingerols, shogaols, paradols and zingerone have also been found in ginger that contributes to the warm pungent sensation in the mouth and their concentration and ratio vary with the form of the ginger (Govindarajan, 1982a, b). Baghel et al., (2017) reported that gingerol, paradol and shogaols etc., are the medicinal properties of ginger. The composition of fresh ginger oil shows that it contains more oxygenated compounds (29%) compared to dry ginger oil (14%).The higher content of geranial and other oxygenated compounds makes fresh ginger oil more potent than dry ginger oil but the content of hydrocarbon compounds is more in dry ginger oil compared to fresh ginger oil and Zingiberene was the major compound from fresh and dried ginger rhizomes volatile oils (Sasidharan et al., 2010). Fresh ginger oil contains geranial (8.5%) as the second main compound and had more oxygenated compounds (29.2%) compared to dry ginger oil (14.4%). The dry ginger oil also contained ar-curcumin (11%), β-bisabolene (7.2%), sesquiphellandrene (6.6%) and δ-cadinene (3.5%). Distillation components of ginger included sulfur compounds and hydrocarbons and α-Zingibirene, β-phellandrene, β- sesquiphellandrene (Ji et al., 1997). Ethanol and aqueous extract possess biologically active chemical compounds such as tannins, Phenols, Saponins, alkaloids, flavonoids and Steroids/ triterpenes (Okigbo et al., 2009). Ethanol and Ethyl acetate extract contains saponins (Ojo et al., 2007). Aqueous, ethanol and acetone extracts contain steroids, triterpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, carbohydrates, amino acids and proteins (Sarda et al., 2017). Ethanol extract of ginger contains flavonoids, alkaloids, saponins, tannins and triterpenes and essential oil by GC-MS shows geranialdehyde (27.42%), nerol (20.11%), 1.8-cineole (13.35%), camphene (4.65%) and E-geraniol (3.92%) (Gonzalez-Guevara et al.,2017), 80% ethanol extract for 40 times and treated with ultrasound for 2 hours yield flavonoids 0.75% (Yu et al., 2009). The major compounds identified in acetone extract were Bicyclo [3.1.1] hept-2-ene,2,6-dimethyl-6-(4-methyl6-pentyl) and 4-(3-hydroxy-2-methoxyphenylbutan-2-one, Gingerol, Furan, 2,5-dibutyl,4-Hexanoyal

resorcinol and the major compounds identified in cyclohexane extract were 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl, trans-.alpha.-Bergamotene, Sesquirosefuran, E-12- Tetradecenal, Gingerol, 2H,6H-Pyrano[3,2-b]xanthen-6- one, 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methyl2-butenyl) (Aziz et al., 2015). Methanol extract of *Zingiber officinale* contain Octanal, 2-Naphthale namine,1,2,4a,5,6,7,8,8a-octahydro-4a-Me, 1-(Cyclopropyl-nitromethyl)- cyclopentanol, Endo-Borneol, Decanal, 1,2-15,16-Diepoxyhexadecane, Propanal,2-methyl-3-Ph, Benzeneacetic acid ,4-(1H-1,2,3,4-tetrazol-1-yl), Ascaridole epoxide, 2-Methoxy-4-vinylphenol, 6-epi-shyobunol, Phenol,2-methoxy-5-(1- propenyl)-, E, Alfa. -Copaene, 8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene. Bicyclo [3.1.0]hexane-6-methanol,2- hydroxy-1,4,4-trimethyl, 7-epi-cis-sesquisabinene hydrate, Alloaromadendrene, Benzene,1-(1,5-dimethyl-4-hexenyl)-4- Me, 1,3-Cyclohexadiene ,5-(1,5-dimethyl-4-hexenyl)-2methyl-, [S-(R*,S*)], Aromadendrene oxide, 1,6,10-Dodecatrien-3- ol,3,7,11-trimethyl-,(E), 4-((1H)-3-Hydroxy-1-propenyl)-2-methoxyphenol. Butan-2-one,4-(3-hydroxy-2-methoxyphenyl), Longipinocarveol, trans, Cholestan-3-ol,2-methylene-, (3β,5a)-, Bicyclo[4.4.0] dec-2-ene-4-ol,2-methyl-9-(prop-1-en-3-ol-2- syl)-, Corymbolone, Estra-1,3,5(10)-trien-17β-ol, 1-Heptatriacotanol. Fenretinide, Folic acid, Spiro [4.5] decan-7-one,1,8- dimethyl-8,9-epoxy-4-isopropyl-, 7H-6,9a-Methano-4H-cyclopenta[9,10] cyclopropa [5,6] cyclodeca[1, Gingerol, 1b,4a- Epoxy-2H-cyclopenta [3,4] cyclopropa [8,9] cycloundec[1,2-b]o, Cyclopropa[5,6]-A-nor-5a-androstane-3,7-dione,3',6β- dihydro-17β-h, Olean-12-ene 3,15,16,21,22,28-hexol,(3β,15α,16α,21β,22α)-, Benz[e]azulen-3(3aH)-one,4,6a,7,8,9,10,10a,10b-octahydro-3a,8,1, Naphthalene, decahydro-1-p (Shareef et al., 2016). Ether extract contains α-Zingibirene, βphellandrene, β-sesquiphellandrene and camphene (Ji et al., 1997). N-hexane-solution extract possess [8]- and [10]-gingerol (Yamada et al., 1992). It has been reported that water and steam distillation yield 2.05% and 2.1% essential oil respectively (Stoyanova et al., 2006). Essential oil of *Z. officinale* rhizome contain β-sesquiphellandrene (27.16%), caryophyllene (15.29%), zingiberene (13.97%), α-farnesene (10.52%) and ar-curcumin (6.62% (El -Baroty et al., 2010) β-bisabolene (4.1%), α-zingiberene (10.3%), β-bisabolene (8.1%), (Stoyanova et al., 2006), eudesmol (8.19%), γ- terpinene (7.88 %), α-curcumene (7.28%), alloaromadendrene (6.56%), α-pinene (5.76 %), δ- cadinene (3.84%), elemol (3.39%), farnesal (3.45%), E-β-farnesene (3.57%) and neril acetate (2.8%) (Lopez et al., 2017), Cineole, 2,2-dimethyl-3-methylenenorbornane, rosefuran epoxide, 2,2-dimethyl-3- methylenenorbornane, β-pinene, β-mircene, cineole, β-citral, α-citral, bornyl acetate, α-curcumene, and

hexadecanoic acid (Hamad et al., 2016), Z-citral (23.332%), citral (18.87%), 1,8-cineole (12.18%), camphene (11.87%), geranyl acetate (3.82%), linalool (2.88%), 5-hepten-2-one, 6-methyl-(2.32%), α-terpineol (2.05%) (Nissa et al.,2014), β- phellandrene (Bonou et al., 2016), valencene (7.61%), β-funebrene (3.09%),selina-4(14), 7(11)-diene (1.03%), citronellyl n-butyrate (19.34%) (Sharma et al., 2016). Guo et al., (2018) also reported two new *Zingiber officinale* monoterpenoid glycosides, trans-1,8-cineole-3,6-dihydroxy-3-O-β-D-glucopyranoside and 5,9-dihydroxy borneol 2-O-β-D-glucopyranoside. Extraction of pulverized ginger roots by using organic solvents, steam distillation, and supercritical CO_2 extraction and subjecting the crude extracts to reversed-phase column chromatogram obtained 6-shogaol, 6-gingerol, and 6-dehydrogingerdione (Wu et al., 2002), Flavonoid 20.3% while the total phenol contents were 19.3% (Son, 2010).

2.5 *Artemisia vulgaris*:

Artemisia vulgaris is a perennial weed belonging to the family Asteraceae, growing wild and abundantly in temperate and cold-temperature zones of the world, native to Asia, Europe and North America. In India, it is generally found growing on uncultivated lands along waysides and wasteland in the hilly areas. *A. vulgaris* possess broadleaf and are perennial, which spreads rapidly upon introduction by a well-developed rhizome system (Barney and Ditommaso, 2003). The herb is a long-stemmed, 70–150 cm highly branched shrub with plenty of heads as well as creeping rhizome without rosette or runners. The rhizome is light brown, up to 1 cm in diameter and can penetrate to a depth of 7–18 cm in the soil. The root is pungent and sweet and the plant is bitter and aromatic. The flowers are red-brown or yellowish and almost glabrous. Flower heads are ovoid, 3–4mm long by 2mm wide. Flowers are arranged in an inflorescence type cluster. The fruit has an indistinct margin. The leaves are 5–20 cm long, sessile and pinnate, dark green, with dense white tomentose hairs on the underside.

Artemisia vulgaris is one of the most important medicinal plant species of the genus Artemisia and is valued for health benefits worldwide due to the rich accumulation of essential oils and other terpenoids. This plant has been used to flavour tea and rice dishes in Asia and as a culinary herb for poultry and pork in Western cultures. In Mexican culture, it was popular for infusion drinks, employed traditionally to treat indigestion, asthma, sprains and wounds, as well as an expectorant, decongestant, anthelmintic and

emmenagogue (Valsaraj et al., 1997; Correa-Ferreira et al., 2014). In Oriental medicine, it has been employed as an analgesic agent and in conjunction with acupuncture therapy (Yoshikawa et al., 1996). It has been reported that *Artemisia vulgaris* is a potential source of natural antioxidants. The essential oil from leaves (Bhatt et al., 2006), aqueous extract (Temraz and El-Tantawy, 2008) and methanol extract of leaves (Melguizo-Melguizo et al., 2014) possess a good amount of antioxidant properties. The methanol extract from leaves possesses antifertility activity. (Shaik et al., 2014) and anti-inflammatory activity (Afsar, 2013). Hydro-alcohol extracts of *A. vulgaris* exhibited a moderate antinociceptive peripheral effect (Pires et al., 2009). Hirschwehr et al., (1998) reported that mugwort pollen contains several cross-reactive allergens and among them, the major mugwort allergen is Art v 1 and also reported that cross-reactive IgE antibodies can lead to clinically significant allergic reactions. Hwang et al., (1985) reported that the compound terpinene-4-ol of *Artemisia vulgaris* possess the most effective repellent activity against yellow fever mosquito *Aedes aegypti* when compared to other compound found in *Artemisia vulgaris*. It has also been reported that essential oil extracted from mugwort stem is a potential larvicide against *Aedes aegypti* (Govindaraj et al., 2013) besides the essential oil also exhibit 90% repellence against *A. aegypti* (Hwang et al., 1985). Wang et al., (2006) also reported that *Artemisia vulgaris* essential oil possess repellent and fumigant activity against the stored-product insect pest, *Tribolium castaneum*.

Blagojevic et al., (2006) reported that the anti-microbial activity of essential oil from mugwort has been attributed to the presence of the compound α-thujone, 1,8-cineole, and camphene, besides these they contain several compounds that play and have great therapeutic value. Leaves essential oil possess antimicrobial properties against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Candida albicans, Propionibacteriumacnes* (Bhatt et al., 2006), *Bacillus subtilis, Bacillus cereus, Salmonella* Typhimurium and *Escherichia coli* (Munda et al., 2009). Leaves and stem essential oil were active against *Candida albicans, Kluyvera cryocrescens, Leminorella ghirmontii, Micrococcus agilis* and *Bacillus* spp. (Singh et al., 2011). Ethanol extract possesses antimicrobial properties against *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Salmonella enterica, Pseudomonas aeruginosa, Yersinia enterocolitica, Candida tropicalis, Candida krusei, Candida glabrata, Candida albicans* (Kačániová et al., 2020). Acetone extract exhibited high antimicrobial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Bacillussubtilis* (Addo-Mensah et al.,

2015). Methanol extract of the plant also possesses antimicrobial properties against *Staphylococcus aureus*, *Ageratina adenophora* and *Rhizopus* spp. (Manandhar et al., 2019).

Abiri et al., (2018) reported that the biological activity of *Artemisia vulgaris* is mainly due to the presence of various classes of secondary metabolites, including flavonoids, sesquiterpene lactones, coumarins, acetylenes, phenolic acids, organic acids, mono- and sesquiterpenes, it exhibits high morphological and phytochemical variability depending on the location where it occurs. It has been reported that the compound which is present in whole plant essential oil was (±)-linalool, (±)-camphor, (+)-camphor, (-)-camphor, isoborneol, (-)-borneol, terpinene-4-ol, isobornyl acetate, Nonanone-3, (α + β)-thujone, and bornyl acetate, β-Pinene, myrcene, α-terpinene, (+)-limonene and cineole terpinene-4-ol (Hwang et al., 1985). The aerial parts essential oil contains Germacrene, trans -Thujone, cis -thujone, Chrysan phenylacetate, 1,8-Cineole, sabinene, β-pinene, artemisia ketone, caryophyllene (Judžentienė and Buzelytė (2006), Isobornyl isobutyrate, Limonene, δ-3-Carene, α-pinene, δ-Terpinene, trans-Rose oxide (Tajadod et al., 2012). Eudesmane acids I and II (Marco et al., 1991), Flowering tops essential oil contains caffeoylquinic acids, 3,5-di-O-caffeoylquinic acid and 1,5-di-O-caffeoylquinic acid (yield 2.0% and 0.3% on dry weight, resp.) (Carnat et al., 2000), camphor (Haider et al., 2003), vulgarole (Nano et al., 1976), heptadeca-1,7,9-trien-11,13,15-triyne, cis-dehydromatricaria ester and tetradeca-6-ene-8,10,12- triyne-3-one (Drake and Lam, 1974), in 1,8-cineole, Borneol and Caryophyllene (Williams, 2012) Leaf essential oil contain 1,8-cineole, α-thujone, camphor, isoborneol (Haider et al., 2003), germacrene D, camphene, β-caryophyllene (Govindaraj et al., 2008), caryophyllene, α-zingiberene, borneol (Williams, 2012), chavicol and myrtenol (Munda et al., 2009). Stem essential contain camphor, camphene, α-thujone, 1,8-cineole, γ-muurolene and β-caryophyllene (Govindaraj et al., 2013). Fruit oil contained α-thujone and artemisia alc (Haider et al., 2003), Root extract contains 7,9,11-triyne and tetradeca-4,6-diene-8,10,12-triyne-1-ol (Drake and Lam, 1974) and leaves and flower contain cineole, 4-terpineol, camphor, borneol, and thujone were present (Carnat et al., 1986).

2.6 *Citrus macroptera*:

The genus *Citrus* (fam. *Rutaceae*) contains many economically important fruits that are grown worldwide for their high nutritional and medicinal value (Su et al. 2014). The flowers and leaves of *Citrus* are usually strong scented and the extracts of these parts along with fruits contain many useful flavonoids and other biologically active compounds effective as insecticides, fungicides, and medicinal agents (Tripoli et al., 2007). Thegenus *Citrus* includes some of the most important cultivated fruit trees worldwide *(*Carbonell-Caballeroet al., 2015). Amongst this genus, *C. macroptera* is a semiwild species native to Malaysia and Melanesia (Li et al., 2006). The fruit of the plant has been reported to possess many biological activities, the important ones of which have been summarized below.

Citrus macroptera peel powder exerts hepatoprotective activity via promoting the antioxidant defence against CCl_4-induced oxidative liver damage (Alam et al., 2018). The fruit pulp juice possesses anti-proliferative activity (Hasan et al., 2019). Fruit rind powder possesses antioxidant properties (DPPH-IC_{50} 87.83 μg/mg, reducing power assay 36.71 μg/mg), anti-inflammatory and cytotoxic agents (Nongalleima et al., 2017). Also, the essential oil of peel and leaves exhibited anti-oxidant and anti-inflammatory activity (Singh et al., 2017). The methanolic extract of fruit pulp had moderate α-amylase inhibitory activity [IC_{50} value = (3.638 ± 0.190) mg/mL] and is reported as a potential source for a hypoglycemic agent (Uddin et al., 2014). The fruit is used as an appetite stimulant and in the treatment of fever, also methanol extract of the fruit is having cardio-protective, moderate hepato-protective and glucose controlling activities (Uddin et al., 2014). The ethanolic extract of *Citrus macroptera* showed significant skeletal muscle relaxant activity (Rahman et al., 2019). Hot methanol extract of stem bark is reported to have a potential antioxidant activity (IC_{50}: 178.96 μg/mL), cold methanol and dichloromethane extracts showed moderate activity (IC_{50}: 242.78 μg/mL and 255.78 μg/mL resp.), whereas mild antioxidant activity was observed with the n-hexane extract of the stem bark of *Citrus macroptera* (IC_{50}: 422.94 μg/mL) (Chowdhury et al., 2008). The *Citrus macroptera* gold nanoparticles (CM-AuNPs) in combination with a sub-MIC dose of gentamicin possess anti-bacterial activities against *P. aeruginosa* bacteria and it could be treated as a potential anti-biofilm and anti-cancer agent (Majumdar et al., 2019). The fruit peels possess considerable antibacterial activity against *Bacillus cereus*, *B.*

subtilis, *Escherichia coli* and *Staphylococcus aureus* with MIC values ranging from 1.25 to 5.0 mg/mL (Miah et al., 2010). The essential oil from the leaves is having activity against *Trichophyton mentagrophytes* var. interdigitale, with a minimal inhibitory concentration (MIC) of 12.5 μg/mL (Waikedre et al., 2010).

Hazarika et al., (2017) reported the fruit parameters of *C. macroptera* individual fruit as; weight; 277.78 to 617.69 g; fruit diam. 9.32-12.52 cm; fruit length 7.74-10.19 cm, fruit vol. 238.33-583.33 cc; pulp weight 153.75- 320.94 g; pulp:peel ratio 1.50-3.24 and seed no. 9.33-23.6. The juice content varied from 13.45 to 32.53 %, ascorbic acid 34.81-73.64 mg/100 mL, TSS 6.15-9.10 %, acidity 5.03-8.75 %, total sugars 5.16-7.97 % and sugar: acid ratio 0.75-1.52.

The major volatile component present in the fruit peels were Bicyclo [4.1.0] heptane, 7-(1- methylethylidene)- (60.03 %), Mentha-2, 8-dien-1-ol(4.0%), Limonene oxide (3.53%), trans Carveol (2.67%) whereas in leaves, essential oil major components were 2-methylaminobenzoic acid methyl ester (57.16 %), bicyclo [4.1.0] heptane, 7-(1-methylethylidene)-(23.23 %), β-pinene (8.79 %), ocimene DB5-519 (3.29 %). The essential oil of peels showed higher volatile oil content than that of leaves (Singh et al.,2017).

The peel powder extract showed caffeic acid and (-) epicatechin (Alam et al., 2018). The methanol extract of fruit pulp contains saponin, steroids and terpenoid (Uddinetal., 2014).The methanol extract of fruit rind powder possesses Quercetin (431.1 μg/mL) and Kaempferol (59.50 μg/mL), while the aqueous methanol extract contains 260.38 μg/mL rutin (Nongalleima et al., 2017).

The essential oils of leaves showed a total of 35 constituents, representing 99.1 % of the total oil (Waikedre et al., 2010). The fruits peels of *Citrus macroptera* var. *annamensis* afforded 120 mg of oil (yield 0.12%) containing 25 terpenoids, predominantly monoterpene hydrocarbons, accounting for 97.0% of the total oil and limonene (73.5%) as the major component (Miah et al., 2010). Two coumarins i.e., Bergamottin and 5-[(6',7'-dihydroxy-3',7'-dimethyl-2-octenyl) oxy]psoralen were isolated from whole dried fruit (Dreyer et al., 1973). The fruit peel contained higher amounts of total polyphenols (620.91±7.75 mg), flavonoids (508.33±5.49 mg), tannins (585.99±4.46 mg) and protein (4.00±0.14 mg). However, the fruit pulps contain 291.06±10.14, 145.02±0.36, 526.08±3.32 mg/100 g and 2.89±0.32 g/100 g, respectively (Paul et al., 2015).

The phenolic contents were (45.62 ±.33 mgGAE/mL) (Rahman et al., 2016). The equivalent weight of pectin extracted from ethanol extract of *Citrus macroptera* was 732.55±18.49 while methoxyl content 1.62±0.24%, anhydrouronic acid content 33.28±1.36% and degree of esterification (DE) were 27.69±3.20%. The pectin has functional groups within the 1740-800 cm^{-1} spectral region and can be used for manufacturing low sugar foods (Rabiul et al., 2017).

Lupeol and Stigmasterol were extracted from the crude extracts of the stem bark (Chowdhury et al., 2008). Similarly, Ribalinine, isoplatydesmine and 5 aromatic compounds, two derived from cinnamic acid as well as syringaldehyde, vanillin and Me vanillate, were isolated from the stem bark (Gaillard et al., 1995).

2.7 *Tithonia diversifolia*:

The genus Tithonia comprises eleven species. *Tithonia diversifolia* belongs to the family Asteraceae and is native to Mexico and Central America. *T. diversifolia* are now found in most parts of the world including Asia and Africa where they have become invasive (Omokhua et al., 2018). Depending on their location area they can be either annual or perennial. They attain 2–3 m (6.6–9.8 ft) height in the form of woody shrubs. Leaves are 10 to 40 cm long, simple or mostly 3-7 lobed, sub-ovate, serrate, acute, somewhat glandular and slightly greyish beneath and arranged alternate. The flowers are yellow to orange in colour and 5–15 cm wide and 10–30 cm long. The seeds are achenes, 4-angled, and 5mm long and are spread through the way of wind, water, and animals.

da Gama et al., (2014) reported that the *Tithonia diversifolia* flower possesses high antioxidant properties and is effective for the prevention of cell ageing. The essential oil of inflorescences possesses cytotoxic effects on A375, MDA-MB 231 and noticeable radical scavenging activity on DPPH and ABTS radicals (Orsomando et al.,2016). The stem part of the plants is endowed with anti-oxidant, anti-cancer, anti-tumour, anti-viral, anti-inflammatory and anti-allergic properties (Essiett and Akpan, 2013). Methanol extract of the dried leaves has anti-inflammatory and analgesic activities (Owoyele et al., 2004). Chiang et al., (2004) reported that the hot water extract has the potential to suppress the replication of HSV-1 and HSV-2 without any cytotoxic effect and is also used for the treatment of type 2 diabetes (Miura et al., 2005). The dichloromethane extract had gastroprotective activity (Sanchez-Mendoza et al., 2011). The methanol

extract of leaves and the compound Tagitinin C have cytotoxic activities against human hepatoma Hep-G2 cells (Liao et al., 2013), anti-proliferative activity against human glioblastoma U373 cells (Lee et al., 2011) and the compound Tagitinin C was claimed as major cytotoxic compound (Wahyuningsih et al.,2015). Tagitanin C can decrease down keloid collagen deposition (Ranti et al., 2018). Tagitanin C also acquire antimalarial, gastroprotective activity, chemotherapeutic adjuvants and toxic activities (Silva et al.,2017), anticancer property (Ikegawa et al., 1990) and is used for treating malignant glioblastoma (Liao et al.,2011), Ruengeler et al., (1988)reported that the pure compounds diversifolin, diversifolin methyl ether, and tirotundin possess anti-inflammatory activity, the same compound is reported to have activity for suppression of parasitaemia in the early and established infection stages and repository test (Elufioye et al., 2004). Chlorogenic acids also comprise a good pool of anti-inflammatory compounds (Chagas-Paula et al., 2011). Wu et al., (2001) andLiao et al., (2013) reported that Acetyl tagitinin E and Tagitinin-F from the leaves possess selective cytotoxicity to Hep G2 human hepatocellular carcinoma cells and also it has been claimed that tagitinin F (2) decreased secretion of inflammatory products (Abe et al., 2015). tagitinin C, 1b, 2a-epoxy tagitinin C showed significant anti-proliferative activity, tithofolinolide, 3b-acetoxy-8b-isobutyryloxyreynosin and 4a,10a-dihydroxy-3-oxo-8b- isobutyryloxyguaia-11(13)-en-12,6a-olide induced HL-60 cellular differentiation and 3b-acetoxy-8b-isobutyryloxyreynosin inhibited lesion formation in the mouse mammary organ culture assay (Gu et al.,2002). Polar extract and leaf rinse extract of leaf part can inhibit oedema and neutrophil migration (Chagas-Paula et al., 2011), and cytotoxic activity against HL-60 leukaemia cells (Kuroda et al., 2007). Tirotundin and tagitinin A exert an anti-diabetic effect (Lin et al., 2012). Diversifolin, diversifolin methyl ether and tirotundin inhibit the activation of NF-kappa B (Rungeler et al., 1988). Saponin from the leaf can enhance immune response and reduce cholesterol and triglycerides in normal rats (Ejelonu et al., 2017). It has been reported that the aqueous extract of the *Tithonia diversifolia* plant was relatively safe at doses lower than 100mg/kg. However, the polar extract and leaf rinse extract demonstrated several adverse effects by damaging the liver and concluded that sesquiterpene lactones and chlorogenic acid derivatives can be toxic in prolonged use at higher doses (Passoni et al., 2013). 80% ethanol extract could reduce blood glucose and also significantly lowered plasma insulin and was found useful for the treatment of type 2 diabetes (Miura et al., 2005). Ether extract from aerial parts possesses cytotoxic properties and antiplasmodial activity (Goffin et al., 2002) and antiamoebic activity (Tona

et al., 1988). Ethanolic extract was enriched with insecticide activity against cephalotes (Hymenoptera: Myrmicinae) (Castano-Quintana et al., 2013). It has also been reported that aqueous extract of the leaf can inhibit parasite infection and possess anti-malarial activities (Nafiu et al., 2014). The essentialoil also showed repellent activities against *Rhipicephalus appendiculatus* (Wanzala et al.,2014). The compound Tagitinin C and 1b-methoxydiversifolin-3-0-methyl ether (Ses-5) a sesquiterpene also possess anti-TMV activities used as bio-pesticides (Zhao et al., 2017), anti-trypanosomal activity (Sut et al.,2018) also active against *Plasmodium* (Goffin et al.,2002). Saponin from leaf possesses leishmanicidal activities (Ejelonu et al., 2017). The compound Tirotundin 3-O-methyl ether, tagitinin F and a guaianolide possess repellent activities against Brown Ear Tick (Wanzala et al., 2014) and they can reduce the internalization of parasites after 48 h (de Toledo et al.,2014). Methanol extract is having phagodeterrent activity against *Bemisia tabaci* and was reported that sesquiterpenic lactones, polyphenolic compounds (flavonoids and tannins) and saponins are the metabolites that cause phagodeterrence (Bagnarello et al., 2009). Dichloromethane extracts of leaf and flower were active against *Plasmodium falciparum* (Muganga et al., 2010) and possess anti-microbial properties against *Escherichia coli* (Kareru et al.,2007). Ethanol and water 9:1 ratio showed activities against *Staphylococcus epidermidis, Enterobacter aerogenes, Bacillus cereus, Escherichia coli* and *Streptococcus g-hemolytic* (Anthoney et al., 2016). Ethyl acetate leaf extract and sesquiterpene lactone 1 showed inhibitory activity against *Bacillus anthracis, Bacillus polymyxa, Bacillus stearothermophilus, Bacillus subtilis, Clostridium sporogenes, Corynebacterium pyogenes, Streptococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Shigella dysenteriae* (Obafemi et al., 2006). Chloroform extract of the plants was active against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus hemolyticus, Bacillus subtilis* (Agustaet al.,1999), *Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli, Staphylococcus aureus, Salmonella* Typhimurium, *Aspergillus fumigatus, Cryptococcus neoformans* and *Candida albicans* (Omokhua et al.,2018). Dichloromethane leaf extract showed inhibitory activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Douglas et al., 2016). The compound diversifolin, diversifolin methyl ether and tirotundin was active against *Bacillus subtilis* (Rungeler et al., 1988). Tithoniaquinone A also possesses antimicrobial properties against *Bacillus megaterium* and *Microbotryum violaceum*, while Psoralen has strong algicidal, fungicidal and antibacterial activities (Bouberte et al.,2006).

The nutritional analysis of *T. diversifolia* revealed that protein content was (10.30%), Fats (1.90%), Fiber (5.80%) and carbohydrate content (61%) respectively. The moisture content of the leaf was (10%), total ash (11.00%), sulfated ash (2.10%) and acid-insoluble ash (1.33%). The stem part of the plant contains moisture (20.6%), total ash (6.55%), acid insoluble ash (0.33%), sulfated ash (14.0%), protein content (9.62%), fat (4.21%), fiber (15.82%), carbohydrate (70.35%), volatile ether sol. (1.66%), water sol. (0.33%) and diluted alcohol solution (5.66%). It was also enriched with the compound flavonoids, tannins, saponins and cardiac glycosides. In all the plant's parts (root, stem and leaf) carbohydrate was found to be highest (41.84 ± 0.19) followed by crude fibre (32.79 ± 0.10%,), moisture (9.19 ± 0.05%), total ash (8.97 ± 0.07%,), crude protein (5.99 ± 0.24%) and crude fat (1.19 ± 0.05%). It also has alkaloids (53.33 ± 6.58 mg/100 g) followed by tannins (382.22 ± 7.58 mg/100 g), flavonoids (338.89 ± 3.50 mg/100 g), saponins (327.78 ± 7.33 mg/100 g), terpenoids (65.00 ± 3.69 mg/100 g) and phenols (48.46 ± 0.32 mg/100 g) (Olayinka et al., 2015). The flower contains phenolic compounds of tannins, flavonoids and total phenols (Essiett et al., 2013). The essential oil of the flower was also enriched with Tagitinin A (I, R = a-OH), C (II), F (III), and tirotundin (I, R = H) (Baruah et al.,1979), Tirotundin (I) (Calzada and Ciccio, 1978), Germacrene D, b-caryophyllene and bicyclogermacrene (Moronkola et al.,2007), α-pinene, (Z)-β-ocimene, limonene and p-mentha-1,5- dien-8-ol. It was reported that the compound of the flower oil was found to be similar to the leaf oil of this species (Menut et al., 1992). Several aliphatic fatty acids, diterpenoid compounds and aracopimaradiene were also present in the flower, which could not be detected in the leaf oil (Moronkolaet al., 2007). Leaf extract was incorporated with germacrene sesquiterpene, 1-acetyltagitinin A and a guaianane sesquiterpene, 8 betaisobutyryloxycumambranolide (Kuo and Chen, 1988), tithoniquinone A and the ceramide, named as tithoniamide B, (Bouberte et al., 2016), caffeic acid derivatives {(E)-3-(((3-(3,4dihydroxyphenyl) acryloyl)oxy)methyl)-2-methyloxyrane-2-carboxylic acid}, (Pulido et el., 2017). The polar extract of the leaf consists primarily of chlorogenic acids (CAs), the leaf rinse extract is rich in sesquiterpene lactones and includes a few flavonoids (Chagas-Paula et al., 2011). Ethyl acetate and methanol extracts of leaves contain tannins, terpenoids, saponins, phenols and flavanoids (Douglas et al.,2016). The leaf essential oil possesses α-pinene, β-caryophyllene, germacrene D, β-pinene and 1,8-cineole (Moronkola et al.,2007). Aqueous extract of the leaves contains saponins, alkaloids and tannins (Nafiu et al., 2014), sesquiterpene lactones i.e.8β-O-(2-methylbutyroyl) tirotundin and 8β-O-(isovaleroyl)

tirotundin (Miranda et al., 2015). Extractnfrom the aerial parts of the plant posess palmitic acid; 9-pentadecadien-1-ol; benzyl benzoate; stearaldehyde; a-methylamine; 1,2,3,5-cyclohexantetrol (Agustaet and jamal1999), Tagitinin A, tagitinin C, 3,5-di-Ocaffeoylquinic (Zhou et al.,2000), sesquiterpenoids ie., 2a- hydroxytirotundin, tithofolinolide, and 3a-acetoxydiversifolol, 3b-acetoxy-8b-isobutyryloxyreynosin, tagitinin C, 1b,2a-epoxytagitinin C , 4a,10a-dihydroxy-3-oxo-8b- isobutyryloxyguaia-11(13)-en-12,6a-olide, 3a-acetoxy-4a-hydroxy-11(13)-eudesmen-12-oic acid Me ester, 17,20- dihydroxygeranylnerol, tagitinin A and tirotundin (Gu et al.,2002), sesquiterpenes of 1a-hydroxytirotundin 3-O-methyl ether, 1a-hydroxydiversifolin 3-O-methyl ether, 4b,10b-dihydroxy-3-oxo-8b-isobutyroyloxyguaia-11(13)-en6,12-olide (Kuroda et al.,2007), Vanillin, ergosterol, ergosterol peroxide, 3-methoxy-4-hydroxy-trans-cinnamaldehyde, p-hydroxybenzaldehyde, 7-Me esculetin, 5-hydroxy Me furfural and 13-hydroxy eupatene (Zhao et al.,2010),monoterpene hydrocarbons (22.2%), oxygenated monoterpenes (4.6%), sesquiterpenes hydrocarbons (58.2%) and oxygenated sesquiterpenes (10.8%) (Gbolade et al.,2008), germacrane sesquiterpenes (1), (2), (3) (Zhao et al., 2012), sesquiterpene lactone 8-(2-methylbutanoyl)-3,10-epoxy-3,8-dihydroxyl-4,11(13)-germacradien-12,6-olide (I) (Elufioye et al., 2004), chromene glycoside, 6-acetyl-2,2-dimethylchromene-8-O-b-D-glucoside (Zhai et al., 2010), monoterpenes: (1S,2R,3R,5S)-2-hydroxymethyl-6,6-dimethylbicyclo[3.1.1]heptane-2,3-diol and (3R)-6,6-dimethyl-4-methylenebicyclo[3.1.1]heptane-1,3-diol-3-O-bglucopyranoside (Liet al., 2013). Ethyl acetate extract of aerial part yielded 8β-O-(2-methylbutyroyl) tirotundin and 8β-O-(isovaleroyl) tirotundin (Miranda et al.,2015). The volatile oil of the aerial part also contains monoterpene hydrocarbons (87.9%), cis-β-ocimene (43.7%), α-pinene (28.6%) and limonene (12.0%) being the main compounds. Sesquiterpenes represented ca. 10% oil (Gbolade et al.,2008), α Pinene (63.81%), Limonene (7.07%), β-Caryophyllene (4.85%), Bicyclogermacrene (2.95%), Sabinene (2.78%) and Spathulenol (2.702%) (Li et al., 2013), α-pinene (63.64%), β-pinene (15.00%), isocaryophyllene (7.62%), nerolidol (3.70%), 1-tridecanol (1.75%), limonene (1.52%) and sabinene (1.00%) (Wanzala et al.,2014). The root part of the plant contains chromone, 6-acetyl-7-hydroxy-2,3-dimethylchromone (Kuo, 1999). It has also been reported that the compound which is present in the leaf oil are monoterpenoids (88.2%) out of which 87.4% are monoterpene hydrocarbons and some sesquiterpene hydrocarbons (8%). The major constituent was (Z)-b-ocimene (40.2%) (Lamaty et al., 1991).

CHAPTER - 3

MATERIALS AND METHODS

To fulfill the objectives of the study, the ethnobotanical survey was carried out in the Aizawl District of Mizoram. The traditional healers were consulted for the traditional remedies for the treatment of skin ailments. The medicinal plants related to the formulations suggested by the traditional healers were collected within the state of Mizoram. The preliminary processing of the medicinal plants for the extraction was done at the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl, while part of the physicochemical analysis, phytochemical analysis and bio-prospection investigation was carried out at the Molecular Bio-prospection Department Laboratory, Council of Scientific and Industrial Research (CSIR) – Central Institute of Medicinal and Aromatic Plants (CIMAP).

The work plans to achieve the objectives were categorized in the following sequence:

1. Collection and categorization of some formulated products, used against skin ailments/ remedies and practices among the tribal communities/ traditional healers in Mizoram,
2. Physicochemical analysis of the selected formulations,
3. Phytochemical analysis of the selected formulations,
4. Bio-prospection against the pathogenic fungi/ Dermatophytes.

3.1Study area:

The study area was confined to the Aizawl district of Mizoram located in the eastern side of NE India, sandwiched between Bangladesh and Myanmar between 23°43′44″N to 92°43′04″E. The topography of the region consists of parallel hill ranges running in the North-South direction, with an altitude varying from 40m to 2157 m above mean sea level. The terrain is highly dissected with streams and rivers. Forest cover is primary and secondary type, comprising tropical wet evergreen, tropical semi-evergreen and sub-tropical type forests. The area of the Aizawl district is 3577sq. km and the weather are pleasant with an equable warm climate throughout the year with moderate to chilly winter during November - January at higher altitudes. The annual temperature ranges between 11°C to 30°C & the average rainfall is 215 cm.

3.2 Ethnobotanical survey:

In the present study, the main emphasis was given to the traditional practitioner(s) / Medicinal Plantusers of the Aizawl district of Mizoram.The information was collected based on interviews, through semi-structured questionnaires(Annexure-I)to decipher the information related to the medicinal plants used in the ethnomedicinal formulae.The dialogue with the traditional healer also emphasized the plant parts used along with the technique of preparation, its dosage, duration, along the method of treatment for its scientific exploration.

3.3Physicochemical analysis of the selected formulations:

2.3.1 Appearance:

The appearance of the essential oils and the extract was recorded based on the visual observation of its colourand its tint about light, dark, bright or paleness.

3.3.2 Texture:

The texture of the samples was recorded by physical characteristics like oily, non-sticky aromatic oil, greasy semi-solid, slimy, sticky, greasyor free-flowing.

3.3.3 Odour:

The odour was recorded randomly through personal observations from volunteers which ranged from lemony, bitter, camphorous, strong, repelling, sweet or pleasant.

3.3.4 Flashpoint:

The flashpoint (T_F) analyser(Ametek, Grabner Instrument, Austria) controlled by Cockpitsoftware was used to measure the flashpoint of each essential oil. About 2.0mL of essential oil was taken from the sample cap and placed in the furnace chamber. To assist the experiment, a standard method ASTM-D6450 was used. During the measurement of T_f (Flashpoint temperature), δ_{max} was set up to 26.5 P_{ka} and $P_{chamber}$ was invariably calibrated at 99.0 KP_a. The T_i (Initial temperature) and T_f (Final temperature) was programmed up to 60°C and 130°C respectively. For the appropriate precision, the nominal value was set at 100°C.

3.3.5 Specific Gravity:

The specific gravity of the essential oils was recorded using the instrument KEM- Density/ Specific gravity meter DA-500.

3.3.6 Optical rotation:

The optical rotation of the essential oils was determined using the Polarimeter (MCP 150, Anton Paar, Austria) as per users instructions. Briefly, the sample in a tube was placed in the polarimeter for determining the dextrorotatory (+) or Laevorotatory (-) characteristic of the oil at 20^0C. Mean values from three repetitions were considered as the result.

3.3.7 Refractive index:

The optical rotation of the essential oils was determined using the Refractometer (Abbemat 3200, Anton Paar, Austria) as per users instructions. Briefly, the instrument was switched on 10-15 minutes before recording the observations and the temperature was set to 20^0C. The instrument was calibrated using distilled water. The sample was placed on the prism and covered with a cover plate. Three subsequent readings were considered to arrive at the mean value of the sample.

3.3.8 Thermogravimetric analysis (TGA):

The thermogravimetric analysisof the essential oils was recorded using the instrument TGA-DSC-1 Mettler toledo.

3.3.9 Solubility in water:

This property plays a significant role in determining the quality of the sample. 1ml/1mg of the sample was introduced into a 10 ml glass stoppered cylinder (calibrated to 0.1 ml). Water was then pipetted into it drop by drop with concurrent shaking of the cylinder after each addition. The solubility of the sample was checked through visual observation.

3.3.10 Solubility in different organic solvents:

Solubility is an important parameter for checking the quality of the essential oil. Generally, the solubility is checked in dilute solutions of absolute alcohol it is of the highest polarity and it gives an idea about the presence of nature of components present in the material. However, the solubility test in alcohol is an important parameter to check the presence of any added

heavy material in essential oil which may sometimes get undetected by analyzing other parameters including GC.

100µL of each of the oil samples were instilled into the glass vials to test the solubility of each oil. Various ratios of ethanol: water and hexane:water from 1:9 to 9:1 were vortexed to check the solubility and the observations were recorded.

3.4 Phytochemical analysis of the selected formulations:

3.4.1 Extraction of the plant secondary metabolites in the form of the essential oil/ extracts:

The selected plants were subjected to extraction of their secondary metabolites (extract/ essential oil). The soxhlet apparatus was used for extraction of the plant extract, using different solvents (viz., Petroleum ether, Chloroform,Benzene, Methanol etc).However, the hydro distillation method, using Clevenger′s apparatus (Clevenger 1928), was used for isolating the essential oil from aromatic plants.Specific plant parts used for the extraction of the secondary metabolites were leaves, fruitsand rhizomes.

3.4.2 Soxhlet Extraction Method:

Soxhlet extractor is a hot continuous extraction device invented by Franz von soxhlet in 1879,designed for processing certain kinds of solids for extraction of secondary metabolites. Soxhlet extractor allows continuous treatment of a sample with a suitable solvent over hours or days to extract the desired compound. Briefly, the plant material was shade dried and chopped into small pieces and weighed. The weighed material is put into a cylindrical porous bag made of muslin cloth. The temperature is adjusted to the boiling point of the solvent being used, such that the solvent boils slowly and vaporizes. The vapours condense which gets directed to the plant material in the chamber from bottom to top. The plant material gets soaked with the solvent and once the solvent level reaches the siphon tube all the solvent gets siphoned back to the boiling flask. This cycle continues until the plant material releases a clear solvent which can be observed coming out of the siphon tube. The boiling flask

containing the extract in the solvent is vacuum dried to get a concentrated extract using a rotary evaporator.

3.4.3 Hydro-distillationMethod:

In hydro-distillation, a Clevenger apparatus is commonly used for the extraction of secondary metabolites.Clevenger apparatus is a glass apparatus invented by Clevenger (1928) and is commonly used for the extraction of volatile plant secondary metabolites/ essential oil. The method was utilized to isolate the essential oils from the aromatic plants. Briefly, the plant material (leaves, stems, fruits/fruit rinds, and rhizomes etc.)were washed, dried and chopped into small pieces. The material was filled into the flask and water was filled in a range of 50-70%, depending upon the type of material. The apparatus is heated to boil the water. The essential oil which gets evaporated gets condensed along with water. The oil being lighter floats on top of condensed water that can be collected at the end of hydro-distillation. The whole process takes about 4-6 hours upon which the essential oil is collected and added to anhydrous sodium sulphate to remove the dissolved water droplets.

In case the yield of the oil is too less and gets solidified with anhydrous sodium sulphate, or even to extract the oil from the left out sodium sulphate, the same is extracted out with dichloromethane to recover the essential oil.

3.4.4 Identification of the active constituents from selected plants:

The plant extract of *Litcea cubeba,Cymbopogon flexuosus, Zingiber officinale, Artemisia vulgaris, Citrus macroptera* and *Tithonia diversifolia* essential oils and ethanol extract were subjected to identification of their active constituents. This was achieved using the following methods and their observations were recorded.

3.4.5 GC and GC-MS analysis:

The essential oil analysis was carried out using GC-FID and GC-MS techniques. Quantification of the essential oil constituents was done on Centurion Scientific Gas Chromatograph (model CS-5800), equipped with flame ionisation detector (FID) and BP-5 fused silica capillary column (5% phenyl)-polymethylsiloxane stationary phase;

30m - 0.25 mm internal diameter; film thickness 0.25 µm). The oven temperature was programmed from 60°C to 240°C with an increase of 3°C min-1 and a final hold time of 10 min. The injector and detector temperatures were 240°C and 250°C, respectively. Nitrogen was used as carrier gas at 1.0 mL min-1. The injection volume was 0.3 µL (diluted in hexane: 1:3) with a split ratio of 1:60. GC-MS analysis was done using a Clarus 680 GC interfaced with a Clarus SQ 8C mass spectrometer of PerkinElmer fitted with Elite-5 MS fused-silica capillary column (5% phenyl)-polymethylsiloxane stationary phase; 30 m × 0.25 mm internal diameter, film thickness 0.25 µm). The oven temperature program was from 60 to 240°C, at 3°C min-1, and programmed to 270°C at 5°C min-1. Injector temperature was 250°C; transfer line and source temperatures were 220°C; injection size 0.03 µL neat; split ratio 1:50; carrier gas He at 1.0 mL min-1; ionization energy 70 eV; mass scan ranges 40-450 amu. Identification of the essential oil constituents was achieved based on retention index (RI), MS Library search (NIST and WILEY), and by comparing RI and mass spectral data with the literature (Adams, 2007). The relative amounts of individual components were calculated based on GC peak area (FID response) without using a correction factor.

3.4.6 HPLC analysis:

Upon screening the most active extracts through *in vitro* and *in vivo* studies, the leadextracts found to be most active were subjected to HPLC analysis to detect the natureor type of compounds present. The RP-HPLC-PDA system was used for thepreparation and characterization of compounds in samples. Waters (Milford MA, USA) HPLC system equipped with a binary pump, manual injector, photodiode array detector(PDA, model 996), Empower Pro software (Waters, USA) were used for the analysis.For the separation and quantification of compounds, the RP Column of Sunfire C18 (4.6mm×250mm, 5µm coating; Waters, USA) was used.

The standards used in the study were Ascorbic acid, Chatechin, Syringic acid, Narigin and Bicalein. Each standard was dissolved in methanol and the stock solution was prepared at a concentration of 1mg/mL. The analysis of the samples was performed by using a gradient solvent system at 30°C.

The mobile phases for gradient elution comprised of solvent A (0.1% TFA withwater) and B (Methanol, 100%), mixed according to the following profile: 25% B to35% B (0-25 min), then 35% B to 50% B (25-50 min), then 50% B to 80% B (50-60min) and 80% to 100% B (60-70 min). 20 µL of injection volume was used forstandards and samples with 0.8 mL/min flow rate. The Signals were detected at 228and 278 nm for the quantification of compounds (Tewari et al., 2017).

3.5 Bioprospection against the pathogenic fungi/ Dermatophytes:

3.5.1 Procurement of test organism from CIMAPLucknow:

Five common dermatophytes viz., *Trichophyton rubrum*; *Microsporum gypseum*,*Malassezia furfur, Candida albicans* and *Staphylococcus aureus*,which cause skininfections in human beings, were selected for the present investigation. The authentic cultures of these dermatophytes were procured from the Microbial Type Culture Collection (MTCC).The strains of the cultures were:

i) *Trichophyton rubrum* (MTCC-296)

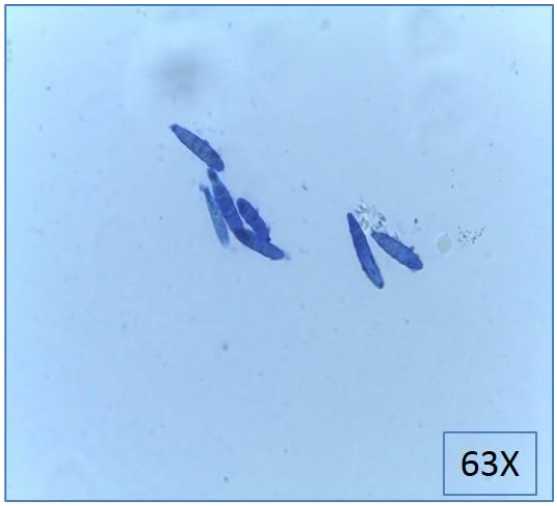
ii) *Microsporum gypseum* (MTCC-2819)

iii) Staphylococcus aureus (ATCC-6538)

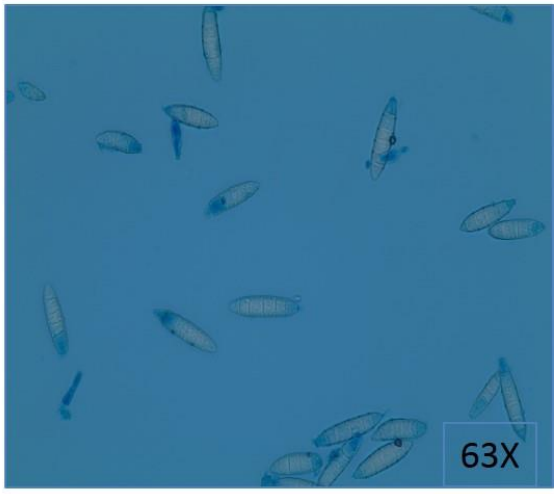
iv) Malassezia furfur (MTCC-1374)

v) Candida albicans (ATCC-0443X-1)

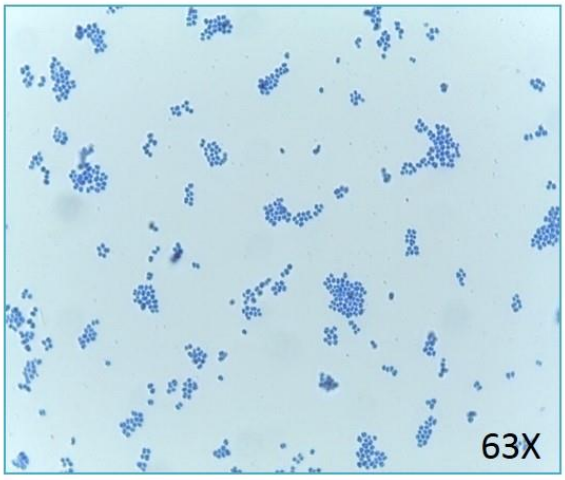

SDA- 22/7/20 - 1 T. rubrum 3/8/20
Trichophyton rubrum in SDA (d 07)


63X

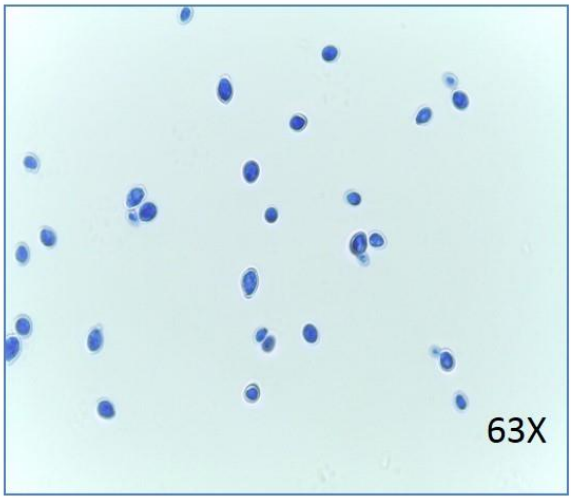

SDA 30/7/20 SB
Microsporum gypseum in SDA (d 07)

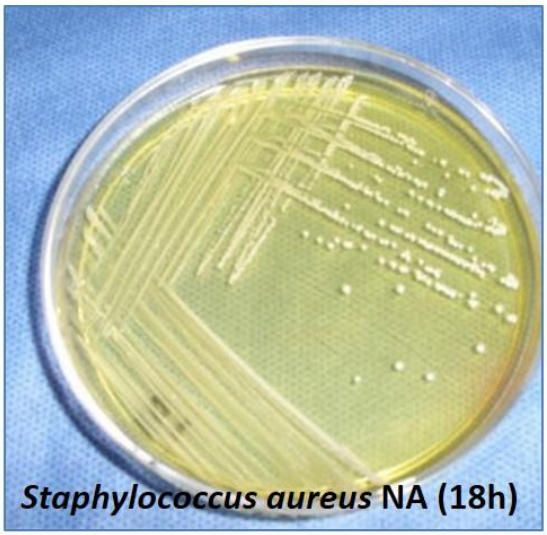

63X

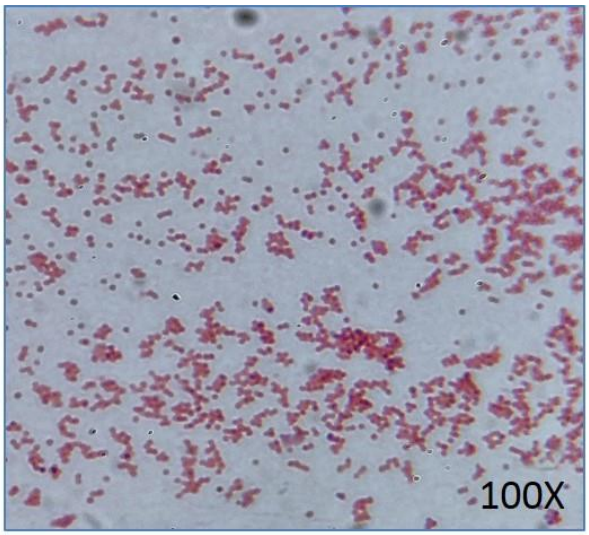

Malasezzia furfur in SDA (72 h)


63X


Candida albicans SDA (72 h)


63X


Staphylococcus aureus NA (18h)


100X

3.5.2 Protocol for Revival of Pathogens:

The cultures thus procured were subjected for the revival of the pathogens, using the following protocols:

- The ampoule wascarefully opened as the contents are in a vacuum.
- With a sharp file the ampoule was markednear the middle of the cotton wool
- The surface around the mark was disinfected with alcohol.
- The ampoule was wrapped with thick cotton around and snapped at the marked area.
- The pointed tip of the ampoule was gently removed. Snap opening could draw the cotton plug to one end; a hasty opening could release fine particles of dried organisms into the air of the laboratory.
- The cotton plug was carefully removed and added about 0.3 to 0.4 ml of SDA / BHI /NA/ Dixon's agar medium to make a suspension of the culture. Avoided frothing or creating aerosols.
- To the medium, a few drops of the suspension was streaked (solidified with agar) in a Petri dish.
- At recommended temperature (25±2^{o}C for fungi and 37±2^{o}C for bacteria),the Petri dishes wereincubated and conditions for the proper culture.
- For *Malasezzia* sp., we used Dixon's agar and incubated the dishes at 30±2^{o}C
- When the ambient conditions were provided, colonies started showing up within 5-7 daysand growth of the culture could be visible.
- Before discarding all the remains of the original, ampoulesare treated as infected and autoclaved.

3.5.3 Multiplication of the pathogens:

The revived cultures were multiplied on the Sabouraud's Dextrose Agar (SDA), Brain Heart Infusion (BHI)and Dixon Agar for further investigations.Throughout thestudy, a routine ofsub-culturing was applied for purification and maintaining the pure culture.

3.5.4 Preservation of the pathogens:

Preservation is required to maintain standard working stock which can be used for further experiments and also they are reliable against loss due to equipment failure or contamination by other microbial organisms. The bacterial strains are preserved by the method of freeze-dried cultures (lyophilization) or at temperatures below -130˚C (cryopreservation). Glycerol and DMSO are used as cryoprotectant agents. The freshly grown bacterial strain under optimal conditions in an appropriate medium was prepared for freezing. The sterile 20% glycerol stock at a final concentration of 10% or 50% DMSO stock at a final concentration of 5% was added in a culture medium. It is then mixed with an equal amount of cell suspension. Aliquot 1 to 1.8 mL of the bacterial suspension in 2 or 3 ml cryovials respectively with appropriate identification code of the bacterial strain and the date. The plastic ampoules were tightly sealed with the screw cap and 'O' ring on them. The cells were equilibrated in the freezing medium at room temperature for a minimum of 15 minutes but no longer than 40 minutes. The vials were placed into a pre-cooled (4°C) chamber and then transfer again into the mechanical freezer at -70°C (or colder) for at least 24 hours. Alternately, a pre-cooled (4°C) programmable freezer unit was used, set to cool the vials at -1°C per minute until a temperature below -40°C is achieved and then set the temperature to abruptly drop to -130°C. Finally, the vials were quickly transferred into liquid nitrogen or a -130°C freezer. A proper record of the location and details of the cryovials were maintained. After 24 hours at -130°C, the cryopreserved strain was tested for viability by removing one vial and restoring it in the culture medium. The recovery of cryopreserved cells requires the rapid thawing of the bacterial suspension in a 37˚C water bath.

3.5.5 Preparation of Stock Solution of the 'Test Sample':

Since, the entire 'test sample' used in the present research were insoluble in water but soluble in organic solvents, hence, DMSO (dimethyl sulphoxide - an organic solvent) was used for preparing their stock solution.

(i) Preparation of stock solution for natural 'test sample':

10mg or higher concentration (100mg) of essential oil/ active constituent was weighed and dissolved in 1 ml DMSO. The stock solution was aliquoted and stored at -4°C.

(ii) Preparation of stock solution for synthetic 'test sample' :

2-10mg of synthetic 'test sample' as a positive control was weighed and dissolved in 1 ml DMSO. The stock solution was aliquot and stored at -20^0C.

3.5.6 Disc Diffusion Assay (DDA):

Disc diffusion method is used for screening the anti-microbial properties of the extract. 0.1ml of microbial suspension containing 1×10^6cfu/ml inoculumwas uniformly spread over the media to form a lawn of the culture. The stock solution was prepared in dimethyl sulfoxide solvent (DMSO). Sterile What man paper discs (6 mm diameter) were onto the media plate with the installation of 20µl of the stock solution. The plates with the discs were placed in an was added and incubated in the incubator. Depending on the microorganism the time and temperature of the incubator were set. Inset of controls and antibiotics/anti-fungal was used as a negative control.The zone of inhibition was calculated by using the following formula:

W=(T-D)/2

Where:

W - Diameter of clear zone of inhibition

T - Total diameter of including disc and clear zone

D - Diameter of the test Disc

3.5.7Minimum Inhibitory Concentration (MIC):

- 'U' bottom 96 well Microtitre culture plates were used. All the wells were filled with 100µl of sterilized media from 1-11 and 200 µl in 12^{th} of the plate using a multichannel pipette.
- 100 µl of test sample{Antibiotic, extract fraction or compound (10mg/ml)} were filled in the first well and serially diluted two folds up to the 10^{th} well. 11^{th} well was used as growth control (broth + inoculum only)

- 100µl of the inoculum was added to all the wells except at the 12^{th} row (acts as a blank control for sterility).
- The plate was incubated overnight at 25-27^0C for fungi and at 35-37^0C for bacteria.
- 40µl of 0.02mg/ml of p-iodonitrotetrazolium chloride wasfilled in each well and again incubated for 2-4 hrs. (Weight 10mg of p-iodonitrotetrazolium chloride and dissolve in 500µl of Ethanol).
- The well containing the lowest dilution that shows colour indicates growth and so the previous well indicates the MIC.

3.5.8 Minimum Fungicidal/Bactericidal concentration(MFC/MBC):

The minimum killing time was performed from the result of minimum inhibitory concentration (MIC). 100µl from each row of MIC were spread on fresh media plates with the help of a spreader. Incubation temperature was adjusted depending on the type of pathogen. A clear plate without any colonies was indicative of the Minimum Fungicidal/Bactericidal concentration.

3.5.9 Minimum Killing Time (MKT):

Briefly, 2X MIC level of the oil was prepared and inoculated with freshly prepared inoculum (1 x 10^6CFU/ml) and incubated at 28°C /37°C. 50 µl samples from the above test tubes were loaded and firmly spread with the help of L-shape spreader onto BHI /SDA/Dixon plates at 0, 4, 6, 9, 12, 18, 24,36 hours intervals and incubated overnight. The activity was observed after overnight incubation of the plates at 28°C and 37°C for bacteria. No growth on the spread plate was considered to be the time required by the oil to kill the organism.

3.5.10 Sorbitol Protection Assay:

In this assay,MIC determinationswere conducted with and without the presence of 0.8M Sorbitol.

3.5.11 Scanning Electron Microscope (SEM):

The inoculum of the pathogen 0.5% McFarland was prepared and was treated with the known 2X MIC value of the samples to investigate the morphological and structural changes.

The cells were harvested after incubation time was over and washed three times with phosphate buffer (0.1M, pH 7.2). Samples were fixed in 3% Glutaraldehyde (pH 7.2) for 12 hours and further rinsed with distilled water. Subsequently, samples were dehydrated with increasing concentrations of acetone (20, 30, 50, 60, 70, 80, 90, and 100%) for 10 min in each graded acetone concentration. Samples were dried in a critical point drier, mounted on aluminium stubs using two-sided adhesive carbon tape and coated with a thin layer of gold using Sputter Coater Unit (Quorum technology, UK). Coated samples were analyzed in a scanning electron microscope (FEI, Netherlands), at an accelerated voltage of 15 - 20 Kv**.**

3.5.12 Confocal microscope:

The tested pathogensat the logarithmic phase were added in tubes containing Samples of essential oil /Extract (2X MIC) and cultured at 28°C/ 37°C for 4-6 hrs. The group without test sampleswere marked as a control. After 6 hrs incubation both the control group and treated groups of pathogen suspension was centrifuged at 10000rpm for 10 minutes. The supernatant was removed and the pellet was incubated with 10 μg/mL−1 4′6-diamidino- 2-phenylindole (DAPI) in dark for 15 min. The pathogen suspension was again centrifuged and reconstituted with Phosphate Buffer Solution (PBS). Prepared samples were further analysed using LSCM (Leica Microsystems CMS GmbH, Mannheim, Germany) with an excitation wavelength of 364 nm and an emission wavelength of 454 nm (TCS SP5 II, Leica, Germany).

3.5.13 Protocol for Skin irritation test:

New Zealand White rabbits were used for the experiment. The fur of the animals was carefully removed and shaved on the lateral sides of the body. Either side of the body of each animal was considered as a control and test site. The animals were kept undisturbed for 2-3 days, to ensure that minor inflammation subsides before the actual experiment. One square inch area was marked and 50µl of the test sample was applied. A non-absorbant patch was placed over the marked area and secured with micropore tape for 4 hours. The same was removed and washed with normal water to remove the test material. Observations were taken after 4, 24, 48 and 72 hrs. The observations were scored (on a scale of 0-4) based on cardinal symptoms of pain and inflammation i.e., erythema (redness), and oedema (swelling). The average score of the observations was used to calculate the primary irritation index (PII) by

subtracting the score of the control site from the test site scores. Federal Health Standards Act was considered to interpret the result.

3.5.14 Cytotoxicity study:

The cell viability studies were undertaken in J774A.1 cells which are macrophages derived from mouse monocytes. These are mostly adherent cells that are active in antibody-dependent phagocytosis. They synthesize large amounts of lysozyme and exhibits minor cytolysis but predominantly antibody-dependent phagocytosis. Interleukin 1 beta (Il1b) is synthesized continuously by this line.

Cell viability was determined by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay.The MTT assay determines the viability of cells by the reduction of yellow soluble MTT in the metabolically active cells. J774A.1 cells were seeded at a density of 0.5×10^6 cells/mL in 96 well plates at 37ºC under 5% CO_2.The adherent cells were treated with different concentrations(0.1µg, 1µg, 10µg) of samples using DMSO as diluent. However, the concentration of DMSO in the final treatment did not exceed 1%. Cells incubated in culture medium alone served as a control for cell viability (untreated wells). After incubation, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and left for incubation again for 4hrs. Following incubation, 200 µL of supernatant culture medium were carefully aspirated and replaced with 200 µL of DMSO to dissolve the formazan crystals, followed by incubation for 10 minutes. The culture plates are then read spectrophotometrically (Molecular Devices, USA) at 550nm. The rise in optical density is directly proportional to the number of viable cells. The results were expressed relative to the control (untreated cells) wells.Cell viability was calculated and expressed as the percentage.

$$\%\, Survival = \frac{(Mean\ experimental\ absorbance)}{(mean\ control\ abosorbance)} x\ 100$$

3.5.15 Maintenance of J774A.1 cell line:

J774A.1 cells are mostly adherent macrophages which are obtained from *Mus musculus*, mouse of BALB/c strain. J774A.1 cells are active in antibody-dependent phagocytosis and their growth is inhibited by dextran sulphate and LPS.They synthesize large amounts of lysozyme and exhibits minor cytolysis but predominantly antibody-dependent phagocytosis. Interleukin 1 beta (IL1β) is synthesized continuously by this line.The cell line was procured

from the National Centre for Cell Sciences, Pune, India and maintained in our cell culture facility. The cells were maintained and grown in Dulbecco's Modified Eagle's Mediumcontaining 10% fetal bovine serum in 75 cm^2 flask, D-glucose, Sodium bicarbonate, 100 IU/ml penicillin and 100 µg/ml streptomycin and fungizone in a humidified incubator, at 37^oC and 5% CO_2 atmosphere. Cryopreservation of the cells is done in a complete growth medium supplemented with 5%(v/v) DMSO and stored in the vapour phase of liquid nitrogen.

3.5.16 *In-vitro* anti-inflammatory profile:

J774A.1 cells were obtained from the National Centre for Cell Sciences (NCCS, Pune, India). Cells were cultured in DMEM (Dulbecco'sModified Eagle'sMedium, Sigma) supplemented with 10% fetal bovine serum (FBS) with 1x stabilized antibiotic–anti-mycotic solution (Sigma) in a CO_2 incubator at 37˚C with 5% CO_2 and 90% relative humidity (Singh et al. 2014). Cells at a concentration of 0.5×10^6 live cells/mLwere used for the experimentation. Cells were treated with test samplesand dexamethasone at the concentration of 1%, 0.1%, 0.01%, 0.001% and 1 µg/ml respectively, followed by stimulation with lipopolysaccharide (LPS) (0.1 µg/ml) for 6 hours. Pro-inflammatory mediators Tumour Necrosis Factor-α (TNF-α)in cell culture supernatant were determined by using Human-specific Enzyme Immuno Assay (EIA) kits (BD Biosciences, USA) following the manufacturer's protocol.

3.5.17 Effect of temperature on the efficacy of oil/ extract:

Effect of temperature on the efficacy of oil *Zingiber officinale, Artemisia vulgaris, Litsea cubeba, Cymbopogon flexuosus* and *Tithonia diversifolia* was also determined. Samples dissolved in DMSO was put in small vials andthey were exposed at 40, 60 and 80^{0}C in a hot water bath, separately. Further, the efficacy of the test sample was tested against the test microorganisms. This was made as per the usual method (NCCLS-2002), and the observations were recorded.

3.5.18 Comparison with some Synthetic Fungicides/ Antifungal drugs:

The efficacy of the essential oil and extract of *Zingiber officinale, Litsea cubeba, Artemisia vulgaris, Cymbopogon flexuosus, Citrus macroptera* and *Tithonia diversifolia* was compared

with some synthetic antifungal drugs, available in the market viz., Clotrimazole, Vancomycin. The observations were recorded by comparing their minimum inhibitory concentrations

3.6 Safety study:

3.6.1 Acute oral toxicity:

The safety profile of the bio-active extract at the dose of 2000 mg/kg body weight was assessed through acute toxicity which was ascertained according to OECD (Organization for Economic Co-operation and Development) guideline (No.420) as reported by *Chanda et al.*, (2009). Swiss albino mice were taken for the acute oral toxicity study and randomly divided into experimental groups (6 mice/group comprising of 3 males and 3 females caged separately) weighing between 20 ± 2g.Before the start of the trial,the experimental mice were keptfasting overnight. Samples were prepared in 0.7% Carboxy-methyl-cellulose (CMC) and administered orally at a single oral dose.

The animals were observed for the first four hours and thereafter overnight for any abnormality in the skin, mucous membrane, eyes, secretions and excretion, lachrymation, piloerection, respiration pattern etc. In addition to the observational study, body weight(s) were recorded and the blood of each experimental mice was collected through the retro-orbital route for haematological (RBC, WBC, and haemoglobin) parameters and serum was separated from the blood for the serum biochemical parameters.The animals were then sacrificed and necropsied for any gross pathological changes. Weight of vital organs like liver, heart, kidney etc. was also recorded.

3.6.1.1 Hematological analysis:

Total White Blood Cell (WBC) and Red Blood Cells (RBC) counting was attainedthrough microscopy (DMLB 2, Leica, Germany) at 40X magnification using a Neubauer's chamber.

3.6.1.2 Red Blood Corpuscles (RBC) counting:

At the ratio of 1 to 200 respectively a dilution of blood and RBC diluting fluid was prepared. 10 µl of the diluted sample was placed in the Neubauer's chamber and the prepared solution was allowed to settle for a couple of minutes. The cells were counted microscopically by using 40X objective. The number of cells in 5 areas of 16 squares was counted.

3.6.1.3 White Blood Corpuscles counting:

At the ratio of1 to 20 blood sample was mixed with WBC diluting fluid.10 µl of the diluted sample(s)was introduced into a Neubauer's chamber and counted under the microscope at 40X magnification. The number of cells from 4 areas of 16 corner squares was counted.

Annexure-I

Ref: MZU/HAMP/PhD/2014

QUESTIONNAIRE

Data acquisition questionnaire for Finished Product, Aizawl District, Mizoram

Informants details:

Name : _____ Sex: M/F Age: _____

Occupation : _____

Education : _____

Location/Residence : _____ Contact no. if any _____

EFFICACY DATA:

Type of Plant : Habitat:

Local Name :

Availability :

Food purpose : Medicinal purpose:

Site of predliction : Skin/ Scalp

Route of application : Dosage:

Response of Patient : Commercial purpose:

Any other comments

RESPONDENTS CONSENT AGREEMENT

I _____, hereby agreed to participate in this study with my full consent and consciousness and declare that to the best of my knowledge, the information that I have provided is true, accurate and complete.

Date:

Signature/thumb impression

AVAILABILITY OF LITERATURE:

_____

(Laldingngheti Bawitlung)
Ph.D. research Scholar

(Dr. Awadhesh Kumar)
Ph.D. Supervisor

Department of Horticulture Aromatic and Medicinal Plants, Mizoram University, Aizawl-796004

3.7 STOCKS, REAGENTS AND BUFFERS:

3.7.1 McFarland Standard:

According to the NCCLS/ CLSI norms the standard inoculums range from 1×10^6 cells /ml to 0.5×10^6 cells /ml. To prepare an inoculum with this range, the best method is- matching of turbidity of the inoculums suspension at 530 nm with 0.5 McFarland standards. Because the absorbance of 0.5 McFarland is equal to the absorbance of inoculums suspension containing 0.5×10^6 cells /ml.

3.7.2 Preparation of McFarland Standard Stocks:

1. 1% H_2SO_4: 2.04ml dissolved in 197.96 ml TDW (Triple Distilled Water)
2. 1% $BaCl_2$: 0.1 gm dissolved in 10ml TDW.

3.7.2 Protocol for preparation of Standard McFarland solution

McFarland	1% H_2SO_4	1% $BaCl_2$
1	9.9	0.1
2	9.8	0.2
3	9.7	0.3
4	9.6	0.4
5	9.5	0.5
6	9.4	0.6
7	9.3	0.7
8	9.2	0.8
9	9.1	0.9
10	9.0	1.0

Optical density was recorded at 530 nm. McFarland was diluted 10 times and corresponding O.D was recorded.

3.7.3Normal Saline Solution (0.9%)

NaCl	0.8gm
D.W	100 ml

The contents were mixed well and then autoclaved at 121^0C, 15 lbs for 15 min.

3.7.4 Phosphate Buffered Saline (PBS)

NaCl	8gm
KCl	0.2gm
KH_2PO_4	1.44gm
Na_2HPO_4	0.24gm
D.W	1000ml

3.7.5 MEDIA AND CHEMICALS:

2.7.5.1 Brain Heart Infusion agar (pH 7.0) Gms / Litre

Beef heart, Infusion	250.0
Disodium phosphate	2.5
Nacl	5.0
Dextrose	2.0
Peptone	10.0
Calf Brain, Infusion	200.0
Agar	15.0
Makeup volume	1000

37.0 grams of the media was suspended in 1000 ml purified/distilled water. The medium was mixed well, and dissolved completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Finally pour into sterile Petri plates.

3.7.5.2 Sabouraud Dextrose Agar (pH 5.6±0.2) Gms/Litre

Dextrose (Glucose)	40.00
Mycological, peptone	10.00
Agar	15.00
Makeup volume	1000

65.0 grams of the media was suspended in 1000 ml purified/distilled water. The medium was mixed well, and dissolved completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Finally pour into sterile Petri plates.

3.7.4.3 Sabouraud's Dextrose Broth (pH 5.6±0.2) Gms/Litre

Dextrose (Glucose)	20.00
Peptone, special	10.00
Makeup volume	1000

30.0 grams of the media was suspended in 1000 ml purified/distilled water. The medium was mixed well and dissolved completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Finally pour into sterile Petri plates.

3.7.4.4 Dixon's Agar (Twin Pack) (pH 6.0±0.2) Gms / Litre

Part A	
Malt extract	36.00
Peptone	36.00
Bile Dessicated	20.00

Agar	14.50
Part B	
Tween	10.00
Glycerol mono-oleate	5.00

15ml of fluid Part B was suspended in 1000 ml purified/distilled water. 106.5 grams of Part A was added. Mix well and heat to boiling to dissolve the medium completely. It was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Finally pour into sterile Petri plates.

3.8 Preparation of RPMI-1640 Media (1X)

Components	Quantity/Volume
RPMI-1640 Media	3.24 gm
Double distilled water	40L

For further use, the prepared solution was filtered through a 0.22µm syringe filter and stored at 4^0C.The prepared 1X solution of media was used as a stock solution.

3.81 Preparation of complete medium for cell lines

Components	**Quantity/Volume**
RPMI-1640 Media (1X)	200mL
Sodium bicarbonate ($NaHCO_3$)	2100mg
Glucose	2000mg
Penicillin-streptomycin	10mL
Fungizone	1mL
FBS	100mL

3.8.2 Cryopreservation buffer

	Components	Quantity/volume
1.	FBS (90%)	90 mL
2.	DMSO (10%)	10 mL
The components mentioned above were mixed and filtered with a 0.22 μm syringe filter to make 100 mL of cryopreservation buffer for cell lines		

3.9 Preparation of coating buffer for ELISA

Components	Quantity
Sodium carbonate (Na2CO3)	3.56 gm
Sodium bicarbonate (NaHCO3)	8.40 gm
The chemicals were mixed in 900 mL of double distilled water and the pH of the solution was adjusted to 9.6. The final volume was achieved up to 1000 mL.	

3.9.1 Preparation of washing buffer for ELISA

Components	Quantity
1X PBS	1000 mL
Tween 20	0.5 mL
For further use, the prepared solution was stored at 4ºC	

3.9.2 Preparation of assay diluent for ELISA

Components	Quantity/volume
1X PBS	1000 mL
FBS	100 mL
For further use, the prepared solution was stored at 4ºC	

CHAPTER - 4

RESULTS

4.1 Collection, categorization, identification and documentation of common ethno medicinal plants, used against skin ailments among the tribal communities:

The present research work was undertaken to decipher the scientific basis of traditional formulations used by tribal communities or the traditional healers of Mizoram for treating skin ailments. A detailed survey was conducted through verbal queries and it was observed that the formulations being prescribed were related to itching of the skin or scalp, pruritus, redness and inflammation. Further, discussions concluded the narrowing down of six formulations for which we presumed the aetiology to be of microbial origin. Analysing the components of the prescribed formulations, it was observed that the herbs used were primarily *Zingiber officinale, Litcea cubeba, Artemisia vulgaris, Cymbopogon flexuosus, Citrus macroptera* and *Tithonia diversifolia*. (Table 4.1).

4.2 Physico-chemical properties of selected essential oils:

The selected essential oils were subjected for various physio-chemical characterization viz., appearance, odour, texture, specific gravity, optical rotation, refractive index, flash point, Thermo gravimetric analysis (TGA), solubility in water and solubility in an organic solvent. The observations thus recorded are given in (Table 4.2)

Table 4.1 Formulations prescribed by the Mizo traditional healers for skin ailments

S.no.	Formulae	Ailment/Indication	Method of use	Suspected aetiology
1		Pimple, Ringworm, sore	Topical application of juice or infusion of *Artemisia vulgaris* and *Tithonia diversifolia* on the affected part of pimple, ringworm and other skin infection.	*Staphylococcus aureus*, *Candida albicans*, *Macrosporum gypseum* and *Trichophyton rubrum*.
2		Ringworm, Pimple and pruritus	Topical application of juice extracted from leaves and fruits of *Litsea cubeba* on the affected part of different skin ailment	*Staphylococcus aureus*, *Candida albican*, *Macrosporum gypseum* and *Trichophyton rubrum*
3		Ringworm, pimple and Dandruff	Paste derived from the homogenization of extracted juice from *Zingiber officinale* and the pulp of *Persea americana* (butter fruit) applied topically on the infected areas of the skin or scalp	*Macrosporum gypseum*, *Trichophyton rubrum*, *Candida albican*s and *Malassezia furfur*
4		Pimple or ringworm	Paste derived from *Zingiber officinale* and essential oil of *Cymbopogon flexuosus* was applied topically on the infected region of pimple and ringworm	*Macrosporum gypseum*, *Trichophyton rubrum*, and *Candida albican*s
5		Dandruff, boil and pimple	The extracted juice of *Tithonia diversifolia*, *Zingiber officinale* and *Citrus macroptera* leaves was used topically for dandruff infection.	*Malassezia furfur* and *Staphylococcus aureus*
6		Skin ailment with itching followed by inflammation	The extracted juice of *Zingiber officinale* and essential oil from the fruit rind of *Citrus macroptera* was applied topically on the infected portion of different skin ailments.	*Malassezia furfur*, *Staphylococcus aureus* and *Candida albican*

Table 4.2. Physio-chemical properties of selected essential oils and extract

Parameter studies	*Zingiber officinale*	*Artemisia vulgaries*	*Litsea cubeba*	*Cymbopogon flexuosus*	*Tithonia diversifolia*	*Cirtus macroptera*
Appearance	Light yellow	Pale green	Pale yellow	Pale yellow	Dark green	Brownish yellow
Odour	Pleasantly pungent aroma	Camphoros, herb-like, and bitter	Sweet fresh lemon citral, green grassy	Citrusy scent	Bitter	Pleasant, Citrusy scent
Texture	Non- sticky aromatic	Non- sticky aromatic	Non -sticky aromatic	Non -sticky aromatic	Greasy semi solid	Non -sticky aromatic
Flash point	59.5°C	66.5°C	84.5°C	88.5°C	NA	51.5
Specific gravity at 25^0C	0.9106	0.9403	0.8952	0.8951	NA	0.8474
Optical rotation at 20^0C	(+)6.28	(-)16.24	(+)0.4	(-)0.32	NA	+93.68
Refractive index at 20^0C	1.4888	1.4804	1.4868	1.4880	NA	1.47196
Thermo gravimetric analysis (TGA)	195°C	200°C	205°C	207°C	NA	146°C
Solubility in water	Insoluble	Insoluble	Insoluble	Insoluble	Soluble	Insoluble
Solubility in organic solvent	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble

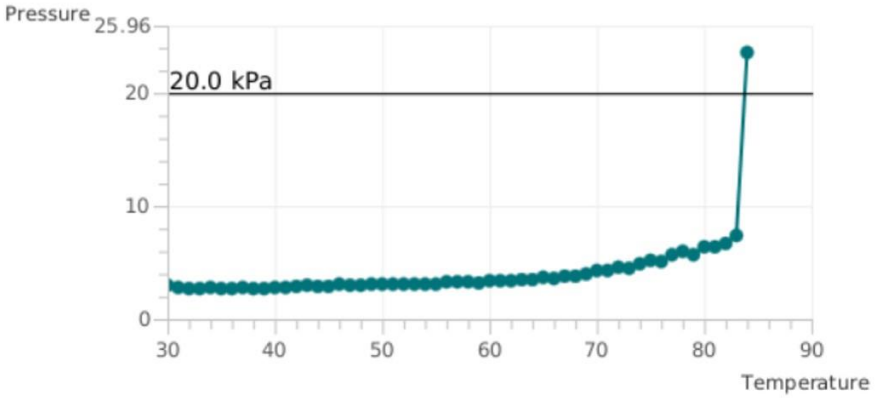
NA-Not applicable (Ethanol extract)

4.2.1 Flash Point of the essential oils

Ti	30.0 °C	Tf	90.0 °C
Ti-T	0.0 °C	Stirrer	true
Cold filling	true	SQC	true
Published Reproducibility	0.00	Accuracy Monitoring	true
ARV	0.00	Pass/Fail-Evaluation	true
Nominal value	70.0 °C		

Tflash = 84.5 °C

DeltaPmax = 23.6 kPa
Pchamber = 99.1 kPa

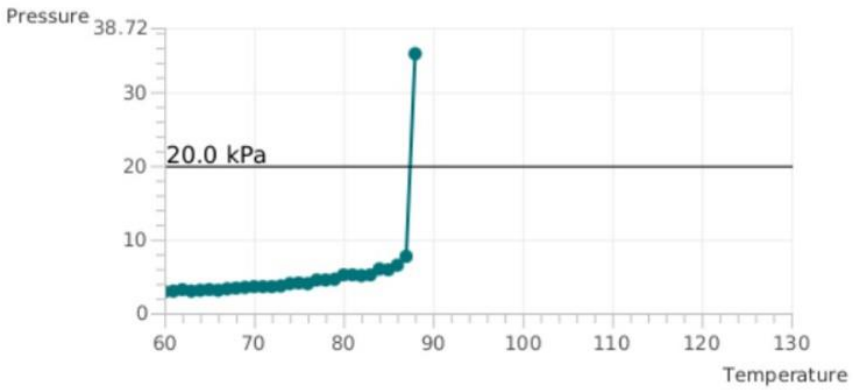


Litsea cubeba

Ti	60.0 °C	Tf	130.0 °C
Ti-T	0.0 °C	Stirrer	true
Cold filling	true	SQC	true
Published Reproducibility	0.00	Accuracy Monitoring	true
ARV	0.00	Pass/Fail-Evaluation	true
Nominal value	100.0 °C		

Tflash = 88.5 °C

DeltaPmax = 35.2 kPa
Pchamber = 99.0 kPa

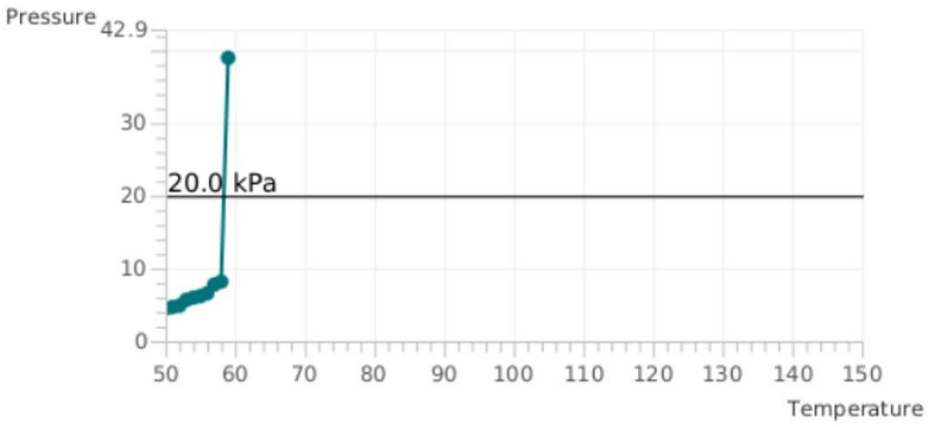


Cymbopogon flexuosus

Ti	50.0 °C	Tf	150.0 °C
Ti-T	0.0 °C	Stirrer	true
Cold filling	true	SQC	true
Published Reproducibility	0.00	Accuracy Monitoring	true
ARV	0.00	Pass/Fail-Evaluation	true
Nominal value	130.0 °C		

Tflash = 59.5 °C

DeltaPmax = 39.0 kPa
Pchamber = 99.1 kPa

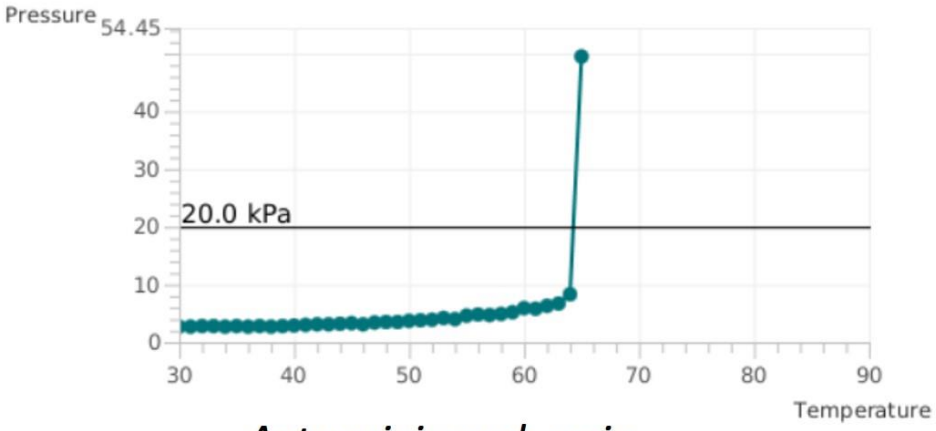


Zingiber officinale

Ti	30.0 °C	Tf	90.0 °C
Ti-T	0.0 °C	Stirrer	true
Cold filling	true	SQC	true
Published Reproducibility	0.00	Accuracy Monitoring	true
ARV	0.00	Pass/Fail-Evaluation	true
Nominal value	70.0 °C		

Tflash = 65.5 °C

DeltaPmax = 49.5 kPa
Pchamber = 99.1 kPa

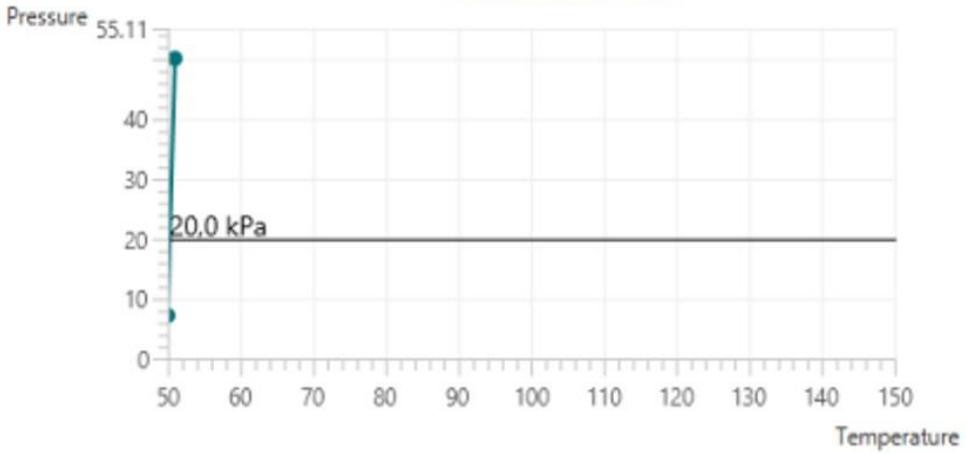


Artemisia vulgaris

Ti	50,0 °C	Tf	150,0 °C
Ti-T	0,0 °C	Stirrer	true
Cold filling	false	SQC	false
Published Reproducibility	0,00	Accuracy Monitoring	true
ARV	0,00	Pass/Fail-Evaluation	true
Nominal value	79,0 °C		

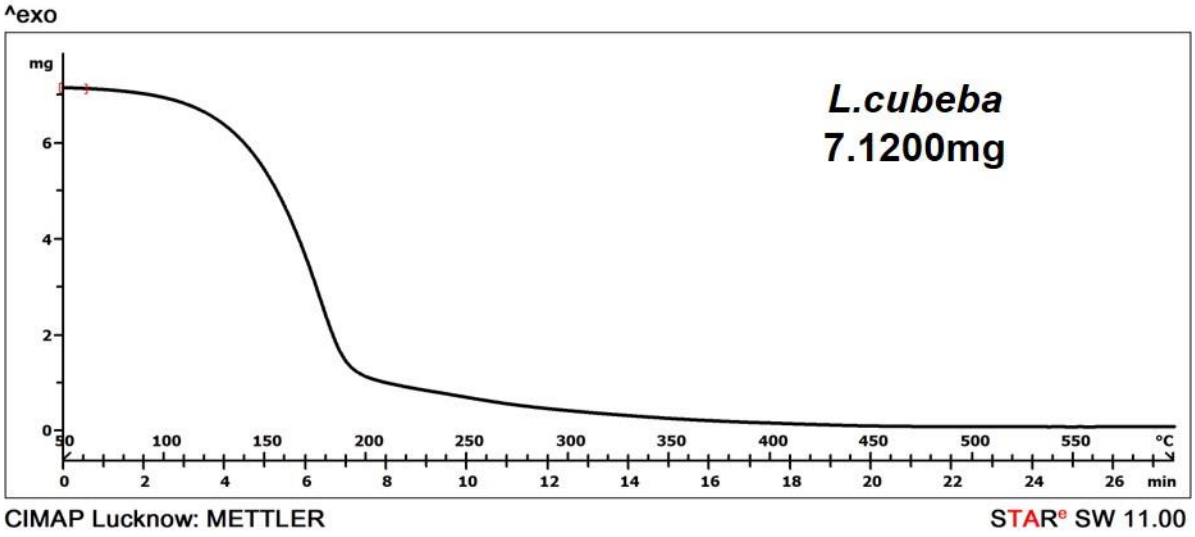
DeltaPmax = 50,1 kPa
Pchamber = 99,5 kPa

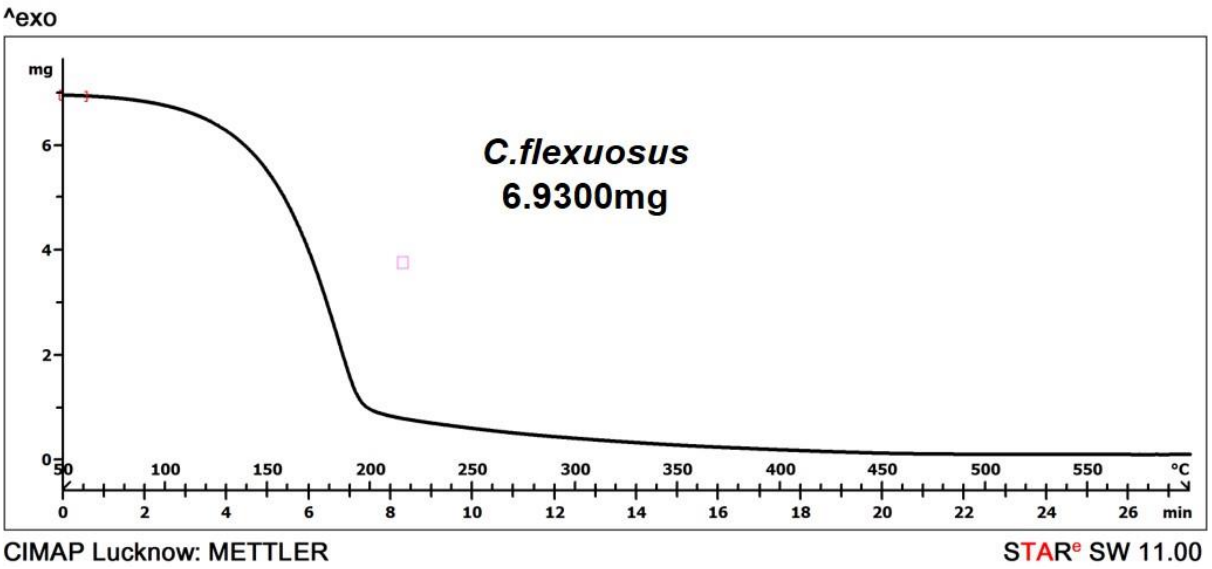
Tflash = 51,5 °C

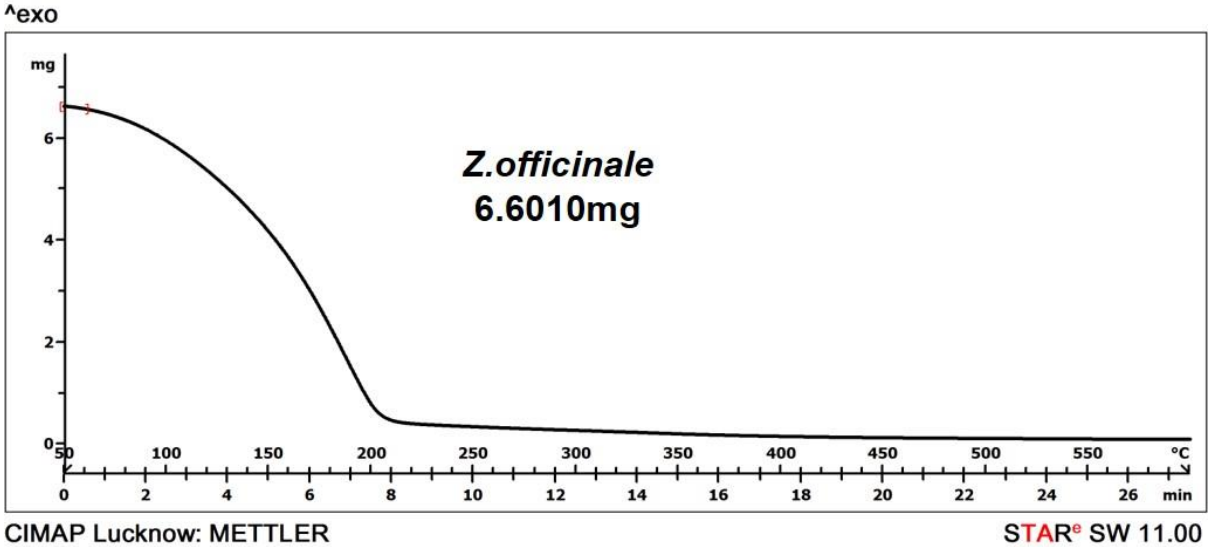


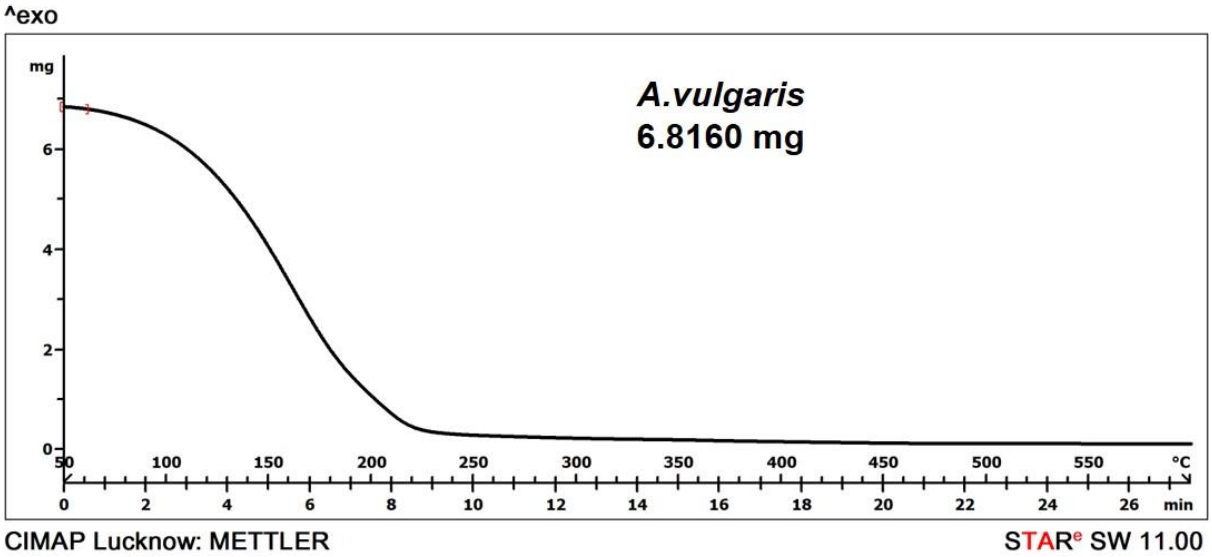
Citrus macroptera

4.2.2 Thermogravimetric analysis(TGA)of bio-active essential oils





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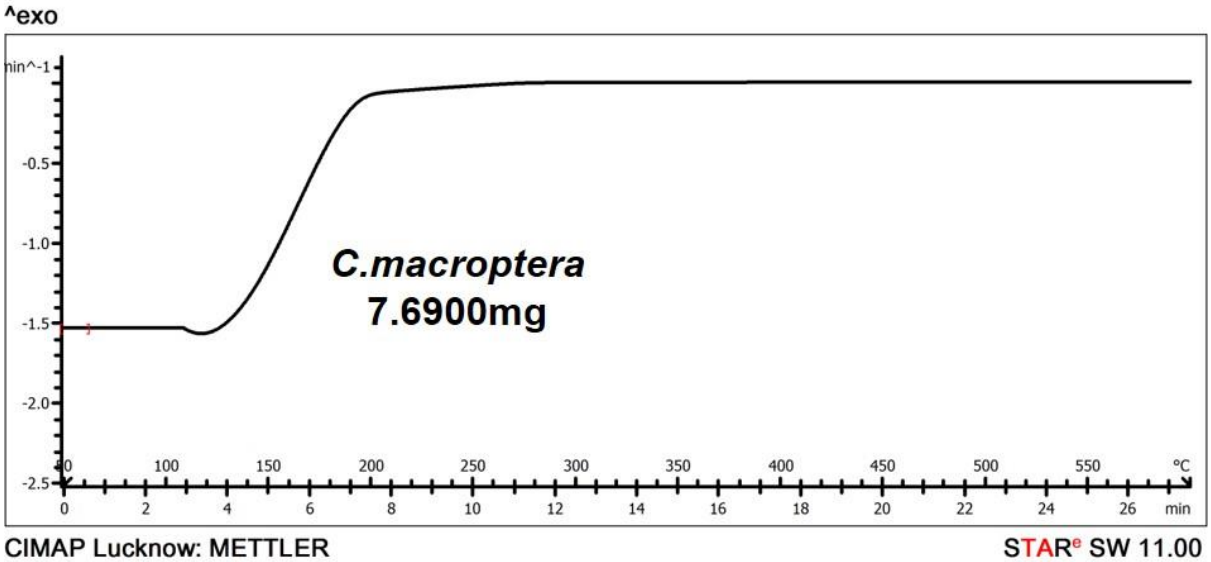
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4.3 Identification of the volatile compounds from selected plant essential oils:

The essential oils of *Litsea cubeba, Cymbopogon flexuosus, Zingiber officinale, Artemisia vulgaris, Citrus macroptera* and *Tithonia diversifolia* were subjected to identification of their active constituents. The observations were recorded as follows:

4.3.1 Volatile compounds of *Litsea cubeba* essential oil using GC MS analysis:

The GC-MS analysis of the essential oil of *Litsea cubeba* revealed the following major compounds; Geranial (=Citral a) (26.64%), Geranyl acetate (20.51%), Neral (=Citral b) (19.52%) and Geraniol (19.51%). Besides, other minor compounds detected and quantified have been mentioned in the (Table 4.3.1)

Table 4.3.1: Volatile compounds of *Litsea cubeba* essential oil

S.no.	Compound	Content (%)
1.	Geranial (=Citral a)	26.64
2.	Geranyl acetate	20.51
3.	Neral (=Citral b)	19.52
4.	Geraniol	19.51
5.	Camphene	0.09
6.	6-Methyl-5-hepten-2-one	0.42
7.	Myrcene	0.48
8.	Limonene	0.41
9.	Linalool	2.32
10.	Citronellal	0.56
11.	(E)-Isocitral (=Isogeranial)	0.76
12.	Citronellol + Nerol	1.97
13.	Citronellyl acetate	0.37
14.	(E)-Caryophyllene	0.36
15.	Caryophyllene oxide	0.19
	Total identified (%)	94.11

4.3.2 Volatile compounds of *Cymbopogon flexuosus* essential oil using GC-MS analysis:

The GC-MS analysis of the essential oil of *Cymbopogon flexuosus* revealed Geranial (=Citral a) (48.10%), Neral (=Citral b) (36.80%), (E)-Isocitral (=Isogeranial) (1.19%), Geraniol (1.78%) and Linalool (1.74%) as the major constituents. The other minor compounds that were quantified have been tabulated below (Table 4.3.2).

Table 4.3.2 Volatile compounds of Lemongrass essential oil

S.no.	Compound	Content (%)
1.	Geranial (=Citral a)	48.10
2.	Neral (=Citral b)	36.80
3.	(E)-Isocitral (=Isogeranial)	1.19
4.	Linalool	1.74
5.	Geraniol	1.78
6.	α-pinene	0.08
7.	Sabinene	0.21
8.	β-pinene	0.07
9.	6-Methyl-5-hepten-2-one	0.60
10.	Myrcene	0.09
11.	Limonene	0.53
12.	1,8-cineole	0.13
13.	(E)-β-Ocimene	0.06
14.	γ-Terpinene	0.03
15.	Citronellal	0.96
16.	(Z)-Isocitral (=Isoneral)	0.64
17.	Nerol	0.23
18.	Dimethoxy-(Z)-citral	0.23
19.	Dimethoxy-(E)-citral	0.62
20.	Geranyl acetate	0.05
21.	(E)-Caryophyllene	0.04

4.3.3 Volatile compounds of *Zingiber officinale* essential oil using GC-MS analysis:

The GC-MS analysis of the essential oil of *Zingiber officinale* shows; Geranial (=Citral a) (17.75%), Neral (=Citral b) (12.35%), Camphene (10.40%) and α-Zingiberene (5.87%) as the major constituents. However, many of the minor compounds were also quantified, all of which have been tabulated in (Table 4.3.3)

Table 4.3.3 Volatile compounds of *Zingiber officinale* essential oil

S.no.	Compound	Content (%)
1.	Geranial (=Citral a)	17.75
2.	Neral (=Citral b)	12.35
3.	Camphene	10.40
4.	α-Zingiberene	5.87
5.	(E,E)-α-Farnesene + β-Bisabolene	5.29
6.	Limonene + β-Phellandrene	4.74
7.	ar-Curcumene	4.86
8.	Geraniol	4.00
9.	1,8-Cineole	3.16
10.	Linalool	2.05
11.	α-Thujene	0.16
12.	α-Pinene	2.89
13.	Sabinene	0.06
14.	β-pinene	0.29
15.	6-Methyl-5-hepten-2-one	0.55
16.	Myrcene	1.47
17.	α-Phellandrene	0.15
18.	δ-3-Carene	0.06
19.	p-Cymene	0.17
20.	γ-Terpinene	0.05
21.	Terpinolene	0.36
22.	Citronellal	0.25
23.	Borneol	2.81
24.	Terpinen-4-ol	0.60
25.	α-Terpineol	0.94
26.	Citronellol + nerol	1.50
27.	Geranyl acetate	0.22
28.	β-elemene	0.25

29.	(E)-Caryophyllene	0.03
30.	β-Sesquiphellandrene	3.45
31.	(E)-Nerolidol	1.21
	Total identified (%)	87.94

4.3.4 Volatile compounds of *Artemisia vulgaris* essential oil using GC-MS analysis:

The GC-MS analysis of the essential oil of *Artemisia vulgaris* shows; 1,8-Cineole (11.89%), Germacrene D (7.21%), Borneol (6.88%) and δ-Cadinene (5.33%) as the major constituents (Table 4.3.4). The minor compounds have also been tabulated.

Table 4.3.4 Volatile compounds of *Artemisia vulgaris* essential oil

S.no.	Compound	Content (%)
1.	1,8-Cineole	11.89
2.	Germacrene D	7.21
3.	Borneol	6.88
4.	δ-Cadinene	5.33
5.	cis-Chrysanthenol	5.02
6.	Sabinene	4.12
7.	α-Thujene	0.05
8.	α-Pinene	1.15
9.	Camphene	1.13
10.	β-pinene	0.60
11.	1-Octen-3-ol	0.13
12.	Myrcene	0.36
13.	α-Terpinene	0.17
14.	p-Cymene	0.72
15.	Limonene	T
16.	Artemisia ketone	2.48
17.	Linalool	0.58
18.	trans-Chrysanthenol + trans-Thujone (minor)	4.47
19.	Chrysanthenone	0.83
20.	Camphor	3.42
21.	Terpinen-4-ol	1.53
22.	α-Terpineol	1.32
23.	Piperitone	1.33
24.	Bornyl acetate	0.68
25.	δ-Elemene	0.72
26.	α-Copaene	0.64
27.	β-Cubebene	0.81
28.	β-Patchoulene	1.11
29.	(E)-Caryophyllene	4.32
30.	α-Humulene	0.98

31.	trans-Muurola-4(14),5-diene	1.18
32.	Bicyclogermacrene	1.72
33.	epi-α-Cadinol	0.56
34.	epi-α-Muurolol	1.59
35.	α-Muurolol	0.44
36.	α-Eudesmol	3.85
	Total identified (%)	79.32

4.3.5 Volatile compounds of *Citrus macroptera* essential oil using GC-MS analysis:

The GC-MS analysis of the essential oil of *Citrus macroptera* shows; Limonene (89.4%), β-Myrcene (1.5%), Nonanal (0.959%) and α-Terpineol (0.573%) as the major constituents. We could also detect other minor compounds and altogether the data has been tabulated below (Table 4.3.5).

Table4.3.5 Volatile compounds of *Citrus macroptera* essential oil

S.no.	Compound	Content (%)
1.	Limonene	89.4
2.	β-Myrcene	1.5
3.	Nonanal	0.959
4.	β-Terpinene	0.416
5.	α-Terpineol	0.573
6.	Linalool	0.491
7.	α-pinene	0.372
8.	Nootkatone	0.248
9.	Terpinene-H-ol	0.235
10.	Cis-ulanol oxide	0.183
11.	Trans-linalol oxide	0.122
12.	α-Terpinene	0.07
13.	Neral	0.210
14.	Geranial	0.293
15.	β-Carylphyllene	0.275

4.3.6 Compounds of *Tithonia diversifolia* using HPLC analysis:

4.3.6 Compounds present in *Tithonia diversifolia* ethanol extract (HPLC)

Sl.no.	Compound
1	Ascorbic acid
2	Catechin
3	Ferulic acid
4	Syringic acid
5	Naringin
6	Bicalein

4.4 Description of the major compounds from bioactive essential oils:

The structure of the major compounds of *Litsea cubeba, Cymbopogon flexuosus, Zingiber officinale, Artemisia vulgaris* and *Citrus macroptera* was made with the help of Chemdraw ver 12.0 The structures were compared and confirmed with Pubchem images (Table 4.4).

4.5 Antimicrobial screening of the extracted essential oil/ extract, against the dermatophytic pathogens:

The antimicrobial activity of the essential oils and extracts were carried out through Disc Diffusion Assay (DDA) followed by the determination of minimum inhibitory concentration (MIC) through either minimum bactericidal/fungicidal concentration(MBC/MFC), and minimum killing time (MKT), the results of which are individually represented below.

4.5.1 Disc diffusion assay:

The antimicrobial screening of these essential oils/extracts (test sample) were carried through DDA. The stock solution of each test sample (at 100µl/ ml for essential oils or 100mg/ml for extract) were prepared separately and subjected to evaluation against the pathogens *T. rubrum* (MTCC-296), *M. gypseum* (MTCC-2819), *M. furfur* (MTCC-1374), *C. albicans* (ATCC-0443X-1) and *S. aureus* (ATCC-6538). The observation was recorded in (Table 4.5.1)

Table 4.4 Bioactive essential oils and their major compounds

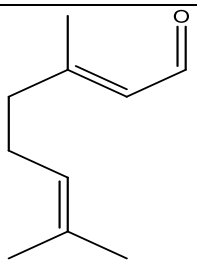
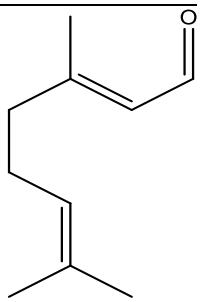
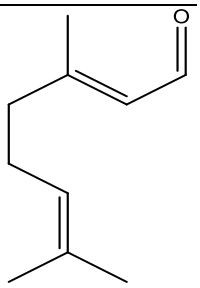
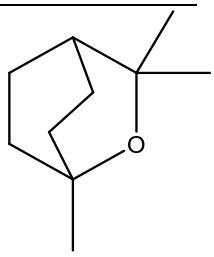
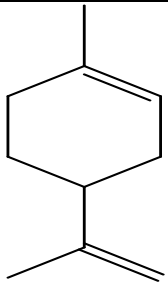
Sl. No.	Bioactive essential oil	Name of Major compound	Composition (%)	Molar Mass (g/mol)	Compound structure
1.	***L.cubeba***	Geranial (= Citral a)	26.64	152.23	
2.	***C.flexuosus***	Geranial (= Citral a)	48.10	152.23	
3.	***Z.officinale***	Geranial (= Citral a)	17.75	152.23	
4.	***A.vulgaris***	1,8-Cineole	11.89	154.25	
5.	***C.macroptera***	Limonene	89.4	154.21	

Table4.5.1 Qualitative antimicrobial activity of the essential oils and extract through Disc Diffusion Assay (DDA) in terms of Zone of Inhibition (ZOI) in mm

Sample code	*T. rubrum*	*M. gypseum*	*M. furfur*	*C. albicans*	*S. aureus*
***L.cubeba*^{o}**	37.33±1.47	31.33±1.76	30.33±1.76	13.66±0.88	17.66±0.88
***C.flexuosus*^{o}**	36.66±1.08	38.33±0.66	24.60±1.76	14.33±0.33	17.33±0.33
***Z.officinale*^{o}**	26.66±0.40	26.33±1.76	20.60±1.76	22.66±2.45	17.33±0.88
***A.vulgaris*^{o}**	13.66±0.40	13.66±0.33	20.33±0.88	14.66±1.20	17.0±0.57
***C.macroptera*^{o}**	23.33±1.08	17.66±1.45	9.66±0.33	10.67±0.33	12.33±0.66
***T.diversifolia*^{e}**	08.34±0.49	08.04±1.34	19.66±1.70	09.04±0.84	16.66±1.85
Clotrimazole (5mg/ml)	39.33±0.40	38.0±0.57	19.00±0.57	39.00±0.58	-
Vancomycin (2mg/ml)	-	-	-	-	17.66±0.33

$^{(O)}$ Essential oil, $^{(e)}$ Ethanol extract
20µl per disc; stock 100µl/ml (essential oil) or 100mg/ml(extract) of DMSO

Based on Disc Diffusion Assay (DDA), the zone of inhibition of the test samples obtained ranges between 38.33±0.66 and 08.04±1.34 against the test pathogens. The maximum inhibition zone was recorded in the case of *L. cubeba* (37.33±1.47 mm) against *T. rubrum*, (30.33±1.76 mm) against *M. furfur* and (17.66±0.88 mm) against *S. aureus*, whereas *C. flexuosus* is found to be most effective against *M. gypseum* and the zone of inhibition was found to be (38.33±0.66 mm). Further, *Z. officinale* is found to be most effective on *C. albicans* and the zone of inhibition was found to be (22.66±2.45 mm).

4.5.2 Minimum inhibitory concentration:

The quantitative evaluation of the anti-dermal pathogen activity of the test sample was carried out through the broth dilution method. The observation was recorded in (Table 4.5.2)

Table4.5.2 Quantitative evaluation of antimicrobial activity through Minimum Inhibitory Concentration (MIC) of various essential oil/ extracts

Sample code	Minimum Inhibitory Concentration (MIC) (µl/ml for oil & mg /ml for extract)				
	T. rubrum	***M. gypseum***	***M. furfur***	***C.albicans***	***S. aureus***
***L.cubeba*[o]**	0.039	0.039	0.078	0.156	0.039
***C.flexuosus*[o]**	0.043	0.086	0.086	0.041	0.043
***Z.officinale*[o]**	0.043	0.086	0.347	0.173	0.086
***A.vulgaris*[o]**	0.086	0.086	0.694	0.346	0.086
***C.macroptera*[o]**	0.195	0.195	0.195	0.781	0.781
***T.diversifolia*[e]**	0.312	0.156	0.312	1.25	0.625
Clotrimazole	0.0009	0.0009	0.015	0.0019	NA
Vancomycin	NA	NA	NA	NA	0.00035

[(o)] Essential oil, [(e)] Ethanol extract

Based on the observations recorded, MIC of the test samples ranged between 0.039 µl and 0.781µl/ml against the test pathogens, the lowest MIC value being that of 0.039 µl/ml from *L. cubeba* essential oil against *T. rubrum*, *M. gypseum*, *M. furfur* and *S. aureus*. Whereas, the lowest MIC value of 0.041 µl/ml was observed from *C. flexuosus* against *C. albicans*.

4.5.3 Minimum bactericidal/fungicidal concentration:

The minimum bactericidal/fungicidal concentration of test samples was performed after achieving the results from MIC. The observation is recorded in (Table 4.5.3)

Table 4.5.3 Minimum bactericidal/fungicidal (MBC/MFC) concentration of various essential oil (µl/ml) and extracts (mg/ml)

Test sample	***T. rubrum***	***M. gypseum***	***M. furfur***	***C.albicans***	***S. aureus***
***L.cubeba*[o]**	0.039	0.039	0.156	0.156	0.156
***C.flexuosus*[o]**	0.043	0.086	0.173	0.173	0.173

***Z.officinale*[o]**	0.086	0.173	0.694	0.346	0.347
***A.vulgaris*[o]**	0.347	0.347	0.694	0.694	0.347
***C.macroptera*[o]**	0.390	0.781	0.125	3.125	3.125
***T.diversifolia*[e]**	0.625	0.625	0.125	2.5	2.500
Clotrimazole	0.003	0.0009	0.031	0.007	NA
Vancomycin	NA	NA	NA	NA	0.0031

[o] Essential oil, [e] Ethanol extract

Based on the observations recorded, the minimum bactericidal/fungicidal concentration (MBC/MFC) of the test samples ranged between 0.039 and 3.125µl/ml against the test pathogens. The lowest MBC/MFC value of 0.039 µl/ml was obtained from *L. cubeba* essential oil against *T. rubrum* and 0.156 µl/ml against *C. albicans* and *S. aureus*. Further, the lowest MBC/MFC value of 0.125µl/ml was obtained from *C. macroptera* against *M. furfur*.

4.5.4 Killing Time of the Essential oil/ Extract, against the Test Pathogens:

The minimum killing time (MKT) of the oil *L. cubeba*, *C. flexuosus*, *Z. officinale*, *A. vulgaris*, *C. macroptera* and ethanol extract of *T. diversifolia*, against the test micro-organisms, *T. rubrum*, *M. gypseum*, *M. furfur*, *C. albicans* and *S. aureus* was determined and the observations recorded are as follows:

Table 4.5.4.1Time kill kinetics of bioactive(s) at 2MIC against *Trichophyton rubrum*

Test sample	**Hours**							
	0	**4**	**6**	**9**	**12**	**18**	**24**	**36**
***L.cubeba*[o]**	-	+	+	+	+	+	+	+
***C.flexuosus*[o]**	-	+	+	+	+	+	+	+
***Z. officinale*[o]**	-	+	+	+	+	+	+	+
***A.vulgaris*[o]**	-	+	+	+	+	+	+	+
***C.macroptera*[o]**	-	-	+	+	+	+	+	+
***T.diversifolia*[e]**	-	-	+	+	+	+	+	+
Vancomycin	-	+	+	+	+	+	+	+

+ : growth inhibition; - : growth observed

Based on the observations, the minimum killing time of *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris* is less than 4 hours, whereas *Citrus macroptera* and *Tithonia diversifolia* require less than 6 hours against *T. rubrum*.

Table 4.5.4.2 Time kill kinetics of bioactive(s) at 2MIC against *Microsporum gypseum*

Test sample	Hours							
	0	**4**	**6**	**9**	**12**	**18**	**24**	**36**
***L.cubeba*^{o}**	-	+	+	+	+	+	+	+
***C.flexuosus*^{o}**	-	+	+	+	+	+	+	+
***Z.officinale*^{o}**	-	+	+	+	+	+	+	+
***A.vulgaris*^{o}**	-	+	+	+	+	+	+	+
***C.macroptera*^{o}**	-	+	+	+	+	+	+	+
***T.diversifolia*^{e}**	-	-	+	+	+	+	+	+
Vancomycin	-	+	+	+	+	+	+	+
+ : growth inhibition; - : growth observed								

Based on the observations, the minimum killing time of *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale* isless than 4 hours, whereas, for *Artemisia vulgaris, Citrus macroptera* and *Tithonia diversifolia* require less than 6 hours against *M. gypseum.*

Table 4.5.4.3Time kill kinetics of bioactive(s) at 2MIC against *Malassezia furfur*

Test sample	Hours							
	0	**4**	**6**	**9**	**12**	**18**	**24**	**36**
***L.cubeba*^{o}**	-	+	+	+	+	+	+	+
***C.flexuosus*^{o}**	-	+	+	+	+	+	+	+
***Z.officinale*^{o}**	-	+	+	+	+	+	+	+
***A.vulgaris*^{o}**	-	-	+	+	+	+	+	+
***C.macroptera*^{o}**	-	+	+	+	+	+	+	+
***T.diversifolia*^{e}**	-	-	+	+	+	+	+	+
Vancomycin	-	+	+	+	+	+	+	+
+ : growth inhibition; - : growth observed								

Based on observation the minimum killing time of *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Citrus macroptera* is less than < 4 hours, whereas *Artemisia vulgaris* and *Tithonia diversifolia* require less than < 4 hours against *M. furfur*.

Table 4.5.4.4 Time kill kinetics of bioactive(s) at 2MIC against *Candida albicans*

Test sample	Hours							
	0	**4**	**6**	**9**	**12**	**18**	**24**	**36**
***L.cubeba*^{o}**	-	+	+	+	+	+	+	+
***C.flexuosus*^{o}**	-	+	+	+	+	+	+	+
***Z.officinale*^{o}**	-	+	+	+	+	+	+	+
***A.vulgaris*^{o}**	-	+	+	+	+	+	+	+
***C.macroptera*^{o}**	-	-	-	-	+	+	+	+
***T.diversifolia*^{e}**	-	-	-	-	+	+	+	+
Vancomycin	-	+	+	+	+	+	+	+
+ : growth inhibition; - : growth observed								

Based on observation the minimum killing time of *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris* is less than 4 hours, whereas *Citrus macroptera* and *Tithonia diversifolia* require less than 12 hours against *C. albicans*.

Table 4.5.4.5 Time kill kinetics of bioactive(s) at 2MIC against *Staphylococcus aureus*

Test sample	Hours							
	0	**4**	**6**	**9**	**12**	**18**	**24**	**36**
***L.cubeba*^{o}**	-	+	+	+	+	+	+	+
***C.flexuosus*^{o}**	-	+	+	+	+	+	+	+
***Z.officinale*^{o}**	-	+	+	+	+	+	+	+
***A.vulgaris*^{o}**	-	+	+	+	+	+	+	+
***C.macroptera*^{o}**	-	+	+	+	+	+	+	+
***T.diversifolia*^{e}**	-	-	+	+	+	+	+	+
Vancomycin	-	+	+	+	+	+	+	+
+ : growth inhibition; - : growth observed								

Further, according to observation minimum killing time of *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Citrus macroptera*, *Artemisia vulgaris* is less than 4 hours and *Tithonia diversifolia* requires less than 6 hours against *S. aureus.*

4.6 Sorbitol Protection Assay:

The assay was carried out to study the effect of the test sample on the integrity of the fungal cell wall in the presence of 0.8M Sorbitol as osmotic support. The observation was recorded in Table 4.6.

(i) Effect of bioactives on *T. rubrum*:

According to the observation on sorbitol assay, MIC of *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris*, *Tithonia diversifolia* increased in the presence of sorbitol against *T. rubrum* except in *Citrus macroptera*.

(ii) Effect of bioactive on *M. gypseum*:

The MIC value of *Litcea cubeba*, *Cymbopogon flexuosus*, *Citrus macroptera*, *Tithonia diversifolia* increased in the presence of sorbitol against *M. gypseum* but no changes were observed in *Zingiber officinale* and *Artemisia vulgaris*.

Table 4.6 Minimum Inhibitory Concentration (MIC) in absence and presence of Sorbitol for assessing the effect of bioactive(s) on the cell wall (µl/ml for oil & mg /ml for extract)

Sample code	*T. rubrum*		*M. gypseum*		*M. furfur*		*C. albicans*	
	Sorbitol		Sorbitol		Sorbitol		Sorbitol	
	(-)	**(+)**	**(-)**	**(+)**	**(-)**	**(+)**	**(-)**	**(+)**
***L.cubeba*[o]**	**0.039**	**0.078**	**0.039**	**0.078**	**0.078**	**0.156**	**0.156**	**0.312**
***C.flexuosus*[o]**	**0.043**	**0.086**	**0.086**	**0.173**	**0.086**	**0.173**	**0.041**	**0.086**
***Z.officinale*[o]**	**0.043**	**0.086**	0.086	0.86	**0.347**	**0.694**	0.173	0.173
***A.vulgaris*[o]**	**0.086**	**0.347**	0.086	0.086	0.694	0.694	**0.346**	**0.694**
***C.macroptera*[o]**	0.195	0.781	**0.195**	**0.781**	0.195	0.195	0.781	0.781
***T.diversifolia*[e]**	**0.312**	**1.250**	**0.156**	**0.156**	0.312	0.312	**1.25**	**2.50**
Clotrimazole	**0.0009**	**0.0019**	**0.0009**	**0.0019**	**0.015**	**0.039**	**0.0019**	**0.0078**

[(o)] Essential oil, [(e)] Ethanol extract, Figures in bold show the changes observed

(iii) Effect of bioactive(s) on *M. furfur*:

The MIC of *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale* increased in the presence of Sorbitol against *M. furfur* but we could not observe any changes in *Artemisia vulgaris*, *Citrus macroptera* and *Tithonia diversifolia*.

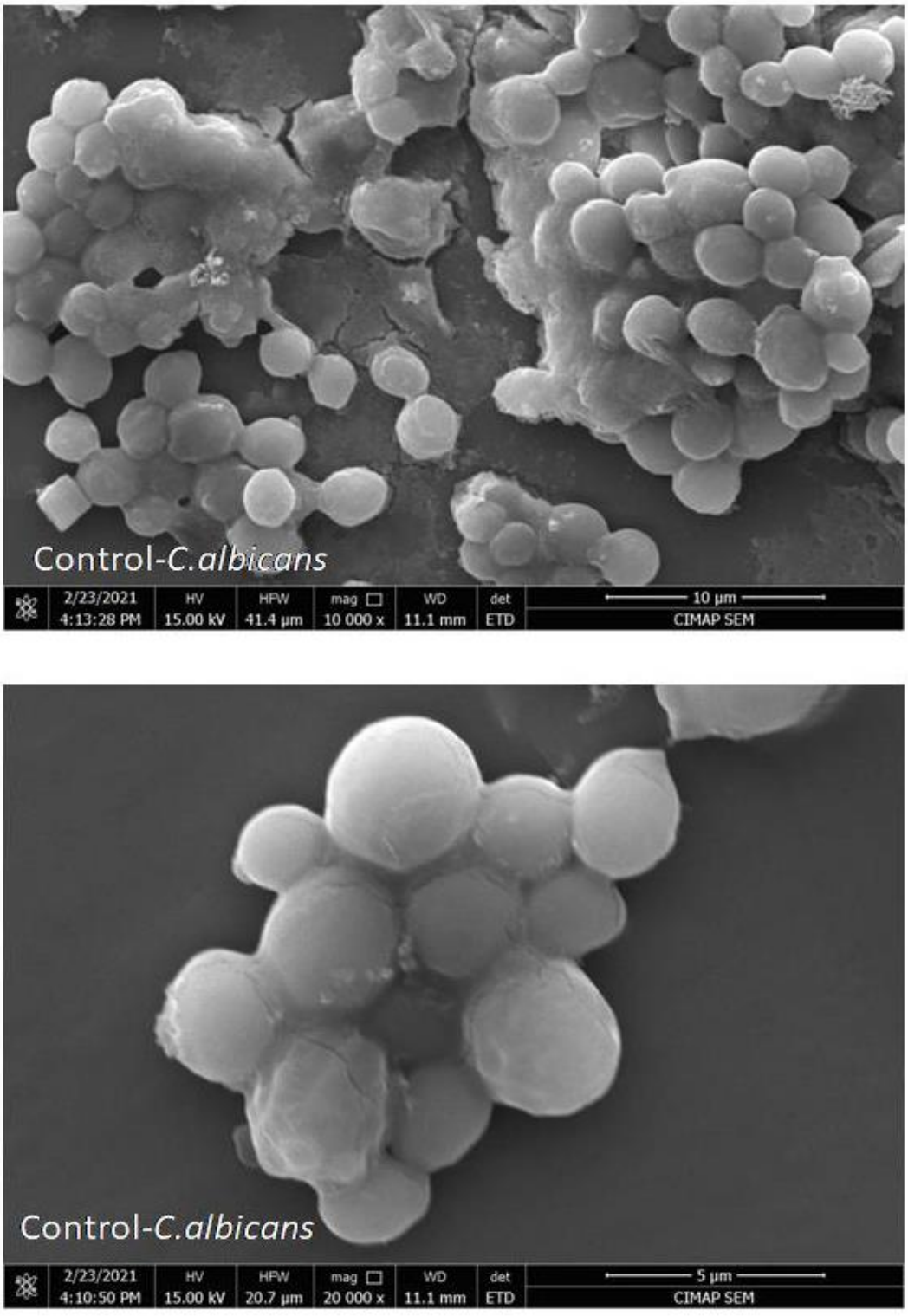
(iv) Effect of bioactive(s) on *C. albicans*:

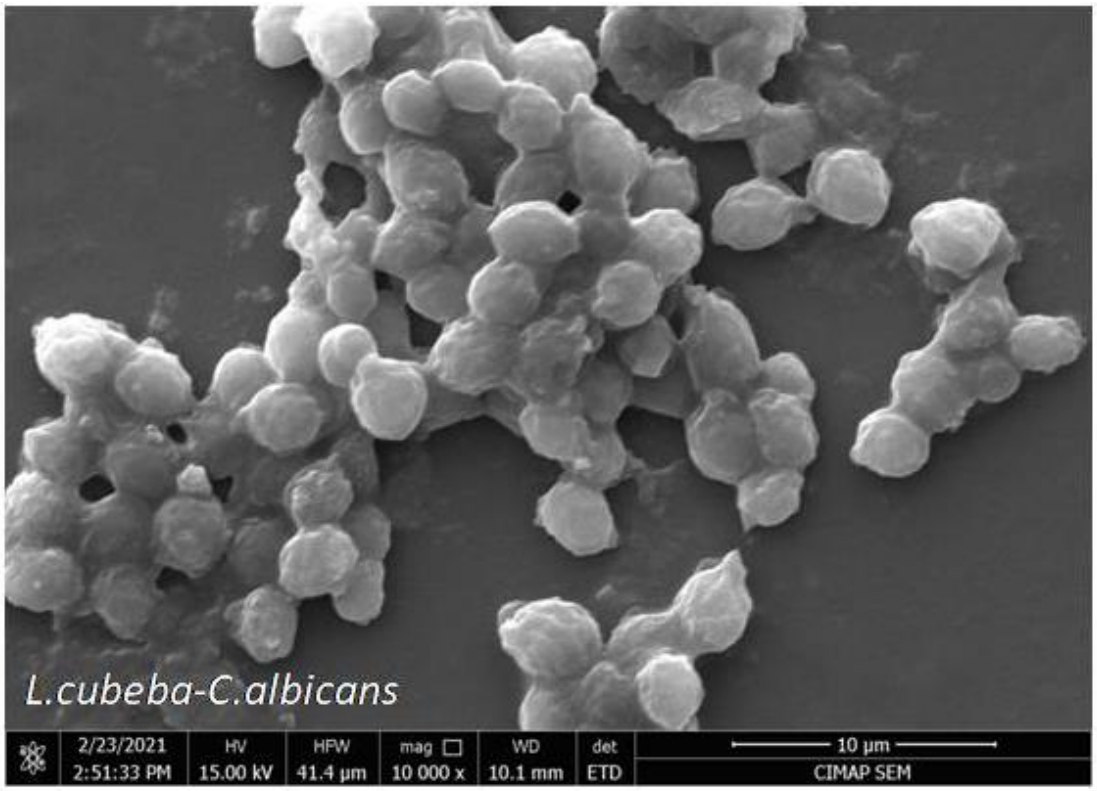
Further, the MIC of *Litcea cubeba*, *Cymbopogon flexuosus*, *Artemisia vulgaris*, *Tithonia diversifolia* increased in the presence of sorbitol against *C. albicans*. However, we could not record any changes in *Zingiber officinale* and *Citrus macroptera*.

4.7 Scanning Electron Microscope (SEM) studies:

The morphological changes of the pathogens that could be visualized upon the treatment (of bioactive(s) which exhibited potent antimicrobial activity) were evaluated at high magnification with a resolution approaching 15 to 20 nm under optimal conditions through SEM. The observations revealed that the surface of the untreated *Malassezia furfur* and *Candida albicans* appeared to be smooth and rounded, whereas degradation, structural and morphological changes in the morphology of cell after treating with bioactive(s). The images from the Scanning Electron Microscope have been arranged below.

Fig 4.7.1 Effect of the bioactive(s) on the surface morphology of *C.albicans* as visualized under Scanning Electron Microscope

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2/23/2021
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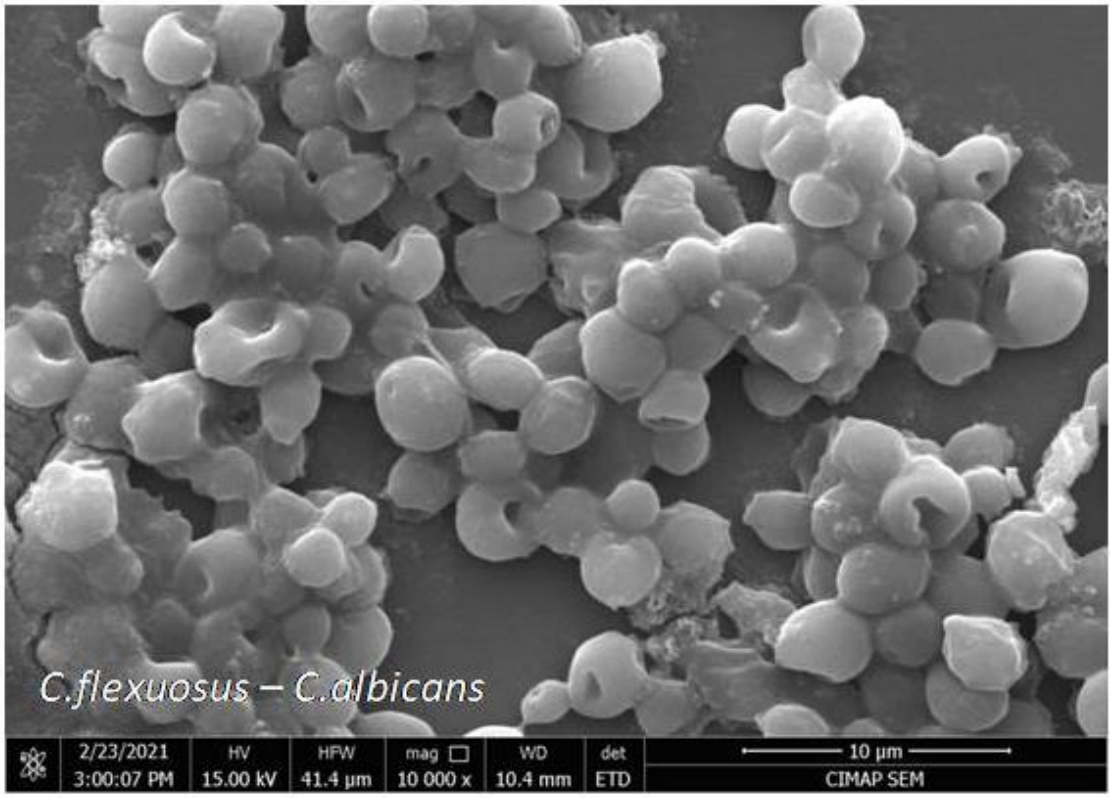
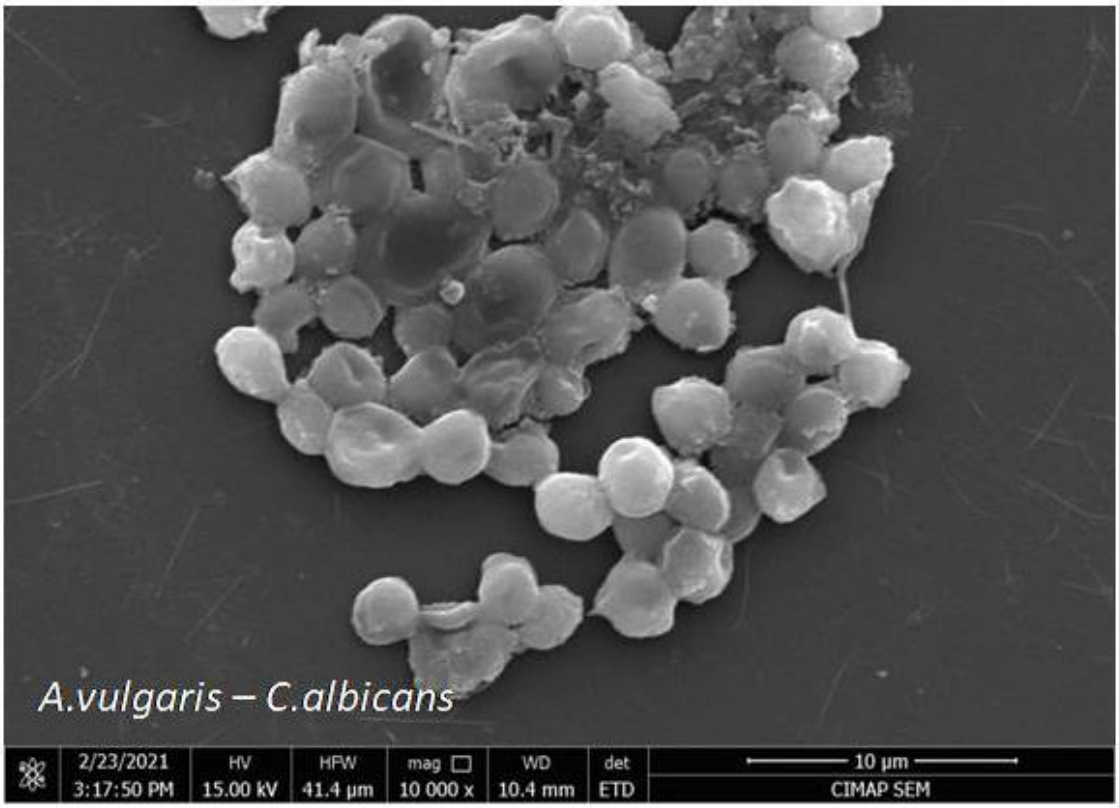
15.00 kV
HFW
41.4 µm
mag
10 000 x
WD
10.1 mm
det
ETD
10 µm
CIMAP SEM


C.flexuosus – C.albicans
2/23/2021
3:00:07 PM
HV
15.00 kV
HFW
41.4 µm
mag
10 000 x
WD
10.4 mm
det
ETD
10 µm
CIMAP SEM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2/23/2021
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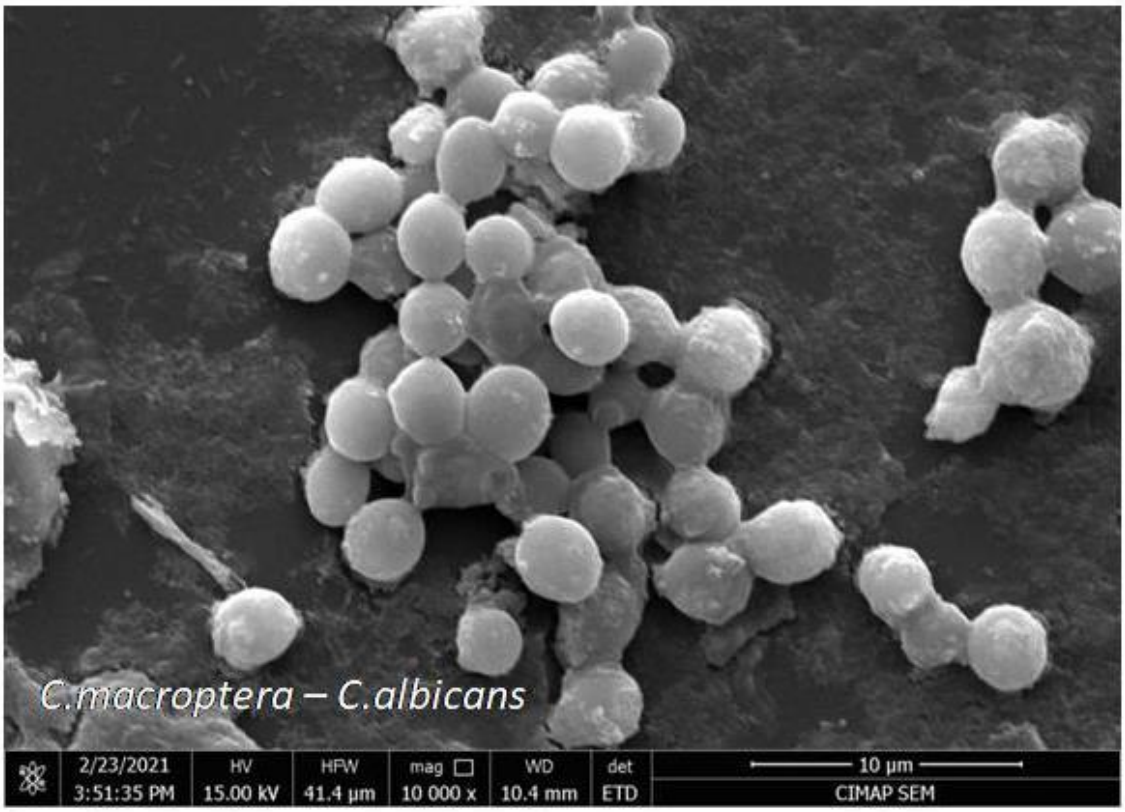
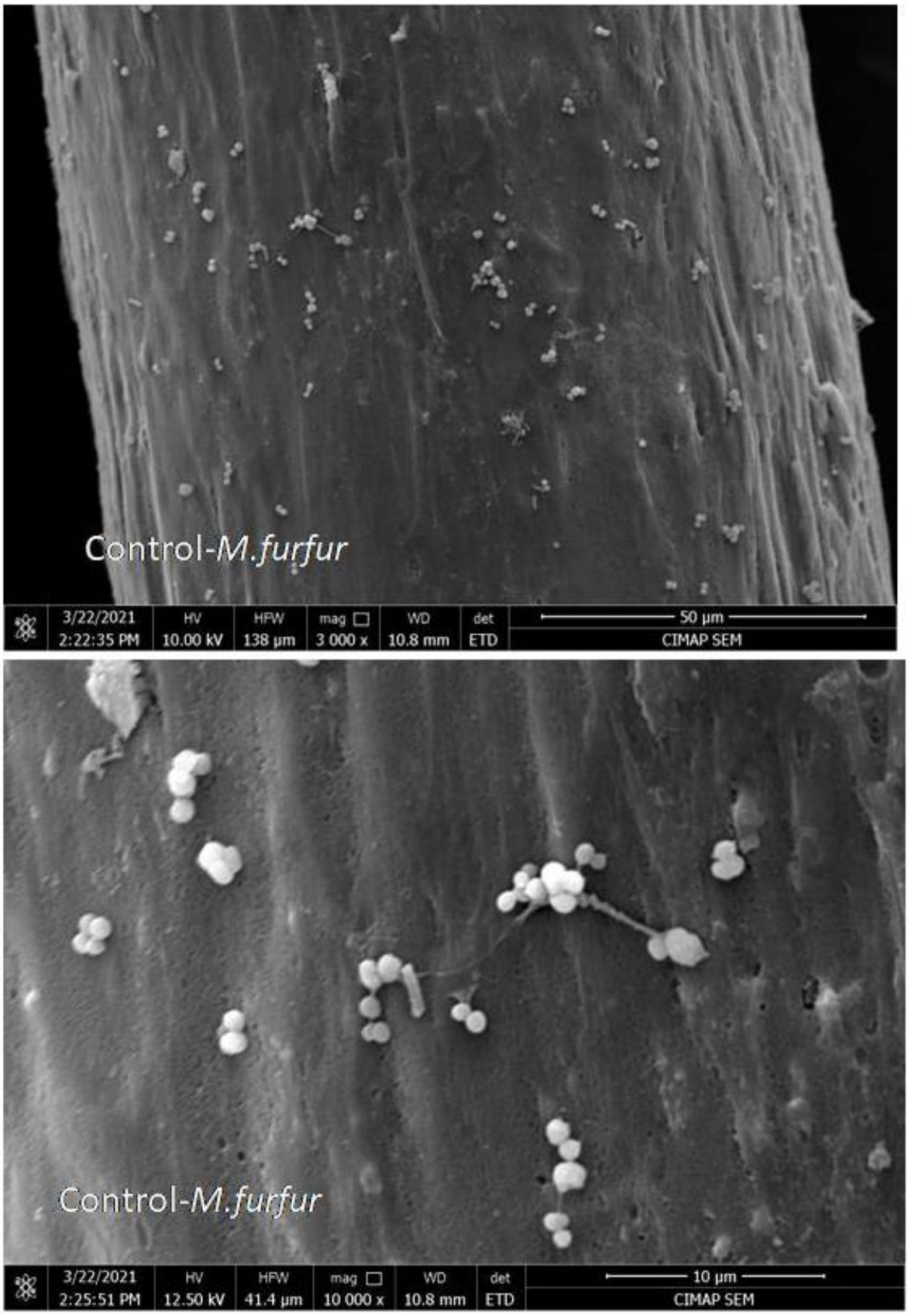
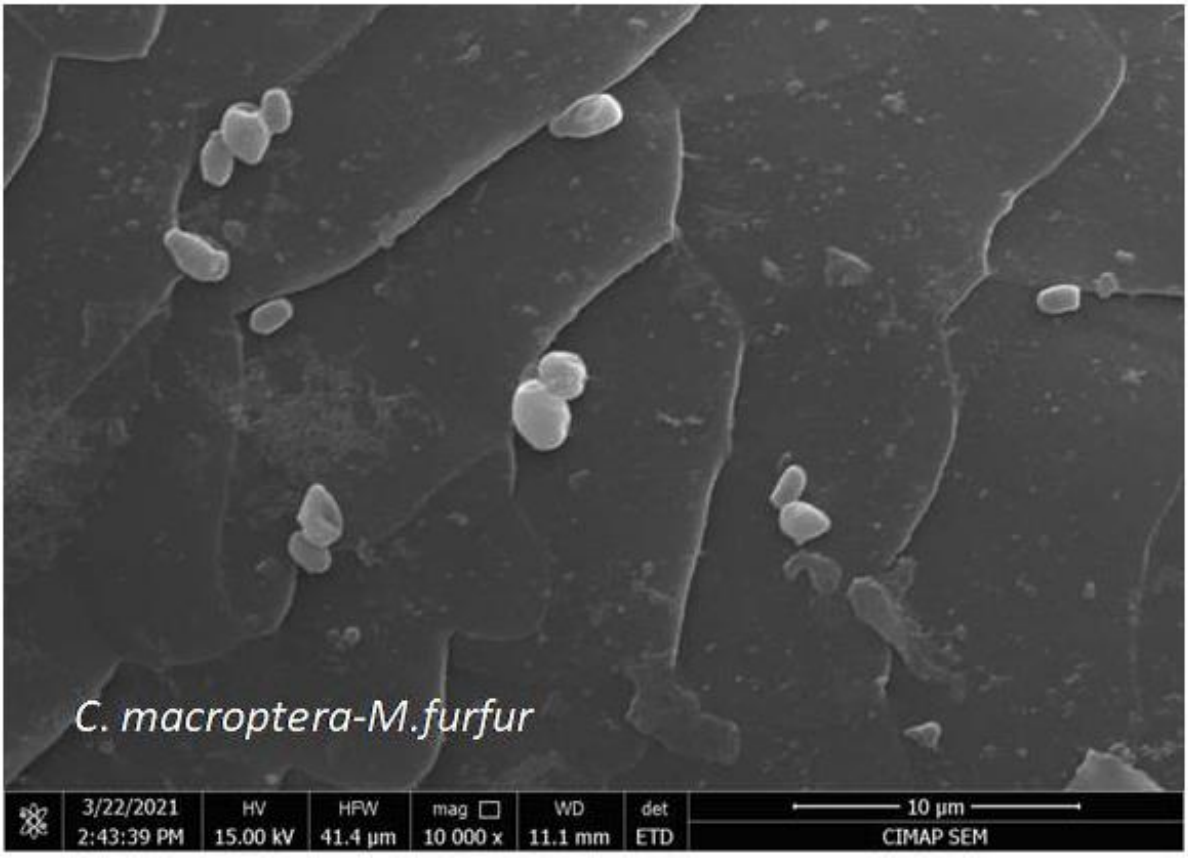
HV
15.00 kV
HFW
41.4 µm
mag
10 000 x
WD
10.4 mm
det
ETD
10 µm
CIMAP SEM


C.macroptera – C.albicans
2/23/2021
3:51:35 PM
HV
15.00 kV
HFW
41.4 µm
mag
10 000 x
WD
10.4 mm
det
ETD
10 µm
CIMAP SEM


T.diversifolia – C.albicans
2/23/2021
3:59:52 PM
HV
15.00 kV
HFW
41.4 µm
mag
10 000 x
WD
11.5 mm
det
ETD
10 µm
CIMAP SEM

Fig4.7.2 Effect of the bioactive(s) on the surface morphology of *M. furfur* colonized on hair surface as visualized under Scanning Electron Microscope

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15.00 kV
HFW
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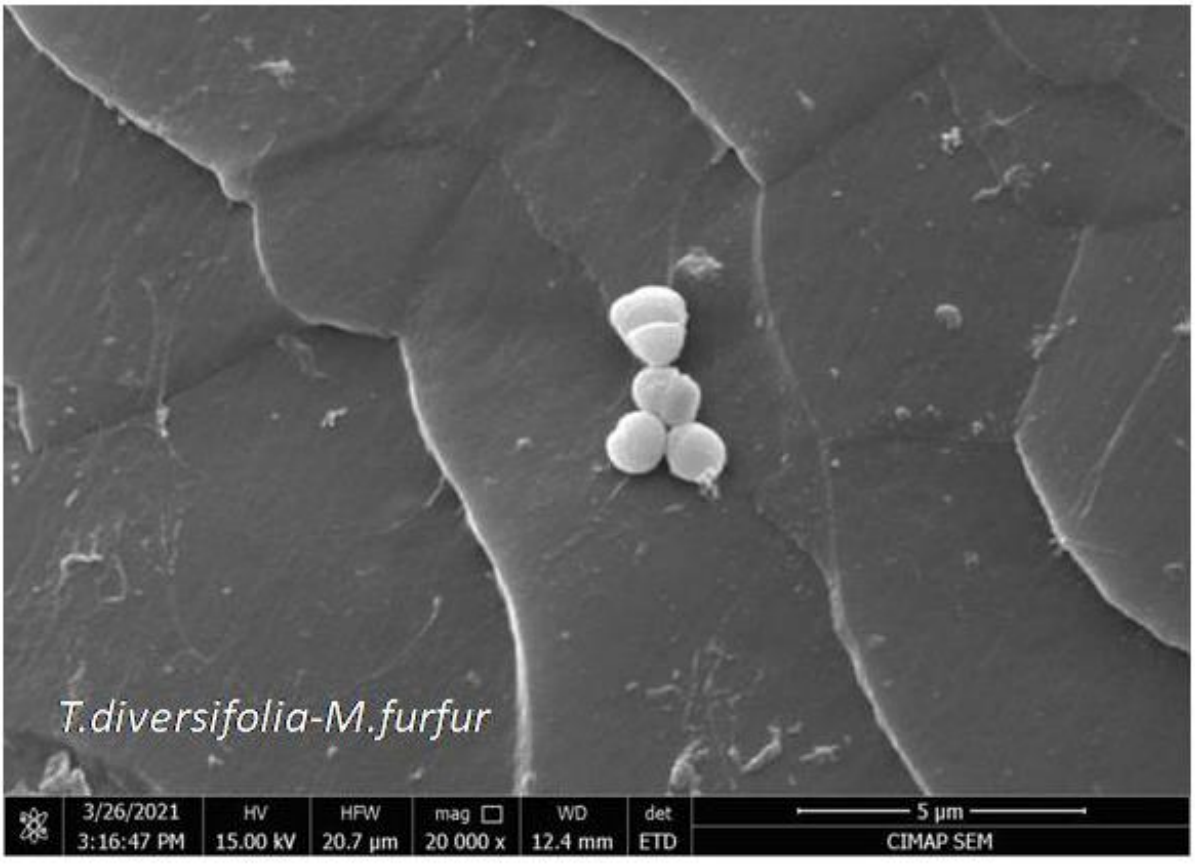
mag
10 000 x
WD
11.1 mm
det
ETD
10 µm
CIMAP SEM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3/26/2021
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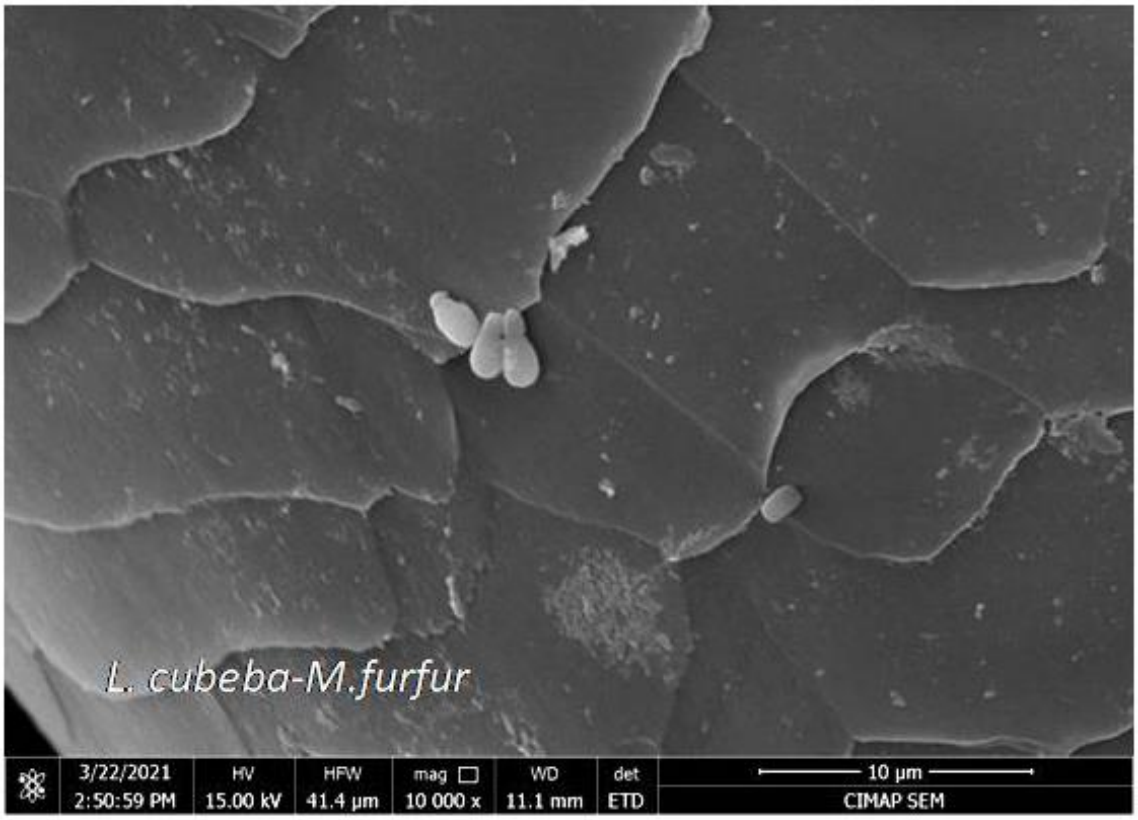
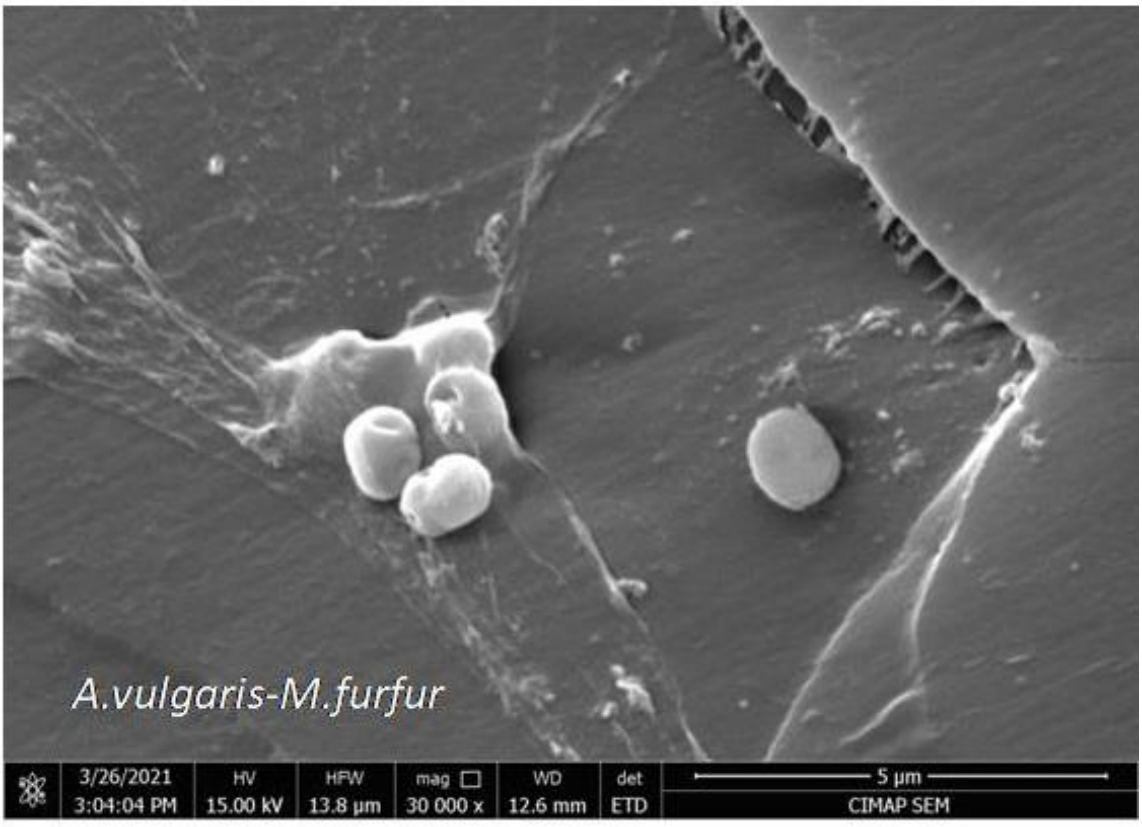
HV
15.00 kV
HFW
20.7 µm
mag
20 000 x
WD
12.4 mm
det
ETD
5 µm
CIMAP SEM

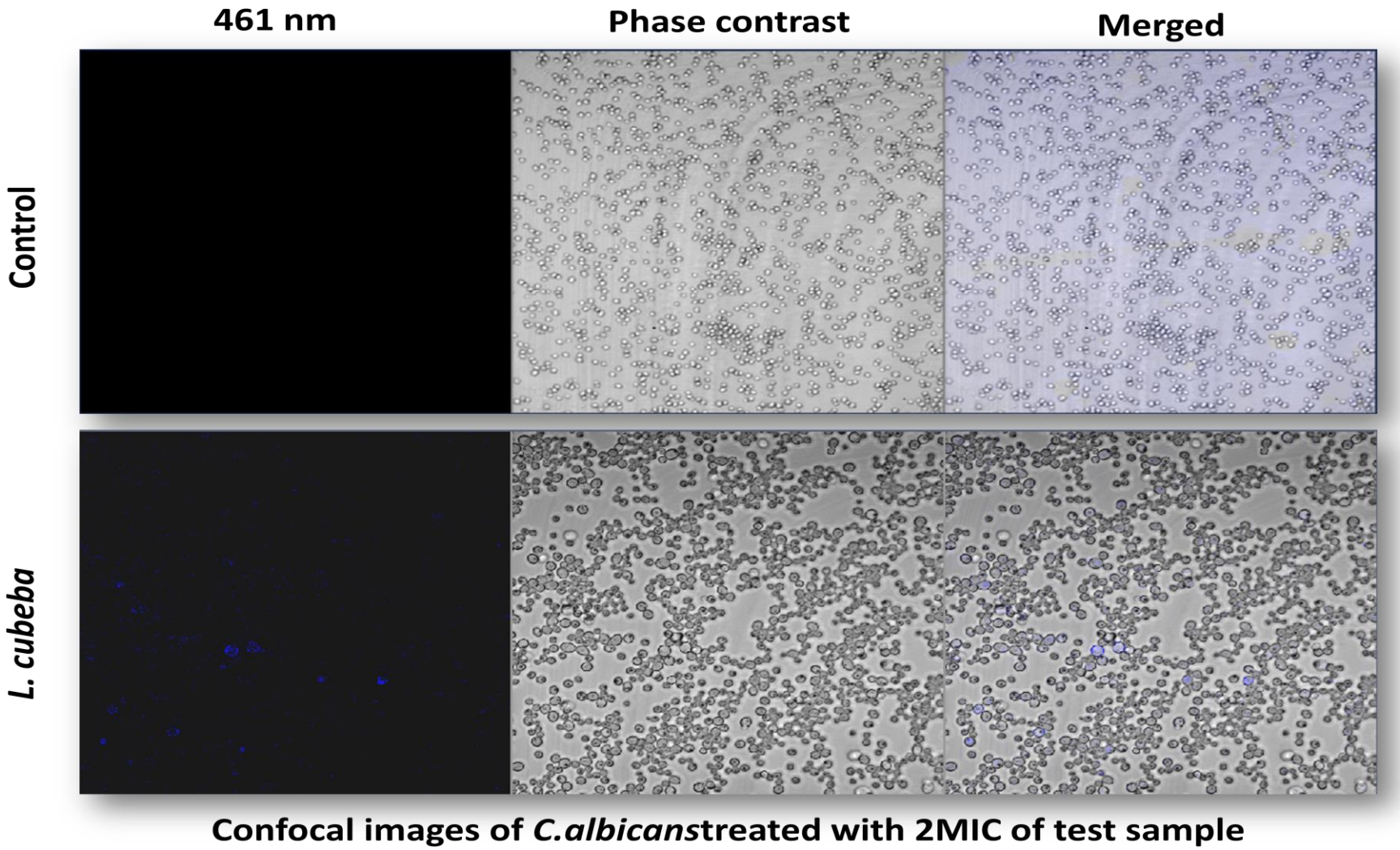

L. cubeba-M.furfur
3/22/2021
2:50:59 PM
HV
15.00 kV
HFW
41.4 µm
mag
10 000 x
WD
11.1 mm
det
ETD
10 µm
CIMAP SEM

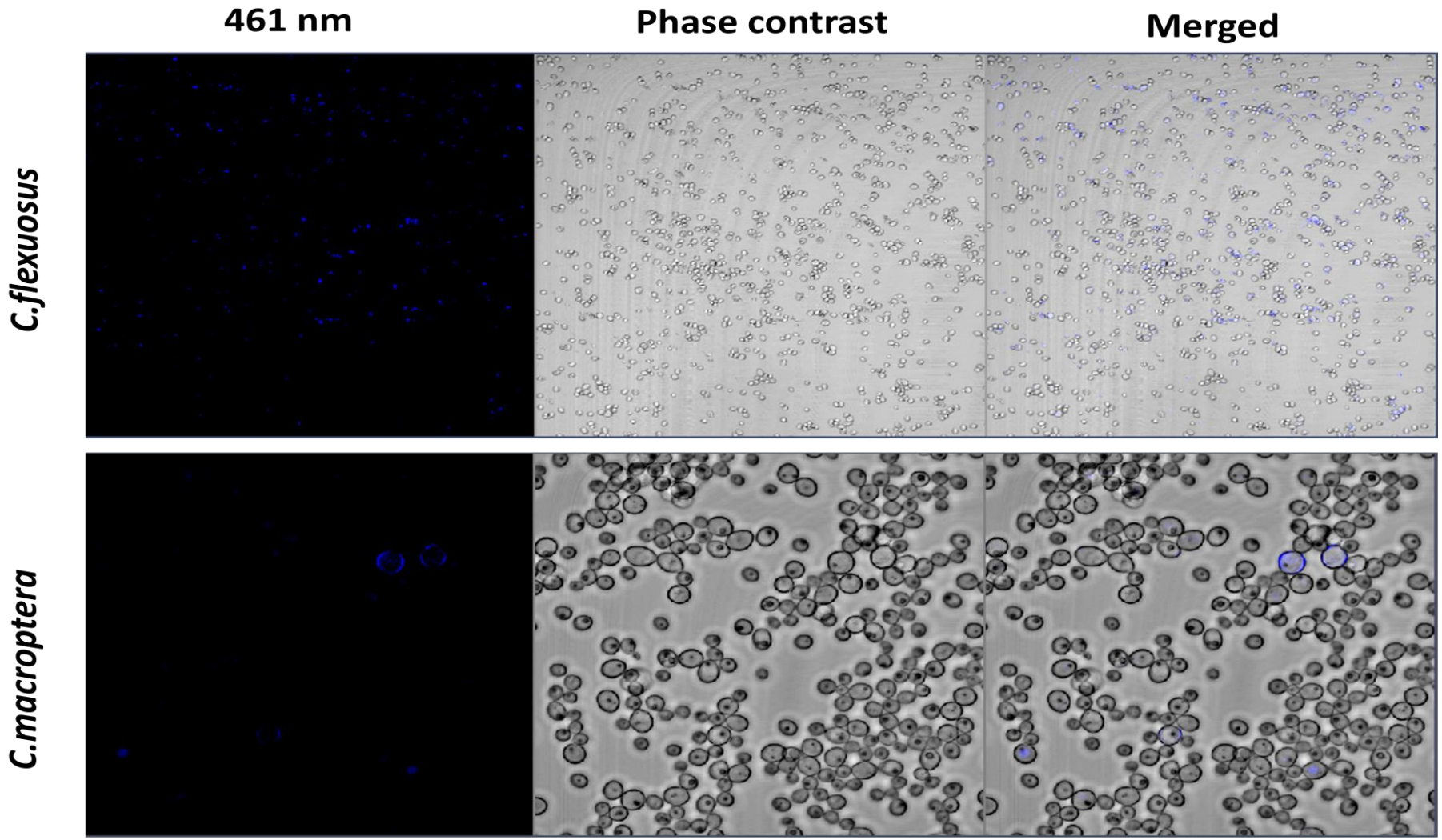

A.vulgaris-M.furfur
3/26/2021
3:04:04 PM
HV
15.00 kV
HFW
13.8 µm
mag
30 000 x
WD
12.6 mm
det
ETD
5 µm
CIMAP SEM

4.8 Confocal microscope study:

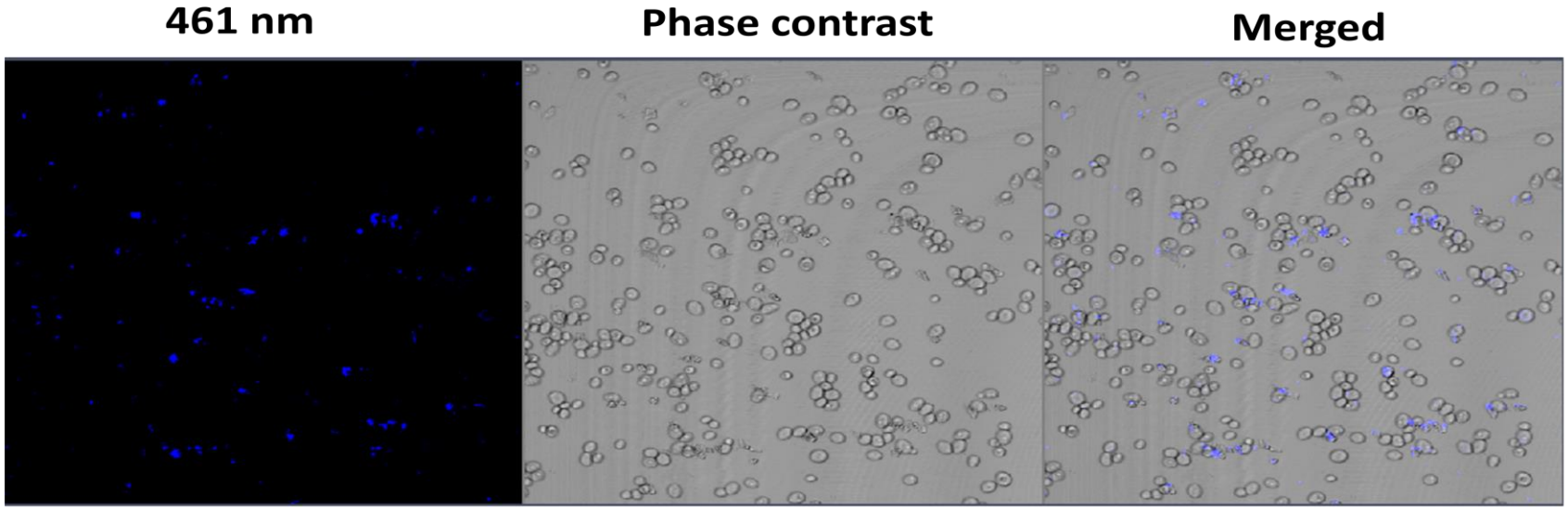
The DAPI (4′,6-diamidino-2-phenylindole) dye which can penetrate and move inside only into the dead cells is indicative of cell wall damage in the pathogen. The luminosity of the fluorescent signal from DAPI can increase almost 20-fold upon binding to the AT regions of double-stranded DNA. We took the advantage of this principle to check if our bioactive acts on the cell wall and visualized the same through the confocal microscope. The pathogens we're exposed to the bioactive(s) and treated with DAPI dye to observe the changes within the cell. The dye after its penetration through the damaged cell wall could be visualized (Fig 4.8.1 &4.8.2) and the dead or damaged cells could be correlated with the activity observed through DDA, MIC, MBC and MKT results. The observations reveal that the bioactive(s) have the potential to kill the test pathogens.

Fig 4.8.1 Confocal Imaging of *C. albicans* treated with the bioactive and stained with 4',6-diamidino-2-phenylindole (DAPI)



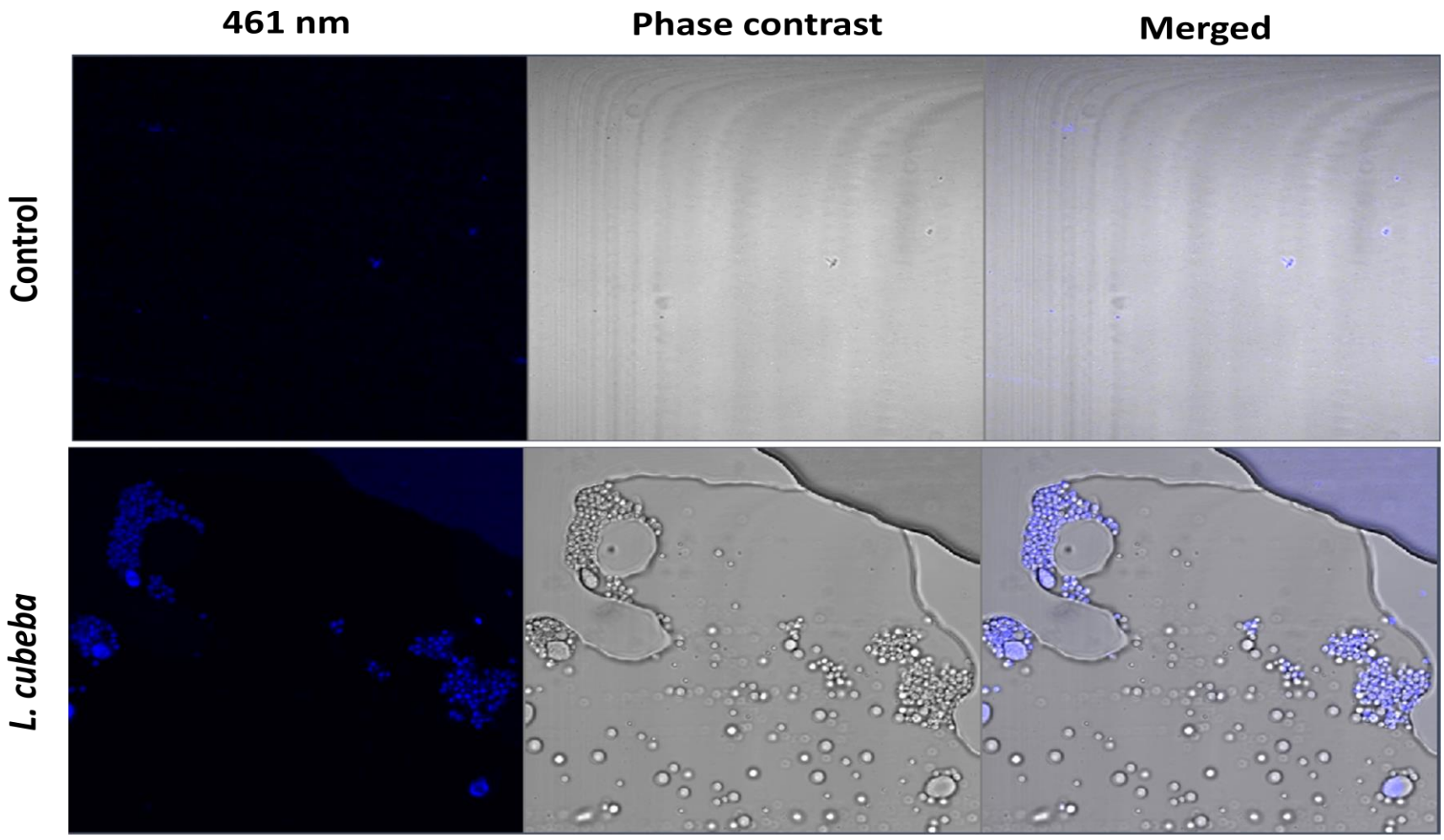


Confocal images of *C.albicans*treated with 2MIC of test sample

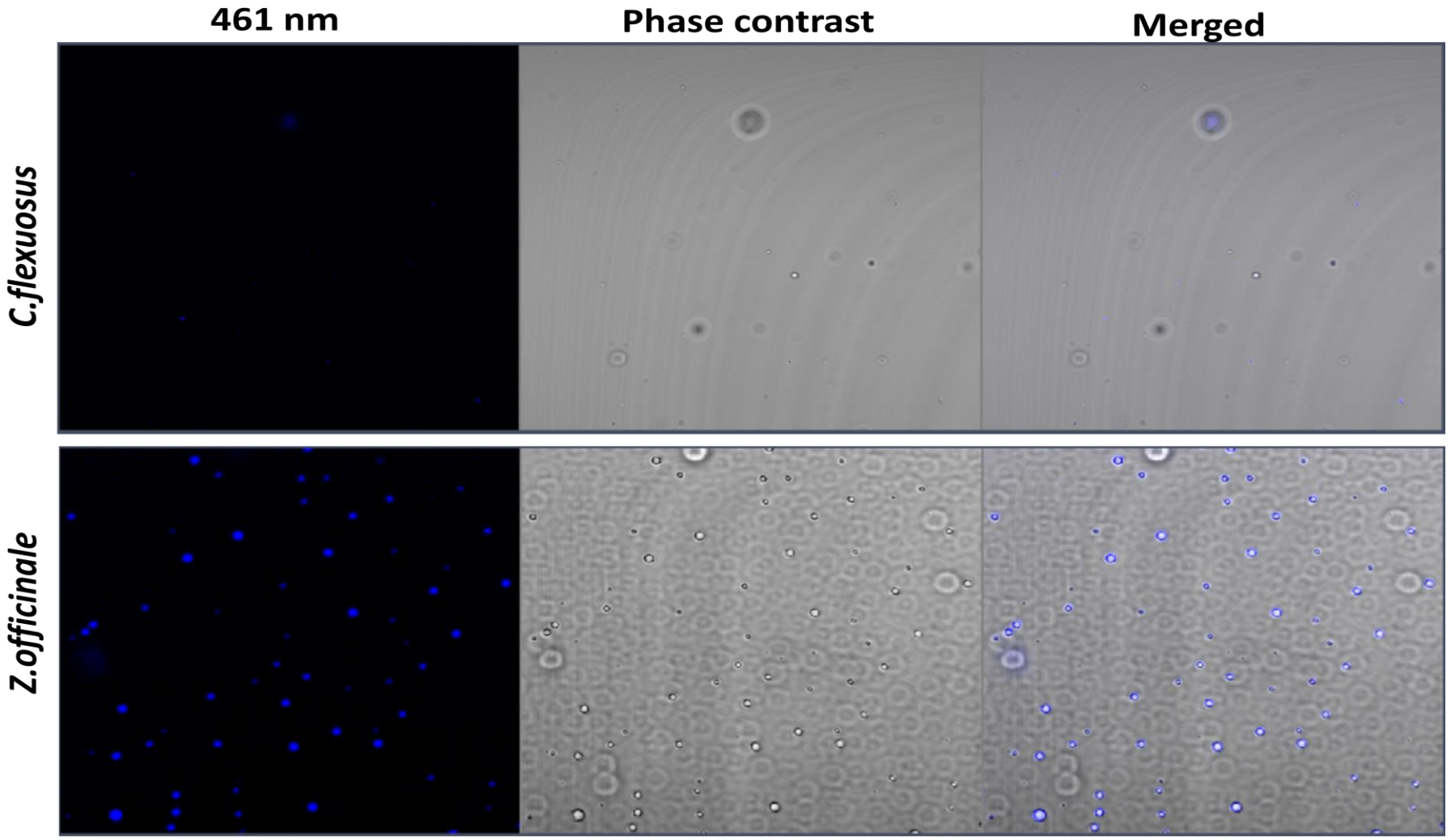


Confocal images of *C.albicans*treated with 2MIC of test sample

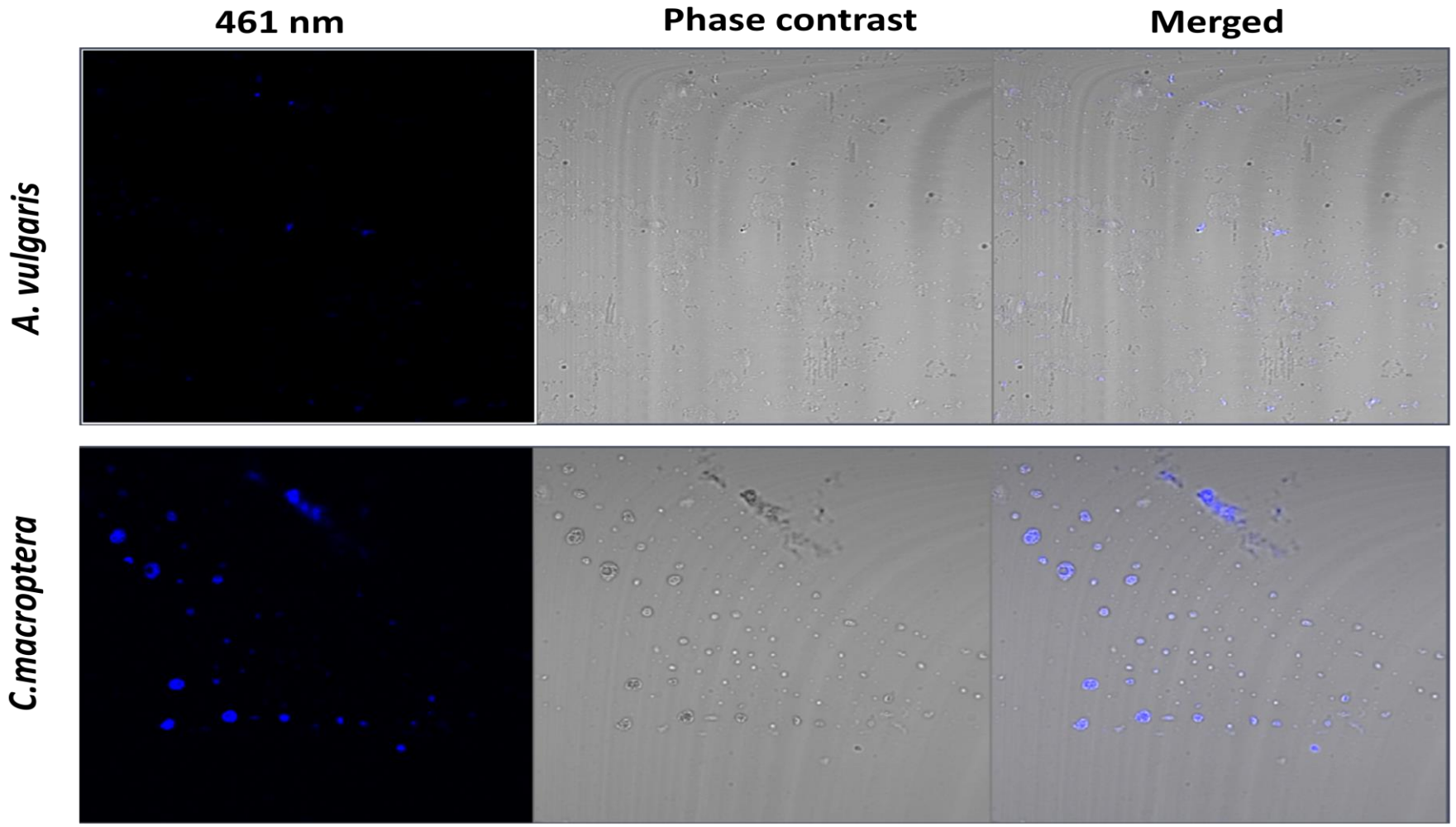
Fig 4.8.2 Confocal Imaging of *S. aureus* treated with the bioactive and stained with 4',6-diamidino-2-phenylindole (DAPI)



Confocal images of *S.aureus* treated with 2MIC of test sample



Confocal images of *S.aureus* treated with 2MIC of test sample



Confocal images of *S.aureus* treated with 2MIC of test sample

4.9 Effect of bioactive(s) on the pro-inflammatory cytokines ((Tumor necrosis factor (TNF)):

The anti-inflammatory activity of *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris*, *Citrus macroptera* at the concentration of 0.1% and *Tithonia diversifolia* at 10µg/ml was performed by exposure to LPS treated macrophages. Upon quantification of TNF, we observed the inhibition of the pro-inflammatory cytokine indicating that the bioactive is having anti-inflammatory activity.

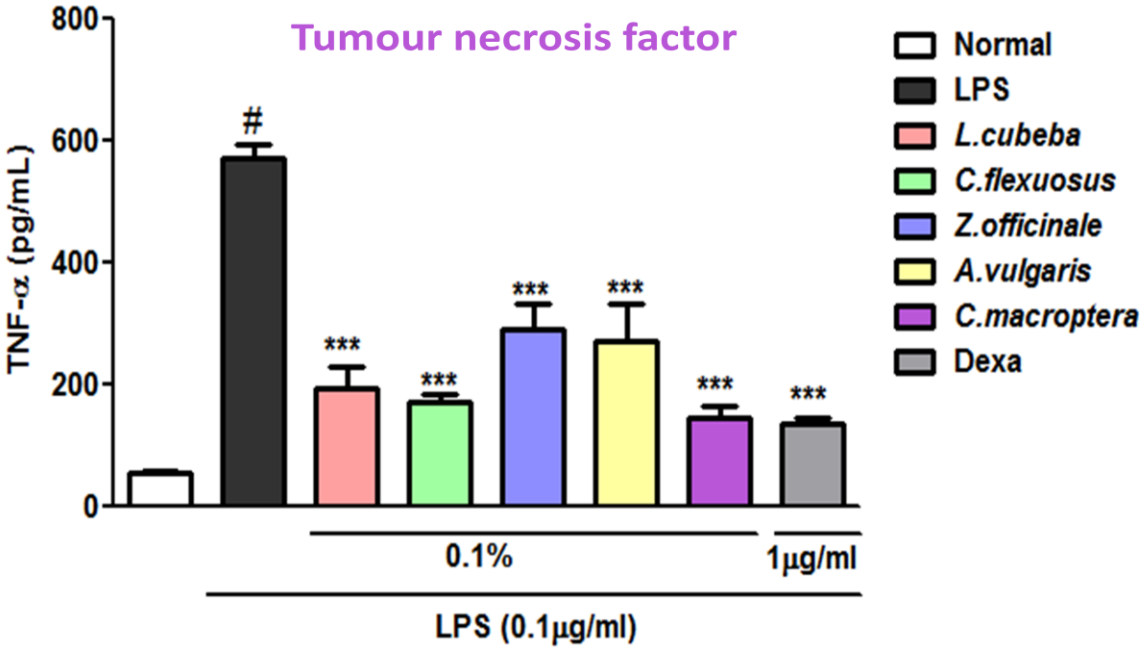


Figure 4.9.1 Effect of essential oils on a pro-inflammatory mediator, Tumor Necrosis Factor (TNF) levels in J774A.1 cell lines stimulated with LPS and treated with *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris* and *Citrus macroptera*.

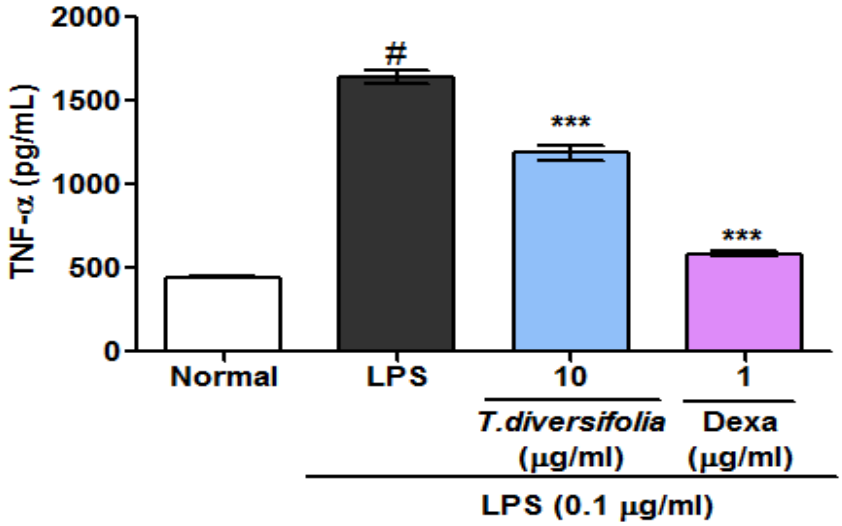


Figure4.9.2 Effect of ethanol extract of *T. diversifolia* on a pro-inflammatory mediator, Tumor Necrosis Factor (TNF) levels in J774A.1 cell lines stimulated with LPS.

4.10 Cell sensitivity assays (the MTT) assay:

The Cytotoxic effect of the test samples *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris*, *Citrus macroptera* and *Tithonia diversifolia* was performed through MTT assay at the concentration of 0.1 µg, 1 µg and 10 µg, our samples did not show any cytotoxic effect up to this concentration as represented in the figure below.

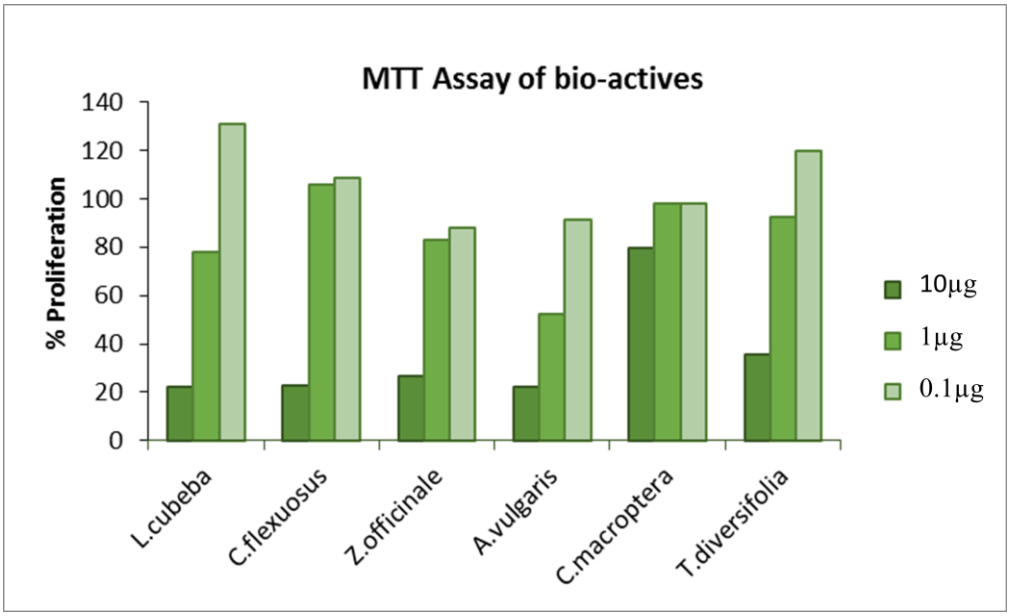


Fig 4.10 Effect of the bioactive(s) on the proliferation of macrophages upon exposure to different concentrations.

4.11 Skin irritation test of bioactive(s):

The skin irritation tests were performed to evaluate the scope of their use in therapeutic formulation(s) or cosmetic preparation(s). According to FHSA (Federal Hazardous Substances Act) standards i.e., PII (Primary irritation index) is 5.0. Less than 5.00 PII is generally not considered a primary irritant to the skin. Separated experiments were undertaken for different essential oils and the extracts and our observations and results indicated that the highest PII of 1.05±0.11 was exhibited by the ethanol extract of *T. diversifolia* and all the other essential oils exhibited lower than 1.0. However, it may be noted that the values below 5.0 are considered to be safe for topical applications. The observations in the form of results have been tabulated in (Table 4.11)

4.12 The safety evaluation study:

The safety evaluation of bioactive(s) of the test samples *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris*, *Citrus macroptera* and *Tithonia diversifolia* was performed in acute oral toxicity study and dose-dependent, no significant variations were found in behavioural or morphometric studies as compared to those of control mice. Moreover, the serum biochemical parameters also did not show any significant changes and the results were found almost comparable to the vehicle control and the result concluded a no-observed-adverse-effect-level (NOAEL) up to 2000mg/kg body weight (Table 4.12.1, 4.12.2, 4.12.3).

Table 4.11 Skin irritation test of bioactive(s)

Sample code	Test PII			Control PII			Test PII-Control PII
	Erythema	Oedema	Erythema + Oedema	Erythema	Oedema	Erythema + Oedema	
***L.cubeba*[o]**	0.45±0.11	0.37±0.08	0.82±0.19	0.33±0.05	0.20±0.07	0.53±0.12	0.29±0.07
***C.flexuosus*[o]**	0.45±0.07	0.33±0.05	0.78±0.12	0.16±0.05	0.12±0.05	0.28±0.10	0.50±0.02
***Z.officinale*[o]**	0.37±0.05	0.33±0.05	0.70±0.10	0.20±0.04	0.16±0.05	0.36±0.09	0.34±0.01
***A.vulgaries*[o]**	0.58±0.05	0.66±0.05	1.24±0.10	0.25±0.00	0.29±0.04	0.54±0.04	0.70±0.06
***C.macroptera*[o]**	0.33±0.05	0.29±0.04	0.62±0.09	0.25±0.00	0.16±0.05	0.41±0.05	0.21±0.04
***T.diversifolia*[e]**	0.75±0.12	0.70±0.07	1.45±0.19	0.20±0.04	0.20±0.04	0.40±0.08	1.05±0.11

[(O)] Essential oil, [(e)] Ethanol extract and [(PII)]Primary Irritation Index

Interpretation: According to FHSA (Federal Hazardous Substances Act) regulations, a material with a PII of less than 5.00 is generally not considered a primary irritant to the skin.

Table 4.12.1 Acute oral toxicity of *Zingiber officinale, Artemisia vulgaris, Litsea cubeba, Cymbopogon flexuosus* essential oil as a single acute oral dose at 2000mg/kg on body weight.

Parameters	Control	*Zingiber officinale*	*Artemisia vulgaries*	*Litsea cubeba*	*Cymbopogon flexuosus*
Body weight (gm)	25.04±1.08	26.28±1.08	24.33±0.60	26.16±1.03	26.90±0.96
Haemoglobin (gm/dL)	21.13±0.89	19.82±0.60	17.46±1.13	17.40±0.73	17.94±0.81
RBC (million/mm^3)	7.70±±0.59	9.20±0.77	8.59±0.83	9.11±0.68	8.26±0.51
WBC (x1000/mm^3)	12.93±2.64	15.59±2.36	18.89±1.98	23.43±2.07	18.18±2.98
ALKP (U/L)	186.45±16.09	216.88±9.91	262.53±16.57	258.23±20.13	198.18±9.26
SGOT (U/L)	42.99±7.10	35.77±2.67	43.63±5.36	35.66±1.86	33.39±5.15
Creatinine (mg/dL)	1.65±0.21	1.54±0.30	1.33±0.16	1.16±0.11	1.12±0.06
Triglycerides (mg/dL)	90.43±8.82	61.16±8.74	109.86±15.79	97.68±9.67	93.91±10.17
Bilurubin (mg/dL)	0.23±0.04	0.27±0.04	0.25±0.03	0.18±0.02	0.24±0.01
Cholesterol (mg/dL)	65.00±5.67	52.50±8.11	49.00±5.40	50.17±2.94	43.38±3.66

Table 4.12. 2 Subacute oral toxicity of *Tithonia diversifolia* ethanol extract upon regular dosing (28 days)

Parameters	Control	5mg	50mg	300mg
Body weight 14 days (gm)	5.20±0.76	2.41±0.67	2.88±0.51	4.31±0.36
Body weight 14 days(gm)	7.61±0.56	8.99±1.03	7.91±0.87	8.01±0.57
Haemoglobin (gm/dL)	19.13±0.89	18.82±0.60	17.46±1.13	19.40±0.73
RBC (million/mm^3)	7.90±0.53	9.20±0.75	8.2±0.93	9.45±0.48
WBC (thousand/mm^3)	13.93±1.64	15.49±1.86	17.89±1.28	21.43±2.12
ALKP (U/L)	189.49±51.01	345.02±43.20	300.38±63.86	333.80±40.27
SGOT (U/L)	41.62±8.76	39.14±7.79	47.34±2.76	51.87±3.04
SGPT(U/L)	23.52±5.04	24.94±4.8	28.04±1.93	29.20±5.69
Creatinine (mg/dL)	1.22±0.25	1.22±0.16	1.06±0.11	1.34±0.12
Triglycerides (mg/dL)	96.97±39.59	127.39±52.01	106.55±43.50	90.65±37.01
Bilurubin (mg/dL)	1.26±0.33	1.53±0.28	1.21±0.12	0.97±0.16
Cholesterol (mg/dL)	86.26±19.12	91.25±12.62	106.83±14.00	72.77±11.93

Table 4.12.3 Safety studies of biologically active *C. macroptera* essential oil for seven days at 4X the effective dose in *S.*Typhimurium infected mice

Parameters	Vehicle	Ofloxacin (100mg/kg)	*C. macroptera* (250mg/kg)
Body weight (gm)	0.97±1.16	3.82±0.75	0.73±0.95
Haemoglobin (gm/dL)	12.96±3.13	17.49±1.33	9.94±4.15
RBC (million/mm^3)	7.58±0.32	7.68±0.30	7.69±0.53
WBC (thousand/mm^3)	7.50±1.36	7.70±0.78	7.41±0.55
ALKP (U/L)	56.66±7.05	87.09±14.32	31.83±10.12
SGOT (U/L)	40.10±4.63	38.58±4.63	41.33±4.77
SGPT (U/L)	7.20±1.26	9.32±2.15	6.57±2.25
Creatinine (mg/dL)	1.73±0.17	1.19±0.10	1.34±0.16
Bilurubin(mg/dL)	0.28±0.04	0.26±0.04	0.01±0.00
Triglycerides (mg/dL)	60.90±10.78	76.55±4.22	54.19±3.12
Cholesterol (mg/dL)	113.90±7.49	114.98±4.91	108.23±5.60

4.13 Stability of the essential oils upon exposure to higher temperatures in terms of efficacy of oil/ extract:

Thermo stability of the essential oil was performed to evaluate the bio-activity of *Litcea cubeba, Cymbopogon flexuosus, Zingiber officinale, Artemisia vulgaris, Citrus macroptera* and *Tithonia diversifolia* upon exposing them to a higher temperature. The activity of the heated samples was compared with the samples stored at 4°C. Upon performing the DDA assay, it was observed that none of the samples lost their activity upon rendering the heat treatment even after exposing to higher temperatures of 40°C, 60°Cand 80°C. The studies were performed following the protocols recommended by NCCLS-2002 and the observations are recorded in the (Table 4.13.1, 4.13.2, 4.13.3, 4.13.4, 4.13.5)

Table 4.13.1 Stability of bioactive(s) upon thermal exposure against *Trichophyton rubrum* through Disc Diffusion Assay (DDA) as Zone of Inhibition (mm)

Test sample	**4ºC**	**40°C**	**60°C**	**80°C**
***L.cubeba*^{o}**	37.33±1.47	36.5±0.5	36.5±1.5	35.0±1.0
***C.flexuosus*^{o}**	36.66±1.08	35.0±1.0	35.5±0.5	34.5±0.5
***Z.officinale*^{o}**	26.66±0.40	27.5±0.5	25.5±0.5	27.0±1.0
***A.vulgaries*^{o}**	13.66±0.40	13.5±0.5	13.5±0.5	13.5±0.5
***C.macroptera*^{o}**	23.33±1.08	22.0±1.0	18.0±1.0	21.0±2.0
***T.diversifolia*^{e}**	08.34±0.49	08.4±0.4	08.3±0.2	07.9±0.07
Clotrimazole (5mg/ml)	39.33±0.40	39.5±0.5	38.0±1.0	37.0±2.0

$^{(O)}$ Essential oil, $^{(e)}$ Ethanol extract
20µl/disc; stock 100µl/ml (essential oil) or 100mg/ml(extract) of DMSO

Table 4.13.2 Stability of bioactive(s) upon thermal exposure against *Microsporum gypseum* through Disc Diffusion Assay (DDA) as Zone of Inhibition (mm)

Test sample	**4ºC**	**40°C**	**60°C**	**80°C**
***L.cubeba*^{o}**	31.33±1.76	34.5±0.5	30.5±0.5	28.0±1.0
***C.flexuosus*^{o}**	38.33±0.66	38.0±1.0	38.0±0.5	37.0±1.0
***Z.officinale*^{o}**	26.33±1.76	27.5±0.5	26.5±1.5	25.5±0.5
***A.vulgaries*^{o}**	13.66±0.33	14.5±0.5	13.5±0.5	12.5±0.5
***C.macroptera*^{o}**	17.66±1.45	19.5±0.5	17.5±0.5	16.5±1.5
***T.diversifolia*^{e}**	08.04±1.34	08.2±1.09	08.4±1.4	08.6±1.9
Clotrimazole (5mg/ml)	38.0±0.57	38.5±0.5	36.5±0.5	30.0±2

$^{(O)}$ Essential oil, $^{(e)}$ Ethanol extract
20µl per disc; stock 100µl/ml (essential oil) or 100mg/ml(extract) of DMSO

Table 4.13.3 Stability of bioactive(s) upon thermal exposure against *Malassezia furfur* through Disc Diffusion Assay (DDA) as Zone of Inhibition (mm)

Test sample	**4ºC**	**40°C**	**60°C**	**80°C**
***L.cubeba*^{o}**	30.33±1.76	27.5±0.5	25.5±0.5	27.5±1.5
***C.flexuosus*^{o}**	24.60±1.76	20.05±1.5	22.0±1.0	16.5±0.5
***Z.officinale*^{o}**	20.60±1.76	19.5±1.5	20.0±1.0	21.5±0.5
***A.vulgaris*^{o}**	20.33±0.88	19.5±1.5	18.5±1.5	18.0±1.0
***C.macroptera*^{o}**	9.66±0.33	8.5±0.5	8.5±1.5	7.5±0.5
***T.diversifolia*^{e}**	19.66±1.70	18.0±1.0	20.0±1.0	17.0±1.0
Clotrimazole (5mg/ml)	19.00±0.57	17.5±0.5	16.5±0.5	15.5±1.5

$^{(O)}$ Essential oil, $^{(e)}$ Ethanol extract
20µl per disc; stock 100µl/ml (essential oil) or 100mg/ml(extract) of DMSO

Table 4.13.4 Stability of bioactive(s) upon thermal exposure against *Candida albicans* through Disc Diffusion Assay (DDA) as Zone of Inhibition (mm)

Test sample	**4ºC**	**40°C**	**60°C**	**80°C**
***L.cubeba*^{o}**	13.66±0.88	13.5±0.5	14.0±1.0	12.5±0.5
***C.flexuosus*^{o}**	14.33±0.33	13.5±0.5	13.5±1.5	13.0±1.0
***Z.officinale*^{o}**	22.66±2.45	21.5±1.5	20.0±1.0	21.0±0.5
***A.vulgaries*^{o}**	14.66±1.20	14.5±1.5	14.5±1.5	13.5±0.5
***C.macroptera*^{o}**	10.67±0.33	11.5±0.5	10.5±0.5	10.5±0.5
***T.diversifolia*^{e}**	09.04±0.84	09.04±0.42	08.94±0.56	08.04±0.34
Clotrimazole (5mg/ml)	39.00±0.58	38.5±0.5	37.5±0.5	36.5±0.5

$^{(O)}$ Essential oil, $^{(e)}$ Ethanol extract
20µl per disc; stock 100µl/ml (essential oil) or 100mg/ml(extract) of DMSO

Table 4.13.5 Stability of bioactive(s) upon thermal exposure against *Staphylococcus aureus* through Disc Diffusion Assay (DDA) as Zone of Inhibition (mm)

Test sample	**4ºC**	**40°C**	**60°C**	**80°C**
***L.cubeba*^{o}**	17.66±0.88	17.0±1.0	16.5±0.5	15.5±0.5
***C.flexuosus*^{o}**	17.33±0.33	17.5±0.5	15.5±0.5	17.5±0.5
***Z.officinale*^{o}**	17.33±0.88	17.5±1.5	17.0±1.0	15.5±0.5
***A.vulgaris*^{o}**	17.0±0.57	15.5±0.5	14.5±0.5	14.5±1.5
***C.macroptera*^{o}**	12.33±0.66	12.5±0.5	11.5±0.5	10.0±1.0
***T.diversifolia*^{e}**	16.66±1.85	16.5±0.5	16.5±1.5	16.5±0.5
Clotrimazole (5mg/ml)	17.66±0.03	17.5±0.5	16.0±1.0	17.5±0.5

$^{(O)}$ Essential oil, $^{(e)}$ Ethanol extract
20µl per disc; stock 100µl/ml (essential oil) or 100mg/ml(extract) of DMSO

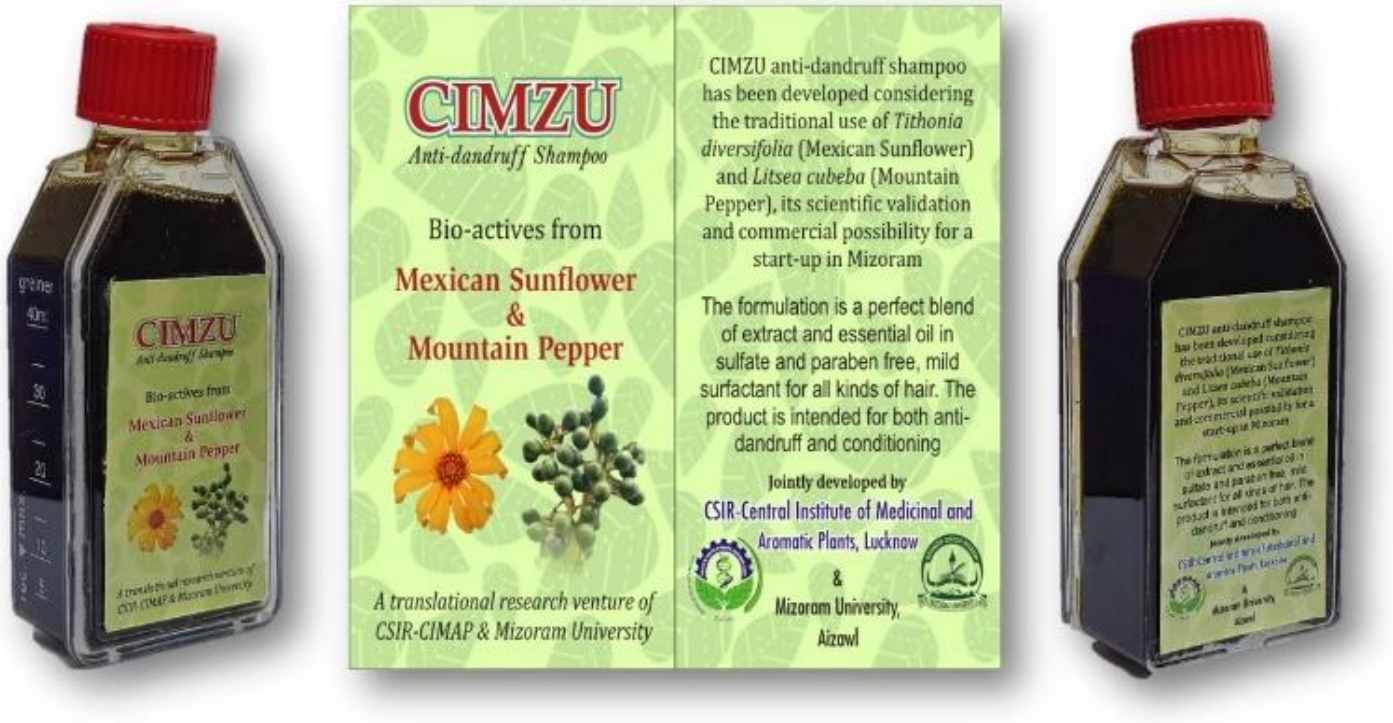
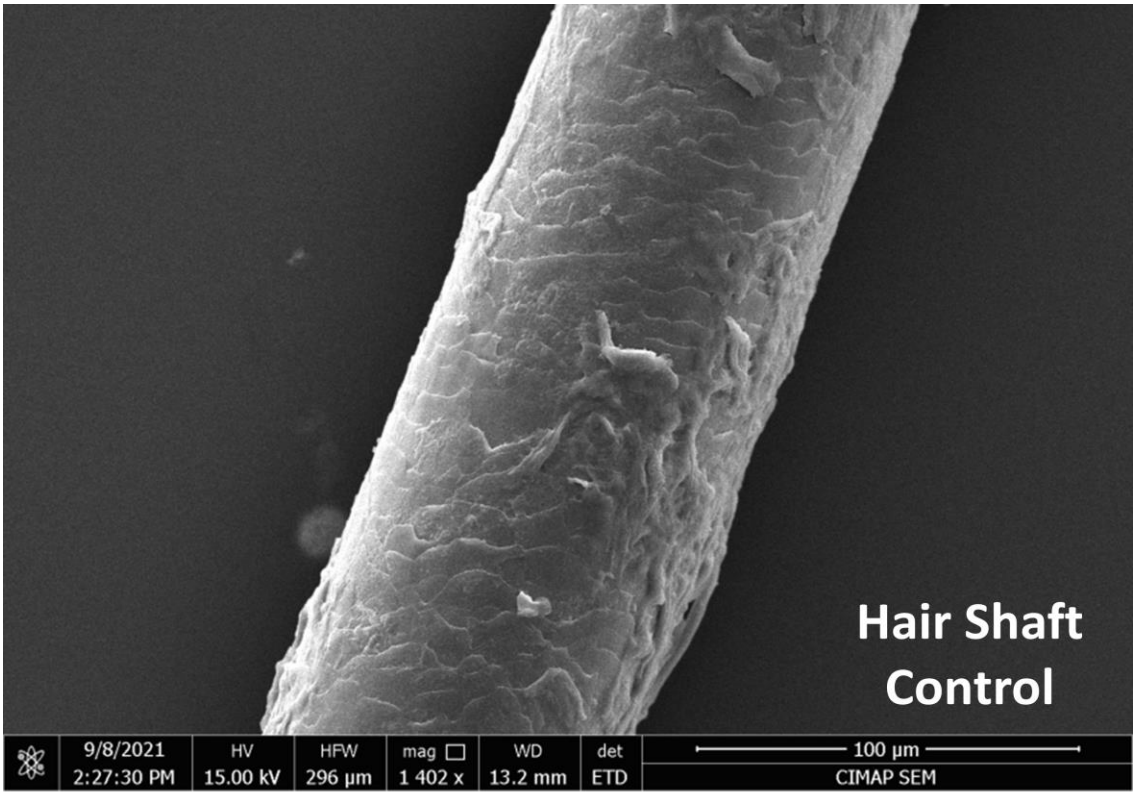


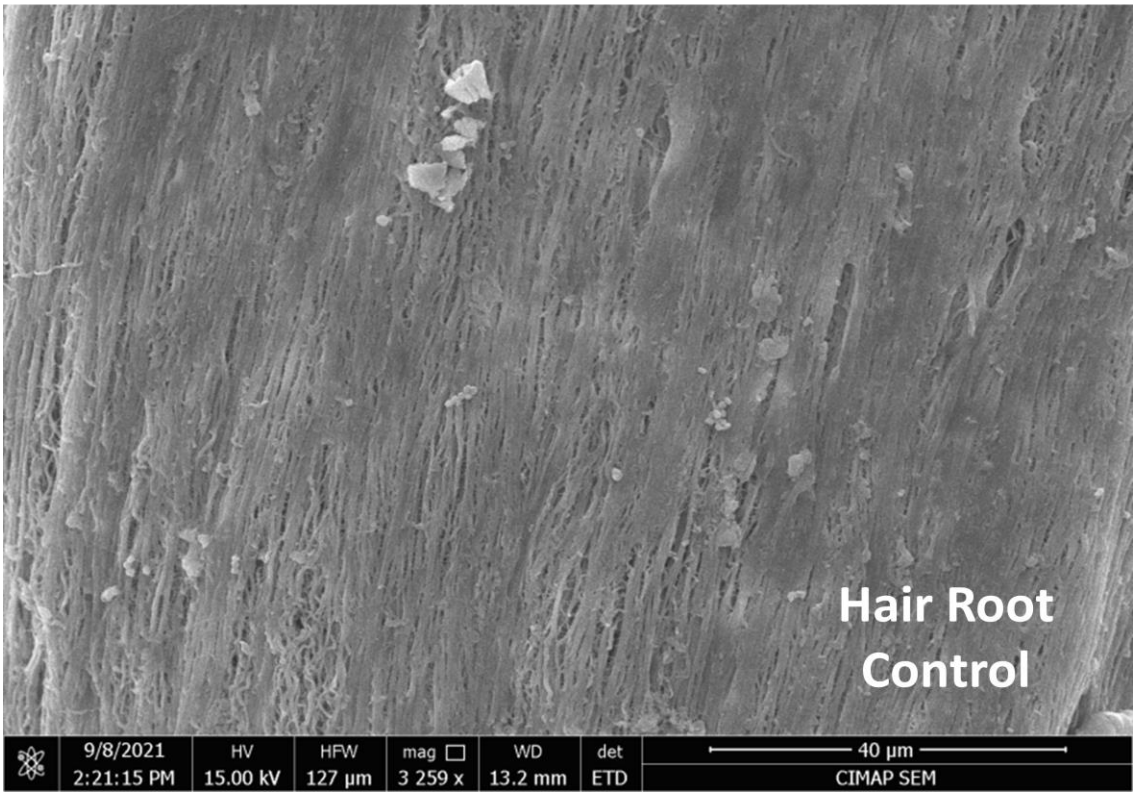
Fig 4.14.1 The developed formulation with bioactive(s)

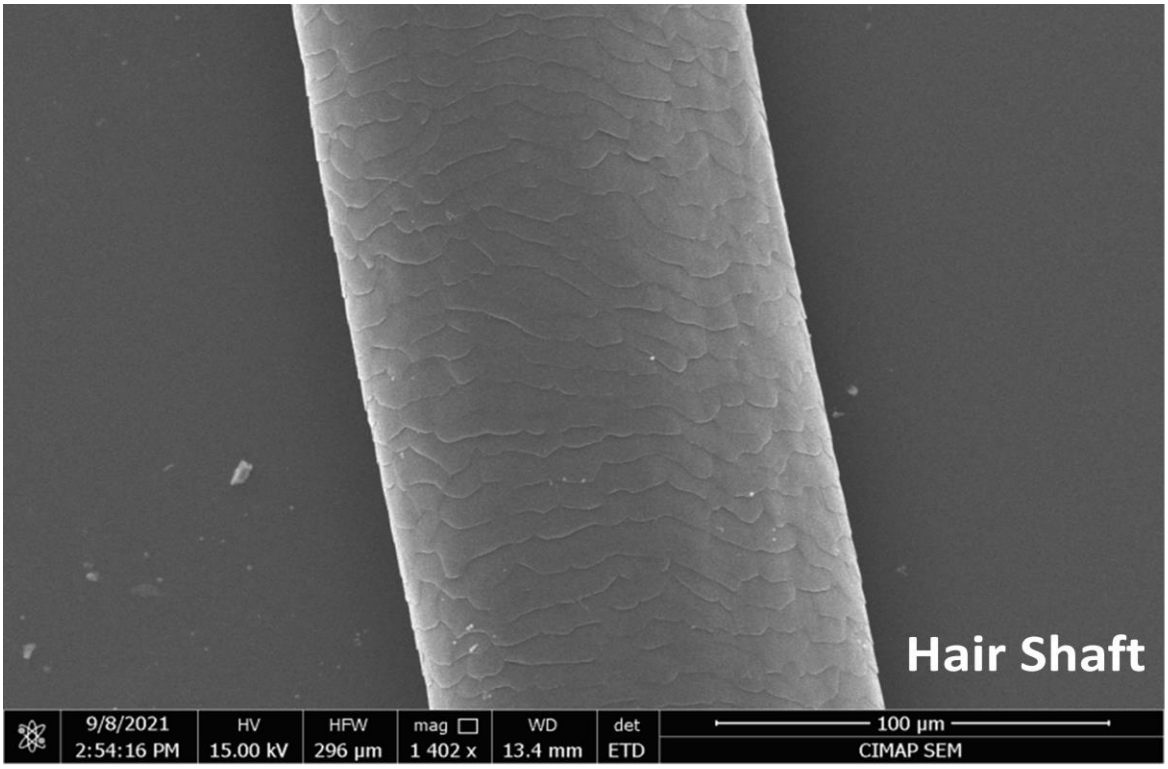
4.14 Effect of the formulation on hair colonized with *Malassezia furfur*:

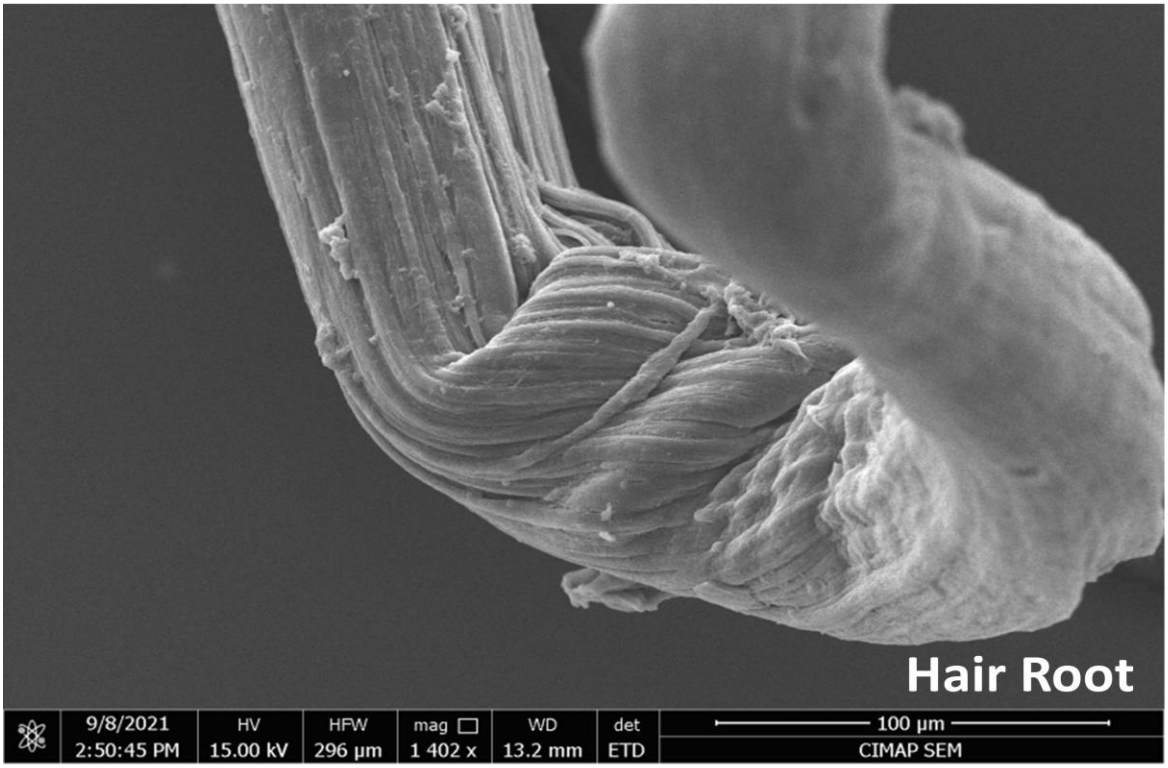
Once the biological activity of the essential oils and the extract were available with us, we developed the formulation which we tried to keep paraben-free. We then exposed sterile hair to *M. furfur* for colonization on its surface and we observed that the colonized hair upon exposure to various concentrations of the formulation for 30 minutes reduced the colonization of the pathogen as visualized through SEM, the images of which are depicted below.

Fig 4.14 2 Effect of the formulation on hair colonized with *M. furfur* visualized through SEM

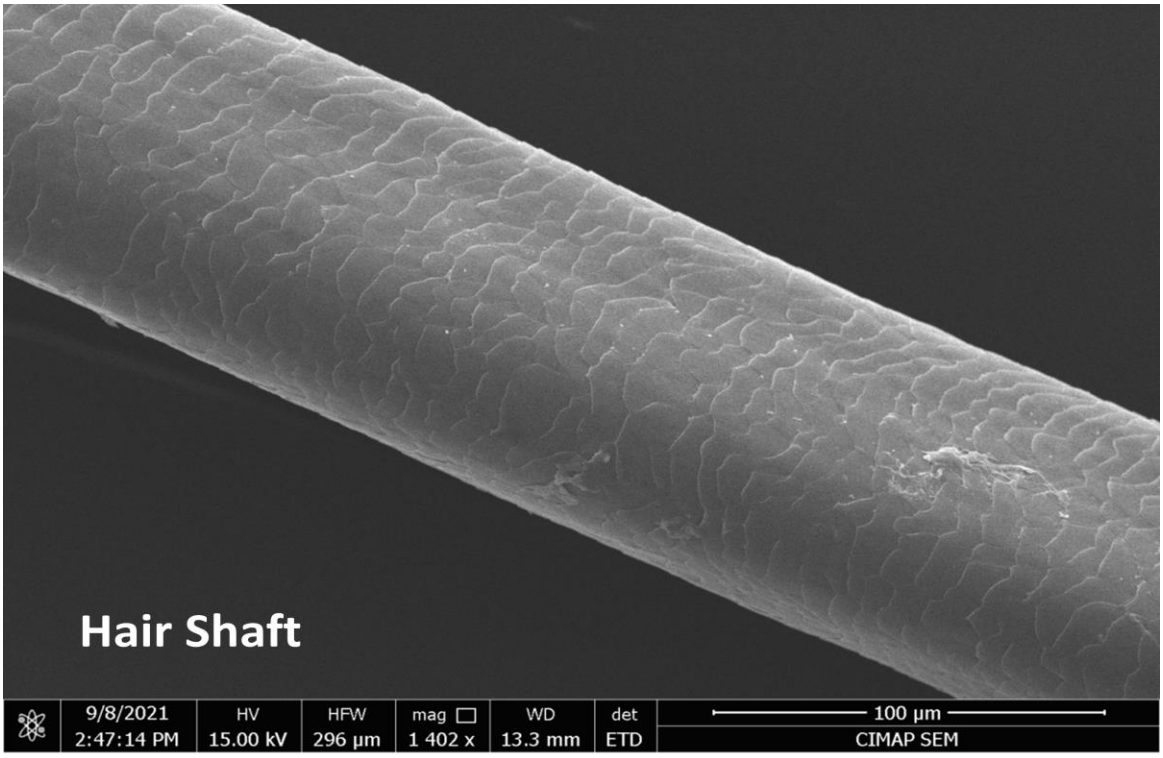


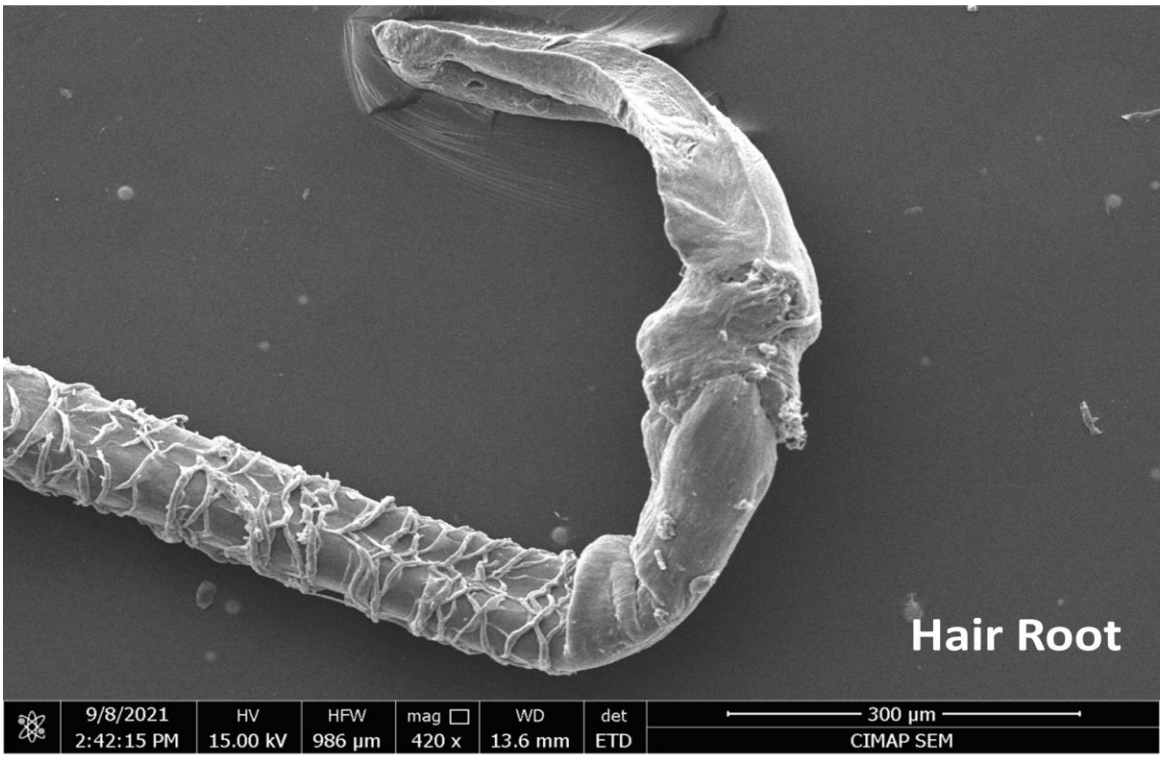




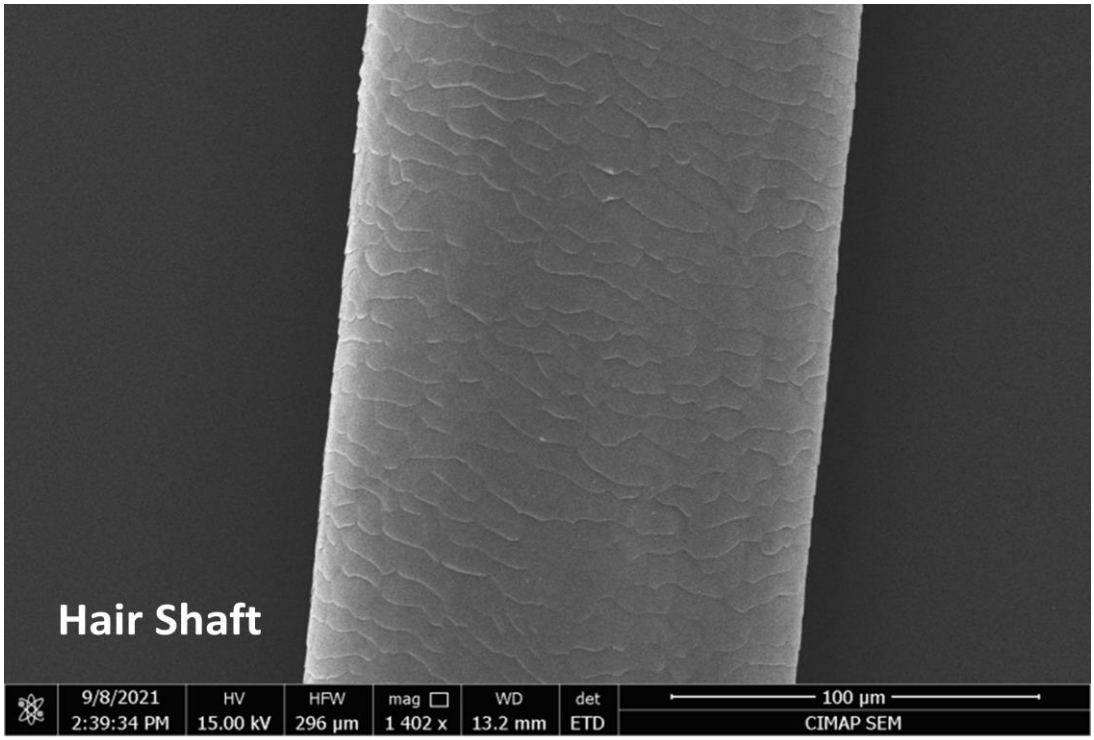


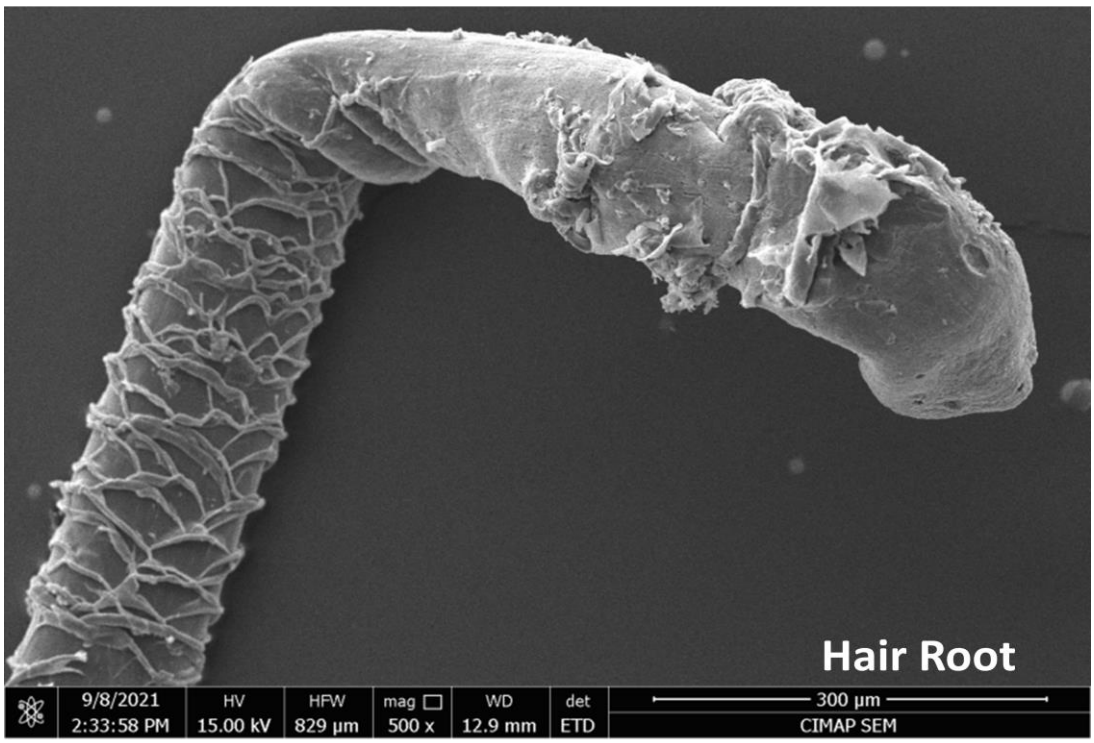
Treatment with 25% CIMZU





Treatment with 50% CIMZU





Treatment with 100% CIMZU

CHAPTER - 5

DISCUSSION

5.1 Collection and Categorization of some formulated products used against skin ailments/remedies and practices among the tribal communities / traditional healers in Mizoram:

India has a rich heritage of the traditional system of medicine as evidenced by Ayurveda, Unani, Siddha systems of medicine. However, naturopathy is another stream, that derives its strength from the practices that have evolved from generation to generation, either through verbal communications or through traditional healers, who have immense knowledge and practical utility of medicinal plants in their vicinity. Most of this knowledge is not documented and is fading out with the increased marketing and acceptance of commercial products even to the remote corners of the country. Unfortunately, this is one of the reasons for the loss of valuable traditional knowledge that could have been tapped for scientifically validated treatments through modern formulations.

In the present study, initial survey, follow-ups, continuous persuasion followed by tete a tete with the traditional healers have resulted in the disclosure of six formulations that the Mizo tribes use for treating skin ailments that are presumed to be caused by the pathogens viz., *Trichophyton rubrum*, *Microsporum gypseum*, *Malassezia furfur, Candida albicans* and *Staphylococcus aureus.*

All six formulations were observed to possess a medicinal aroma upon application and as per the conversation with some of the patients, the formulations were found to be therapeutically active. Conversations with the traditional healers and the analysis of the samples as shown by them followed by their identification by the botanists revealed the plants to be primarily; *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris*, *Citrus macroptera* and *Tithonia diversifolia*. With these oservations, we took up studies to validate the biological activity of these plants against the pathogens that primarily cause skin infections amongst the locals in Mizoram.

5.2.Physicochemical analysis of the selected formulations:

Plants are a rich source of therapeutic compounds that have tremendous applications inthe pharmaceutical industry. Besides, medicinal plants have been used to treat human

diseases for thousands of years because they have a vast and diverse assortment of organic compounds that can produce a definite physiological action on the human body. The most important of such compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inayatullah et al., 2012). Several such compounds have been isolated from plants which could be used for the development of new drugs to inhibit the growth of bacterial and fungal pathogens and to quench ROS with possibly novel mechanisms of action and low toxicity to the host cell (Ahmad and Aqil, 2007). In the recent past, pharmaceutical laws oblige drug manufacturers to assess the compatibility of active substances, excipients, and medicinal products with established standards. However, characterization of the active pharmaceutical ingredients (APIs) improves the quality parameters of all raw materials used during the manufacturing process of pharmaceuticals, as well as those in the final products(Singh,2015).

The formulations that were narrowed down in our study were observed to be mostly aromatic in nature (*Litcea cubeba, Cymbopogon flexuosus, Zingiber officinale, Artemisia vulgaris, Citrus macroptera*) except one of them i.e., *Tithonia diversifolia*. Evaluation and observation of raw drugs material is a must for identification and maintenance of the quality of the articles.From our finding, we observe that the refractive index of the plant sample *Litcea cubeba, Cymbopogon flexuosus, Zingiber officinale, Artemisia vulgaris, Citrus macroptera* and *Tithonia diversifolia* were 1.4868 (20°C), 1.4860(20°C), 1.4888(20°C), 1.4804(20°C) and 1.4719(20°C) respectively, Optical rotation were + 0.4(20°C), -0.32(20°C), + 6.28(20°C), - 16.24(20°C) and + 93.68(20°C) respectively, Specific gravity was 0.8952(28.4°C), 0.8951(28.4°C), 0.9106(28.4°C), 0.9403(28.4°C) and 0.8474(28.4°C), thermogravimetric were 205°C, 207°C, 195°C, 200°C and 146°C respectively and the flashpoint were 84°C, 88°C, 59°C and 66°C respectively. We also observe that all test samples were soluble in an organic solvent but are insoluble in water except ethanol extract of *Tithonia diversifolia.*

5.3. Phytochemical analysis of the selected formulations:

Formulations developed from medicinal plants have bioactive compounds that prove beneficial for curing various human diseases including skin ailments upon topical application. The phytochemicals present in the medicinal plants instead could be either the primary or secondary metabolites. The primary metabolites are those of chlorophyll,

proteins, sugars or amino acids while the secondary metabolites could be terpenoids and alkaloids. These secondary metabolites are responsible for various biological activities like antibacterial, antifungal or anti-inflammatory activity. The phytochemical analysis is very important from the perspective of pharmaceutical companies and commercial aspects.

The formulations that were narrowed down mostly had the aromatic plants, the essential oils of which are known for their biological activities. The secondary metabolites from different aromatic and medicinal plants show different levels of antimicrobialactivity. Investigation of plant bioactive compounds can be evident in the detection of a significant number of therapeutic properties. The quantitative analysis of the compounds through GC-MS and HPLCinferred that they are enriched with monoterpenoids. It has been reported that terpenes are a group of antimicrobial compounds that are active against a broad spectrum of microorganisms, including yeasts and moulds (Fillipe et al., 2014).The major components which are present in the test samples were Geranial (=Citral a), Geranyl acetate, Neral (=Citral b),Geraniol, Camphene, α-Zingiberene, 1,8-Cineole, Germacrene, Limonene, β-Myrcene, Ascorbic acid and Catechin etc.According to our observation,Geranial (=Ciral a) was the major constituent among *Litsea cubeba*, *Cymbopogon flexuosus* and *Zingiber officinale* attaining 26.64%, 48.10%and 17.75%. Coutinho et al.,(2015) and Marei et al., (2012) reported that geraniol (lipophilic character) has the ability to adhere to cell membrane lipids of the microorganism; interacting with its components, making it more permeable, and binding essential intracellular sites, to thus destroy their structures. We also found that 1,8-Cineole (11.89%) was the major constituent of *Artemisia vulgaris*.Vilela et al.,(2009) reported that in their studies 1, 8-cineole alone has low antifungal power, but their findings reveal that the synergistic effect of other components in the antifungal activity.From our experimental work, we also observed that Limonene (89.4%) was the major constituent of *Citrus macroptera* essential oil.Limonene is a widely cited compound in the literature for its bioactivities, including its antimicrobial activity (Duarte et al., 2005). Further, the compound present in *Tithonia diversifolia* were Ascorbic acid, Catechin, Ferulic acid etc.

5.4 Bioprospection against the pathogenic fungi/ Dermatophytes:

Aromatic and medicinalplants play important role in drugs development. Their qualities are augmented by the complex nature of their secondary metabolites. Several scientific studies have proven that they are reliable for therapeutic uses for different ailments. Burt

(2004) reported that essential oils, as well as compounds derived from aromatic plants, possess a wide range of activities and their antimicrobial properties are the most studied concern. Several studies have demonstrated that numerous essential oils exhibit antimicrobial properties due to the presence of components such as thymol, eugenol, 1,8-cineole, α- and β- pinenes, linalool, α- terpineol etc. (Srivastava et al., 2000; Sinha and Gulati, 1990).

Farag et al.,(1989); Arfa et al.,(2006) also reported that the antimicrobial properties of essential oils are due to the presence of phenolic compounds. It has also been reported that terpene of essential oil can act individually, additively or synergistically to improve the therapeutic efficiency of other drugs (Coutinho et al., 2015). The monoterpenes and sesquiterpenes of essential oil being lipophilic in nature can cause the membrane to become more permeable to protons and ions due to structural disarrangement, including that of efflux proteins, which may lead to greater interaction with the substances and cause pathogen death (Oluwatuyi et al., 2004; Cristani et al., 2007).

From our experimental studies, we observed that all the test samples i.e., *Zingiber officinale, Litcea cubeba, Artemisia vulgaris, Cymbopogon flexuosus, Citrus macroptera* and *Tithonia diversifolia* possess antimicrobial activities against *T. rubrum* (MTCC-296), *M. gypseum* (MTCC-2819), *M.furfur* (MTCC-1374), *C.albicans* (ATCC-0443X-1) and *S.aureus* (ATCC-6538). The *in vitro* qualitative analysis was performed through Disk Diffusion Assay (DDA). Our results demonstrated that the zone of inhibition of the test samples wasobtained range between 38.33±0.66 and 08.04±1.34 against the test pathogens and the sample *L.cubeba* was the most potent leads against *T.tubrum*, *M.furfur* and *S.aureus*, whereas *C.flexuosus*was found to be most effective against *M.gypseum*. Further, *Z.officinale* was also found to be the most effective against *C.albicans*. The qualitative evaluation reveals that Minimum Inhibitory Concentration (MIC) ranges between0.039 µl and 0.781µl/ml against the test pathogens and *L.cubeba* essential oil was the most potent lead against *T.rubrum, M.gypseum, M.furfur* and *S.aureus*. Whereas, the lowest MIC was observed from *C.flexuosus* against *C.albicans*. In search of the cidal concentration of the test sample we also observed that the minimum bactericidal/fungicidal concentration (MBC/MFC) of the test samples ranged between 0.039 and 3.125µl/ml against the test pathogens. The sample of *L.cubeba* essential oil possesses the lowest MBC/MFC against *T.rubrum, C.albicans* and *S.aureus*. Further,

C.macroptera possess the lowest MBC/MFC against *M.furfur*. The killing time studies from our observation also disclosed the minimum killing time was in the range of 4 hours to 9 hours. The minimum time killing was found similar to most of the test samples which are less than 4 hours, whereas the maximum times taken was recorded from *C.macroptera* and *T. diversifolia* against *C.albicans* which took 9 hours under standard conditions.

The probable mechanism of action which are carried out through Sorbitol protection assay revealed that in the case of *L.cubeba* and *C.flexuosus* the MIC values increased against all the test pathogens, but in the case of *Z.officinale* the MIC value increased against only to *T.rubrum* and *M.furfur*. Similarly, inthe case of *A.vulgaris* the MIC value increased against *T.rubrum* and *C.albicans*, while the MIC value of *C.macroptera* changed only against to *M.gypseum*, whereas in the case of *T.diversifolia* the MIC value increased against all the test pathogensexcept in *M.furfur*.

The morphological changesstudied through Scanning Electron Microscope upon bioactive treated and control group illustrated that the bioactive act on the cell wall resulting in the degradation and causing them to have shrinkingappearance. Whilethe surface of the untreated pathogens appeared to be smooth and rounded.Burt (2004) explainedthat the mechanism of action for essential oil against the pathogenis thought to bedegradation of the cell wall, damage to cytoplasmic membrane proteins, the binding of proteins, leakage of cell contents and coagulation of cytoplasm and depletion of the proton motive force.

The confocal microscopy studies carried out on the test samples revealed that the fluorescent dye (DAPI) penetrated the test pathogens that had a damaged cell wall. DAPI binds strongly to A-T rich regions in DNA and can inefficiently pass through the intact cell membrane and therefore preferentially stain the dead cells (Wallberg et al., 2016). It may be observed from the untreated sample that DAPI does not enter the cells since their cell membranes are intact and hence the fluorescence is almost negligible when compared to the treated groups.

Our research findings forthe probable anti-inflammatory ability of the test sample has revealed that 0.1% of essential oil and 10µg/ml of plant ethanol extractcould alleviatethe LPS induced inflammation significantly by decreasing the production of pro-inflammatory cytokines (TNF-α). Our resultsare similar to the previously reported studies

(Boukhatem et al., 2014, Afsar 2013),which revealed thatthe constituents of essential oils and plant ethanol extract could inhibit the production of pro-inflammatory cytokines in LPSactivated macrophage cells. It may be speculated that the incorporation of these essential oils in any of the formulations could have anti-inflammatory activity in the areas of pathogenesis caused by the pathogen.

The viability of J774A.1 cells was determined through MTT assay. The test samples that exhibited anti-microbial activity i.e., *Litcea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris*, *Citrus macroptera* and *Tithonia diversifolia* were found to be non-cytotoxic and seemed cytoprotective as evident through the modulation of inflammatory mediators. The samples at the higher doses were also able to lessen the cell damage caused by LPS, which might be due to the presence of phenols on the test sample (Ruengeler et al., 1988;Yang et al., 2018; Haris et al., 2018).

The Dermal Irritation test in terms of PII was observed to be 1.05±0.11 as the highest, which is less than FHSA standards i.e., PII of 5.0. The experimental results reveal that the bioactive oils did not exhibit any skin irritation and were found to be safe as per FHSA standards.

In the acute oral toxicity study, the test sampledid not show any haematological and biochemical alterations in the treated animals. The testing of drugs beyond the dose of 2000 mg/Kg is discouraged by OECD guidelines for oral toxicity. Since no significant changes were observed in haematological and biochemical parameters at this particular oral dose, the test sample result concluded a no-observed-adverse-effect-level (NOAEL) upto 2000mg/kg body weight.

The thermal stability of the oils was performed with the intention of its incorporation into a suitable pharmaceutical formulation for use against skin ailments. It was observed that all the test samples when exposed up to a temperature of 80°C for up to 4 hrs is quite stable as evidenced by the activity which were performed through DDA assay.

CHAPTER - 6

SUMMARY AND CONCLUSION

Evidence-based traditional medicine can be a prospective and alternative source of safe, affordable and effective medicines for developing countries especially in interior hamlets of North East India or any similar locations around the world, deprived of modern medical facilities.

The present study was undertaken to narrow down on the validation of traditional formulations for skin ailments and translate the scientific pieces of evidence into a formulation with therapeutic and commercial value. Five traditional formulations were narrowed down based on the conversations with the traditional healers and their ingredients were analysed. The plants used commonly in the formulations were that of *Litsea cubeba*, *Cymbopogon flexuosus*, *Zingiber officinale*, *Artemisia vulgaris*, *Citrus macroptera* and *Tithonia diversifolia*. All the plants were subjected to either hydrodistillation process except *T.diversifolia* which was solvent extracted.

The essential oils and extract were found to be active against dermal pathogens viz., *Trichophyton rubrum*, *Microsporum gypseum*, *Melasezzia furfur*, *Candida albicans* and *Staphylococcus aureus* as evidenced through Disc Diffusion Assay, Minimum Inhibitory Concentration, Minimum Bactericidal/Fungicidal Concentration and Minimum Killing Time. The bioactive(s) were subjected to the mechanism of action studies which concluded their activity to be upon the cell wall of the pathogens. This was concluded through Sorbitol assay, Scanning Electron Microscopy and Confocal Microscopy studies. These bioactive(s) were also subjected to studies involving cell lines and it was found to impart an anti-inflammatory activity while they did not exhibit any cytotoxic effect even at concentrations much higher than the ones they were biologically active. These essential oils and extract were also subjected to thermogravitimetric analysis to check their stability at higher temperatures. Corroborating all the data on the efficacy and the safety of the bioactive(s), a formulation (shampoo) was developed for the management of dandruff. SEM images of hair colonized with *M.furfur* confirmed the anti-dandruff activity.

The series of studies concluded that the scientific validation of traditional formulations can be a good source of leads that could be converted into commercially viable formulations for the betterment of the local population.

Future Prospects

- Based on the leads obtained from the study, different other formulations cold be explored for development specific to pathogens like *Staphylococcus aureus* or
- Formulation specific to skin infections can be targeted from the results obtained from the study
- Observational studies in a bigger population suffering from dandruff or scalp infection

Conclusions

- Traditional medicines or formulations have a scientific background as evidenced in our study can be a source of potential therapeutic leads
- The bioactive(s) isolated from the medicinal plants were found to be active against dermal pathogens
- Scientific validation studies can lead to the reformulation leading to a product that could be standardized based on the phytochemical parameters

Abd El-Kalek, H. H., & Mohamed, E. A. (2012). Synergistic effect of certain medicinal plants and amoxicillin against some clinical isolates of methicillin–resistant *Staphylococcus aureus* (MRSA). *Int J Pharm Appl*, 3, 387-398.

Abdalla, A. S. A. H. (2000). Use of plant-derived aromatic oils as insecticides in agriculture and the home. *Turk. Pat. Appl*, TR 9801333 A2 20000921.

Abe, A. E., Oliveira, C. E. D., Dalboni, T. M., Chagas-Paula, D. A., Rocha, B. A., Oliveira, R. B. D., Gasparoto, T. H.; Da Costa, F. B., & Campanelli, A. P. (2015). Anti-inflammatory sesquiterpene lactones from *Tithonia diversifolia* trigger different effects on human neutrophils. *Revista brasileira de farmacognosia*, 25, 111-116.

Abiri, R., Silva, A. L. M., de Mesquita, L. S. S., de Mesquita, J. W. C., Atabaki, N., de Almeida Jr, E. B., Mesquita, L. S., Shaharuddin, N. A. & Malik, S. (2018). Towards a better understanding of *Artemisia vulgaris*: Botany, Phytochemistry, pharmacological and biotechnological potential. *Food Research International*, 109, 403-415.

Addo-Mensah, A., Garcia, G., Maldonado, I. A., Anaya, E., Cadena, G., & Lee, L. G. (2015). Evaluation of the antibacterial activity of *Artemisia vulgaris* extracts. *Research Journal of Medicinal Plants*, 9, 234-240.

Ademuyiwa, A. J., & Grace, O. K. (2015). The effects of *Cymbopogon citratus* (Lemongrass) on the antioxidant profiles Wistar albino rats. *Merit Research Journal of Environmental Science and Toxicology* (ISSN: 2350-2266), 3,051-058.

Afsar, S. K., Kumar, K. R., Gopal, J. V., & Raveesha, P. (2013). Assessment of anti-inflammatory activity of *Artemisia vulgaris* leaves by cotton pellet granuloma method in Wistar albino rats. *journal of pharmacy research*, 7, 463-467.

Agusta, D. A., & Jamal, Y. (1999). Chemical components and an antibacterial assay of Kirinyu (*Tithonia diversifolia*) leaves extract. Indonesian *J. Pharm. (Majalah Farmasi Indonesia)*, 10, 113-120.

Ahmad, I., & Aqil, F. (2007). In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. *Microbiological Research*, 162, 264-275.

Alam, M. A., Sagor, A. T., Tabassum, N., Ulla, A., Shill, M. C., Rahman, G. M. S., Hossain, H., & Reza, H. M. (2018). Caffeic acid-rich *Citrus macroptera* peel powder supplementation prevented oxidative stress, fibrosis and hepatic damage in CCl 4 treated rats. *Clinical Phytoscience*, 4, 1-10.

Ali, B. H., Blunden, G., Tanira, M. O., & Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. *Food and chemical Toxicology*, 46, 409-420.

Ali, M., Sahrawat, I., & Singh, O. (2004). Volatile constituents of *Cymbopogon citratus* (DC.) Stapf. leaves. *Journal of Essential Oil Bearing Plants*, 7, 56-59.

Alzate, D. A., Mier, G. I., Afanador, L., Durango, D. L., & García, C. M. (2009). Evaluation of phytotoxicity and antifungal activity against *Colletotrichum acutatum* of

essential oils of thyme (*Thymus vulgaris*), lemongrass (*Cymbopogon citratus*), and its main constituents. *Vitae*, 16,116-12

Amer, A., & Mehlhorn, H. (2006). Repellency effect of forty-one essential oils against Aedes, Anopheles, and Culex mosquitoes. *Parasitology Research*, 99, 478-490.

Anal, J. M. H. (2014). Trace and essential elements analysis in *Cymbopogon citratus* (DC.) stapf samples by graphite furnace atomic absorption spectroscopy and its health concern. *Journal of toxicology*, 2014690758

Anitha, R., Ramachandran, T., Rajendran, R., & Mahalakshmi, M. (2011). Microencapsulation of lemongrass oil for mosquito repellent finishes in polyester textiles. *Elixir Bio Phys*, 40, 5196-5200.

Anthoney, S. T., Jackie, O. K., Miyogo, E., & Lasiti, T. T. (2016). Bioassay screening of the ethanolic extract of *Tithonia diversifolia* leaves on selected microorganisms. *Int J Bioass*, 5, 4794-8.

Arfa AB, Combes S, Preziosi-Belloy L, Gontard N, Chalier P. 2006. Antimicrobial activity of carvacrol related to its chemical structure. *Lett Appl Microbiol*. 43, 149–154.

Asakawa, Y., Tomiyama, K., Sakurai, K., Kawakami, Y., & Yaguchi, Y. (2017). Volatile compounds from the different organs of *Houttuynia cordata* and *Litsea cubeba* (*L. citriodora*). *Journal of oleo science*, 66, 889-895.

Avcioglu, N. H., Sahal, G., & Bilkay, I. S. (2016). ANTIBIOFILM EFFECTS of Citrus limonum and *Zingiber officinale* Oils on BIOFILM FORMATION of *Klebsiella ornithinolytica*, *Klebsiella oxytoca* and *Klebsiella terrigena* SPECIES. *African Journal of Traditional, Complementary and Alternative Medicines*, 13, 61-67.

Awan, U. A., Ali, S., Shahnawaz, A. M., Shafique, I., Zafar, A., Khan, M. A. R., Ghous, T., & Andleeb, S., Saleem, A. (2017). Biological activities of *Allium sativum* and *Zingiber officinale* extracts on clinically important bacterial pathogens, their phytochemical and FT-IR spectroscopic analysis. *Pakistan Journal of pharmaceutical* sciences, 30(3).

Aziz, D. M., Wsoo, M. A., & Ibrahim, B. M. (2015). Antimicrobial and antioxidant activities of extracts from medicinal plant ginger (*Zingiber officinale*) and identification of components by gas chromatography. *African Journal of plant science*, 9, 412-420.

Baghel, P., Chauhan, A., Namdeo, A., & Chauhan, S. (2017). Quantitative estimation of bioactive compound present in *Zingiber officinale* extract and its antioxidant and antimicrobial activity**.** *International Journal for Scientific Research and Development*, 5(9), 1-5.

Bagnarello, G., Hilje, L., Bagnarello, V., Cartín, V., & Calvo, M. (2009). Phagodeterrent activity of the plants *Tithonia diversifolia* and *Montanoa hibiscifolia* (Asteraceae) on adults of the pest insect *Bemisia tabaci* (Homoptera: Aleyrodidae). *Revista de biologia tropical*, 57, 1201-1215.

Bajpai, A.B., Verma, N. K., Sharma, V., & Akhtar, J. (2018). Evaluation of the antimicrobial activity of the aqueous extract of lemongrass against selected pathogenic bacteria. *Journal of Plant Development Sciences*, 10, 133-135.

Banu, M. A. F., Geetha, R. V. (2015). Evaluation of antimicrobial efficacy of essential oils on streptococcus mutans. *International Journal of Pharmaceutical Sciences Review and Research*, 33, 119-121.

Barney, J. N., & DiTommaso, A. (2003). The biology of Canadian weeds. 118. *Artemisia vulgaris* L. *Canadian journal of plant science*, 83, 205-215.

Bell-Sayer, S. E. M., Hart, R., Crawford, F., Tyrrel, W., & Torgerson, D. (1998).Systematic Treatments for Fungal Infections of the Skin of the Foot, Cochrane Review Protocol. *The Cochrane Library*, 3.

Bhatt, L. R., Lim, J. A., Chai, K. Y., Kang, J. I., Oh, H. K., & Baek, S. H. (2006). Antioxidative and antimicrobial activities of essential oil from *Artemisia vulgaris*. *Natural Product Sciences*, 12, 226-231.

Blagojevic, P., Radulovic, N., Palic, R., & Stojanovic, G. (2006). Chemical composition of the essential oils of Serbian wild-growing *Artemisia absinthium* and *Artemisia vulgaris*. *Journal of Agricultural and Food Chemistry*, 54, 4780–4789.

Bonou, J., Baba-Moussa, F., Adéoti, Z., Ahouandjinou, H., Dougnon, V., Dossa, D., Gbenou, J. D., Toukourou, F., & Baba-Moussa, L. (2016). Antimicrobial activity of essential oils of *Lippia multiflora*, *Eugenia caryophyllata*, *Mentha piperita* and *Zingiber officinale* on five oral-dental microorganisms. *Journal of Pharmacognosy and Phytochemistry*, 5, 271.

Bouberte, M.Y., Krohn, K., Hussain, H., Dongo, E., Schulz, B., & Hu, Q. (2006). new anthraquinone and a new ceramide from leaves of *Tithonia diversifolia* Zeitschrift fuer Naturforschung, *B: Chemical Sciences*, 61, 78-82.

Boukhatem, M. N., Ferhat, M. A., Kameli, A., Saidi, F., & Kebir, H. T. (2014). Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs. *Libyan Journal of Medicine*, 9, 25431

Burt, S., (2004). Essential oils: their antibacterial properties and potential applications in foods da review. *International Journal of Food Microbiology*, 94, 223-253.

Cakir, U., Tayman, C., Serkant, U., Yakut, H. I., Cakir, E., Ates, U., Koyuncu, I & Karaogul, E. (2018). Ginger (*Zingiber officinale* Roscoe) for the treatment and prevention of necrotizing enterocolitis. *Journal of ethnopharmacology*, 225, 297-308.

Calzada,J. G., & Ciccio, J. F. (1978). Isolation of tirotundin from *Tithonia diversifolia* (Hemsl.) Gray. *Revista Latinoamericana de Quimica*, 9, 202-3.

Campos, J., Schmeda-Hirschmann, G., Leiva, E., Guzmán, L., Orrego, R., Fernández, P., Radojkovic, C., Zuniga, F. A., Lamperti, L., & Gonzalez M. (2014). Lemon grass (*Cymbopogon citratus* (DC) Stapf) polyphenols protect human umbilical vein endothelial cells (HUVECs) from oxidative damage induced by high glucose, hydrogen peroxide and oxidised low-density lipoprotein. *Food Chemistry*, 151, 175-181.

Carbonell-Caballero, J., Alonso, R., Ibañez, V., Terol, J., Talon, M., & Dopazo, J. (2015). A phylogenetic analysis of 34 chloroplast genomes elucidates the relationships between wild and domestic species within the genus Citrus. Molecular biology and evolution, 3, 2015-2035.

Carnat, A., Heitz, A., Fraisse, D., Carnat, A. P., & Lamaison, J.L. (2000). Major dicaffeoylquinic acids from *Artemisia vulgaris*. *Fitoterapia*, 71, 587-589.

Castaño-Quintana, K., Montoya-Lerma, J., & Giraldo-Echeverri, C. (2013). Toxicity of foliage extracts of *Tithonia diversifolia* (Asteraceae) on *Atta cephalotes* (Hymenoptera: Myrmicinae) workers. *Industrial Crops and Products*, 44, 391-395.

Chagas-Paula, D. A., de Oliveira, R. B., da Silva, V. C., Gobbo-Neto, L., Gasparoto, T. H., Campanelli, A. P., Faccioli, L. H., & Da Costa, F. B. (2011). Chlorogenic acids from *Tithonia diversifolia* demonstrate a better anti-inflammatory effect than indomethacin and its sesquiterpene lactones. *Journal of Ethnopharmacology*, 136, 355-362.

Chahal, K. K., Kumari, M., Joia, B. S., & Chhabra, B. R. (2007). Chemistry and potential of lemongrass oil as stored grain protectant. *PesticideResearch Journal*, 19, 141-144.

Chakotiya, A. S., Tanwar, A., Narula, A., & Sharma, R. K. (2017). *Zingiber officinale*: Its antibacterial activity on *Pseudomonas aeruginosa* and mode of action evaluated by flow cytometry. *Microbial pathogenesis*, 107, 254-260.

Chakravarti, K. K., & Bhattacharyya, S. C. (1954). Chemical examination of the oil of Malabar lemon-grass oil. *I. Perfumery and Essential Oil Record*, 45, 184-5.

Chandarana, H., Baluja, S., & CHANDA, S. (2005). Comparison of antibacterial activities of selected species of Zingiberaceae family and some synthetic compounds. *Turkish Journal of Biology*, 29, 83-97.

Chang-jiang, Y. U., Xiao-ping, S. O. N. G., Xin, Z. H. A. N. G., Chang-ri, H. A. N., & Lin-hua, Z. H. U. 2013. Chemical components of essential oil in peel from Hainan Litsea cubeba. *Guangzhou Huagong*, 4, 102-104.

Chang-jiang, Y. U., Xiao-ping, S. O. N. G., Xin, Z. H. A. N. G., Chang-ri, H. A. N., & Lin-hua, Z. H. U. (2014). Chemical components and antitumor activity of essential oil *of Litsea cubeba* seed from Hainan. natural product research and development, 26, 1849.

Chen, C. J., Tseng, Y. H., Chu, F. H., Wen, T. Y., Cheng, W. W., Chen, Y. T., Tsao, N. W., & Wang, S. Y. (2012). Neuropharmacological activities of fruit essential oil from *Litsea cubeba* Persoon. *Journal of wood science*, 58, 538-543.

Chen, H. C., Chang, W. T., Hseu, Y. C., Chen, H. Y., Chuang, C. H., Lin, C. C., Lee, M. S., & Lin, M. K. (2016). Immunosuppressive effect of *Litsea cubeba* L. essential oil on dendritic cell and contact hypersensitivity responses. International journal of molecular sciences, 17, 1319.

Chen, Y., Wang, Y., Gao, M., & Wu, L. (2018). *Litsea cubeba* fruit essential oil, preparation method and application thereof. *Faming Zhuanli Shenqing*, CN 108,

Chen, Y., Zhou, X., Li, S., & Cai, T. (2001). Antimicrobial activity of ginger oleoresins. *Shipin Yu Fajiao Gongye*, 27, 30-34.

Chen, Z., Liu, X., Bi, H., & Xiao, B. (2012). Determination of total flavonoid content in the stems and leaves of *Litsea cubeba*. *Hainan Shifan Daxue Xuebao, Ziran Kexueban*, 25, 409-411.

Cheng, H., Chen, J., Watkins, P. J., Chen, S., Wu, D., Liu, D., & Ye, X. (2018). Discrimination of aroma characteristics for cubeb berries by sensomics approach with chemometrics. *Molecules*, 23, 1627.

Cheng, M. C., & Cheng, Y. S. (1983). Compositions of the essential oils of *Litsea Klosterman* in Chang, *Litsea garciae* Vidal and *Litsea cubeba* (Lour.) Persoon. *Journal of the Chinese Chemical Society*, 30, 59-62.

Choi, E. M., & Hwang, J. K. (2004). Effects of methanolic extract and fractions from *Litsea cubeba* bark on the production of inflammatory mediators in RAW264. 7 cells. *Fitoterapia*, 75, 141-148.

Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1956). Glossary of Indian Medicinal Plants Council of Scientific and Industrial Research. *New Delhi*, 89.

Choudhury, S. N., Deka, D. K., Baruah, A. K. S., Rao, P. G., Dube, M. F., & Riggins, A. (2009). Constituents of essential oil of *Litsea cubeba* from North East. *Indian germplasm. Indian Perfumer*, 53, 38-43.

Choudhury, S., Ahmed, R., Barthel, A., & Leclercq, P. A. (1998). Composition of the stem, flower and fruit oils of *Litsea cubeba* Pers. from two locations of Assam, India. *Journal of Essential Oil Research*, 10, 381-386.

Chowdhury, S. A., Sohrab, M. H., Datta, B. K., & Hasan, C. M. (2008). Chemical and antioxidant studies of *Citrus macroptera*. *Bangladesh Journal of Scientific and Industrial Research*, 43, 449-454.

Chukwuocha, U. M., Fernández-Rivera, O., & Legorreta-Herrera, M. (2016). Exploring the antimalarial potential of whole *Cymbopogon citratus* plant therapy. *Journal of ethnopharmacology*, 193, 517-523.

Chungsamarnyart, N., & Jiwajinda, S. (1992). Acaricidal activity of volatile oil from lemon and citronella grasses on tropical cattle ticks. *Kasetsart Journal*, 26, 46-51.

Corrêa-Ferreira, M. L., Noleto, G. R., & Petkowicz, C. L. O. (2014). *Artemisia absinthium* and *Artemisia vulgaris*: A comparative study of infusion polysaccharides. *Carbohydrate polymers*, 102, 738-745.

Coutinho, H.D.M., Freitas, M.A., Gondim, C.N.F.L., Albuquerque, R. S., Ferreira, J.V.A., & J.C. Andrade (2015). In vitro antimicrobial activity of Geraniol and Cariophyllene against *Staphylococcus aureus*. *Revista Cubana de Plantas Medicinales*, 20, 98–105.

Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., & Trombetta, D. (2007). Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. *Journal of agricultural and food chemistry*, 55, 6300-6308.

da Gama, R. M., Guimarães, M., de Abreu, L. C., & Armando-Junior, J. (2014). Phytochemical screening and antioxidant activity of ethanol extract of *Tithonia diversifolia* (Hemsl) A. Gray dry flowers. *Asian Pacific Journal of Tropical Biomedicine*, 4, 740-742.

da Silva, F. T., da Cunha, K. F., Fonseca, L. M., Antunes, M. D., El Halal, S. L. M., Fiorentini, Â. M., ... & Dias, A. R. G. (2018). The action of ginger essential oil (*Zingiber officinale*) encapsulated in proteins ultrafine fibres on the antimicrobial control in situ. *International journal of biological macromolecules*, 118, 107-115.

Dalimunthe, A. M. I. N. A. H., Zaitun Hasibuan, P. A., Silalahi, J., & Satria, D. (2018). Antioxidant activity of alkaloid fractions of *Litsea cubeba* lour. Fruits. *Asian Journal of Pharmaceutical and Clinical Research*, 11, 31.

Dalimunthe, A., Achmad, S., & Satria, D. (2016). Phenolic, flavonoid content and antioxidant activities of ethylacetate extract of *Litsea cubeba* (lour.) pers. barks. *Der Pharma Chemica*, 8, 466-8.

Dalimunthe, A., Hasibuan, P. A. Z., Silalahi, J., Sinaga, S. F., & Satria, D. (2018). Antioxidant activity of alkaloid compounds from *Litsea cubeba* Lour. *Oriental Journal of Chemistry*, 34,1149-1152.

Dangkong, D., & Limpanasithikul, W. (2015). Effect of citral on the cytotoxicity of doxorubicin in human B-lymphoma cells. *Pharmaceutical biology*, 53, 262-268.

Daniewski, W., Strojny, T. (1962). Product of lemon-grass oil hydrogenation. *Przemysl Chemiczny*, 41, 43-4.

De Silva, B. C. J., Jung, W. G., Hossain, S., Wimalasena, S. H. M. P., Pathirana, H. N. K. S., & Heo, G. J. (2017). Antimicrobial property of lemongrass (*Cymbopogon citratus*) oil against pathogenic bacteria isolated from pet turtles. *Laboratory animal research*, 33, 84-91.

De Silva, M. G. (1959). Lemongrass oil from Ceylon. *Manufacturing Chemist*, 30, 415-16.

De Toledo, J. S., Ambrósio, S. R., Borges, C. H., Manfrim, V., Cerri, D. G., Cruz, A. K., & Da Costa, F. B. (2014). In vitro leishmanicidal activities of sesquiterpene lactones from *Tithonia diversifolia* against *Leishmania braziliensis* promastigotes and amastigotes. *Molecules*, 19, 6070-6079.

Dhiman, A. (2017). Comparison of antimicrobial, larvicidal and anthelmintic activity of *Zingiber officinale* Rose. cow urine extract. *International Journal of Green Pharmacy (IJGP)*, 11(02).

Dong, H. B., Yang, M. Y., Jiang, J. Z., & Wang, M. A. (2013). Total synthesis of 3, 7-dimethyl-7-hydroxy-2-octen-1, 6-olide and 3, 7-dimethyl-2, 6-octadien-1, 6-olide. *Journal of Asian natural products research*, 15, 880-884.

Douglas, K., & Jeruto, J. (2016). Phytochemistry and antimicrobial activity of extracts from medicinal plants *Tithonia diversifolia* and *Olea africana*. *Journal of Pharmaceutical Research International*, 12, 1-7.

Drake, D., & Lam, J. (1974). Polyacetylenes of *Artemisia vulgaris*. *Phytochemistry*, 13, 455-457.

Dreyer, D. L., & Huey, P., (1973). Chemotaxonomy of the Rutaceae. IX. Coumarins of *Citrus macroptera*. *Phytochemistry*,12, 3011-13.

Duarte, M. C. T., Figueira, G. M., Sartoratto, A., Rehder, V. L. G., & Delarmelina, C. (2005). Anti-Candida activity of Brazilian medicinal plants. *Journal of ethnopharmacology*, 97, 305-311.

Dudai, N., Weinstein, Y., Krup, M., Rabinski, T., & Ofir, R. (2005). Citral is a new inducer of caspase-3 in tumor cell lines. *Planta medica*, 71, 484-488.

Eggimann, P., Garbino, J., & Pittet, D. (2003). Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. *The Lancet infectious diseases*, 3, 685-702.

Eissa, H. A., Abd-Elfattah, S. M., & Abu-Seif, F. A. (2008). Anti-microbial, anti-browning and anti-mycotoxigenic activities of some essential oil extracts in apple juice. *Polish journal of food and nutrition sciences*, 58(4).

Ejelonu, O. C., Elekofehinti, O. O., & Adanlawo, I. G. (2017). *Tithonia diversifolia* saponin-blood lipid interaction and its influence on the immune system of normal Wistar rats. *Biomedicine & Pharmacotherapy*, 87, 589-595.

Elufioye, T. O., & Agbedahunsi, J. M. (2004). Antimalarial activities of *Tithonia diversifolia* (Asteraceae) and *Crossopteryx febrifuga* (Rubiaceae) on mice in vivo. *Journal of ethnopharmacology*, 93, 167-171.

Elufioye, T. O., Agbedahunsi, J. M., & Adesanya, S. A. (2004). 8-(2-methylbutanoyl)-3, 10 epoxy-3, 8-dihydroxyl-4, 11 (13)-germacradien-12, 6-olide a new sesquiterpene lactone from *Tithonia diversifolia*. *Nigerian Journal of Natural Products and Medicine*, 8, 74-76.

Endo, K., Kanno, E., & Oshima, Y. (1990). Structures of antifungal diarylheptenones, gingerenones A, B, C and isogingerenone B, isolated from the rhizomes of *Zingiber officinale*. *Phytochemistry*, 29(3), 797-799.

Essiett, U., & Akpan, E. (2013). Proximate composition and phytochemical constituents of *Aspilia africana* (Pers) CD Adams and *Tithonia diversifolia* (Hemsl) a. grey stems (Asteraceae). *Indian Journal of Pharmaceutical and Biological Research*, 1, 23-30.

Fan, L., Wang, Z.G., Xing, C. P., & Wang, Y. M. (2002). *Synthesis of β-ionone. Jingxi Huagong*, 19, 133-136.

Fang, F., Zhaoping, L. V., Wang, Z., Huang, Y., & Hongbin, L. I. (1994). Antifungal Susceptibility of Candida spp. to *Litsea cubeba* Oil: a Broth Microdilution Tests and Electron Microscopy. *Chinese Journal of Dermatology*. 35, 349-351.

Farag, R.S., Daw, Z.Y., & Abo-Raya, S. H. (1989). Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *J Food Sci*. 54, 74–76.

Fillipe de.O. P., Juliana, M. M., Igara, O. L, Kelly, S. D. L. M., Wylly, A. D. O., & Edeltrudes de, O. L. (2014). Antifungal activity of geraniol and citronellol, two monoterpenes alcohols, against *Trichophytonrubrum* involves inhibition of ergosterol biosynthesis. *Pharmaceutical biology* 53, 228–234.

Friedman, M., Henika, P. R., & Mandrell, R. E. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes,* and *Salmonella enterica*. *Journal of food protection*, 65, 1545-1560.

Friedman, M., Henika, P., Levin, C., & Mandrell, R. E. (2002). Bactericidal activities of plant essential oils and their components against *Escherichia coli* O157: H7 and *Salmonella enterica* in apple juice. Abstracts of Papers, 224th ACS National Meeting, Boston, MA, United States, August 18-22, AGFD-165.

Fujita, Y. (1951). Genesis of herbs and their essential oils. *Koryo*, 16, 27-30.

Gaillard, E., Muyard, F., Bevalot, F., Regnier, A., & Vaquette, J. (1995). Rutaceae from New Caledonia. Chemical composition of stem bark of *Citrus macroptera* Montr. *AnnalesPharmaceutiquesFrancaises*, 53, 75-8.

Gayathri, K., Jayachandran, K. S., Vasanthi, H. R., & Rajamanickam, G. V. (2011). Cardioprotective effect of lemongrass as evidenced by biochemical and histopathological changes in experimentally induced cardiotoxicity. *Human & experimental toxicology*, 30, 1073-1082.

Gbolade, A. A., Biondi, D. M., & Ruberto, G. (2008). Comparative analysis of the essential oils from two Asteraceous plants found in Nigeria, *Acanthospermum hispidum* and *Tithonia diversifolia*. *Natural Product Communications*, 3, 1735-1738.

Ghasemzadeh, A., Jaafar, H. Z., & Rahmat, A. (2016). Changes in antioxidant and antibacterial activities as well as phytochemical constituents associated with ginger storage and polyphenol oxidase activity. *BMC complementary and alternative medicine*, 16, 1-11.

Ghasemzadeh, A., Jaafar, H. Z., Baghdadi, A., & Tayebi-Meigooni, A. (2018). Formation of 6-, 8-and 10-shogaol in ginger through the application of different drying methods: altered antioxidant and antimicrobial activity. *Molecules*, 23, 1646.

Goffin, E., Ziemons, E., De Mol, P., de Madureira, M. D. C., Martins, A. P., da Cunha, A. P., Philippe, G., Tits, M., Angenot, L., & Frederich, M. (2002). In vitro antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent: tagitinin *C. Planta medica*, 68, 543-545.

Gogoi, P., Baruah, P., & Nath, S. C. (1997). Antifungal Activity of the Essential Oil of *Litsea cubeba* Pers. *Journal of Essential Oil Research*, 9, 213-215.

Gogoi, R., Loying, R., Sarma, N., Munda, S., Pandey, S. K., & Lal, M. (2018). A comparative study on antioxidant, anti-inflammatory, genotoxicity, anti-microbial activities and chemical composition of fruit and leaf essential oils of *Litsea cubeba* Pers from North-east India. *Industrial Crops and Products*, 125, 131-139.

Govindaraj, S., & RanjithaKumari, B. D. (2013). Composition and larvicidal activity of *Artemisia vulgaris* L. stem essential oil against Aedes aegypti. *Jordan Journal of Biological Sciences*, 6, 11-16.

Govindaraj, S., Kumari, B. D. R., Cioni, P. L., & Flamini, G. (2008). Mass propagation and essential oil analysis of *Artemisia vulgaris*. *Journal of bioscience and bioengineering*, 10, 176-183.

Govindarajan, V.S. (1982a). Ginger-Chemistry, Technology and Quality Evaluation: Part I-CRC Critical Reviews in Food Science and Nutrition. Quensland, 17, 1-98.

Govindarajan, V.S. (1982b). Ginger-Chemistry, Technology and Quality Evaluation: Part 2-CRC Critical Reviews in Food Science and Nutrition. *Quensland*, 17, 189-258.

Gu, J. Q., Gills, J. J., Park, E. J., Mata-Greenwood, E., Hawthorne, M. E., Axelrod, F., Chavez, P. I., Fong, H. S.; Mehta, R. G., & Pezzuto, J. M. (2002). Sesquiterpenoids from *Tithonia diversifolia* with potential cancer chemopreventive activity. *Journal of natural products*, 65, 532-536.

Guo, D., Li, Z., Wang, P., Chen, S., Fan, G., & Wang, Z. (2016). Inhibition effect of active components from *Litsea cubeba* (Lour.) Pers. oil on wood fungi. *Chemistry and Industry of Forest Products*, 36, 88-94.

Guo, Q., Bai, R. F., Su, G. Z., Zhu, Z. X., Zeng, K. W., Tu, P. F., & Chai, X. Y. (2016). Chemical constituents from the roots and stems of *Litsea cubeba*. *Journal of Asian natural products research*, 18, 51-58.

Guo, T., Tan, S. B., Wang, Y., & Chang, J. (2018). Two new monoterpenoid glycosides from the fresh rhizome of Tongling White Ginger (*Zingiber officinale*). *Natural product research*, 32, 71-76.

Gupta, A. K., & Cooper, E. A. (2008). Update in antifungal therapy of dermatophytosis. *Mycopathologia*, 166,353-367.

Gupta, S., & Ravishankar, S. (2005). A comparison of the antimicrobial activity of garlic, ginger, carrot, and turmeric pastes against *Escherichia coli* O157: H7 in laboratory buffer and ground beef. *Food bourne Pathogens & Disease*, 2, 330-340.

Haider, F., Dwivedi, P. D., Naqvi, A. A., & Bagchi, G. D. (2003). The essential oil composition of *Artemisia vulgaris* harvested at different growth periods under Indogangetic Plain conditions. *Journal of Essential Oil Research*, 15, 376-378.

Halabi, M. F., & Sheikh, B. Y. (2014). Anti-proliferative effect and phytochemical analysis of *Cymbopogon citratus* extract. *BioMed research international*, 2014906239

Hamad, A., Alifah, A., Permadi, A., & Hartanti, D. (2016). Chemical constituents and antibacterial activities of crudeextract and essential oils of *Alpinia galanga* and *Zingiber officinale*. *International Food Research Journal*, 23(2).

Hammid, S. A., & Ahmad, F. (2015). Chemotype of *Litsea cubeba* essential oil and its bioactivity. *Natural product communications*, 10, 1301-4,

Haris, P., Mary, V., & Sudarsanakumar, C. (2018). Probing the interaction of the phytochemical 6-gingerol from the spice ginger with DNA. *International journal of biological macromolecules*, 113, 124-131.

Hasan, M. M., Islam, M. S., Hoque, K. M. F., Haque, A., & Reza, M. A. (2019). Effect of *Citrus macroptera* fruit pulp juice on alteration of caspase pathway rendering anti-proliferative activity against Ehrlich's ascites carcinoma in mice. *Toxicological research*, 35, 271-277.

Hassan, A. M., Abutalib, A. A., Almagboul, A. Z., & Kabbashi, A. S. (2017). Antimicrobial activity of the rhizome essential oil of *Zingiber officinale* Roscoe. *Adv Med Plant Res*, 5, 5-10.

Hata, T. (1939). Seed oils of Formosan plants. XVI. The seed oil of Litsea cubeba Pers. *Nippon Kagaku Kaishi*, 60, 122-5.

Hazarika, T. K., Lalchhanmawia. J., Chhangte, L., Chhangte, L., Shukla, A. C., & Nautiyal, B. P. (2017). Assessment of genetic variability in the endangered *Citrus macroptera* Mont. ("hatkora") from Mizoram, north-east India. *Genetic Resources and Crop Evolution*, 64, 321-330.

He, j. m., Xiao, y. h., Pan, c. x., & Zhang, z. m. (2012). The analysis of fruit and volatile oil traits on *Litsea cubeba* (lour) Pers. in Shaoguao. *Journal of Inner Mongolia Agricultural University* (Natural Science Edition), 33, 30-35.

Heikal, H. M. (2015). Studies on the Occurrence, Identification and Control of House Dust Mites at Rural Houses of Shebin El-Kom Locality, *Egypt. Pakistan journal of biological sciences: PJBS*, 18, 179-184.

Hirschwehr, R., Heppner, C., Spitzauer, S., Sperr, W. R., Valent, P., Bergerd, U., Horak, F., Jager, S., Kraft, D., & Valenta, R. (1998). Identification of common allergenic structures in mugwort and ragweed pollen. *Journal of allergy and clinical immunology*, 101, 196-206.

Ho, C. L., Jie-Ping, O., Liu, Y. C., Hung, C. P., Tsai, M. C., Liao, P. C., Wang, E. I., Chen, Y. L., & Su, Y. C. (2010). Compositions and in vitro anticancer activities of the leaf and fruit oils of *Litsea cubeba* from Taiwan. *Natural Product Communications*, 5, 1934578X1000500425.

Hsu, W.S., Yen, J, H., & Wang, Y. S. (2013). Formulas of components of citronella oil against mosquitoes (*Aedes aegypti*). *Journal of environmental science and health*. Part. B, Pesticides, food contaminants, and agricultural wastes,48, 1014-9

Hu, L., Wang, Y., Du, M., & Zhang, J. (2011). Characterization of the volatiles and active components in ethanol extracts of fruits of *Litsea cubeba* (Lour.) by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O). *Journal of Medicinal Plants Research*, 5, 3298-3303.

Hu, W., Li, C., Dai, J., Cui, H., & Lin, L. (2019). Antibacterial activity and mechanism of *Litsea cubeba* essential oil against methicillin-resistant *Staphylococcus aureus* (MRSA). *Industrial Crops and Products*, 130, 34-41.

Huang, C. H., Huang, W. J., Wang, S. J., Wu, P. H., & Wu, W. B. (2008). Litebamine, a phenanthrene alkaloid from the wood of *Litsea cubeba*, inhibits rat smooth muscle cell adhesion and migration on collagen. *European journal of pharmacology*, 596, 25-31.

Huargliang, Y., Su, M., & Chen, P. (1994). The analysis of components of essential oil from fruit of *Litsea cubeba* and the study of antifungal activity/decay-free activity. *Tianran Chanwu Yanjiu Yu Kaifa*, 6, 1-5.

Hube, B., Hay, R., Brasch, J., Veraldi, S., & Schaller, M. (2015). Dermatomycoses and inflammation: The adaptive balance between growth, damage, and survival. *Journal de mycologie medicale*, 25, 44-58.

Hwang, J. K., Choi, E. M., & Lee, J. H. (2005). Antioxidant activity of *Litsea cubeba***.** *Fitoterapia*, 76, 684-6.

Hwang, Y. S., Wu, K. H., Kumamoto, J., Axelrod, H., & Mulla, M. S. (1985). Isolation and identification of mosquito repellents in *Artemisia vulgaris*. *Journal of Chemical Ecology*, 11, 1297-1306.

Ikegawa, T., Ikegawa, N., Okuma, A., (1990). Anticancer agents containing tagitinin C. *Jpn. Kokai Tokkyo Koho, JP* 022.

Imayoshi, R., Okamoto, T., Nagao, J., Cho, T., Matsunaga, K., Akaike, T., & Kaminishi, H. 2011. Experimental study of citral on the preventive effect of influenza virus infection. *Igaku to Seibutsugaku*, 155, 489-494.

Inouye, S., Takizawa, T., & Yamaguchi, H. (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *Journal of antimicrobial chemotherapy*, 47, 565-573.

Irkin, Reyhan; Korukluoglu, Mihriban. 2009. Effectiveness of *Cymbopogon citratus* L. essential oil to inhibit the growth of some filamentous fungi and yeasts. *Journal of Medicinal Food*, 12, 193-197

Jacob, J., Peter, G., Thomas, S., Haponiuk, J. T., & Gopi, S. (2019). Chitosan and polyvinyl alcohol nanocomposites with cellulose nanofibers from ginger rhizomes and its antimicrobial activities. *International journal of biological macromolecules*, 129, 370-376.

Jagetia, G. C., Baliga, M. S., Venkatesh, P., & Ulloor, J. N. (2003). Influence of ginger rhizome (*Zingiber officinale* Rosc) on survival, glutathione and lipid peroxidation in mice after whole-body exposure to gamma radiation. *Radiation research*, 160, 584-592.

Jain, N., & Sharma, M. (2017). Phytochemical screening and anti-dermatophytic activity of *Cymbopogon citratus* leave essential oil and their fractions. *Journal of Essential Oil Bearing Plants*, 20, 1107-1116.

Jain, S. K., & Prakash, V. (1995). Zingiberaceae in India: phytogeography and endemism. *Rheedea*, 5, 154-169.

Jayanthi, A. (2010). Evaluation of antimicrobial activity of herbal extracts against Salmonella. *Journal of Pharmacy Research*, 3, 1981-1983.

Jeon, Y. J., & Lee, H. S. (2016). Chemical composition and acaricidal activities of essential oils of *Litsea cubeba* fruits and *Mentha arvensis* leave against house dust and stored food mites. *Journal of Essential Oil Bearing Plants*, 19, 1721-1728.

Ji, W. D., Jeong, M. S., Chung, H. C., Lee, S. J., & Chung, Y. G. (1997). Antimicrobial activity and distilled components of garlic (*Allium sativum* L.) and ginger (*Zingiber officinale* Roscoe). *Applied Biological Chemistry*, 40, 514-518.

Jian, Z., Chen, S., Liu, S., Zhang, J., Yang, Y., & Hu, H. (1986). Chemical constituents of the essential oil from the root of *Litsea cubeba* (Lour.) pers. *Huazhong Shifan Daxue Xuebao, Ziran Kexueban*, 20, 169-73.

Joshua, A. A., Usunomena, U., Lanre, A. B., Amenze, O., & Gabriel, O. A. (2012). Comparative studies on the chemical composition and antimicrobial activities of the ethanolic extracts of lemongrass leaves and stems. *Asian Journal of medical sciences*, 4, 145-148.

Judžentienė, A., & Buzelytė, J. (2006). Chemical composition of essential oils of *Artemisia vulgaris* L.(mugwort) from North Lithuania. *Chemija*, 17, 12-15.

Jyotsna, D., Neelam, A., & Viveka, N. (2017). A Review on *Zingiber officinale*. *Journal of pharmacognosy and phytochemistry*, 6: 174-184.

Kačániová, M., Terentjeva, M., Mňahončáková, E., Ivanišová, E., Kunová, S., Rovná, K., Žiarovská, J., Borotová, P., Válková, V., & Galovičová, L. (2020). Medicinal Plants as Antimicrobial Agents Against Pathogenic Bacteria. Scientific Papers: *Animal Science & Biotechnologies/Lucrari Stiintifice: Zootehnie si Biotehnologii*, 53, 181-187.

Kahuku, K., & Kato, R. (1938). Essential oil of the fruits of *Litsea cubeba*, Persoon. I. *Nippon Kagaku Kaishi*, 59, 1096-8.

Kamboj, V. P. (2000). Herbal medicine. *Current science*, 78, 35-39.

Kanatt, S. R., Chawla, S. P., & Sharma, A. (2014). Antioxidant and radio-protective activities of lemongrass and star anise extracts. *Food bioscience*, 6, 24-30.

Kareru, P. G., Keriko, J. M., Kenji, G. M., Thiong'o, G. T., Gachanja, A. N., & Mukiira, H. N. (2010). Antimicrobial activities of skincare preparations from plant extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 7(3).

Katewa, S. S., Chaudhary, B. L., & Jain, A. (2004). Folk herbal medicines from tribal area of Rajasthan, India. *Journal of ethnopharmacology*, 92, 41-46.

Kato, R. (1951). Essential oil of *Litsea cubeba*. II. Constituents of the volatile oil from the leaf of *Litsea cubeba*. *Kogyo Kagaku Zasshi*, 54, 465-6.

Kato, R. (1951). The essential oil of Litsea cubeba. III. The constituents of the volatile oil from the trunk of *Litsea cubeba*. *Kogyo Kagaku Zasshi*, 54, 517-19.

Khaliq, S., Haider, S., Siddiqui, M., Khan Sherwani, S., Sajid, I., Mustafa, S., & Ejaz, T. (2017). Enhancement in spatial and recognition memory functions following long term oral administration of ginger extract in rats. *Pakistan journal of pharmaceutical sciences*, 30(6).

Kimutai, A., Ngeiywa, M., Mulaa, M., Njagi, P. G., Ingonga, J., Nyamwamu, L. B., Ombati, C., & Ngumbi, P. (2017). Repellent effects of the essential oils of *Cymbopogon citratus* and *Tagetes minuta* on the sandfly, *Phlebotomus duboscqi*. *BMC research notes*, 10, 1-9.

Ko, K., Juntarajumnong, W., & Chandrapatya, A. (2009). Repellency, fumigant and contact toxicities of *Litsea cubeba* (Lour.) Persoon against *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). *Agriculture and Natural Resources*, 43, 56-63.

Kokate, C. K., & Varma, K. C. (1971). Antimicrobial activity of volatile oils of *Cymbopogon nardus* and *Cymbopogon citrates*. *Science and Culture*, 37, 196-8.

Krishnaveni, G., Rekha, C. B., Rajendran, P., Nithyakalyani, V., & Vithiyavani, P. (2016). Antidermatophytic Potential of Selected Medicinal Plants against *Microsporum* Species. *International Journal of Current Research in Biosciences and Plant Biology*, 3, 102-106.

Kumar, V., Tewari, R., & Singh, K. (2015). Comparative studies of drying methods on yield and composition of the essential oil of *Cymbopogon citratus*. *Journal of Essential Oil Bearing Plants*, 18, 744-750.

Kumari, P., Kumari, N., Singh, K. P., & Prasad, K. (2018). *Cymbopogon flexuosus* Leaves Mediated Synthesis of ZnO Nanoparticles: Cytotoxicity Assay Against THP-1 Human Leukemia Cell Line. *Journal of Bionanoscience*, 12, 683-688.

Kuo, Y. H., & Chen, C. H. (1998). Sesquiterpenes from the leaves of *Tithonia diversifolia*. *Journal of natural products*, 61, 827-828.

Kuo, Y. H., & Lin, B. Y. (1999). A new dinorxanthane and chromone from the root of *Tithonia diversifolia*. *Chemical and pharmaceutical bulletin*, 47, 428-429.

Kuroda, M., Yokosuka, A., Kobayashi, R., Jitsuno, M., Kando, H., Nosaka, K., Ishii, H., Yamori, T., & Mimaki, Y. (2007). Sesquiterpenoids and flavonoids from the aerial parts of *Tithonia diversifolia* and their cytotoxic activity. *Chemical and Pharmaceutical Bulletin*, 55, 1240-1244.

Kyle, A. A., & Dahl, M. V. (2004). Topical therapy for fungal infections. *American journal of clinical dermatology*, 5, 443-451.

Lamaty, G., Menut, C., Zollo, P. H. A., Kuiate, J. R., Bessière, J. M., & Koudou, J. (1991). Aromatic plants of tropical Central Africa. III. Constituents of the essential oil of the leaves of *Tithonia diversifolia* (Hemsl.) A. Gray from Cameroon. *Journal of essential oil research*, 3, 399-402.

Lean, L. P., & Mohamed, S. (1999). Antioxidative and antimycotic effects of turmeric, lemongrass, betel leaves, clove, black pepper leaves and *Garcinia atriviridis* on butter cakes. *Journal of the Science of Food and Agriculture*, 79, 1817-1822.

Lee, M. Y., Liao, M. H., Tsai, Y. N., Chiu, K. H., & Wen, H. C. (2011). Identification and anti-human glioblastoma activity of tagitinin C from *Tithonia diversifolia* methanolic extract. *Journal of agricultural and food chemistry*, 59, 2347-2355.

Li, B., & Li, Y. (1998). Quantitative Analysis of Citral in *Litsea cubeba* Oil by Gas Chromatography. Chemical world-shanghai-, 39, 99-100.

Li, F., Zhang, M., Holness, H., & O'Shea, K. (2013). Effects of different solvent extract and fractions from *Litsea cubeba* on antioxidant activity and its GC-MS analysis. *Xuzhou Gongcheng Xueyuan Xuebao, Ziran Kexueban*, 28, 28-32.

Li, H., Wang, Q., Zhang, J., & Shi, Q. (1997). Study on preparation of alkanol amide from *Litsea cubeba* Kernel waste oil. *Shanghai Daxue Xuebao, Ziran Kexueban*, 3, 676-681.

Li, J. M., Che, C. T., Lau, C. B., Leung, P. S., & Cheng, C. H. (2006). Inhibition of intestinal and renal Na+-glucose cotransporter by naringenin. The *international journal of biochemistry & cell biology*, 38, 985-995.

Li, W. R., Shi, Q. S., Liang, Q., Xie, X. B., Huang, X. M., & Chen, Y. B. (2014). Antibacterial activity and kinetics of *Litsea cubeba* oil on *Escherichia coli*. *PLoS One*, 9(11), e110983.

Li, W. R., Shi, Q. S., Mo, C. Y., Ouyang, Y., Chen, Y., & Duan, S. (2013). Chemical composition and antimicrobial activity of several typical essential oils. *Microbiology China*, 40, 2128-2137.

LI, X. J., & LIU, Y. M. (2015). Study on antioxidant activity of lemongrass essential oil by stage extraction. *Shipin Gongye Keji*, 36, 237-241.

Li, X., Huang, G., Zhao, G., Chen, W., Li, J., & Sun, L. (2013). Two new monoterpenes from *Tithonia diversifolia* and their anti-hyperglycemic activity. *Records of Natural Products*, 7, 351.

Li, X., Shen, Y., Fan, Z., Huang, Q., Cheng, H., & Liu, L. (2013). Chemical constituents and allelopathy of *Tithonia diversifolia* volatile oil. Guangxi Zhiwu/Guihaia, 33, 878-882.

Li, X., Xia, H., Wang, L., Xia, G., Qu, Y., Shang, X., & Lin, S. (2019). Lignans from the Twigs of *Litsea cubeba* and their Bioactivities. *Molecules*, 24, 306.

Liao, M. H., Lin, W. C., Wen, H. C., & Pu, H. F. (2011). *Tithonia diversifolia* and its main active component tagitinin C induce survivin inhibition and G2/M arrest in human malignant glioblastoma cells. *Fitoterapia*, 82(3), 331-341.

Liao, M. H., Tsai, Y. N., Yang, C. Y., Juang, C. L., Lee, M. Y., Chang, L. H., & Wen, H. C. (2013). Anti-human hepatoma Hep-G2 proliferative, apoptotic, and antimutagenic activity of tagitinin C from *Tithonia diversifolia* leaves. *Journal of natural medicines*, 67(1), 98-106.

Lin, B., Sun, L. N., Xin, H. L., Nian, H., Song, H. T., Jiang, Y. P., Wei, Z. Q., Qin, L,P., & Han, T. (2016). Anti-inflammatory constituents from the root of *Litsea cubeba* in LPS-induced RAW 264.7 *macrophages. Pharmaceutical biology*, 54, 1741-1747.

Lin, H. R. (2012). Sesquiterpene lactones from *Tithonia diversifolia* act as peroxisome proliferator-activated receptor agonists. *Bioorganic & medicinal chemistry letters*, 22(8), 2954-2958.

Lin, Z., & Hua, Y. (1987). Chemical constituents of 14 essential oils from Lauraceae growing in Yibin area, Sichuan Province. *Chemistry & Industry of Forest Products*, 7, 46-64

Lin, Z., & Zhang, Y. (2017). Application of lauraceae plant extract as detergent. *Faming Zhuanli Shenqing*, CN 106.

Liu, H., Peng, B., & Wang, W. (2015). A method for extraction of *Litsea cubeba* oil and citral from *Litsea cubeba* fruit. *Faming Zhuanli Shenqing*, CN 104450197 A 20150325.

Liu, T. T., & Yang, T. S. (2012). Antimicrobial impact of the components of essential oil of *Litsea cubeba* from Taiwan and antimicrobial activity of the oil in food systems. *International journal of food microbiology*, 156, 68-75.

López, E. I. C., Balcázar, M. F. H., Mendoza, J. M. R., Ortiz, A. D. R., Melo, M. T. O., Parrales, R. S., & Delgado, T. H. (2017). Antimicrobial activity of essential oil of *Zingiber officinale* Roscoe (Zingiberaceae*). American Journal of Plant Sciences*, 8, 1511-1524.

Luo, A. R., She, J. M., Xie, X. Z., Liu, H. W., & Xiao, Y. (2012). Analysis of Essential Oil in Flower Buds of *Litsea cubeba*. *Guangzhou Chemical Industry*, 12.

Luo, M., Jiang, L. K., & Zou, G. L. (2005). Acute and genetic toxicity of essential oil extracted from *Litsea cubeba* (Lour.) Pers. *Journal of food protection*, 68, 581-588.

Luo, M., Jiang, L. K., Huang, Y. X., Xiao, M., Li, B., & Zou, G. L. (2004). Effects of citral on *Aspergillus flavus* spores by quasi-elastic light scattering and multiplex microanalysis techniques. *Acta Biochimica et Biophysica Sinica*, 36, 277-283.

Madankumar, A., Tamilarasi, S., Premkumar, T., Gopikrishnan, M., Nagabhishek, N., & Devaki, T. (2017). Geraniol attenuates 4NQO-induced tongue carcinogenesis through downregulating the activation of NF-κB in rats. *Molecular and cellular biochemistry*, 434, 7-15.

Maekawa, L. E., Rossoni, R. D., Barbosa, J. O., Jorge, A. O. C., Junqueira, J. C., & Valera, M. C. (2015). Different extracts of *Zingiber officinale* decrease *Enterococcus faecalis* infection in *Galleria mellonella*. *Brazilian dental journal*, 26, 105-109.

Majumdar, M., Biswas, S. C., Choudhury, R., Upadhyay, P., Adhikary, A., Roy, D. N., & Misra, T.K. (2019). Synthesis of Gold Nanoparticles Using *Citrus macroptera* Fruit Extract: Anti-Biofilm and Anticancer Activity". *ChemistrySelect*, 4, 5714-5723.

Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of tropical medicine*, 2019.

Marco, J. A., Sanz, J. F., & Hierro, P. D. (1991). Two eudesmane acids from *Artemisia vulgaris*. *Phytochemistry* (United Kingdom). 30, 2403-4.

Marei, G. I. K., Rasoul, M. A. A., & Abdelgaleil, S. A. (2012). Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. *Pesticide biochemistry and physiology*, 103, 56-61.

Marongiu, B., Piras, A., Porcedda, S., & Tuveri, E. (2006). Comparative analysis of the oil and supercritical CO_2 extract of *Cymbopogon citratus* Stapf. *Natural product research*, 20, 455-9.

Martin, A. C. B., Fuzer, A. M., Becceneri, A. B., da Silva, J. A., Tomasin, R., Denoyer, D., & Pouliot, N. (2017). [10]-gingerol induces apoptosis and inhibits metastatic dissemination of triple-negative breast cancer in vivo. *Oncotarget*, 8, 72260.

Melguizo-Melguizo, D., Diaz-de-Cerio, E., Quirantes-Piné, R., Švarc-Gajić, J., & Segura-Carretero, A. (2014). The potential of *Artemisia vulgaris* leaves as a source of antioxidant phenolic compounds. *Journal of functional foods*, 10, 192-200.

Menut, C., Lamaty, G., Zollo, P. H. A., Kuiate, J. R., & Bessière, J. M. (1992). Aromatic plants of tropical central Africa. IX. Chemical composition of flower essential oils of *Tithonia diversifolia* (Hemsl.) A. Gray from Cameroon. *Journal of essential oil research*, 4(6), 651-653.

Miah, M. N., Bachar, S. C., Nahar, L., Rahman, M. S., Rashid, M. A., Hadiuzzaman, S., & Sarker, S. D. (2010). Composition of the Volatiles of *Citrus macroptera* var. annamensis and Evaluation of Bioactivity. *Journal of Essential Oil-Bearing Plants*,13, 211-218.

Miranda, M. A., Varela, R. M., Torres, A., Molinillo, J. M., Gualtieri, S. C., & Macías, F. A. (2015). Phytotoxins from *Tithonia diversifolia*. *Journal of Natural Products*, 78, 1083-1092.

Miura, T., Nosaka, K., Ishii, H., & Ishida, T. (2005). Antidiabetic effect of Nitobegiku, the herb *Tithonia diversifolia*, in KK-Ay diabetic mice. *Biological and Pharmaceutical Bulletin*, 28, *2152-2154.*

Monod, M. (2008). Secreted proteases from dermatophytes. *Mycopathologia*, 166, 285.

Moronkola, D. O., Ogunwande, I. A., Walker, T. M., Setzer, W. N., & Oyewole, I. O. (2007). Identification of the main volatile compounds in the leaf and flower of *Tithonia diversifolia (Hemsl) Gray. Journal of Natural Medicines*, 61, *63-66.*

Muganga, R., Angenot, L., Tits, M., & Frederich, M. (2010). Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria. *Journal of ethnopharmacology*, 128, 52-57.

Munawar, T. M., & Rao, D. M. (2017). Phytochemical analysis and antioxidant activity of ethanolic extracts of *Tagetes erecta* and *Zingiber officinale*. *World Journal of Pharmaceutical Research*, 6(11Spec.Iss.), 478-490.

Munda, S., Pandey, S. K., Dutta, S., Baruah, J., & Lal, M. (2019). Antioxidant activity, antibacterial activity and chemical composition of essential oil of *Artemisia vulgaris* L. leave from Northeast India. *Journal ofEssential Oil Bearing Plants*, 22, 368-379.

Murata, C., Yoshizawa-Fujita, M., Rikukawa, M., & Usuki, T. (2017). extraction of essential oils of Lemon Grass using ionic liquid. *Asian Journal of Chemistry*, 29, 309.

Nafiu, M. O., Akanji, M. A., Raji, Z. A., & Abdulsalam, T. A. (2014). Phytochemical analysis and in vivo anti-malarialactivities of aqueous extracts of *Tithonia diversifolia* and *Parquetina nigrescens* leaves in mice. *Biochemistry*, 26, 63-68.

Nagoshi, C., Shiota, S., Kuroda, T., Hatano, T., Yoshida, T., Kariyama, R., & Tsuchiya, T. (2006). Synergistic effect of [10]-gingerol and aminoglycosides against vancomycin-resistant enterococci (VRE). *Biological and Pharmaceutical Bulletin*, 29, 443-447.

Nano, G. M., Bicchi, C., Frattini, C., & Gallino, M. (1976). On the composition of some oils from *Artemisia vulgaris*. *Planta medica*, 30, 211-215.

Nardoni, S., D'Ascenzi, C., Rocchigiani, G., Papini, R. A., Pistelli, L., Formato, G., Najar, B., & Mancianti, F. (2018). Stonebrood and chalkbrood in *Apis mellifera* causing fungi: in vitro sensitivity to some essential oils. *Natural product research*, 32, 385-390.

Nardoni, S., Najar, B., Fronte, B., Pistelli, L., & Mancianti, F. (2019). In vitro activity of essential oils against *Saprolegnia parasitica*. *Molecules*, 24, 1270.

Nardoni, S., Tortorano, A., Mugnaini, L., Profili, G., Pistelli, L., Giovanelli, S., Pisseri, F. Papini, R., & Mancianti, F. (2015). Susceptibility of *Microsporum canis* arthrospores to a mixture of chemically defined essential oils: a perspective for environmental decontamination. *Zeitschrift für Naturforschung* C, 70, 15-24.

Nath, S. C., Hazarika, A. K., Baruah, A., & Sarma, K. K. (1996). Essential oils of *Litsea cubeba* Pers.—an additional chemotype of potential industrial value from northeastern India. *Journal of essential oil research*, 8, 575-576.

Nayak, K., & Dutta, N. K. (1961). Role of Essential Oils and Allied Drugs in Experimental Cholera of the Rabbit. *Indian Journal of Medical Research*, 49, 51-4.

Nguyen, H. V., Chu-Ky, S., Caruso, D., Lebrun, M., Nguyen, N. T., Trinh, T. T., Meile, J. C., & Sarter, S. (2016). Antibacterial activity of *Litsea cubeba* (Lauraceae, May Chang) and its effects on the biological response of common carp *Cyprinus carpio* challenged with *Aeromonas hydrophila*, *Journal of Applied Microbiology*, 121, 341-351.

Nguyen, H. V., Meile, J. C., Lebrun, M., Caruso, D., Chu-Ky, S., & Sarter, S. (2018). *Litsea cubeba* leaf essential oil from Vietnam: chemical diversity and its impacts on antibacterial activity, *Letters in applied microbiology*, 66, 207-214.

Nissa, A., Utami, R., Sari, A. M., & Nursiwi, A. (2018). Combination effect of nisin and red ginger essential oil (*Zingiber officinale* var. rubrum) against foodborne pathogens and food spoilage microorganisms. In AIP *Conference Proceedings* (Vol. 2014, No. 1, p. 020023). AIP Publishing LLC.

Nongalleima, K., Ajungla, & T., Singh, C. B. (2017). Determination of antioxidant activity and simultaneous RP-HPLC analysis of quercetin, rutin and kaempferol in *Citrus macroptera* Montruz. *Journal of Pharmacognosy and Phytochemistry*, 6, 474-478.

Nongalleima, K., Ajungla, T., Singh, C.B. (2017). Anti-oxidant, anti-inflammatory and cytotoxic activity of *Citrus macroptera* montruz extracts. *International Journal of PharmTech Research*, 10, 200-210.

Noosidum, A., Chareonviriyaphap, T., & Chandrapatya, A. (2014). Synergistic repellent and irritant effect of combined essential oils on *Aedes aegypti* (L.) mosquitoes, *Journal of vector ecology*. 39. 298-305.

Obad, K., Ali, K. S., Lakal, A. M., Bekshokov, K. S., & Saidov, M. S. (2016). antibacterial activity of some essential oils against *Staphylococcus aureus a*nd *E. coli*. *World Journal of Pharmaceutical Research*, 5, 1774-1779.

Obafemi, C. A., Sulaimon, T. O., Akinpelu, D. A., & Olugbade, T. A. (2006). Antimicrobial activity of extracts and a germacranolide type sesquiterpene lactone from *Tithonia diversifolia* leaf extract. *African Journal of Biotechnology*, 5(12).

Oboh, G., & Elusiyan, C. A. (2004). Nutrient composition and antimicrobial activity of sorrel drinks (soborodo). *Journal of medicinal food*, 7, 340-342.

Oh, T. I., Jung, H. J., Lee, Y. M., Lee, S., Kim, G. H., Kan, S. Y., ... & Lim, J. H. (2018). Zerumbone, a tropical ginger sesquiterpene of *Zingiber officinale* roscoe, attenuates α-MSH-induced melanogenesis in B16F10 cells. *International journal of molecular sciences*, 19, 3149.

Ojo, O., Ajayi, A., & Anibijuwon, I. (2007). Antibacterial properties of extracts of some Nigerian spices. *Journal of Pure and Applied Microbiology*, 1, 119-122.

Okamoto, H., & Okada, F. (2005). Sterilizing/antimicrobial agents containing 1,2-octanediol and lemongrass extract, and their uses. *Jpn. Kokai Tokkyo Koho*, JP 2005232013 A 20050902

Okere, O. S., Sangodele, J. O., Tade, O. G., Obafemi, O. T., & Falode, J. A. (2015). Anti-diarrhoea potential and acute toxicity studies of methanolic extract of *Vernonia amygdalina* and *Cymbopogon citratus* against castor oil-induced diarrhoea model in rats. *International Journal of Biochemistry Research & Review*, 6, 46-52.

Okigbo, R. N., Anuagasi, C. L., Amadi, J. E., & Ukpabi, U. J. (2009). Potential inhibitory effects of some African tuberous plant extracts on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. *International journal of integrative biology*, 6, 91-98.

Olayinka, B. U., Raiyemo, D. A., & Etejere, E. O. (2015). Phytochemical and proximate composition of *Tithonia diversifolia* (Hemsl.) A. Gray. *Annals Food Science and Technology*, 16, 195-200.

Oliveros-Belardo, L., & Aureus, E. (1978). Essential oil from *Cymbopogon citratus* (D.C.) Stapf growing in the Philippines. *Asian Journal of Pharmacy*, 3, 14-17.

Oluwatuyi, M., Kaatz, G. W., & Gibbons, S. (2004). Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. *Phytochemistry*, 65, 3249-3254.

Omokhua, A. G., Abdalla, M. A., Van Staden, J., & McGaw, L. J. (2018). A comprehensive study of the potential phytomedicinal use and toxicity of invasive *Tithonia* species in South Africa. *BMC complementary and alternative medicine*, 18, 1-15.

Onawunmi, G. O. (1988). In vitro studies on the antibacterial activity of phenoxyethanol in combination with lemongrass oil. *Die Pharmazie*, 43,42-44.

Orsomando, G., Agostinelli, S., Bramucci, M., Cappellacci, L., Damiano, S., Lupidi, G., & Petrelli, R. (2016). Mexican sunflower (*Tithonia diversifolia*, Asteraceae) volatile oil as a selective inhibitor of *Staphylococcus aureus*, nicotinate mononucleotide adenylyltransferase (NadD). *Industrial Crops and Products*, 85, 181-189.

Owoyele, V. B., Wuraola, C. O., Soladoye, A. O., & Olaleye, S. B. (2004). Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. *Journal of ethnopharmacology*, 90, 317-321.

Oyeleke, G. O. (2009). Some nutritive elements and antinutrient compositions of three medicinal plant leaves. *International Journal of Chemistry* (Calcutta, India), 19, 147-150.

Pandit, R., Gaikwad, S., & Rai, M. (2017). Biogenic fabrication of CuNPs, Cu bioconjugates and in vitro assessment of antimicrobial and antioxidant activity. *IET nanobiotechnology*, 11, 568-575.

Park, M., Bae, J., & Lee, D. S. (2008). Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 22, 1446-1449.

Passoni, F. D., Oliveira, R. B., Chagas-Paula, D. A., Gobbo-Neto, L., & Da Costa, F. B. (2013). Repeated-dose toxicological studies of *Tithonia diversifolia* (Hemsl.) A. gray and identification of the toxic compounds. *Journal of Ethnopharmacology*, 147, 389-394.

Patel, G. A., & Schwartz, R. A. (2011). Tinea capitis: still an unsolved problem? *Mycoses*, 54 183-188.

Paul, S., Hossen, M. S., Tanvir, E. M., Islam, M. A., Afroz, R., Ahmmed, I., Saha, M., Gan, S. H., & Khalil, M. I. (2015). Antioxidant properties of *Citrus macroptera* fruit and its in vivo effects on the liver, kidney and pancreas in Wistar rats. *International Journal of Pharmacology*, 11, 899-909.

Pazinato, R., Volpato, A., Baretta, D., Stefani, L. M., Da Silva, A. S., Baldissera, M. D., Vaucher, R. A., Santos, R. C. V., Giongo, J. L., & Boligon, A. A. (2016). In vitro effect of seven essential oils on the reproduction of the cattle tick *Rhipicephalus microplus*. *Journal of advanced research*, 7, 1029-1034.

Peres, N. T. D. A., Maranhão, F. C. A., Rossi, A., & Martinez-Rossi, N. M. (2010). Dermatophytes: host-pathogen interaction and antifungal resistance. *Anais brasileiros de dermatologia*, 85, 657-667.

Phillipson, J. D., & Anderson, L. A. (1989). Ethnopharmacology and western medicine. *Journal of ethnopharmacology*, 25, 61-72

Piaru, S. P., Perumal, S., Cai, L. W., Mahmud, R., Majid, A. M. S. A., Ismail, S., & Man, C. N. (2012). Chemical composition, anti-angiogenic and cytotoxicity activities of the essential oils of *Cymbopogan citratus* (lemon grass) against colorectal and breast carcinoma cell lines. *Journal of Essential Oil Research*, 24, 453-459.

Pires, J. M., Mendes, F. R., Negri, G., Duarte-Almeida, J. M., & Carlini, E. A. (2009). Antinociceptive peripheral effect of *Achillea millefolium* L. and *Artemisia vulgaris* L.: both plants known popularly by brand names of analgesic drugs. Phytotherapy Research: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 23, 212-219.

Puatanachokchai, R., Kishida, H., Denda, A., Murata, N., Konishi, Y., Vinitketkumnuen, U., Nakae, D. (2002). Inhibitory effects of lemongrass (*Cymbopogon citratus*, Stapf) extract on the early phase ofhepatocarcinogenesis after initiation with diethylnitrosamine in male Fischer 344 rats. *Cancer letters*, 183, 9-15.

Pulido, K. D. P., Dulcey, A. J. C., & Martínez, J. H. I. (2017). New caffeic acid derivative from *Tithonia diversifolia* (Hemsl.) A. Gray butanolic extract and its antioxidant activity. *Food and Chemical Toxicology*, 109, 1079-1085.

Punyarajun, S., & Nandhasri, P. (1981). Volatile oil from *Litsea cubeba* in Thailand. Mahidol Univ. *J. Pharm. Sci*, 8, 65-71.

Rabe, T., & Van Staden, J. (1997). Antibacterial activity of South African plants used for medicinal purposes. *Journal of ethnopharmacology*, 56, 81-87.

Rabiul, I. G. M., & Abdur R. U. N. A. K. M. (2017) Isolation, characterization and industrial prospects of pectin isolated from *Citrus macroptera* and *Citrus assamensis*. *Nutrafoods*, 16, 41-47.

Rahman, H., & Eswaraiah, M.C. (2019). Study of skeletal muscle relaxant activity of ethanolic extract of *Oryza sativa* var. Joha rice and *Citrus macroptera* var. Annamensis; two indigenous medicinal plants of Assam. *Pharma Innovation*, 8, 48-53.

Rahman, M. M., Khan, F. E., Das, R., & Hossain, M. A. (2016). Antioxidant activity and total phenolic content of some indigenous fruits of Bangladesh. *International Food Research Journal*, 23, 2399-2404, 2016.

Raj, F. A. A., & Jayalakshmy, E. (2015). Effect of zinc oxide nanoparticle produce by *Zingiber officinale* against pathogenic bacterial. *Journal of Chemical and Pharmaceutical Sciences*, 8, 124-127.

Ramroop, P., & Neetoo, H. (2018). Antilisterial activity of *Cymbopogon citratus* on crabsticks. *AIMS Microbiology*, 4, 67-84.

Ranade, S. S., & Thiagarajan, P. (2015). Lemon grass. Int. J. Pharm. Sci. Rev. Res, 35, 162-167.

Ranti, I., Wahyuningsih, M. S. H., & Wirohadidjojo, Y. W. (2018). The antifibrotic effect of isolate tagitinin C from *Tithonia diversifolia* (Hemsley) A. Gray on keloid fibroblast cell. *The Pan African Medical Journal*, 30.

Rao, B. L., Sobti, S. N., Sarin, Y. K., & Kaul, B. L. (1993). CKP-25 is an additional source for citral. Newer Trends Essent. Oils Flavours, Sel. Pap. *Int. Symp*, 322-6.

Rao, V. S., Menezes, A. M., & Viana, G. S. (1990). Effect of myrcene on nociception in mice. *The Journal of pharmacy and pharmacology*, 42, 877-8.

Rawal, P., & Adhikari, R. S. (2016). Evaluation of the antifungal activity of *Zingiber officinale* against *Fusarium oxysporum* f. sp. lycopersici. *Adv. Appl. Sci. Res*, 7, 5-9.

Remya, S., Mohan, C. O., Bindu, J., Sivaraman, G. K., Venkateshwarlu, G., & Ravishankar, C. N. (2016). Effect of chitosan-based active packaging film on the keeping quality of chilled stored barracuda fish. *Journal of Food Science and* Technology, 53, 685-693.

Revati, S., Bipin, C., Chitra, P. B., & Minakshi, B. (2015). In vitro antibacterial activity of seven Indian spices against high-level gentamicin resistant strains of enterococci. *Archives of medical science*: *AMS*, 11, 863.

Riazipour, M., Tavakoli, H. R., & IMANI, F. A. (2011). Assessment of carboxylesterase activity in clinical isolates of *Candida albicans*. *Jundishapur J Microbiol*. 4:43–8.

Rovesti, P., Variati, G. L. (1960). Lemongrass extract in *Italian Somaliland*. *France et Ses Parfums*, 3, 39-44.

Rüngeler, P., Castro, V., Mora, G., Pahl, H. L., & Merfort, I. (1998). Study of three sesquiterpene lactones from *Tithonia diversifolia* on their anti-inflammatory activity using the transcription factor NF-κB and enzymes of the arachidonic acid pathway as targets. *Planta medica*, 64, 588-593.

Saikia, A. K., Chetia, D., D'Arrigo, M., Smeriglio, A., Strano, T., & Ruberto, G. (2013). Screening of fruit and leaf essential oils of *Litsea cubeba* Pers. from north-east

India–chemical composition and antimicrobial activity. *Journal of Essential Oil Research*, 25, 330-338.

Samusenko, A. L. (2008). Comparative evaluation of antioxidant properties of spice-aromatic plant essential oils. *Oxidation Communications*, 31, 275-284.

Sánchez-Mendoza, M. E., Reyes-Ramírez, A., Cruz Antonio, L., Martínez Jiménez, L., Rodríguez-Silverio, J., & Arrieta, J. (2011). Bioassay-guided isolation of an anti-ulcer compound, tagitinin C, from *Tithonia diversifolia*: role of nitric oxide, prostaglandins and sulfhydryls. *Molecules*, 16, 665-674.

Santo Grace, U., & Sankari, M. (2017). Antimicrobial activity of ethanolic extract of *Zingiber officinale* an in vitro Study. *Journal of Pharmaceutical Sciences and Research*, 9, 1417.

Saraswathi, K., Vidhya, J., Mohanapriya, L., & Arumugam, P. (2016). Green synthesis of silver nanoparticles using *Zingiber officinale* extract and evaluation of their antioxidant, antimicrobial and anti-inflammatory effect. *WJPPS*. 5: 1219–1233.

Saravanan, M., & Dhurai., B. (2017). Investigation of the effect of natural antibacterial agents on kitchen borne bacteria. *International Research Journal of Pharmacy*, 8, 119-125.

Sarda, P. D., Nagve, A. D., Salve, B. V., Prashar, K., & Warkhade, B. B. 2017. *Zingiber officinale*: phytochemical analysis activity in combination with commercial antibiotics. *International Journal of Current Research*, 9, 1-5.

Sargenti, S. R., & Lancas, F. M. (1997). Supercritical fluid extraction of *Cymbopogon citratus* (DC.) *Stapf. Chromatographia*, 46, 285-290.

Sasidharan, I., & Menon, A. N. (2010). Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (*Zingiber officinale* Roscoe). *International Journal of Current Pharmaceutical Research*, 2, 40-43.

Sepahpour, S., Selamat, J., Abdul Manap, M. Y., Khatib, A., Abdull Razis, A. F. & Khatib, A. (2018). Comparative analysis of the chemical composition, antioxidant activity and quantitative characterization of some phenolic compounds in selected herbs and spices in different solvent extraction systems. *Molecules*, 23, 402.

Shahzadi, P., Muhammad, A., Mehmood, F., & Chaudhry, M. Y. (2014). Synthesis of 3, 7-Dimethyl-2, 6-Octadienal Acetals from Citral Extracted from Lemon Grass, Cymbopogon citrates L. *J Antivir Antiretrovir* 6: 028-031.

Shaik, A., Kanhere, R. S., Cuddapah, R., Nelson, K. S., Vara, P. R., & Sibyala, S. (2014). Antifertility activity of *Artemisia vulgaris* leaves on female Wistar rats. *Chinese journal of natural medicines*, 12, 180-185.

Shaikh, M. N., Suryawanshi, Y. C., & Mokat, D. N. (2019). Volatile profiling and essential oil yield of *Cymbopogon citratus* (DC.) Stapf treated with rhizosphere fungi and some important fertilizers. *Journal of Essential Oil Bearing Plants*, 22, 477-483.

Shaker, E. S. (1999). Antioxidant activity of some spice essential oils. *In abstracts of papers of the American Chemical Society* (Vol. 218, pp. U51-U52). 1155 16TH ST, NW, Washington, DC 20036 USA: Amer Chemical Soc.

Shareef, H. K., Muhammed, H. J., Hussein, H. M., & Hameed, I. H. (2016). Antibacterial effect of ginger (*Zingiber officinale* roscoe) and bioactive chemical analysis using gas chromatography-mass spectrum. *Oriental Journal of Chemistry*, 32, 20-40.

Sharma, P. K., Singh, V., & Ali, M. (2016). Chemical composition and antimicrobial activity of fresh rhizome essential oil of *Zingiber officinale* Roscoe. *Pharmacognosy Journal*, 8, 185-190.

She, Q. H., Li, W. S., Jiang, Y. Y., Wu, Y. C., Zhou, Y. H., & Zhang, L. (2020). Chemical composition, antimicrobial activity and antioxidant activity of *Litsea cubeba* essential oils in different months. *Natural product research*, 34, 3285-3288.

Shin, J. Y., Park, S. C., Kim, K. H., Shin, D. H., Kim, S. H., & Kim, J. C. (2005). Primary dermal irritation study of lemongrass (*Cymbopogon citratus*) essential oil in rabbits. *Toxicological Research*, 21, 249-253.

Shinde, A. B., & Mulay, Y. R. (2015). Phytochemical analysis and antibacterial properties of some selected Indian medicinal plants. *International Journal of Current Microbiology and Applied Sciences*, 4, 228-235.

Shuai-wen, Y. I. N., Feng, Z. H. U., Li-ping, L. I. U., Chun-xia, C. H. E. N., Xiao-yan, G. A. O., Bing-tao, D. A. N. G., & Xu-mei, H. E. (2011). Screening Antifungal Component from *Litsea cubeba*. *Natural Product Research & Development*, 23, 734-738.

Shukla., N. K., & S., U. (2016). Antibacterial activity of carom seed/thyme (*Trachyspermum ammi*), anise (*Pimpinella anisum*) and dry ginger (*Zingiber officinal*) against *V. cholerae, V. parahaemolyticus* and *V. vulnificus.World Journal of Pharmacy and Pharmaceutical Sciences*, 5, 1621-1631.

Si, L., Chen, Y., Han, X., Zhan, Z., Tian, S., Cui, Q., & Wang, Y. (2012). Chemical composition of essential oils of *Litsea cubeba* harvested from its distribution areas in China. *Molecules*, 17, 7057-7066.

Silva, A. M., Ferreira, N. L., Oliveira, A. E., Borges, L. L., & Conceição, E. C. (2017). Comparison of ultrasound-assisted extraction and dynamic maceration over the content of tagitinin C obtained from *Tithonia diversifolia* (Hemsl.) A. gray leaves using factorial design. *Pharmacognosy magazine*, 13, 270.

Singh, B. R., Singh, V., Singh, R. K., Toppo, S., Haque, N., & Ebibeni, N. (2011). Antimicrobial effect of *Artemisia vulgaris* essential oil. *Natural Products*, 7, 5-12.

Singh, C. B., Khumukcham, N., Ajungla, T. (2017). GCMS based metabolic profiling of essential oil of *Citrus macroptera* Montruz. leaves and peel, assessment of in vitro antioxidant and anti-inflammatory activity. *International Journal of Pharmacy and Pharmaceutical Sciences*, 9, 107-114.

Singh, G., Kapoor, I. P. S., Singh, P., de Heluani, C. S., de Lampasona, M. P., & Catalan, C. A. (2008). Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. *Food and chemical toxicology*, 46, 3295-3302.

Singh, J. (2015). International conference on harmonization of technical requirements for registration of pharmaceuticals for human use. *Journal of pharmacology & pharmacotherapeutics*, 6, 185.

Singh, N. K., Singh, H., Mehta, N., & Rath, S. S. (2019). In vitro assessment of synergistic combinations of essential oils against *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae). *Experimental parasitology*, 201, 42-48.

Sinha, G. K., & Gulati, B. C. (1990). Antibacterial and antifungal study of some essential oils and some of their constituents. *Indian perfumer*, 34, 126-129.

Somparn, N., Saenthaweesuk, S., Naowaboot, J., & Thaeomor, A. (2014). Effects of *Cymbopogon citratus* Stapf water extract on rat antioxidant defence system. *Journal of the Medical Association of Thailand Chotmaihet Thangphaet*, 97, S57-63.

Son, J. Y. (2010). Antioxidant and antimicrobial activities of methanol extract from spices. *Journal of the Korean Society of Food Science and Nutrition*, 39, 648-654.

Śpiewak, R., & Szostak, W. (2000). Zoophilic and geophilic dermatophytoses among farmers and non-farmers in Eastern Poland. *Ann Agric Environ Med*, 7, 125-129.

Spoon, W. (1954). Lemongrass oil from Surinam. *Koninkl. Inst. Tropen Amsterdam*, 244, 8.

Squibbs, F. L. (1938). Work of the department [for the year 1936]. Ann. Rept. Dept. Agr. *Seychelles*, 10-28

Srivastava, A., Shukla, Y.N., & Kumar, S. (2000). Recent development in plant-derived antimicrobial constituents-A Review. *J Med Arom Plant Sci*, 22, 349-405.

Stenlake, J. B., & Williams, W. D. (1957). The Spectrophotometric Determination of Aβ-Unsaturated Aldehydes and Ketones With Girard-T Reagent: Part I. Essential Oils. *Journal of Pharmacy and Pharmacology*, 9, 900-907.

Stoyanova, A., Konakchiev, A., Damyanova, S., Stoilova, I., & Suu, P. (2006) Composition and antimicrobial activity of ginger essential oil from Vietnam.*Journal of Essential Oil-Bearing Plants*, 9, 93-98.

Su, H. J., Hogenhout, S. A., Al-Sadi, A. M., & Kuo, C. H. (2014). Complete chloroplast genome sequence of Omani lime (*Citrus aurantifolia*) and comparative analysis within the rosids. *PloS one*, 9, e113049.

Su, Y. C., & Ho, C. L. (2016). Essential oil compositions and antimicrobial activities of various parts of *Litsea cubeba* from Taiwan. *Natural product communications*, 11, 1934578X1601100425.

Suaeyun, R., Kinouchi, T., Arimochi, H., Vinitketkumnuen, U., & Ohnishi, Y. (1997). Inhibitory effects of lemongrass (*Cymbopogon citratus* Stapf) on the formation of azoxymethane-induced DNA adducts and aberrant crypt foci in the rat colon. *Carcinogenesis*, 18, 949-955.

Sukandar, E. Y., Neng, F. K., Pratiwi, W., & Deani, A. (2016). Antibacterial interaction of a combination of ethanolic extract of *Zingiber officinale* var rubrum rhizome, *Boesenbergia pandurata* rhizome, and *Stevia rebaudiana* leaves with certain antibiotics against infectious mouth microbial. *Asian J Pharm Clin Res*, 9, 332-335.

Sut, S., Dall'Acqua, S., Baldan, V., Kamte, S. L. N., Ranjbarian, F., Nya, P. C. B., & Petrelli, R. (2018). Identification of tagitinin C from *Tithonia diversifolia* as antitrypanosomal compound using bioactivity-guided fractionation. *Fitoterapia*, 124, 145-151.

Tajadod, G., Mazooji, A., Salimpour, F., Samadi, N., & Taheri, P. (2012). The essential oil composition of *Artemisia vulgaris* L. in Iran. *Annals of Biological Research*, 3, 385-389.

Taneja, S. C., Koul, S., Dhar, K. L. (2009). A process for the preparation of geranyl nitrile from lemongrass oil. *Indian*, IN 195658 A1 20090731

Tang, B., Tu, H., Long, H. A., Du, J., Guo, J. M., Liu, H. J., Hu, X. Y., & Du, X. (2017). A new N-methoxyl-carbonyl benzylisoquinoline from *Litsea cubeba*. *Journal of Asian natural products research*, 19, 941-945.

Taweechaisupapong, S., Aieamsaard, J., Chitropas, P., & Khunkitti, W. (2012). Inhibitory effect of lemongrass oil and its major constituents on Candida biofilm and germ tube formation. *South African Journal of Botany*, 81, 95-102.

Temraz, A., & El-Tantawy, W. H. (2008). Characterization of antioxidant activity of extract from *Artemisia vulgaris*. *Pakistan journal of pharmaceutical sciences*, 21, 321-326

Tona, L., Kambu, K., Ngimbi, N., Cimanga, K., & Vlietinck, A. J. (1998). Antiamoebic and phytochemical screening of some Congolese medicinal plants. *Journal of ethnopharmacology*, 61, 57-65.

Tripathi, P., Yami, H., & Shukla, A. K. (2016). Evaluation of the antifungal activity of *Artimesia*, *Litsea* and *Mikani*a essential oils against post-harvested fungal diseases of kiwifruits. *International Journal of Current Microbiology and Applied Sciences*, 5, 19-29

Tripoli, E., La Guardia, M., Giammanco, S., Di Majo, D., & Giammanco, M. (2007). Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chemistry*, 104, 466-479.

Trisonthi, P., Sato, A., Nishiwaki, H., & Tamura, H. (2014). A new diterpene from *Litsea cubeba* fruits: Structure elucidation and capability to induce apoptosis in HeLa cells. *Molecules*, 19, 6838-6850.

Tyagi A. K., Malik, A. (2010). Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: microscopic observations and chemical characterization of Cymbopogon citrates. *BMC complementary and alternative medicine*, 1065.

Tyagi, A. K., & Malik, A. (2012). Bactericidal action of lemongrass oil vapours and negative air ions. *Innovative Food Science & Emerging Technologies*, 13, 169-177.

Tyagi, A. K., & Malik, A. (2012). Morphostructural damage in food-spoiling bacteria due to the lemongrass oil and its vapour: SEM, TEM, and AFM investigations. *Evidence-Based Complementary and Alternative Medicine*, 2012.

Tyagi, A.K., Malik, A. (2010). In situ SEM, TEM and AFM studies of the antimicrobial activity of lemongrass oil in liquid and vapour phase against *Candida albicans*. *Micron*, 41, 797-805.

Uddin, N., Hasan, M. R., Hasan, M. M., Hossain, M. M., Alam, M. R., Hasan, M. R., Islam, A. F. M. M., Rahman, T., & Rana, M. S. (2014). Assessment of toxic effects of the methanol extract of *Citrus macroptera* Montr. fruit via biochemical and haematological evaluation in female Sprague-Dawley rats. *PLoS One*, 9, e111101.

Uddin, N., Hasan, M. R., Hossain, M. M., Sarker, A., Hasan, A. N., Islam, F.A. M. M., Chowdhury, M. M. H., & Rana, M. S. (2014). In vitro α–amylase inhibitory activity and in vivo hypoglycemic effect of methanol extract of *Citrus macroptera* Montr. fruit. *Asian Pacific journal of tropical biomedicine*, 4, 473-479.

Utakod, N., Laosripaiboon, W., Chunhachart, O., & Issakul, K. (2017). The efficiency and the correlation between testing methods on antimicrobial and antioxidant activities of selected medicinal essential oils. *International Food Research Journal*, 24, 2616-2624.

Valera, M. C., Cardoso, F. G. D. R., Maekawa, L. E., Camargo, C. H. R., De Oliveira, L. D., & Carvalho, C. A. T. (2015). In vitro antimicrobial and anti-endotoxin action of *Zingiber Officinale* as auxiliary chemical and medicament combined to calcium hydroxide and chlorhexidine. *Acta Odontologica Scandinavica*, 73, 556-561.

Valsaraj, R. P., Pushpangadan, U. W., Smitt, A., Adsersen & Nyman, U. (1997). Antimicrobial screening of selected medicinal plants from India. *J. Ethnopharmacol*, 58, 75-83.

Vasala, P. A. (2004). Ginger. (ed) Peter, KV Handbook of Herbs and Spices. Cochin India.

Vellore, M., Komathi, S., & Rajalakshmi, G. (2016). Preliminary phytochemical screening of different solvent extracts from *Cymbopogon citratus* leaf. *European Journal of Biomedical and Pharmaceutical Sciences*, 3, 367-370.

Vilela, G. R., de Almeida, G. S., D'Arce, M. A. B. R., Moraes, M. H. D., Brito, J. O., da Silva, M. F. D. G., Silva S.C., De Stefano P. S.M., Calori-Domingues M.A., & da Gloria, E. M. (2009). Activity of essential oil and its major compound, 1, 8-cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. *Journal of Stored Products Research*, 45, 108-111.

Vinitketkumnuen, U., Puatanachokchai, R., Kongtawelert, P., Lertprasertsuke, N., & Matsushima, T. (1994). Antimutagenicity of lemongrass (*Cymbopogon citratus* Stapf) to various known mutagens in salmonella mutation assay. *Mutation Research/Genetic Toxicology*, 341(1), 71-75.

Vongsombath, C., Pålsson, K., Björk, L., Borg–Karlson, A. K., & Jaenson, T. G. (2014). Mosquito (Diptera: Culicidae) repellency field tests of essential oils from plants traditionally used in Laos. *Journal of Medical Entomology*, 49, 1398-1404.

Wahyuningsih, M. S. H., Wijayanti, M. A., Budiyanto, A. R. I. E. F., & Hanafi, M. U. H. A. M. M. A. D. (2015). Isolation and identification of potential cytotoxic compound from kembang bulan *[Tithonia diversifolia* (Hemsley) A. Gray] leaves. *Int J Pharm Pharm Sci*, 7, 298-301.

Waikedre, J., Dugay, A., Barrachina, I., Herrenknecht, C., Cabalion, P., & Fournet, A. (2010). Chemical Composition and Antimicrobial Activity of the Essential Oils from New Caledonian *Citrus macroptera* and *Citrus hystrix*. *Chemistry & Biodiversity*, 7, 871-877.

Wallberg, F., Tenev, T., & Meier, P. (2016). Analysis of apoptosis and necroptosis by fluorescence-activated cell sorting. *Cold Spring Harbor Protocols*, 4, pdb-prot087387.

Wang, C. Chen, J., Lu, S., Xia, Z., & Zhou, M. (1985). Studies on the active principles of *Litsea cubeba* for the treatment of coronary heart disease. *Zhongyao Tongbao*, 10, 414-16.

Wang, C. X., Lin, G., & Zhou, Z. (2011). The chemical constituent's analysis of the volatile oils from the leaves of *Litsea cubeba* (Lour.) Pers. By GC-MS. *Zhonghua Zhongyiyao Xuekan*, 29, 1898-1899.

Wang, F., Yang, D., Ren, S., Zhang, H., & Li, R. (1999). Chemical composition of essential oil from leaves of *Litsea cubeba* and its antifungal activities. Zhong Yao cai Zhongyaocai *Journal of Chinese medicinal materials*, 22, 400-402.

Wang, H. M., Chen, C. Y., Chen, H. A., Huang, W. C., Lin, W. R., Chen, T. C., & Chen, Y. H. (2010). *Zingiber officinale* (ginger) compounds have tetracycline-resistance modifying effects against clinical extensively drug-resistant *Acinetobacter baumannii*. *Phytotherapy research*, 24, 1825-1830.

Wang, J., Zhu, F., Zhou, X. M., Niu, C. Y., & Lei, C. L. (2006). Repellent and fumigant activity of essential oil from *Artemisia vulgaris* to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Journal of Stored Products Research*, 42, 339-347.

Wang, L. Y., Qu, Y. H., Li, Y. C., Wu, Y. Z., Li, R., Guo, Q. L., Li. R., Wang S. J., Wang Y. N., Yang, Y. C., & Lin, S. (2017). Water-soluble constituents from the twigs of *Litsea cubeba*. Zhongguo Zhong Yao za zhi Zhongguo Zhong Yao zazhi China journal of Chinese materia medica, 42, 2704-2713.

Wang, L. Y., Qu, Y. H., Wu, Y. Z., Li, Y. C., Li, R., Lin, P. C., Shang, X, Y., & Lin, S. (2018). Two new terpenoid ester glycosides from the twigs of *Litsea cubeba*. *Journal of Asian natural products research*, 20, 1129-1136.

Wang, Q., Ni, X., & Shi, J. (1993). Manufacturing technology of capric acid and hydroxyalkyl amide from *Litsea cubeba* nucleolus oil. *Huaxue Shijie*, 34, 84-7.

Wang, X., Hao, Q., Chen, Y., Jiang, S., Yang, Q., & Li, Q. (2015). The effect of chemical composition and bioactivity of several essential oils on *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Journal of Insect Science*, 15(1).

Wang, Y., Jiang, Z. T., & Li, R. (2012). Antioxidant activity, free radical scavenging potential and chemical composition of *Litsea cubeba* essential oil. *Journal of Essential Oil Bearing Plants*, 15, 134-143.

Wang, Y., Lu, X., Yuan, P., & You, M. (2003). *Oleum litseae* extracting from *Litsesa cubeba* (lours) pers having parasiticide effect on ectozoa of human and animals. *Faming Zhuanli Shenqing*, CN 1426811 A 20030702.

Wannissorn, B., Jarikasem, S., & Soontorntanasart, T. (1996). Antifungal activity of lemongrass oil and lemongrass oil cream. *Phytotherapy Research*, 10, 551-554.

Wanzala, W., Hassanali, A., Mukabana, W. R., & Takken, W. (2014). Repellent activities of essential oils of some plants used traditionally to control the brown ear tick, *Rhipicephalus appendiculatus. Journal of parasitology research*, 2014.

Wariyar, N. S. (1954). Lemon-grass oil. *Bulletin of the Research Institute, University of Kerala, Trivandrum, Series A: Physical Sciences*, 3, 165-204.

Weitzman, I., & Summerbell, R.C. (2001). The dermatophytes. *Clin Microb Rev*; 8, 240–259.

White, B. (2007). Ginger: an overview. *American family physician*, 75, 1689-1691.

White, B., Evison, A., Dombi, E., & Townley, H. E. (2017). Improved delivery of the anticancer agent citral using BSA nanoparticles and polymeric wafers. *Nanotechnology, science and applications*, 10, 163.

Williams, J. D., Saleh, A. M., & Acharya, D. N. (2012). Composition of the essential oil of wild-growing *Artemisia vulgaris* from Erie, Pennsylvania. *Natural product communications*, 7, 637-640.

Wu, K. Zhao, S., Chai, X., & Zhao, X. X. (2013). Antibacterial effect of plant essential oils against *Pseudomonas aeruginosa*. *Xiandai Shipin Keji*, 29(12), 2830-2833.

Wu, T. S., Shi, L. S., Kuo, P. C., Leu, Y. L., Liou, M. J., Wu, P. L., Wu, Y.C., Iou, S.C., Chen, Y., & Chang, H. C. (2001). Cytotoxic principles from the leaves of *Tithonia diversifolia*. *Chinese Pharmaceutical Journal*, 53, 217-223.

Yamada, Y., Kikuzaki. H., Nakatani, N. (1992). Identification of antimicrobial gingerols from ginger (*Zinglber officinale* Roscoe). *J. Antibact. Antifung. Agent*, 20, 309-311.

Yang, K., Wang, C. F., You, C. X., Chun, X., Geng, Z. F., Sun, R. Q., Guo, S. S., Du, S. S., Liu, Z. L., & Deng, Z. W. (2014). Bioactivity of essential oil of *Litsea cubeba* from China and its main compounds against two stored product insects. *Journal of Asia-Pacific Entomology*, 17, 459-466.

Yang, T. S., Liou, M. L., Hu, T. F., Peng, C. W., & Liu, T. T. (2013). Antimicrobial activity of the essential oil of *Litsea cubeba* on cariogenic bacteria. *Journal of Essential Oil Research*, 25, 120-128.

Yang, X., Gao, X., Cao, Y., Guo, Q., Li, S., Zhu, Z., Zhao, Y., Tu, P., & Chai, X. (2018). Anti-inflammatory effects of boldine and reticuline isolated from *Litsea cubeba* through JAK2/STAT3 and NF-κB signaling pathways. *Planta medica*, 84, 20-25.

Yang, Y. S., & Hwang, G. A. (2000). Germicide composition has natural perfume substance. *Repub. Korean Kongkae Taeho Kongbo*, KR 2000061074 A 20001016

Yang, Y., Jiang, J., Qimei, L., Yan, X., Zhao, J., Yuan, H., Qin, Z., & Wang, M. (2010). The fungicidal terpenoids and essential oil from *Litsea cubeba* in Tibet. *Molecules*, 15, 7075-7082.

Yeh, P. H. (1973). Essential oils. XV. Essential oil of Malabar lemongrass roots. *Huaxue*, 3, 88-93.

Yokogawa, Y., Tanaka, N., Yagi, E., & Sakamoto, O. (1997). Skin-lightening cosmetics containing *Litsea cubeba* extracts. *Jpn. Kokai Tokkyo Koho*, JP 09030952 A 19970204

Yoshikawa M, Shimada H, Matsuda H, Yamahara J, Murakami N. (1996). Bioactive constituents of Chinese natural medicines. New sesquiterpene ketones with vasorelaxant effect from Chinese moxa, the processed leaves of *Artemisia argyi* Levlet Vant: Moxartenone and moxartenolide. *Chem Pharm Bull*, 44, 1656–1662.

Yu, B., & Liu, D. (1998). Antioxidant activity of *Litsea cubeba* oil and residues remaining after oil extraction. *Shipin Kexue* (Beijing), 19, 9-11.

Yu, J., Chen, Y. R., Wang, S. X., & Zheng, X. H. (2009). Ultrasonic Assisted Extraction, Antioxidant and Antibacterial Activities of Ginger Flavonoids. *Journal of Food Science*, 24.

Yu, J., Zhang, N., Li, W., & Li, H. 2018. Method for extracting citronellal from *Litsea cubeba* oil. *Faming Zhuanli Shenqing*, CN 108383702 A 20180810.

Yu, P., Lin, X., & Liu, Y. (1999). Bacteriostatic effects of water-soluble oligo-chitosan, chitosan, and *Litsea cubeba* oil. *Yaowu Shengwu Jishu*, 6, 171-173.

Yu, S. H., Chen, X. H., & Bi, K. S. (2008). GC determination of palmitic acid, oleic acid and linoleic acid in *Litsea cubeba* (Lour.) Pers. *Chinese Journal of Pharmaceutical Analysis*, 28, 1485-1488.

Yu, S., Chen, X., Tong, L., & Bi, K. (2007). Simultaneous determination of the 6-methyl-5-heptane-2-one, limonene, linalool and citral in essential oil from *Litsea cubeba* (Lour.) Pers by capillary gas chromatography. *Asian Journal of Traditional Medicines. 2, 66-69.*

Zhai, H. L., Zhao, G. J., Yang, G. J., Sun, H., Yi, B., Sun, L. N., ... & Zheng, S. Q. (2010). A new chromene glycoside from *Tithonia diversifolia*. *Chemistry of natural compounds*, 46, 198-200.

Zhan, Y., Long, Y., Ye, F., & Zhou, J. (1985). Analysis of *Litsea cubeba* oil in Hunan by gas chromatography. *Sepu*. 2, 190-4.

Zhang, F. (1985). Effects of the essential oil of mountain spicy tree (*Litsea cubeba*) against experimentally-induced cardiacarrhythmia. *Zhongcaoyao*, 16, 254-5.

Zhang, H. J., Zheng, L. H., Zhao, K., Chen, Y., & Yi, Z. (2017). Insecticidal activities of constituents of *Litsea cubeba* fruit extract effective against the maize weevil (Coleoptera: Curculionidae). *Journal of Insect Science*, 17, 103.

Zhang, J., Chen, X. H., Liu, Y., Xie, P., & Bi, K. S. (2009). Determination of the content of rutin in *Litsea cubeba* by RP-HPLC. Shenyang Yaoke Daxue Xuebao. 26, 633-635.

Zhang, J., Chen, X. H., Zhang, D., Xie, P., & Bi, K. S. (2010). GC simultaneous determination of six components in *Litsea cubeba* volatile oil. *Chinese Journal of Pharmaceutical Analysis*. 30, 424-427.

Zhang, M., & Shen, Y. 2010. Application of *Litsea cubeba* or its extract in medicines for treating biliary tract diseases. *Faming Zhuanli Shenqing*, CN 101,

Zhang, S. Y., Guo, Q., Gao, X. L., Guo, Z. Q., Zhao, Y. F., Chai, X. Y., & Tu, P. F. (2014). A phytochemical and pharmacological advance on medicinal plant *Litsea cubeba* (Lauraceae). Zhongguo Zhong Yao za zhi Zhongguo Zhong Yao zazhi *China journal of Chinese materia medica*, 39, 769-776.

Zhang, S., Zhang, Q., Guo, Q., Zhao, Y., Gao, X., Chai, X., Tu, P., Zhang, S., Z. S., & Guo, Q. (2015). Characterization and simultaneous quantification of biological aporphine alkaloids in *Litsea cubeba* by HPLC with hybrid ion trap time-of-flight mass spectrometry and HPLC with diode array detection. *Journal of separation science*, 38, 2614-2624.

Zhang, W. Hu, J. F., Lv, W. W., Zhao, Q. C., & Shi, G. B. (2012). Antibacterial, antifungal and cytotoxic isoquinoline alkaloids from *Litsea cubeba*. *Molecules* (Basel, Switzerland), 17, 12950-60.

Zhao, G., Li, X., Chen, W., Xi, Z., & Sun, L. (2012). Three new sesquiterpenes from *Tithonia diversifolia* and their anti-hyperglycemic activity. *Fitoterapia*, 83, 1590-1597.

Zhao, G., Zhang, C., Wu, Z., Li, X., Yang, Y., Sun, L., & Zheng, S. (2010). Chemical constituent analysis of EtOAc fraction of *Tithonia diversifolia* (HEMSL.) A. Gray. *Academic Journal of Second Military Medical University*, 31, 189-192.

Zhao, H., Xu, H., Qiao, S., Lu, C., Wang, G., Liu, M., Liu, M., Ju, D., Tan, Y., Guo, B & Xiao, C. (2017). Boldine isolated from *Litsea cubeba* inhibits bone resorption by suppressing the osteoclast differentiation in collagen-induced arthritis. *International immunopharmacology*, 51, 114-123.

Zhao, L., Dong, J., Hu, Z., Li, S., Su, X., Zhang, J., & Chen, H. (2017). Anti-TMV activity and functional mechanisms of two sesquiterpenoids isolated from *Tithonia diversifolia*. *Pesticide biochemistry and physiology*, 140, 24-29.

Zhao, M. (2001). Method for using lemongrass*aiming Zhuanli Shenqing*, CN 1290493 A 20010411

Zhao, O., Zhou, J. W., & Ban, D. M. (2010). Analysis of volatile oil from different parts of *Litsea cubeba*. Zhong Yao cai Zhongyaocai *Journal of Chinese medicinal materials*, 33

Zhao, Z., Chen, H., Shi, Y., Chen, Q., & Zou, X. (2015). Process for extracting methyl heptane from *Litsea cubeba*. *Faming Zhuanli Shenqing*, CN 104478680 A 20150401,

Zhong, R. M., Xiao, Z. J., Liu, J. N., & Luo, Y. J. (2011). Antimicrobial Activities of Two Edible Essential Oils. *Modern Food Science and Technology*, 27, 250-253.

Zhong, S. A., Cai, H. Q., & Ai, H. D. (2009). Preparation of biodiesel from high-acid value *Litsea cubeba* kernel oil. *Chemical Engineering* (China), 37, 63-66.

Zhong, Y.M., Tu, D., & Peng, Y.L. (2013). Study on antioxidant activity of *Litsea cubeba* oil and its application in chilled pork preservation. *Anhui Nongye Kexue*, 41, 6857-6861.

Zhou, H., Peng, L., Jiang, B., Hou, A., Lin, Z., & Sun, H. (2000). Chemical constituents from *Tithonia diversifolia*. *Acta Botanica Yunnanica*, 22, 361-364.

Zhou, X., Cheng, J., Zhang, J., & Fu, W. (2019). Natural plant source preservative and preparation method and application thereof. *Faming Zhuanli Shenqing*, CN 109966223 A 20190705.

Zhu, C., & Yang, P. (2007). Isolation and structure identification of chemical constituents from the root of *Litsea cubeba*. *Zhongguo Yiyao Gongye Zazhi*, 38, 558-560.

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TITLE OF THESIS **:** SCIENTIFIC EXPLORATION OF ETHNOMEDICINAL FORMULATIONS AGAINST SKIN AILMENT IN MIZORAM AND THEIR ANTI FUNGAL EFFICACY.

DATE OF ADMISSION **:** 08.08.2014

APPROVAL OF RESEARCH PROPOSAL **:**

1. BOS : 12th May, 2015
2. SCHOOLBOARD : 19th May, 2015

REGISTRATION NO. & DATE: MZU/Ph.D/739 of 19.05. 2015

EXTENSION (IF ANY): 16-2/MZU (Acad)/20/391-393
Dt. 11th February 2021

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Area of Interest/Specialization

- *In-vitro* and *in-vivo* Pre-clinical validation studies for natural bio-actives with anti-microbial potential, Production development, Ethnomedicine.

Publications Profile

Number of publications: **05**