Anatomical Investigation and Isolation of Leaf Essential Oils from *Cinnamomum zeylanicum* and *Murraya koengii* for Evaluation of their Antioxidant Potential and Mosquito Larvicidal Activity

A Dissertation for

Course Code and Title: **BOT- 651 (DISCIPLINE SPECIFIC DISSERTATION)**

Credits: 16

Submitted in partial fulfilment of Masters Degree

M.Sc. in Botany

by

MS. SAMRUDHI SANJIV NAIK GAUNEKER

Roll No: 22PO480012

ABC ID : 434-111-003-327

PR. NO : 201708798

Under the Supervision of

PROF. S. KRISHNAN, M.Phil, Ph.D.

School of Biological Sciences and Biotechnology

Botany Discipline

GOA UNIVERSITY

Date: April 2024

GOA UNIVERSITY
SBSB
TALEIGAO
GOA
BOTANY DISCIPLINE

Seal of the School

Examined by:

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "**Anatomical investigation and isolation of leaf essential oils from *Cinnamomum zeylanicum* and *Murraya koengii* for evaluation of their antioxidant potential and mosquito larvicidal activity**" is based on the results of investigations carried out by me in the Botany Discipline at the School of Biological Sciences and Biotechnology, Goa University, under the Supervision of **Prof. S. Krishnan** and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations /experimental or other findings given the internship report/work.

I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

Signature and Name of Student
(Ms. Samrudhi Sanjiv Naik Gauneker)
Seat No:22P0480012

Date: 08.04.2024

Place: Goa University

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "**Anatomical investigation and isolation of leaf essential oils from *Cinnamomum zeylanicum* and *Murraya koengii* for evaluation of their antioxidant potential and mosquito larvicidal activity**" is based on the results of investigations carried out by me in the Botany Discipline at the School of Biological Sciences and Biotechnology, Goa University, under the Supervision of **Prof. S. Krishnan** and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations /experimental or other findings given the internship report/work.

I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

Signature and Name of Student
(Ms. Samrudhi Sanjiv Naik Gauneker)
Seat No:22P0480012

Date: 08.04.2024

Place: Goa University

CONTENTS

PREFACE

As an alternative to synthetic chemicals, which can be detrimental to the environment and public health, there is growing interest in the use of natural compounds with insecticidal and antioxidant qualities. The two plants that are studied and presented in this dissertation are *Murraya koenigii* (curry leaf) and *Cinnamomum zeylanicum* (cinnamon), both of which are noted for their fragrant and therapeutic qualities. The anatomical, antioxidant, and larvicidal properties of these plants essential oils are investigated during this study. Anatomical characteristics such as oil glands, oxalate crystals, and stone cells indicate high levels of bioactive compounds. In both plants, efficient transport of nutrients and water is facilitated by well-developed vascular bundles. Strong larvicidal and antioxidant properties were shown by *C. zeylanicum* essential oil, whereas *M. koenigii* showed only modest effects. These results demonstrated how essential oils derived from plants can be used as sustainable and natural remedies.

ACKNOWLEDGEMENTS

To begin with, I want to express my sincere gratitude to the Lord for his guidance and blessings. I thank all the people who have guided and helped me in the completion of my dissertation.

I express my sincere gratitude to my guide, Prof. S. Krishnan, Senior Professor of Botany & Vice-Dean (Research), School of Biological Sciences and Biotechnology (SBSB), Goa University, for his guidance, encouragement, and motivation rendered to me for the successful completion of this dissertation work. I thank all the teachers, Prof. B.F. Rodrigues, Dr. Rupali Bhandari, Dr. Siddhi Jalmi, and Dr. Aditi Naik for their advice, help, and support.

I would like to sincerely acknowledge the tremendous support and guidance provided by Dr. Ajeet Kumar Mohanty, Assistant Research Scientist & Officer-In-Charge of the ICMR- National Institute of Malaria Research Field Unit, Health Services Building, Campal, Panaji, Goa, for providing me with the laboratory facilities and guiding me with the mosquito larvicidal studies. I am thankful to every member of NIMR for their cooperation and willingness to help whenever approached. I am very grateful to Research Scholar Ms. Vaishali Gaonkar for being the source of motivation and her constant support in making this project successful. I sincerely thank research scholars Mrs. Annie Nadar, Mrs. Divyarani Revankar, and Ms. Arti Dabolkar for their help and advice. I also thank Mr.Samrat Gaonkar, Mrs. Sahara Baby, and Miss Shanta Baganawar from the Botany discipline for helping me complete my dissertation. I am grateful to my Father, mother, and brother for all the love, support, and encouragement during my dissertation work.

Ms. Samrudhi Sanjiv Naik Gauneker

TABLES

FIGURES

PLATES

Plate No	Description
1.1	*Cinnamomum zeylanicum* (a) Habit, (b) Bark of the tree, (c) Leaf arrangement, (d) inflorescence.
1.2	Macroscopic images of *Cinnamomum zeylanicum* (a) Mature leaf showing the presence of oil glands, (b) Young leaf showing the presence of oil glands, (c) Outer layer of Bark of the tree, (d) Inner layer of bark of the tree, (e, f) Flower.
1.3	*Murraya koenigii* (a) Habit, (b) Bark of the tree, (c) Leaf arrangement, (d) Infloresence.
1.4	Macroscopic images of *Murraya koenigii* (a) Young leaves showing the presence of oil glands, (b) Stem showing the presence of trichomes, (c) Flower, (d) Petals showing the presence of oil glands, (e) Fruits, (f) Cut open fruit showing accumulation of oil.
1.5	Mosquito larval stages of *Culex quinquefasciatus* species, (a) 1^{st} Instar larvae, (b) 2^{nd} Instar larvae, (c) 3^{rd} Instar larvae, (d) 4^{th} Instar larvae, (e) Pupa stage.
3.1	Process of essential oil extraction from *C. zeylanicum* and *M. Koenigii* (a) Clevenger apparatus , (b) Plant extract , (c) Essential oil extracted from the leaves of *M. koenigii* , (d) Essential oil extracted from the leaves of *C. zeylanicum.*

4.1	Anatomy of *C. zeylanicum* leaf, (a) Overview of leaf (4X), (b) T.S. Of leaf showing presence of secretory cavities and oil glands along with vascular strands (c) leaf section showing oil glands and secretorty cavities (d-e) presence of secretory cavities and vasular strands , (f) Anomocytic stomata(10X).
4.2	Anatomy of *C. zeylanicum* petiole and stem. (a)Overview of petiole (4X), (b) Section showing presence of secretory cavities, (c)Section of petiole showing presence of stone cells, (d)Overview of *C. zeylanicum* stem (4X), (e)Section of stem showing presence of secretory cavities, (f)Showing presence of secretory cavities and stone cells.
4.3	Anatomy of *M. koenigi* leaf , (a) Overview of leaf(10X) , (b) Leaf section showing presence of trichome along with epidermal layer , (c-d) section showing the presence of Schizolysigenous oil glands are present close to the upper epidermis , (e) Trichome (40X) , (f) Leaf showing anisocytic stomata.
4.4	Anatomy of *M. koenigii* stem and petiole (a) Overview of stem (4X), (b) Section of stem showing trichome along with epidermal layer , (c) Section of stem showing presence of schizolysigenous oil glands and calcium oxalate crystals , (d) Petiole showing presence of Trichome along with epidermal layer (10X) , (e) Petiole showing presence of trichome , (f)T.S. of Petiole showing presence of secretory gland (40X).

4.5	Antioxidant studies using DPPH method: (a) L-Ascorbic acid as standard, (b) *C. zeylznicum.* (c) *M. koenigii*.
4.6	Larvicidal activity (a) Bowl containing 3rd instar stage larvae , (b) Larvae in different concentration of essential oil , (c) 3rd instar *Culex quinquefasciatus* (untreated) (4X), (d) 3rd instar *Culex quinquefasciatus* (Treated) (4X).

ABBREVIATIONS USED

ENTITY	ABBREVIATION
1,1- diphenyl-2-picrylhydrazyl	DPPH
Absorbance of control	A_0
Absorbance of sample	A_1
ABTS	2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid
Cinnamomum leaf essential oil	CLEO
Degree Celsius	◦C
Dimethyl sulfoxide	DMSO
Essential oils	EOs
Global Positioning System	GPS
Lethal concentration killing 50%	IC_{50}
Lethal concentration killing 90%	LC_{90}
Lower confidence limit	LCL
microgram	µg
microlitre	µl
milligram	mg
milliliters	ml
millimetre	mm
Murraya koenigii leaf essential oil	MKLEO
Nanometer	nm
National Institute of Standards and	NIST

Technology	
Parts per million	PPM
Percent	%
Reactive oxygen species	ROS
Replicates	R
Room temperature	RT
Transverse section	TS
Upper confidence limit	UCL
Weight in volume	w/v
World health organization	WHO
Statistical Package for Social Sciences	SPSS

ABSTRACT

The purpose of the study was to evaluate the anatomical, larvicidal, and antioxidant qualities of leaf essential oils that were extracted from *Murraya koenigii* (Family Rutaceae) and *Cinnamomum zeylanicum* (Family Lauraceae). The study used anatomical characterisation to pinpoint unique characteristics found in the plants, including oxalate crystals, oil glands, and stone cells. The efficacy of the essential oils bioactive qualities and the plants capacity to produce them may be influenced by these anatomical features. The stems and leaves of both species showed evidence of well-developed vascular bundles, which aid in the effective movement of nutrients and water throughout the plant. The entire health and growth of the plant are supported by its interior structure, which may have an impact on the type and amount of essential oils that are produced.

Due to the high content of Phenolic compounds and other bioactive chemicals including cinnamonaldehyde, the essential oil from *C. zeylanicum* demonstrated remarkable antioxidant potential. These ingredients support the oil's capacity to scavenge free radicals, delivering possible health advantages and uses in goods high in antioxidants. Nevertheless, the essential oil of *M. koenigii* showed comparatively less antioxidant activity, which may have resulted from a distinct phytochemical profile with reduced amounts of phenolics and other bioactive components. When it came to *Culex quinquefasciatus* mosquito larvae, both essential oils worked well. Because it contains a high concentration of cinnamaldehyde and other bioactive substances with insecticidal qualities, the essential oil of *C. zeylanicum* exhibited the best larvicidal efficacy.

The effectiveness of *M. koenigii* essential oil was also notable, though slightly less pronounced than *C. zeylanicum*. The observed differences in larvicidal activity could be linked to variations in the chemical compositions of the oils and the mosquito larvae's developmental stages. The study suggests that these essential oils have potential applications in natural mosquito control strategies and in developing antioxidant formulations for various industries. Further research is recommended to isolate and identify the specific active compounds responsible for the antioxidant and larvicidal properties observed in both plant species. Understanding their mechanisms of action could lead to the development of more targeted and effective applications in health, agriculture, and pest control.

Keywords: Anatomy, Antioxidant, Essential oil, Larvicidal.

CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

Throughout the world, medicinal plants have been widely employed for their diverse therapeutic advantages and cost-effectiveness in treating and preventing diseases. Notably, these plants possess many pharmacological properties, aligning with their abundance of bioactive compounds. These bioactive compounds are found to be distributed in various parts of the plants (Agour *et al*., 2023).

1.1.1. ESSENTIAL OILS AND THEIR ROLE

Essential oils are volatile, natural compounds that aromatic plants form in the form of plant secondary metabolites. Aromatic plants, especially spices, are the primary sources of essential oils, which can act against various bacteria, fungi, etc. Also, they are effective against larvicidal activity and are known for their medicinal properties and fragrance. They are also used as preservatives (Bakkali *et al*., 2007).

Essential oils are generally liquid, volatile compounds that are rarely coloured and lipid-soluble. They are primarily soluble in organic solvents containing lower density than water. Essential oils are synthesized from most plant organs such as buds, flowers, leaves, barks, twigs, seeds, barks, woods, etc., and are stored chiefly in cavities, canals, secretory cells, glandular trichomes, and epidermal cells (Idaomar *et al*., 2007). Various methods for extracting essential oils from plants include liquid carbon dioxide or microwaves and mainly low or high-pressure hydro or steam distillation. They are obtained mainly by steam or hydro-distillation, which Arabs first developed in the Middle Ages. Due to their bactericidal and fungicidal properties, they are widely used in

the pharmaceutical and food industries as an alternative to chemical products (Fernandez and Vivuda, 2018).

In nature, essential oils help plants protect themselves from bacterial, fungal, and viral attacks. They also protect the plants from herbivores by reducing their desire to eat that particular plant; their ability to draw in bees and butterflies facilitates the transfer of pollens and seeds between different areas (Javad *et al*., 2017).

Essential oils are complex natural mixtures containing 20-60 components at different concentrations. The leading group comprises terpenes and terpenoids; the other group comprises aromatic and aliphatic constituents. The demand for essential oils as natural additives to extend the shelf-life of foods and food products is rising (Javad *et al*., 2017).

Nowadays, the younger generation is more health conscious and is afraid of consuming food that uses chemical or synthetic preservatives. Therefore, one of the central or significant emerging technologies is the extraction of essential oils from various plant parts and their use in food. Essential oils mainly have antioxidant and antimicrobial properties and are used for shelf-life extension in food (Fernandez and Vivuda, 2018). Plants with essential oils belong to broad genera scattered around 60 families, such as Lauraceace, Rutaceae, Lamiaceae, Asteraceae, and Myrtaceae. They are known for their potential to produce essential oils of commercial and medicinal value (Fernandez and Vivuda, 2018).

1.1.2. FAMILY: LAURACEACE

The Lauraceae family is widely distributed in tropical and subtropical climates and has more than 2500 species; from the taxonomic point of view, it is one of the most challenging families to identify species (Goncalves *et al*., 2018).

Lauraceae, the laurel family of flowering plants (order Laurales), comprises 50 genera and more than 2,500 species of mostly evergreen shrubs and trees, being the complex family of angiosperms (Goncalves *et al.*, 2018). Lauraceae is distributed throughout tropical and subtropical regions, principally Southeast Asia and tropical America, particularly Brazil. This aromatic spice family contains some economically and medicinally essential trees, including avocado, cinnamon, bay, and a variety of valuable timber trees, the wood of which sometimes remains fragrant for decades after it is cut. The family includes 67genera 2747 species (Rogimon *et al*., 2014). Around 26 species are found in India. Out of 12 are from the North East and southeast India (Akanksha *et al*., 2017). The family also stands out for its economic importance in the pharmaceutical, medicinal, wood, and food industries (Judd *et al*., 1999).

Some genera are used in carpentry and construction, as they offer good quality wood; other genera are widely used in the food industry (cinnamon and bay), and some genera have great value in the aromatic and cosmetic industries as well as in the medical industry (Marques, 2001). The leaves of the members of the Lauraceae family are arranged alternatively or whorled. Few have opposite leaves. The leaves are primarily leathery and contain oil cavities. Many species have oil in the wood and bark. Flowers are arranged in clusters that are green, yellow, or white. The fruits are berries or drupes, which are fleshy (Judd *et al*., 1999).

1.1.2.1. *Cinnamomum zeylanicum* blume (Judd *et al*., 1999)

Classification:

Taxonomical Rank	**Taxon**
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Laurales
Family	Lauraceae
Genus	*Cinnamomum*
Species	*zeylanicum*

1.1.2.1. BOTANICAL DESCRIPTION

C. zeylanicum (Dalchini) is an evergreen tropical shrub with thick, smooth, reddish-brown bark that grows to a height of around 6-8 meters. The opposite or sub-opposite leaves are glabrous, ovate, lanceolate, complex, and coriaceous. The leaves are brightly coloured above and pale beneath, with 3-5 significant nerves. Petiole flattened to ½inch in length. Axillary or sub-terminal cymes or panicles produce flowers. The fruit is ovate or oblong, about 1.5-2 cm long, minutely apiculate, dry or somewhat fleshy, and dark purple (Goncalves *et al*., 2018).

1.1.2.1. ORIGIN AND DISTRIBUTION

It is a tree indigenous to Sri Lanka and southern India, growing semi-widely in moist lowlands. Currently, Sri Lanka and Madagascar are the primary producers of *Cinnamomum*. It is a well-known flavouring ingredient that is used in a variety of dishes. In the Ayurveda system, *Cinnamomum* bark is used in medicinal preparation for

PLATE 1.1 : *Cinnamomum zeylanicum* (a) Habit, (b) Bark of the tree, (c) Leaf arrangement, (d) inflorescence.

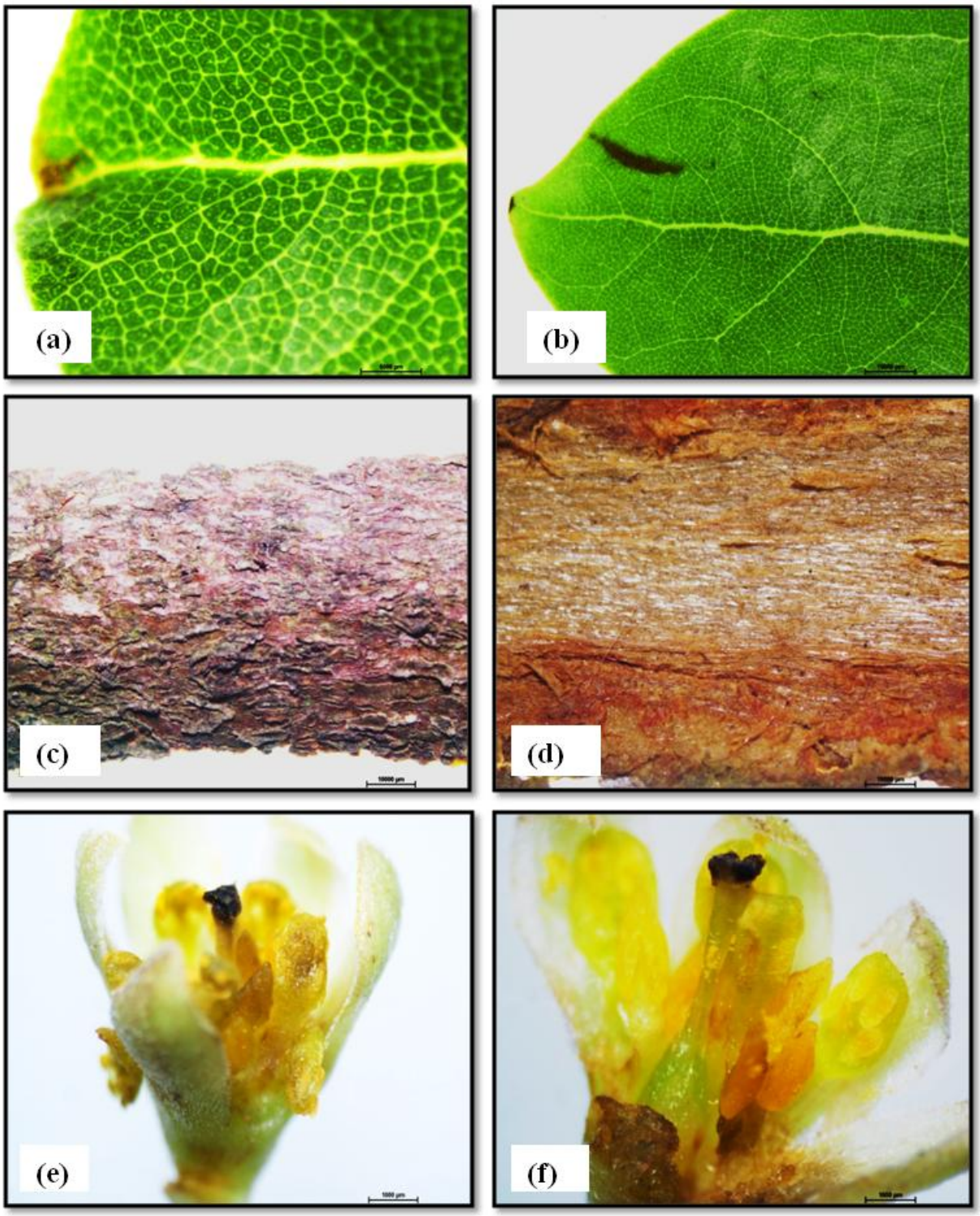


PLATE 1.2 : Macroscopic images of *Cinnamomum zeylanicum* (a) Mature leaf showing the presence of oil glands, (b) Young leaf showing the presence of oil glands, (c) Outer layer of Bark of the tree, (d) Inner layer of bark of the tree, (e, f) Flower.

indigestion, mouthwashes, and flu-prevention treatments. The leaves, panicles, and bark of species have aromatic oils (Judd *et al*., 1999).

It is a spice plant well-known for its medicinal and pharmacological qualities. The old botanical synonym for this tree, *C. zeylanicum*, is derived from Sri Lanka's former name, Ceylon *C. verum, a spice plant* well-known for its medicinal and pharmacological qualities (synonym *C. verum* J. S. Presl). *Cinnamomum* is among the most traditional and popular spices that humanity uses. Cinnamomum's commercial presentation is the tree's dried inner bark, used as an essential spice in every household; Indian cuisine is incomplete without *Cinnamomum* (Judd *et al*., 1999).

Cinnamomum comprises about 250 species, of which 20 occur in India (Anon., 1950). The essential sources of volatile oils in cinnamon are *C. zeylanicum* bark and leaf oils, *C.cassia* (cassiaoil),and *C.canephora*. However, several other *Cinnamomum* species are distilled on a smaller scale, and the oils are used either locally or exported to regional markets.

The leaf and bark of *C. zeylanicum* are used as spices and for producing volatile oils. Leaves have a spicy odour when brushed and a hot taste. Volatile compounds are low molecular weight compounds (<300 Da) that vaporize readily at room temperature (Jayprakashan *et al.,* 2011)

Cinnamon has been utilized in various culinary applications for thousands of years. Due to its high healing importance, it has been employed as an antiemetic, antidiarrheal, ant-flatulent, and stimulant in Ayurvedic medicine. It was employed for mummification by the Egyptians (Pathak and Sharma, 2021).

1.1.2.1. TRADITIONAL USES (Pathak and Sharma, 2021)

True cinnamon is made from the bark of the *C. zeylanicum* tree and is one of the most often used spices. *Cinnamomum* has antifungal, antibacterial, antitermitic, larvicidal, nematicidal, and insecticidal effects. This plant balances the Vata and Pitta energies in the body. It helps to relieve menstruation pain.

1.1.2.4.1. SOME AYURVEDIC MEDICINAL USES (Pathak and Sharma, 2021).

- It relieves sore throats, influenza, the common cold, and headaches.
- It has antitubercular properties and is used as an expectorant.
- In the case of rheumatoid arthritis, it is a natural treatment.
- It is suitable for lowering cholesterol and strengthening the cardiac muscles.
- It provides relief for menstrual pain. A study says women should drink a cup of warm cinnamon water every day. It helps them experience less pain during menstruation for a short duration.

1.1.3. FAMILY: RUTACEAE

The Rutaceae family, also known as the "Rue family" or "Citrus family," comprises mainly perennial trees (*Aegle*, *Citrus*), Shrubs (*Murraya*, *Limonia*, *Zanthoxylum*), and herbs (*Ruta*, *Boenninghausenia*) frequently with volatile aromatic oil contained in glands visible at the surface of the leaves, young branches, inflorescence, flowers, fruits, or seeds. Members of this family have a high percentage of vitamin C and several alkaloids. It comprises about 60 genera and about 2,070 species, of which India contributes 71 species. It is distributed worldwide, especially in warm temperate and tropical regions. The most enormous numbers are in Africa and Australia, often semi-arid woodlands (Roy *et al*., 2016).

The Rutaceae exhibits woody, erect, solid, cylindrical, branched, gland-dotted stems, often with thorns or spines. Leaves are alternate (*Citrus, Murraya)* or opposite (*Evodia*), petiolate. Petiole may be winged (*Citrus aurantium*), simple or compound-pinnate (*Murraya*), palmate (*Aegle* and *Citrus*), smooth gland-dotted with essential oils, ex-stipulate, with entire margin or serrate, unicostate reticulate venation.

In *Citrus*, the petiole is winged (Groppo *et al*., 2008). The inflorescence is terminal or axillary cymes or panicles, sometimes racemose or solitary. Flowers are conspicuous for their colour, fragrance, and nectar; they are bisexual or unisexual (*Zanthoxylum*). This aromatic family consists of numerous economically significant fruit trees and ornamental species. The essential genus in this family is *Citrus*, which includes *C. sinensis, C. limon, C. paradise, C. aurantifolia, C. latifolia*, and *C. reticulate*. *Citrus* is edible and rich in vitamin *C.* Lemon oil is an effective mosquito repellent. *Barosmabetulina* produces buchu from its leaves, which helps treat urinary diseases. Leaves of *Alurniyakoenigii* are used in flavouring Luvunga *scandens*, *Ruta*, *Ptelea*, *Calodendrum*, and *Murraya* are cultivated in gardens for their fragrant flowers and leaves (Hussain, 2021).

1.1.3.1. *Murraya koenigii* L. Spreng (Jain *et al*., 2017)

Taxonomical Rank	**Taxon**
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Rutaceae
Genus	*Murraya*
Species	*koenigii*

PLATE 1.3 : *Murraya koenigii* (a) Habit, (b) Bark of the tree, (c) Leaf arrangement, (d) Inflorescence

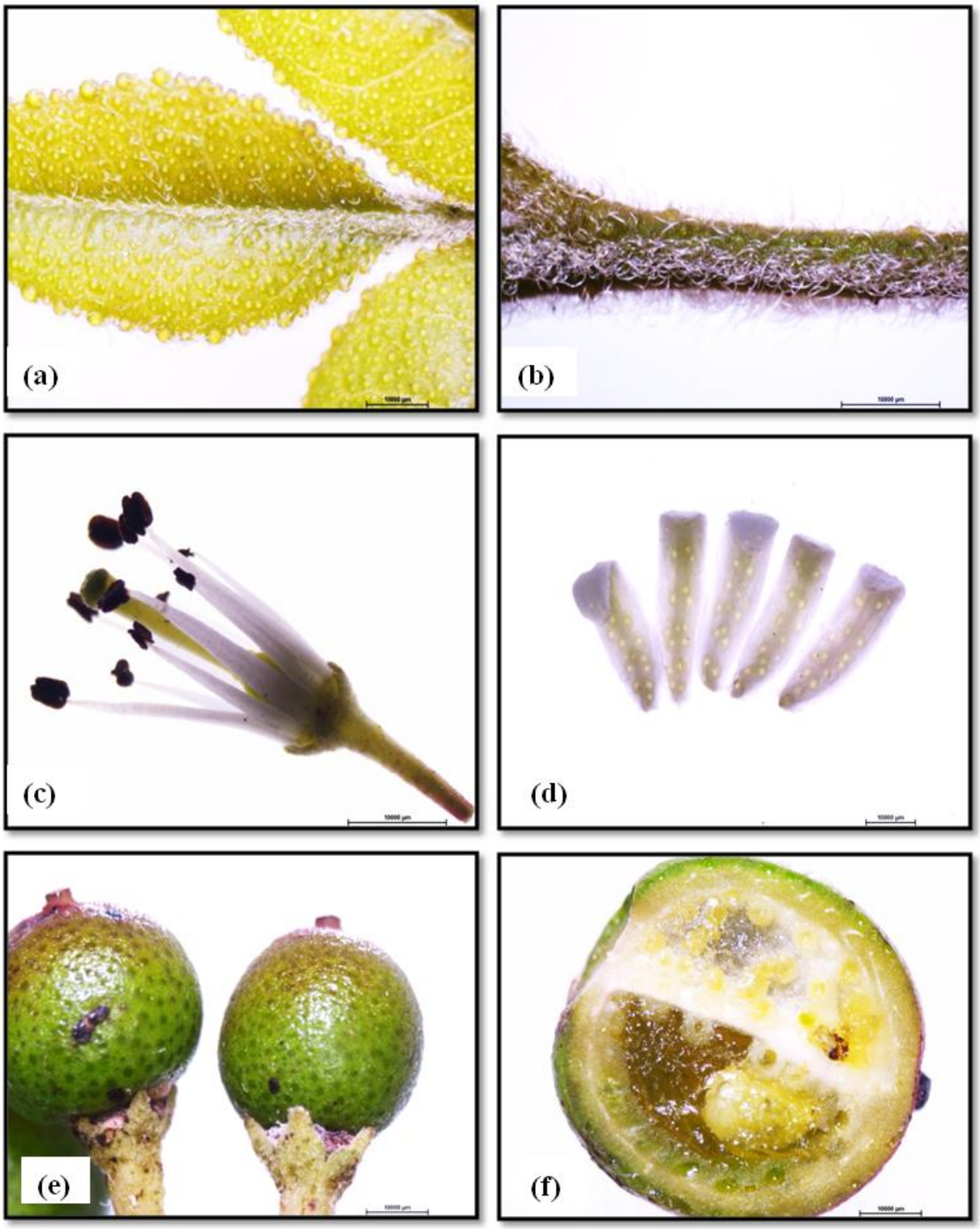


PLATE 1.4 : Macroscopic images of *Murraya koenigii* (a) Young leaves showing the presence of oil glands, (b) Stem showing the presence of trichomes, (c) Flower, (d) Petals showing the presence of oil glands, (e) Fruits, (f) Cut open fruit showing accumulation of oil.

1.1.3.1. BOTANICAL DESCRIPTION

M. koenigii belongs to the family Rutaceae, commonly known as the curry-leaf tree, and is a native of India, Sri Lanka, and other South Asian countries. It is found almost everywhere in the Indian subcontinent; it shares aromatic nature, more or less deciduous shrub or tree up to 6 m in height and 15-40 cm in diameter with a short trunk, thin, smooth grey or brown bark, and dense shady crown (Jain *et al*., 2017).

The whole plant has a solid, peculiar smell, with grey bark and longitudinal striations. Leaves are bipinnately compound, 15-30 cm long, each bearing 11-25 leaflets alternate on rachis, 2.5-3.5 cm long ovate-lanceolate with an oblique base. Margins irregularly creatate, petioles 2-3 mm long, flowers are bisexual, white, funnel-shaped, sweetly scented, stalked, complete, ebracteate, regular with an average diameter of the fully opened flower being in average 1.12 cm inflorescence, terminal cymes each bearing 60-90 flowers. Fruits are ovoid to subglobose, wrinkled, or rough with glands. It is 2.5 cm long and 0.3 cm in diameter and gets purplish black when ripening. Fruits are generally biseeded. Seeds generally occur in spinach green colour, 11 mm long, 8 mm in diameter and weighs up to 445 mg (Harish *et al*., 2012).

1.1.3.1. DISTRIBUTION

The history of curry leaves is seen in the early 1st to 4th century AD. Tamil and Kannada literature updated it as the word ‘kari’ with its uses. The word now popularly used for the *M. koenigii* is curry leaf, which originated from the Tamil word Kari, which means ‘spiced sauce.’ In the early literature of Tamil and Kannada, *M. koenigii* is described as the flavouring agent for vegetables. They are grown as a cultivated crop in India, Sri Lanka, Southeast Asia, Australia, the Pacific Islands, and Africa as flavouring agents for food (Jain *et al*., 2017).

M. koenigii originates from the east and south parts of India, Pakistan, Sri Lanka, China, and Hainan but is widely cultivated in Southeast Asia and some parts of the United States and Australia. It grows up to 1500 to 1655m from sea level and in the Andaman Islands throughout India. It is also available in other parts of Asia, like in moist forests of 500-1600m in height in Guangdong, Shainan, Bhutan, Laos, Nepal, Pakistan, Sri Lanka, Thailand, and Vietnam. With South Indian immigrants, curry leaves reached Malaysia, South Africa, and Reunion Island.3,4 Of the 14 global species that belong to the genus *Murraya*, only two are known to be found in India: *Murraya koenigii* (Spreng) and *Murraya paniculata* (Jack). It can grow in full sun or light shade (Jain *et al*., 2017).

1.1.3.3. USES (Jain *et al*., 2017)

1. Essential oil *M. koenigii* is used as sun protection and an erythema agent in the formulation.
2. Curry leaf oil in your regular skin care cream or lotion helps by applying it on the affected area to cure skin problems such as pimples, athlete's foot, ringworm, itches, acne, boils, and septic wounds and burns.
3. The study evaluated essential oils of *M. koenigii* for toxicity and repellent activity against *Callosobruchus maculatus* due to having active constituents α-pinene and caryophyllene.
4. Curry leaves and their essential oil are used internally and externally for healthy, long, strong, lustrous hair. A balanced diet requires equal vitamins, minerals, iron, and other nutrients to maintain healthy hair.
5. Curry leaf oil helps contract the muscles and tissues.
6. Curry leaf extract helps in pigmentation and reduces the white patches all over the body.

7. Richness of vitamin A and calcium in Curry leaf oil is used for strengthening the bone, osteoporosis, calcium deficiency, and radiotherapy and chemotherapy treatments
8. Fresh leaves, dried leaf powder, and essential oil of curry leaf are widely used as flavouring soups, curries, fish, meat dishes, egg dishes, traditional curry powder blends, seasoning, and ready-to-use other food preparations for cancer.
9. The essential oil of *M. koenigii* is utilized in the soap and cosmetic industry for aromatherapy.
10. The *M. koenigii* is beneficial for bruises and eruptions and for treating bites of poisonous animals.

1.1.4. ANATOMY

The entire plant comprises multiple elements that together form a complicated structure. Given the frequent correlation between the location of an oil's therapeutic activity and its plant, anatomical studies aid aromatherapists in comprehending the significance of plant parts and identifying the section of the plant from which an essential oil is extracted (Raut and Karuppa, 2014).

The Rutaceae family is characterized by secretory cavities, particularly in the leaves and reproductive structures (Groppo *et al*., 2008). Pellucid glands are found on the leaves and are responsible for the aromatic smell of Rutaceae family members (Muntoreanu *et al*., 2011).

1.1.5. ESSENTIAL OIL

Since the beginning of human civilization, essential oils—also called volatile oils—have been used extensively in fragrance, flavoring, and pharmaceutical applications. It blends volatile, fragrant, and hydrophobic substances from particular plant parts, including the rind, bark, leaves, flowers, and seeds. Bryophytes, such as liverworts, are frequently discovered to contain these chemicals. From a biological perspective, they are considered secondary metabolites for the plants. Each constituent of an essential oil has different solubility in water. Although essential oils have a slight solubility in water, it is generally assumed that substances with more polar functional groups would have a higher solubility in water than other substances (Poonkodi *et al*., 2019).

Essential oils can impact the environment when it comes to drought resistance, pollinator attraction, fire resistance, seed distribution, or plant-to-plant biosemiotics (Sadagrove and Jones, 2015). They function as chemical defenses against microbial, fungal, and viral diseases, combat infection, and include molecules that resemble hormones. They also trigger cellular regeneration (Rao and Pandey, 2007).

The medicinal qualities of essential oils were altered in the past when extraction was done with alcohol and a fermentation process. Techniques for extracting essential oils include steam distillation, hydro distillation, turbo distillation, carbon dioxide and supercritical carbon dioxide, and microwave-assisted hydro-distillation (Rao and Pandey, 2007).

Variations in methods results in differences in the yield and composition of resulting essential oil , both qualitatively and quantitatively (Poonkodi et al., 2019).

Gas chromatography combined with mass spectrometry is used to characterize essential oils (GC-MS) chemically. This method determines the molecular mass and

volatility of a mixture of organic molecules to determine their structure (Sadgrove and Jones, 2015).

The body must receive its antioxidants from outside sources because they lower the risk of heart disease and boost immunity. In order to combat the highly pathological condition known as "oxidative stress," research is concentrated on extracting natural antioxidants that are less toxic and more potent than synthetic antioxidants that are frequently utilized (Zafar *et al*., 2017). i.e., Within living things, antioxidant enzymes, reduced glutathione, vitamins, pigments, phenols, and polyphenols provide defense against free radicals and reactive oxygen species. Well-known for their capacity to halt the cascades of lipid peroxidation are synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Schmidt *et al.,* 2013).

Antioxidants are substances that, when applied in minimal amounts (<1%, typically 1–1000 mg/L) relative to the amount of material they have to protect, can slow or delay the oxidation of an oxidizable material (Riccardo *et al.,* 2013).

1.1.6. MOSQUITO LARVICIDAL ACTIVITY

The Culicidae family includes mosquitoes. They resemble midges and pose a threat to human safety. These genera include the species *Aedes*, *Anopheles*, and *Culex*. Female mosquitoes only spread the illness (Ohri *et al*., 2023).

The difficult challenge of controlling mosquito species primarily depends on chemical insecticides because no other practical method has been able to close this gap in control. Phytochemicals are a class of chemicals derived from plants that contain components that effectively and economically reduce mosquito populations (Singh *et al*., 2023).

Strategies to stop the spread of vector-borne diseases must include developing appropriate methods and instruments to manage the vector population. Because of Southeast Asia's tropical or subtropical climate, inadequate drainage, particularly during the rainy seasons, and the region's abundance of fish ponds, irrigation ditches, and rice fields—all of which serve as mosquito breeding grounds—mosquito-borne diseases like malaria, dengue, filariasis, chikungunya, and other illnesses are serious public health concerns in these nations (Das *et al*., 2015).

The WHO has designated mosquitoes as "public enemy number one" since they are the primary source of infections that cause various diseases (WHO, 1998). Synthetic insecticides negatively impact the ecology (Mahanta *et al*., 2017).

Products made from plants, particularly essential oils, have drawn much interest since they are a rich source of bioactive components that effectively inhibit mosquito development stages and naturally biodegrade into non-toxic products (Mahanta *et al*., 2017).

Larviciding is an efficient way to lower mosquito populations before adulthood, as the movement of mosquitoes is very slow during the larval stage,with effective control (osanloo *et al*., 2017 ; Ishtiquetal *et al*., 2019).

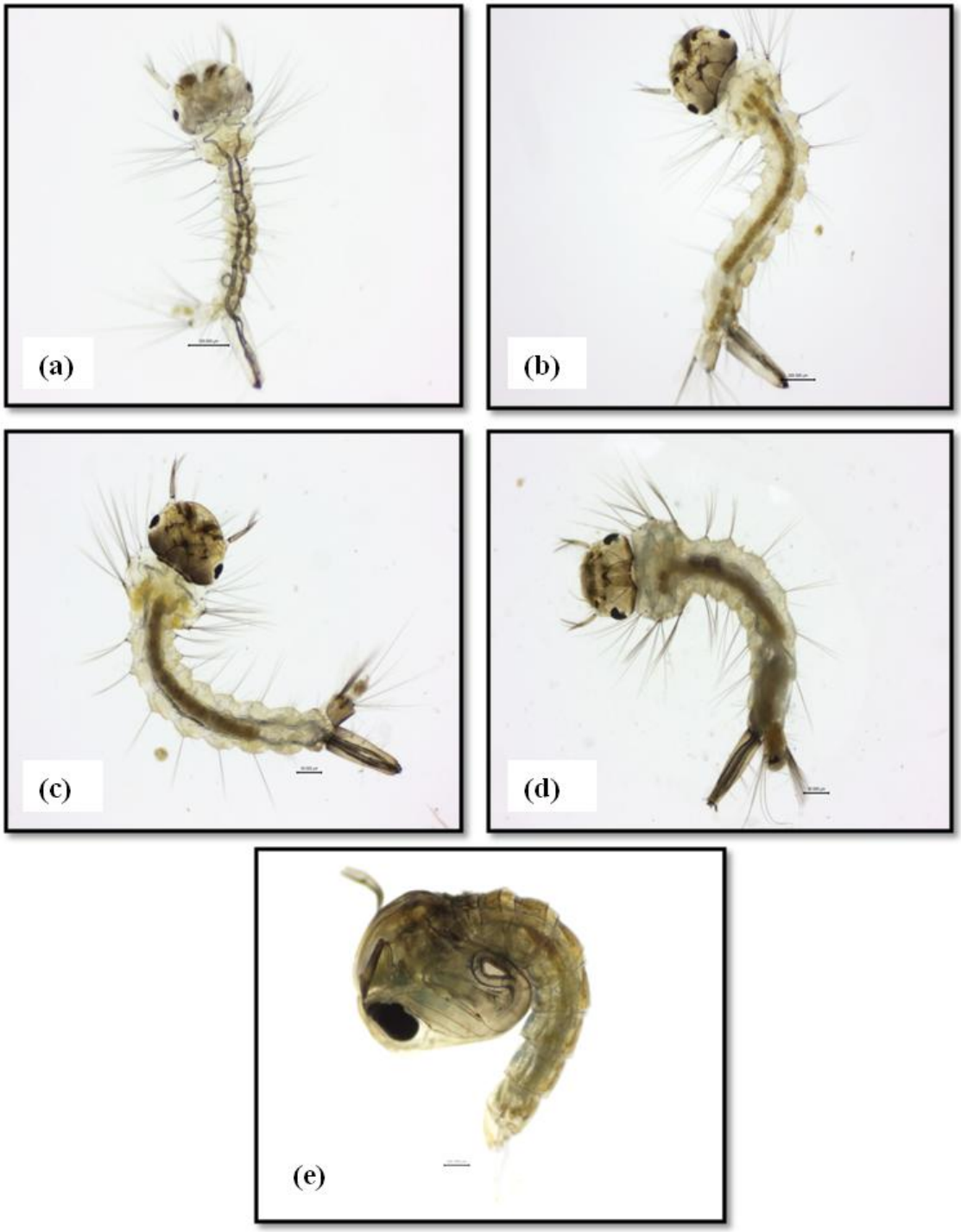


PLATE 1.5 : Mosquito larval stages of *Culex quinquefasciatus* species, (a) 1st Instar larvae, (b) 2nd Instar larvae, (c) 3rd Instar larvae, (d) 4th Instar larvae, (e) Pupa stage

1.2. AIM AND OBJECTIVES

AIM: Anatomical investigation and isolation of leaf essential oils from *Cinnamomum zeylanicum* and *Murraya koengii* for evaluation of their antioxidant potential and mosquito larvicidal activity

OBJECTIVES: Most plants from Lauraceae and Rutaceae are economically important. Members of the Lauraceae and Rutaceae families have significant value due to their various uses in industries such as food, medicine, and cosmetics, and they also have antioxidant, antimicrobial, and insecticidal properties.

Cinnamomum zeylanicum is a tropical evergreen tree that is indigenous to Sri Lanka. It is commonly referred to as natural cinnamon or Ceylon cinnamon. It is well-known for its tasty and fragrant bark, frequently used as a spice in traditional medicine and cookery. Since ancient times, Ceylon cinnamon, also known as genuine cinnamon, has been used in various traditional methods and is being used today in several sectors.

M. koenigii is a native of India, Sri Lanka, and other South Asian nations. It is a member of the Rutaceae family and is also referred to as the curry-leaf tree. It is widespread throughout the Indian subcontinent and has a similar fragrant character to deciduous shrubs and trees.

The present study was conducted to understand the plant species' anatomical characterization and antioxidant activity. The essential oil was extracted from *C. zeylanicum* and *M. koenigii* leaves. Also, the larvicidal activity was studied using essential oil.

The specific objectives of this study are as follows:

1. Collection of different parts of *Cinnamomum zeylanicum* and *Murraya koengii* for anatomical characterization.

2. Extraction and isolation of the leaf essential oil from the selected plant species.

3. Evaluation of the Antioxidant potential of selected plant species.

4. Determination of larvicidal activity of essential oils on selected mosquito species

1.3. HYPOTHESIS / RESEARCH QUESTION

The hypothesis for the study could be as follows: The anatomical features of the two plant species significantly influence the composition of their essential oils, which in turn affects the biological activities such as antioxidant and larvicidal properties. Essential oils from *Cinnamomum zeylanicum* will exhibit higher antioxidant and larvicidal activities than those from *Murraya koenigii* due to differences in their chemical compositions and the specific active components present in each oil. The developmental stage of the mosquito larvae may also play a role in the observed differences in larvicidal activity between the two essential oils.

1.4. SCOPE

The scope of the study encompasses the anatomical features, antioxidant properties, and larvicidal activities of leaf essential oils from two plant species, *Cinnamomum zeylanicum* (family Lauraceae) and *Murraya koenigii* (family Rutaceae). The research explores how the distinct anatomical features, such as the presence of oil glands, stone cells, and oxalate crystals, contribute to the chemical compositions of the essential oils derived from these plants and how these oils perform against *Culex quinquefasciatus* larvae.

CHAPTER 2: LITERATURE REVIEW

The global market trades more than 250 different varieties of essential oils. They are found in small amounts in specific plant structures like resin ducts, glandular trichomes, and oil cells. They are recognized for their volatile nature—the chemical components of an essential oil to determine its purity. A quick overview of the pertinent literature on the subject was conducted to comprehend the various facts of the study.

2.1. ANATOMY

The anatomical studies of vegetative organs of plant species can provide additional data regarding morphological characteristics and may help solve taxonomic issues.

A member of the Lauraceae family of plants is *Cinnamomum zeylanicum*. According to studies, *C. zeylanicum* leaves are oval, simple, and opposite, with three ribbeds starting just above the base. Young leaves have a reddish hue, which matures from light green to dark green (Hari *et al.,* 2006).

According to anatomical investigations, the radial epidermal cells of *C. zeylanicum* leaves are uniseriate and have a thick cuticle covering them (Al-Safa *et al*., 2016). The leaf's transverse portion passes through the midrib. The surfaces on top and below are both conical and wide. *C. zeylanicum* has an elongated, somewhat ellipsoidal xylem with 28–30 radial rows of vessels; collenchymatous cells comprise the lower cortex. The petiole of *C. zeylanicum* has a cordate transverse section with a tiny projection within the top surface's depression, while the stomata are anomocytic. There is a description of the structure and arrangement of mucilage and oil cells in the leaves of 150 different *Cinnamomum* species. Idioblasts can always be found in the spongy parenchyma and the palisade. Both mucilage and oil cells are typically found together,

but only one kind of cell is found in certain species. Idioblasts of both kinds have a suberized wall layer. The size of the idioblasts varies between species. form, durability, and quantity. It is possible to partially explain variations in the distribution pattern by considering the oil and mucilage cells' hypothesized similarity (Bakker *et al*., 2006).

Scharaschkin and Damayanthi (2019) claimed that the form and outline of the petiole, the presence of winged extensions, surface grooves on the upper surface, the presence or absence of trichomes, the structure of the vascular bundle, and the stone cell characters (shape, structure, pits, and fissure size) are the unique, significant, and essential taxonomic characteristics of the *Cinnamomum* petiole. According to a study on the petiole structure of *Cinnamomum* species, there are variances across species. The samples under examination exhibited notable variations in petiole morphologies. Every petiole was bifacial and had unique structural characteristics. The petiole can be classified as Type I, II, III, IV, V, VI, and VII. The complete petiole contour has seven distinct shapes in cross-section, ranging from circular/sub-circular to fairly reniform. Cross-pollination occurs in all known species of *Cinnamomum*, with synchronized protogynous dichogamy serving as the primary mechanism (Hathurusinghe *et al*., 2023).

The morphoanatomical features of the leaf and stem, as well as the trichome and secretory gland structures that may be related to the plant's medicinal properties, are described in the paper by Soundappan *et al*. (2018). Transverse slices of the leaf and stem reveal the positions of the oil glands (schizolysigenous glands) and other constituent cells. Near the epidermis are oil glands used for secretory discharge.

A study by Handral *et al*. (2010) revealed that the *Murraya koenigii* leaf exhibited an asymmetric base, dentate border, and reticulate venation. There were stomata on both sides. It was observed that the stomata were anomocytic. The uniseriate multicellular trichomes were seen on both surfaces but more frequently on the upper surface of the midrib part—the petioles measured between 20 and 30 centimeters in length.

The leaves are very rhomboid or obliquely ovate, having an acuminate obtuse or acute apex, according to Jain *et al*. (2017). Asymmetric base, dentate border, and reticulate venation characterize the leaves of the 20–30 cm long petiole. The microscopic analyses demonstrated that the stomata are distributed across the adaxial surface, that no stomata are present on the adaxial surface, and that the stomata that were found belonged to the anomocytic type. The epidermal layer of the transverse section of leaves comprises rectangular cells.

2.2. ANTIOXIDANT

M. koenigii leaf and stem essential oil was the subject of research by Iqbal *et al*. (2017). DPPH determined the antioxidant activity of both essential oils, and the results varied depending on the concentration. When comparing the essential oils of leaves and stems to ascorbic acid, the maximum activity was determined to be 78% and 59% at 100 µl, respectively.

The study by Schmidt *et al*. (2013) found that *cinnamon* essential oil exhibited the most potent inhibitory action among the antioxidants examined, with a peak of 94.42% at 8.0 µg/ml. In addition to acting as an iron chelator, the essential cinnamon oil demonstrated an intense inhibitory action on hydroxyl radicals. *Cinnamon* leaf oil

effectively reduced the synthesis of conjugated dienes and the development of secondary products from lipid peroxidation at a concentration equal to that of conventional BHT.

In order to determine antioxidant activity, Tripathi *et al*. (2018) used MKLEO. They noted that the percentage of DPPH scavenging capacity and reducing power was inversely related to the IC50 and RP50 values, meaning that the lower the IC50 and RP50 values, respectively, the higher the scavenging and reducing power rate. Mathew and Abraham (2006) found that the CLEO had free radical scavenging activity, particularly against the DPPH and ABTS radical cations. Together with hydroxyl radical scavenging activity, they also demonstrated reducing power and metal ion chelating activity. Using the linoleic acid emulsion technique, the peroxidation inhibitory activity of CLEO demonstrated excellent antioxidant efficacy.

Significant antioxidant activity of *C. zeylanicum* was identified for essential oil in the DPPH free radical scavenging assay (IC50= 234.7 µg/mL), leaf, and reducing power assay (0.907 nm in 48 µg/mL leaf essential oil), according to investigations conducted by Gogoi *et al.,* (2021).

2.3. MOSQUITO LARVICIDAL ACTIVITY

In their investigation, Seenivassan *et al*. (2019) reported that, for 24 hours, various concentrations (50, 100, 200, and 400µg/mL) of essential oils were used to screen for larvicidal efficacy against *Culex quinquefasciatus*, the primary urban filarial vector. According to the early screening results, at 400µg/mL, *Cinnamon* oil showed the most potent larvicidal efficacy, resulting in a hundred percent larval death.

The larvicidal activity of these essential oils against *Aedes aegypti* was studied by Romano *et al*. (2023). They discovered that the phenological stage more influenced the chemical composition of the essential oil than climatic conditions. Lethal concentrations of *Ae. aegypti* were not significantly altered by any of the essential oil samples.

Murraya koenigii has been discovered to have insecticidal qualities and to help control pest populations, according to a study done by Singh *et al*. (2023). The third and fourth larval instars of the *Aedes mosquito*, a significant dengue disease vector, have been tested against the leaves and stems of this plant.

At concentrations ranging from 250 ppm to 900 ppm, the acetone and petroleum ether extracts of *M. koenigii* leaves have demonstrated larvicidal action against *A. aegypti* larvae (Ajay *et al*., 2011).

2.4. ESSENTIAL OIL

According to Wang *et al*. (2008), they were able to extract the essential oil of *Cinnamomum zeylanicum*, which they had gathered from India and Sri Lanka. Hydro distillation was used to prepare the essential oil using cleavenger-style equipment.

Ismail *et al*. (2014) examined the essential oils of *Murraya koengii*, also called curry leaves, cultivated in the vicinity of Malaysia. The oil was extracted from the leaves using a traditional hydro distillation method. After nine hours of extraction time, the maximum yield from the process is expected to be 0.22%.

The essential oil from the *Cinnamomum zeylanicum* plant was extracted in this work by Wong *et al*. (2014) utilizing two different techniques: Steam distillation and

Soxhlet extraction. Steam distillation, using a separatory funnel, provided high-quality essential oil extraction. A rotary evaporator was used in the Soxhlet extraction process to create crude essential oil and then purify the extracted product. The primary ingredient in cinnamon, cinnamaldehyde, is present in high concentrations in *Cinnamon* essential oil. In the work conducted by Imen *et al*. (2019), essential oil was isolated using steam distillation, and the surface-response methodology was utilized to optimize its conditions.

2.5. GC-MS ANALYSIS

Using a GC-2010 gas chromatography system (Shimadzu, Suzhou, China) outfitted with a GCMS-QP2010 Plus mass spectrometer, Wang *et al*. (2008) conducted GC/MS analysis following the methodology of Wu *et al*. (2008). The separation was performed using a Rxi-5MS capillary column (30 m × 0.25 mm, i.e., film thickness 0.25 μm, Shimadzu, Japan)—the volatile compounds extracted from *C. zeylanicum* essential oil. Aldehydes, alcohols, alkanes, alkenes, ketones, ethers, and sulfides were among the twenty-two volatile substances found. Rather than trans-cinnamaldehyde (16.25%), eugenol (79.75%) was the predominant volatile component.

Iqbal *et al*. (2017) reported that GC-MS analysis was carried out for chemical components. α-pinene 2.7%, spathulenol 19.10%, caryophyllene 18.4%, caryophyllene oxide 17.6%, α-caryophyllene 10.2%, β-eleminene 9.1%, and germacerene 4.3% were the main components of the leaf essential oil. In contrast, the significant components of the stem essential oil were α-pinene 4.0%, β-terpineol 2.9%, α-caryophyllene 7.2%, epiglubolol 5.4%, spathulenol 6.6%, clarene oxide 1.2%, heptatrioctanol 2.3%, and phytol 1.9%, respectively.

According to Imen *et al*. (2017), the GC-MS study of volatile elements showed the existence of 4 unknown and 7 identified main components. The components that have been discovered and those that have not are, respectively, 99.1089% and 0.1932%. pinene (3.4155%), caryophyllene (15.8231%), sabinene (59.7176%), pinene (10.9345%), phellandrene (5.2556%), terpinene (2.7974%), and myrcene (1.8632%) were the compounds that were found.

CHAPTER 3: MATERIALS AND METHODS

3.1. COLLECTION OF PLANT SAMPLES

The fresh Samples of *Cinnamomum zeylanicum* and *Murraya koenigii* were collected from the natural habitat of Sanguem, Goa. Mature and healthy leaves were collected in Zip-lock Polythene bags and brought to the laboratory for further studies.

Table 3.1: Plant collection sites and their GPS coordinates.

Sr. no	Plant name	Collection site and location
1	*Cinnamomum zeylanicum* Blume	Uguem, Sanguem- Goa Lat 15.21 71 84° Long 74.17 44 32°
2	*Murraya koenigii* (L.) Spreng	Sanguem Goa Lat 15.22 43 84° Long 74.14 54 66°

3.2. ANATOMICAL STUDIES (Hari *et al*., 2021)

Anatomical studies of selected plant species were conducted to understand the leaf, petiole, and stem structure. Fresh leaves, petiole, and stems of *C. zeylanicum* and *M. koenigii* were brought to the laboratory. Free-hand thin sections of all the above parts were taken and stained with 0.1% safranin stain. Then, the sections were thoroughly washed with distilled water and mounted on the glass slide using 10% glycerine. Sections were observed and photographed using bright-field microscopy.

The following standard procedure was adopted for the preparation of stains.

PREPARATION OF SAFARANIN: 0.1% w/v Safaranin was prepared by dissolving 0.1g of Safaranin in 100 mL of distilled water. Thin free-hand sections were placed in distilled water and then in the stain for 2-3 minutes. The excess stain was removed by washing the sections in distilled water. Then, the sections were mounted on a clean,

stain-free glass slide using 10% glycerine and observed using a bright-field Nikon Eclipse E200 microscope under 4X,10X, and 40X magnification.

3.3. ISOLATION OF ESSENTIAL OIL (Zafar *et al*., 2017)

Fresh leaves of both plants were cut separately into small pieces.100g of each sample and 1000 ml of distilled water were subjected to hydro-distillation using a Clevenger-type apparatus with 5L capacity for 3 hours at 90°C. The extraction of essential oil from its aqueous phase was obtained using n-hexane. The extract was dried over anhydrous sodium sulphate and stored in vials at 4°C until further analysis. **(Plate 3.1)**.

Essential oil yield was calculated using the formula:

$$\textbf{Yield (\%)} = \frac{\textbf{Weight of oil extracted (g)}}{\textbf{Weight of the sample taken}} \times 100$$

3.4. ANTIOXIDANT ACTIVITY (Iqbal *et al*., 2017)

The antioxidant studies on leaf essential oil were carried out on the selected plant species using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.

A. Preparation of DPPH: The stock solution was prepared by dissolving 24mg of DPPH in 100 mL of ethanol in the dark and stored in an Amber-coloured bottle. The working solution was prepared by adding 10mL of the Stock solution to 45mL of ethanol.

B. Preparation of L-ascorbic acid solution: 10mg of ascorbic acid was dissolved in 10 mL of distilled water. Serial dilution was performed to prepare the solution with different concentra(12.5µg/mL-200µg/mL)

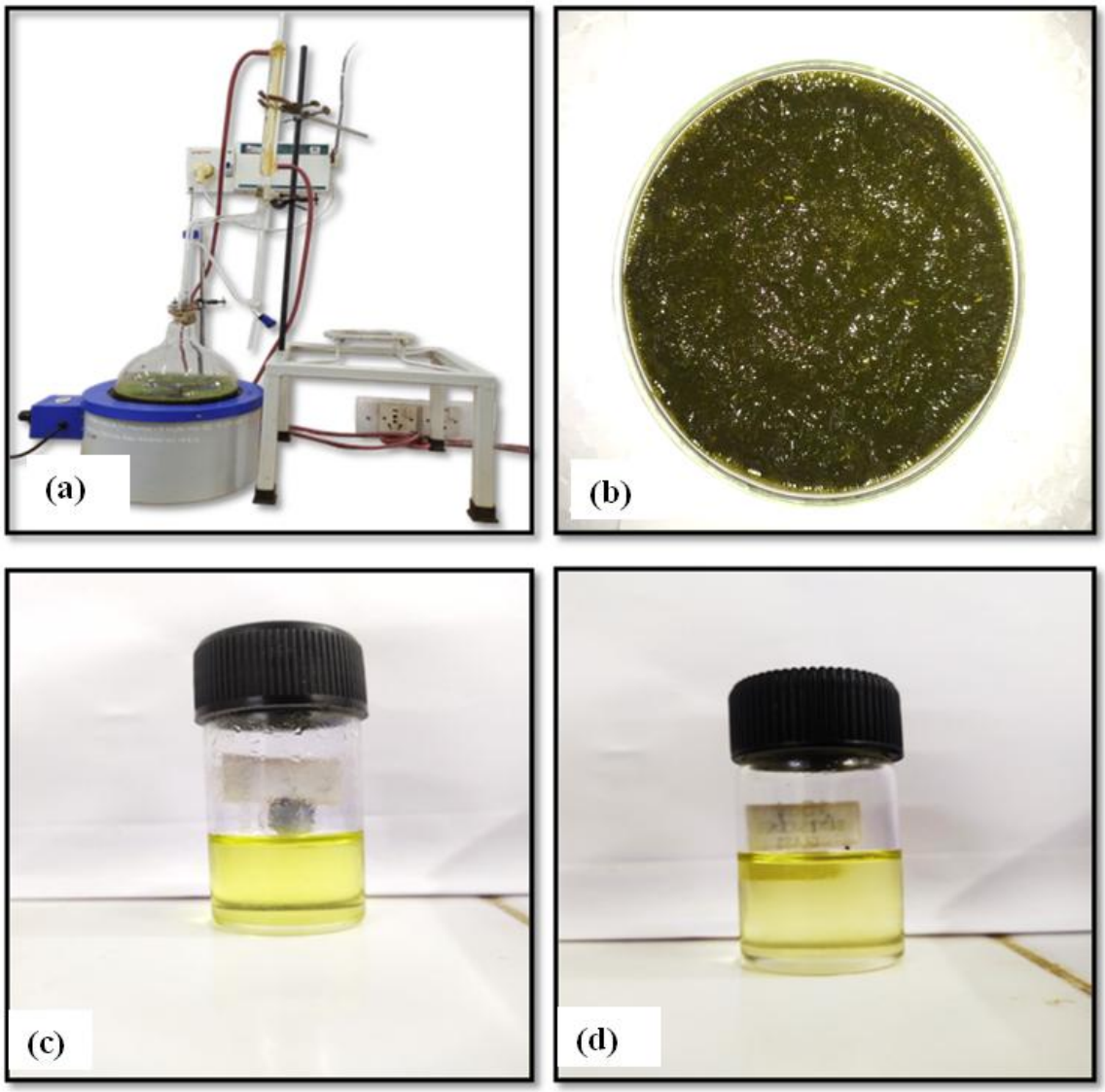


PLATE 3.1 : (a) Clevenger apparatus , (b) Plant extract , (c) Essential oil extracted from the leaves of *M. koenigii* , (d) Essential oil extracted from the leaves of *C. zeylanicum*

C. Preparation of Test solution:1:1 ratio of essential oil with ethanol was prepared and used as stock solution. Serial dilution was performed to prepare the solution with different concentrations (12.5µg/mL-200µg/mL).

D. Preparation of control: 3mL DPPH was used as a negative control.

In the reaction mixture, 3mL of DPPH working solution was added to 100µl of a sample of different concentrations and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517nm. The following equation calculated the percent inhibition.

$(A0-A1)/A0 \times 100$

Here, Ao is the absorbance of the control, and As is the absorbance of the extract. The antioxidant assay was performed in triplicate, and the mean value was expressed.

3.5. MOSQUITO LARVICIDAL ACTIVITY (Cheng *et al*., 2004)

3.5.1. Source of mosquito larvae

Mosquito larvae of *Culex quinquefasciatus* were screened for larvicidal activity. The ICMR-National Institute of Malaria Research, Campal, Panjim, Goa, maintained the cyclic colony of this mosquito species in their insectary. 3rd instar larvae were used for the bioassay. Fish flake was used in the laboratory to keep mosquito larvae.

3.5.2. Larval Bioassay

Detection of susceptibility of larvae to the essential oil of *C. zeylanicum* and *M. koenigii* leaves. Twenty larvae of *Culex quinquefasciatus* were selected and placed in separate bowls containing 100ml distilled water. The essential oil was dissolved in Dimethyl

sulfoxide (DMSO) to prepare various concentrations (50ppm, 100ppm, 150ppm, 200ppm, 300ppm).

The control was prepared using 20µl of DMSO in 100ml of distilled water. For each concentration, four replicates were maintained. Larval mortality was assessed by direct observations of larvae movements 24 and 48 hours after exposure at room temperature.

The percentage of mortality was calculated using the following formula.

$$\textbf{Percentage mortality} = \frac{\textbf{Average of dead larva}}{\textbf{Total number of larvae exposed/treated}} \times 100$$

3.5.3. Calculation of LC_{50} and LC_{90} Value

The activity of essential oil against test mosquito larvae in terms of LC_{50} and LC_{90} values was calculated using SPSS Software.

CHAPTER 4: RESULTS AND DISCUSSION

4.1. ANATOMICAL STUDIES

4.1.1. *Cinnamomum zeylanicum* Blume

4.1.1.1. Leaf Anatomy

Leaves simple, opposite, ovate or ovate-lanceolate—glabrous leaves. The young leaves are reddish, turning to lighter green and deep green. The transverse section of the lamina showed the presence of a uniseriate epidermal layer, which is covered with a thick cuticle. Similar to that observed by Al-Safa *et al*. (2016). A sclerenchymatous ring in the midrib of the leaves surrounds the vascular bundle. Lamina consists of a single layer of vertically elongated parenchyma cells. The spongy parenchyma cells show sclerification, oil, and mucilage cells (Hari *et al*., 2021). Oil glands are found embedded in the mesophyll layer in the form of secretory cavities **(Plate 4.1-d)**. The vascular strands are present in the leaf section. The presence of sclerenchymatous bundle sheath extensions of the vertical transcurrent lower-order veins. Transfusion tissues are present in the lamina (Ricardo *et al*., 2018). The stomata are anomocytic.

4.1.1.2. Petiole Anatomy

The transverse section of the petiole is reniform shaped. The adaxial surface of the petiole is convex, and the abaxial surface is concave. The cross-section of the petiole shows the presence of single-layer epidermis cells that are barrel-shaped and covered with a thick layer of cuticle. Presence of parenchymatous cells in the cortical region. Volatile oils containing cavities are present below the epidermal layer. There is a discontinuous layer of sclerenchymatous around the vascular bundle. The shape of the vascular bundle is an open arc with a slight curve towards the center, similar to that observed by (Pushpa and Tanya 2019). Stone cells (sclereids) were observed in the cortex, mainly around the vascular bundle **(Plate 4.2-a,b,c)**.

4.1.1.3. Stem Anatomy

The stem of *C. zeylanicum* is thicker. The transverse section of the stem shows the presence of a uniseerate epidermis with a thick-walled cuticle. The stem section shows the presence of secretory cavities such as oil glands. The cortical region shows the presence of brown tannin deposits of sclerenchyma in the pericycle region, which is the characteristic feature of the stem (Shylaja and Manilal, 1992). Vascular bundles are conjoint collateral, and the Secondary xylem forms the bulk of the stem, whereas the primary xylem is present towards the pith (Hari *et al*., 2021). Mucilage cells are in the pith region **(Plate 4.2-d,e,f).**

4.1.2. *Murraya koenigii* L. Spreng

4.1.2.1. Leaf Anatomy

The transverse section of the leaf of *M. koenigii* is flat towards the adaxial surface and ridged towards the abaxial surface. Unicellular, non-glandular trichomes arise from the abaxial epidermis. The adaxial hypodermis is bi-seriate or tri-seriate and comprises isodiametric collenchymatous cells. Schizolysigenous oil glands are present close to the upper epidermis in the leaf section where oil is stored. Both the surfaces of collenchymatous cells show the presence of single and twinned rhomboid calcium oxalate crystals. The parenchymatous cells in the ground tissue are isodiametric, thick-walled, and loosely arranged. The adaxial xylem and abaxial phloem vascular bundle form an arc (Chandrul and Singh, 2016). Secretory cells and lysigenous cavities were found in the leaf section. Anisocytic stomata are observed **(Plate 4.3)**

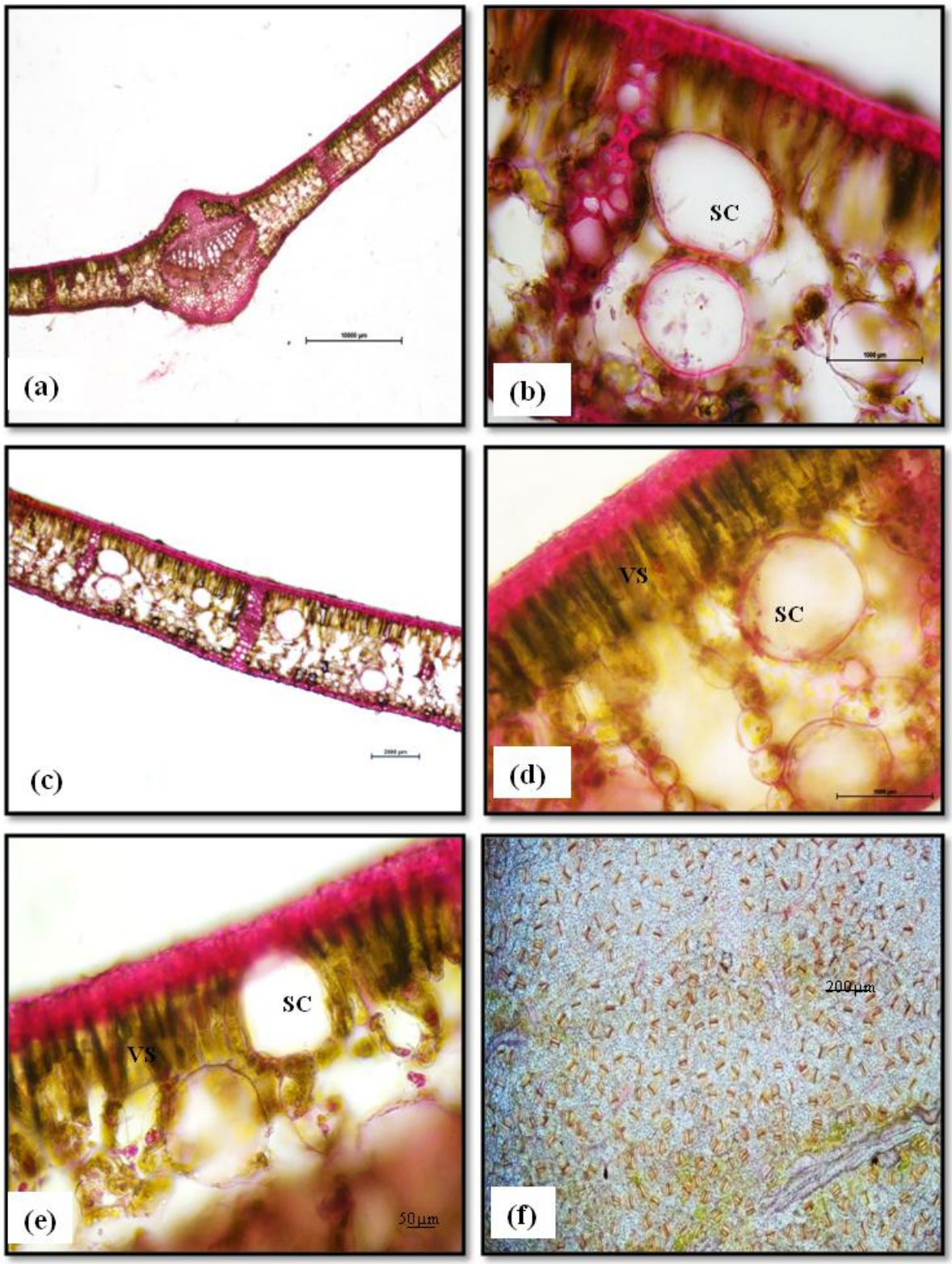


PLATE 4.1 : Anatomy of *C. zeylanicum* leaf, (a) Overview of leaf (4X), (b) T.S. Of leaf showing presence of secretory cavities and oil glands along with vascular strands (c) leaf section showing oil glands and secretorty cavities (d-e) presence of secretory cavities and vasular strands , (f) Anomocytic stomata(10X).
SC= secretory cells

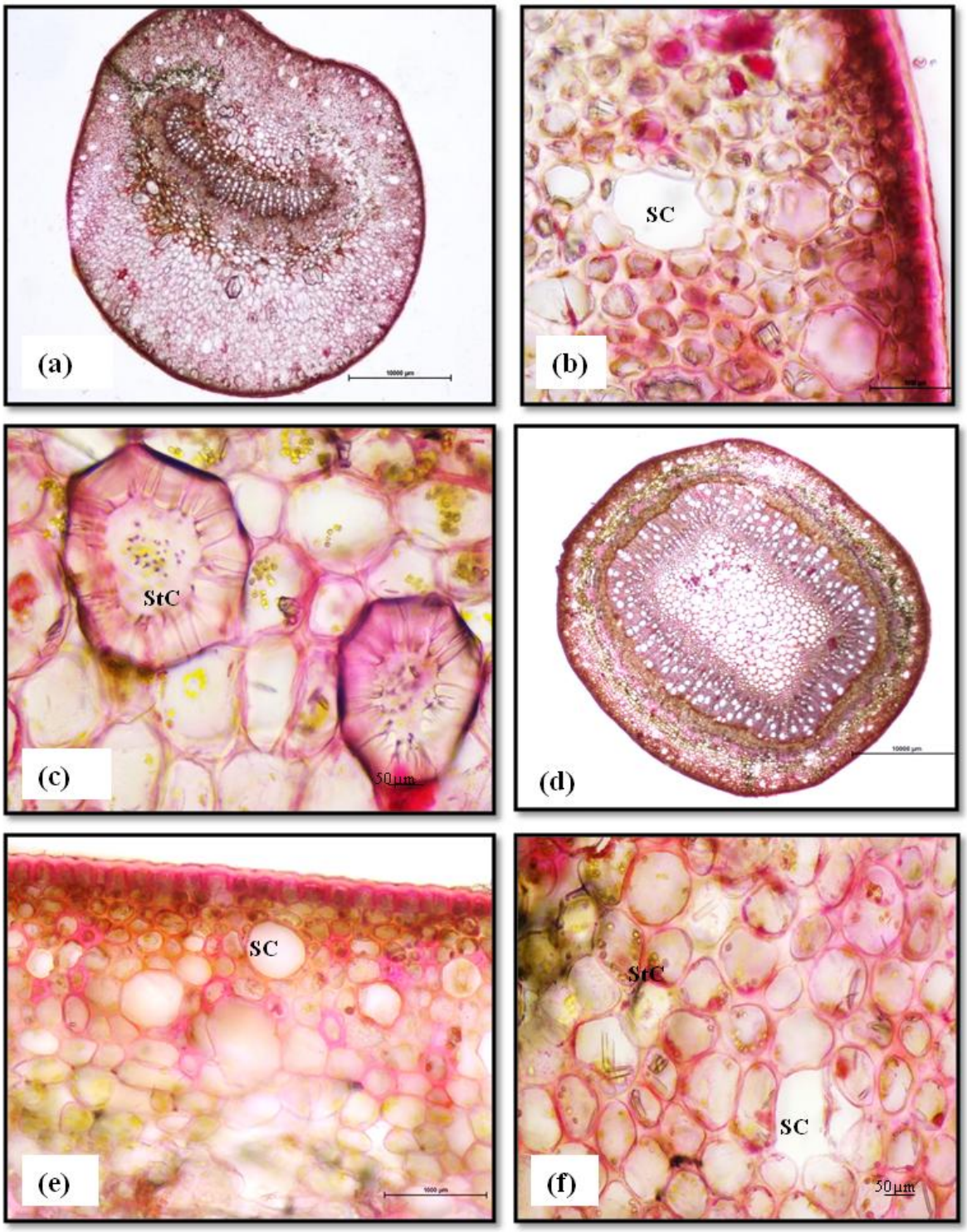


PLATE 4.2 : Anatomy of *C. zeylanicum* petiole and stem. (a)Overview of petiole (4X), (b) Section showing presence of secretory cavities, (c)Section of petiole showing presence of stone cells, (d)Overview of *C. zeylanicum* stem (4X), (e)Section of stem showing presence of secretory cavities, (f)Showing presence of secretory cavities and stone cells.
SC= secretory cells ,StC = stone cells TR= trichome

4.1.2.2. Petiole Anatomy

The transverse section of the petiole shows the presence of a layer of epidermis covered with a cuticle. Petiole shows the presence of secretory cavities, which contain oil glands. The section shows the presence of vascular bundles surrounded by parenchymatous cells. Also, the petiole shows a non-glandular type of trichomes on the surface of the petiole **(Plate 4.4)**.

4.1.2.3. Stem Anatomy

The epidermis's transverse section shows a single-layer epidermis followed by a thick cuticle and cortex region. In the center of the section, a thin-walled parenchymatous pith is present, and vascular bundles are arranged between the pith and cortex. The stem section shows the presence of schizolysigenous oil glands and calcium oxalate crystals in the cortex region, similar to what was observed by Soundappan (2018). The transverse section of the epidermis shows the presence of a single-layer epidermis. Also, the stem shows the presence of non-glandular trichomes and secretory cavities **(Plate 4.4)**.

4.2. ESSENTIAL OIL EXTRACTION

The essential oil of fresh leaves of *C. zeylanicum* and *M. koenigii* was extracted using a Clevenger hydro-distillation apparatus. The oil yield from *C. zeylanicum* was 1.65%, whereas *M. koenigii* yielded 0.3%. The essential oil extracted from leaves of *C. zeylanicum* was pale yellow to amber yellow with a strong, spicy aroma and a spicy, pungent taste, whereas *M. koenigii* leaf essential oil was pale yellow in colour with a citrusy, spicy, slightly bitter aroma and a bitter, pungent taste

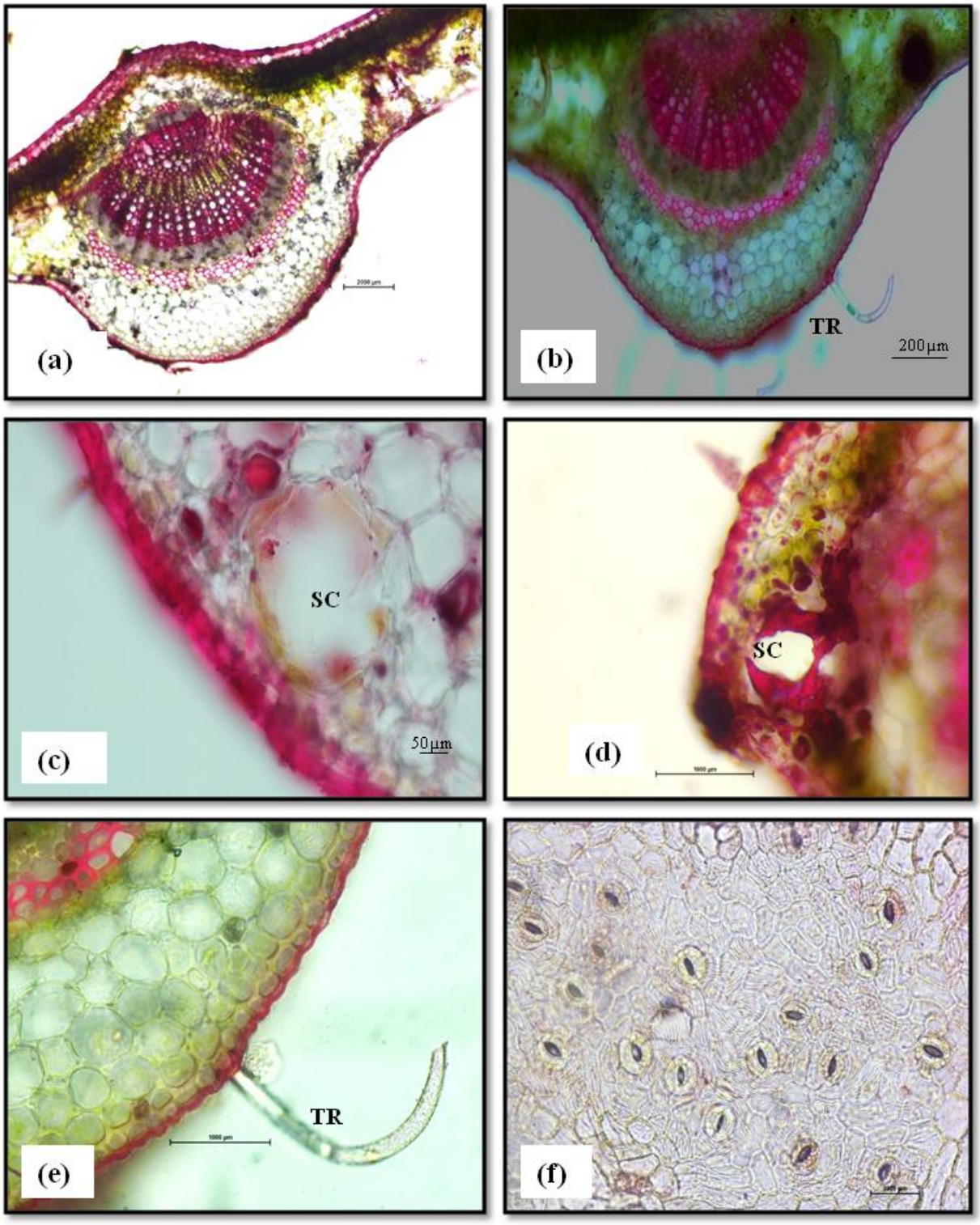


PLATE 4.3 : Anatomy of *M. koenigi* leaf , (a) Overview of leaf(10X) , (b) Leaf section showing presence of trichome along with epidermal layer , (c-d) section showing the presence of Schizolysigenous oil glands are present close to the upper epidermis , (e) Trichome (40X) , (f) Leaf showing anisocytic stomata.

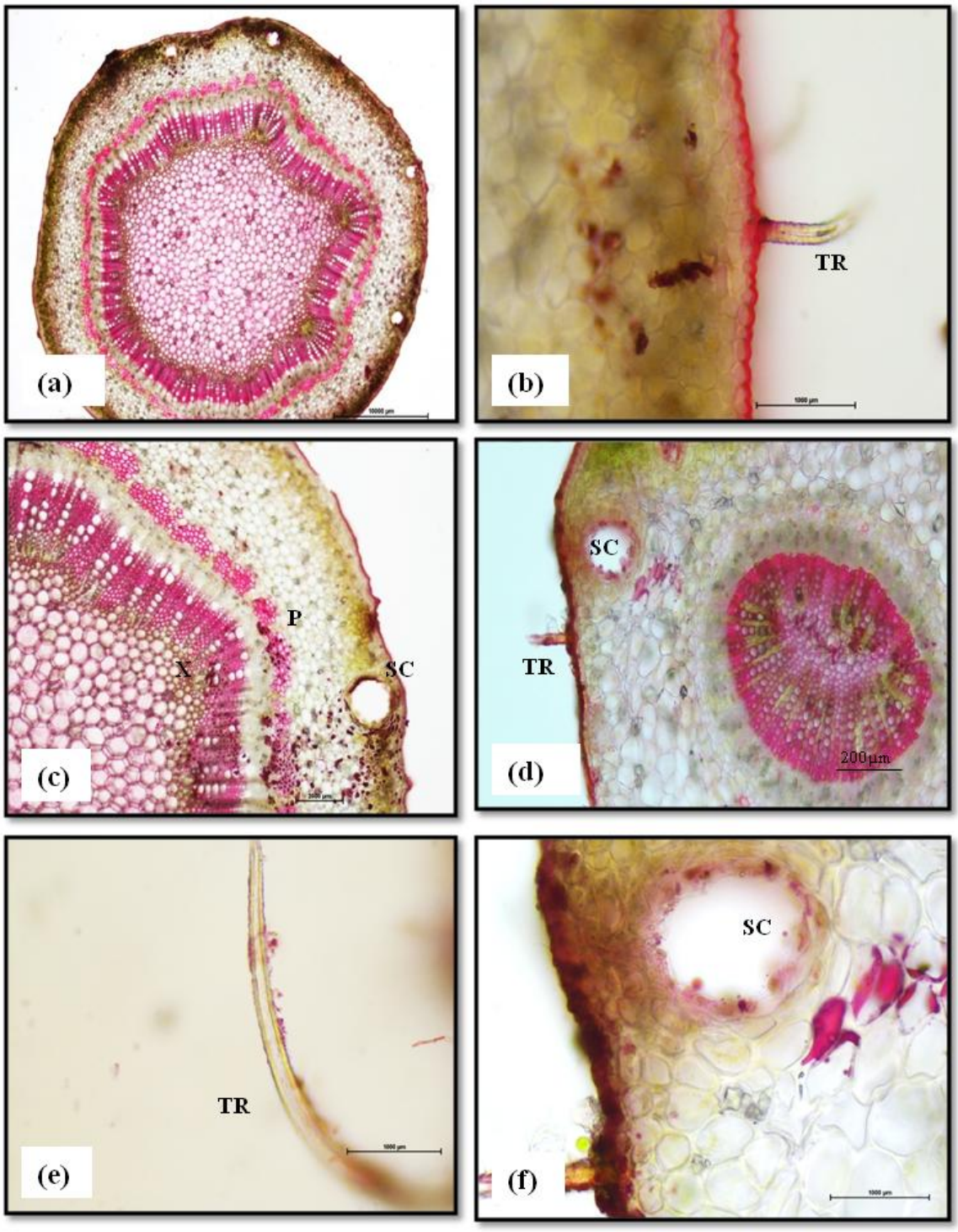


PLATE 4.4 : Anatomy of *M. koenigii* stem and petiole (a) Overview of stem (4X), (b) Section of stem showing trichome along with epidermal layer , (c) Section of stem showing presence of schizolysigenous oil glands and calcium oxalate crystals , (d) Petiole showing presence of Trichome along with epidermal layer (10X) , (e) Petiole showing presence of trichome , (f)T.S. of Petiole showing presence of secretory gland (40X)

SC= Secretoty gland, TR=Trichome , X=Xylem, P= Pholem

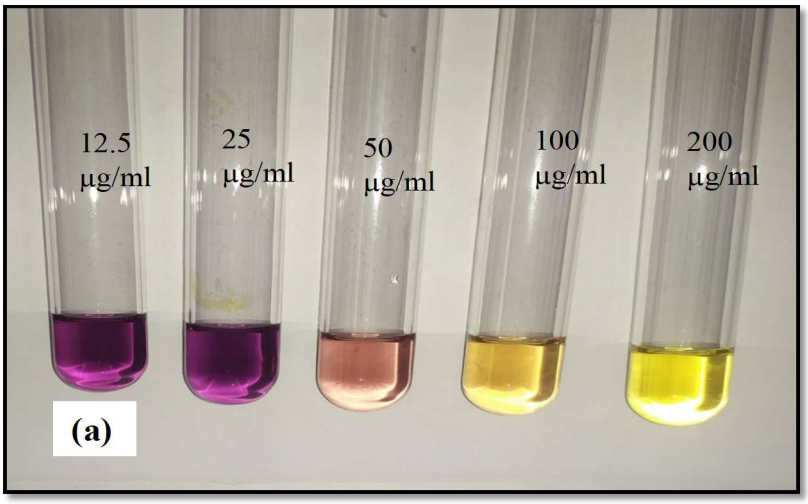
The essential oil yield from plants can vary widely depending on factors such as plant species, plant part used for extraction, the method used for extraction, environmental conditions, age, and plant health condition. Also, the yield may vary depending upon the leaves used, whether the young leaves are used or the older ones are used, although extreme care was taken while harvesting the leaves.

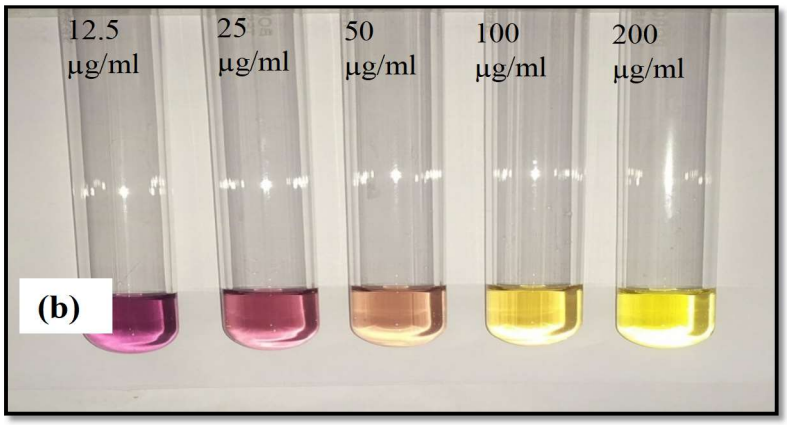
Because of their biological properties, essential oils have been employed for centuries in a wide variety of traditional medical systems around the globe. Though there are few human studies, essential oils have the potential to be safe and effective phytotherapeutic agents due to their demonstrated antimicrobial, antioxidant, anti-inflammatory, and anticancer activities in a variety of cell and animal models, as well as the elucidation of their mechanism of action and pharmacological targets (Javad *et al*., 2017).

4.3. Antioxidant activity

Antioxidants are essential for preventing illnesses in humans. They can act as reducing agents, pro-oxidant metal complexes, free radical scavengers, and singlet oxygen quenchers (Raja and Khan, 2017). Free radicals can lessen the oxidative damage linked to various illnesses, including cancer, AIDS, cataracts, and neurological conditions.

Cinnamomum zeylanicum and *Murraya koenigii* leaf essential oil were subjected to 1, 1-diphenyl-2-picrylhyrazyl (DPPH) test to determine their potential as free radical scavengers. This method measures the decrease in the absorption of the DPPH solution after adding an antioxidant at 517 nm **(Table 4.1)**. The standard was ascorbic acid. The experiment was carried out in triplicate **(Plate 4.5**





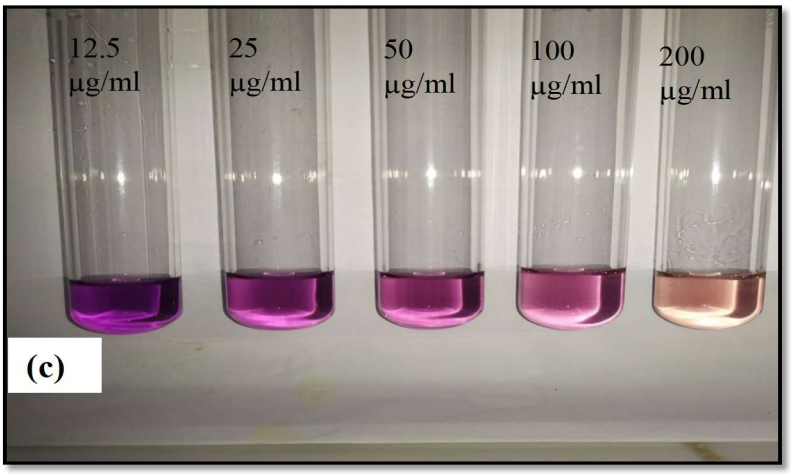


PLATE 4.5 :Antioxidant studies using DPPH method: (a) L-Ascorbic acid as standard, (b) *C. zeylznicum.* (c) *M. koenigii*

As the quantity of oil increased, the absorbance decreased as the antioxidant reacted with the DPPH to reduce it to DPPH-H. This demonstrated the antioxidant component in the oil's capacity to scavenge hydrogen-donating compounds.

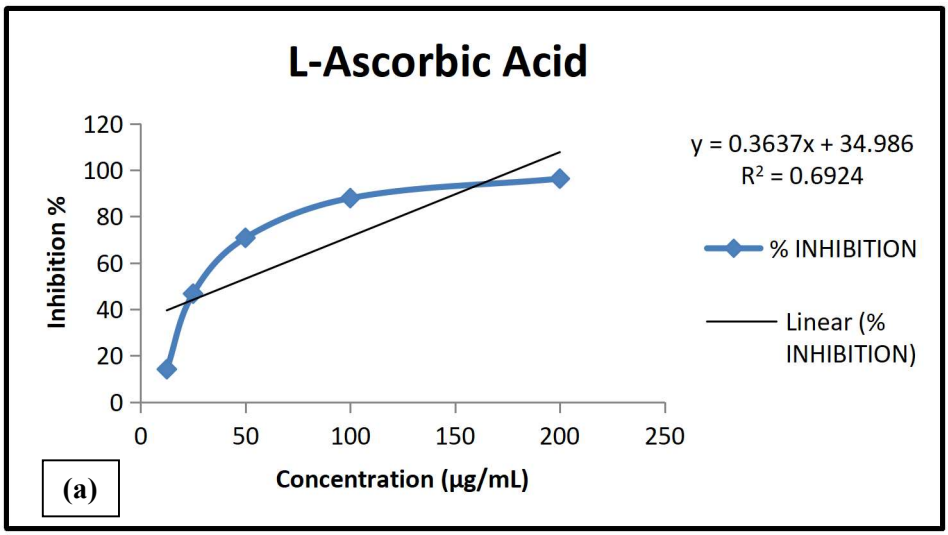
The maximum free radical scavenging activity was observed in the leaf essential oil of *Cinnamomum zeylanium* with an IC50 value of 39.42µg/mL. In contrast, the free radical scavenging activity observed in the leaf essential oil of *M. koenigii* had an IC_{50} value of 171.85µg/mL, respectively. This shows that the *C. zeylanicum* has the highest anti-oxidant activity **(Fig 4.3 Table 4.2)**.

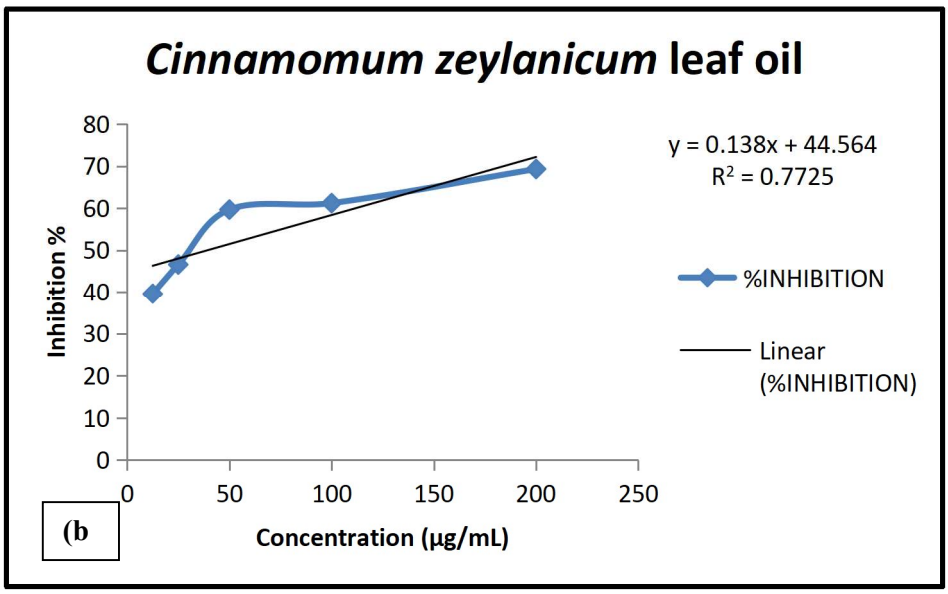
Leopold *et al*. (2006) concluded that *Cinnamomum* essential oil had the highest inhibitory antioxidant activity compared to the others, with an IC50 value of 0.245 µg/ml. According to the study conducted by Soghra *et al.* (2015), the concentration of CEO resulting in 50% inhibition of the free radical (IC_{50}) was 79.54 µL.

DPPH and ascorbic acid were used as standards in the study by Zafar *et al*. (2017) to assess the antioxidant activity of *M. koenigii* leaf essential oil at a concentration of 100 ul, they were 78% active. Less effective leaf essential oil of *M. koenigii* may be caused by this, as the antioxidant activity primarily depends on the oil's phenolic levels.

Table 4.1: DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and leaf essential oil of *C. zeylanicum* and *M. koenigii.*

Sr. No	Concentration (µg/mL)	L-Ascorbic acid (µg/mL)	Leaves essential oil (µg/mL)	
			C. zeylanicum	***M. koenigii***
1	**12.5**	14.15±0.001	39.57±0.005	3.52±0.002
2	**25**	46.71±0.001	46.58±0.006	9.09±0.002
3	**50**	70.80±0.001	59.65±0.004	21.69±0.001
4	**100**	87.91±0.001	61.19±0.004	40.71±0.002
5	**200**	96.31±0.001	69.30±0.004	52.66±0.001





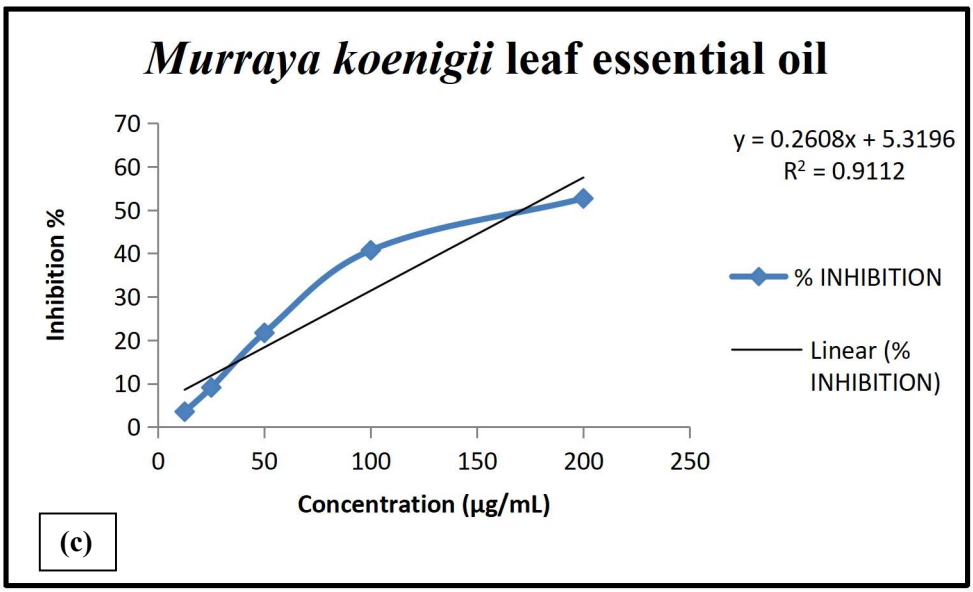


Figure 4. 1: DPPH Radical Scavenging activity of (a) L-Ascorbic acid, (b) *Cinnamomum zeylanicum* leaf essential oil, (c) *Murraya koenigii* leaf essential oil.

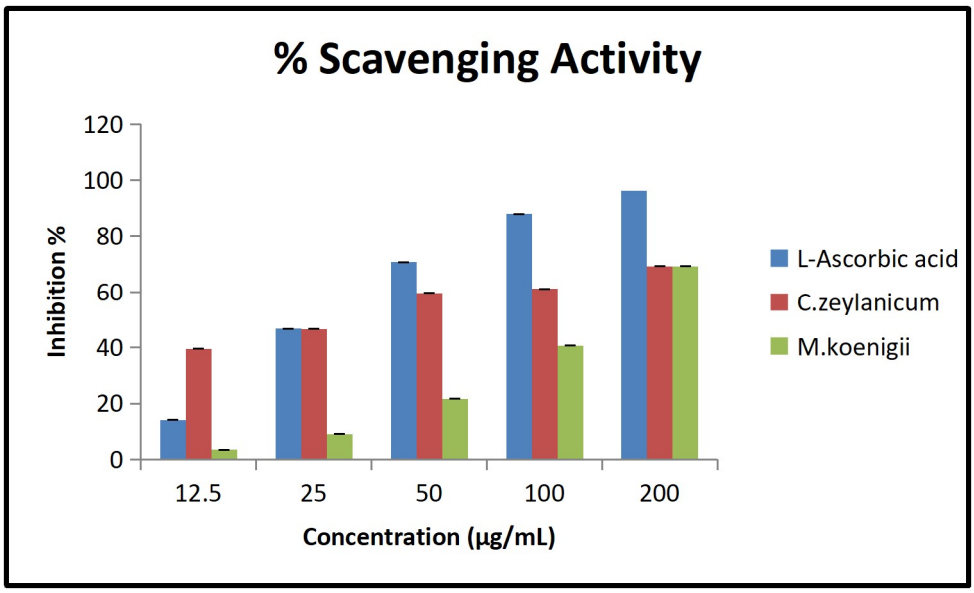


Figure 4.2: Percentage Scavenging activity of L-Ascorbic acid (standard) with *C. zeylanicum* leaf essential oil and *M. koenigii* leaf essential oil

Table 4.2 : IC_{50} value of leaf essential oil of *C. zeylanicum* and *M. koenigii*

Sr.No	Plant Name	IC_{50} Value of leaf essential oil (µg/ml)
1	*Cinnamomum zeylanicum*	39.42
2	*Murraya koenigii*	171.85

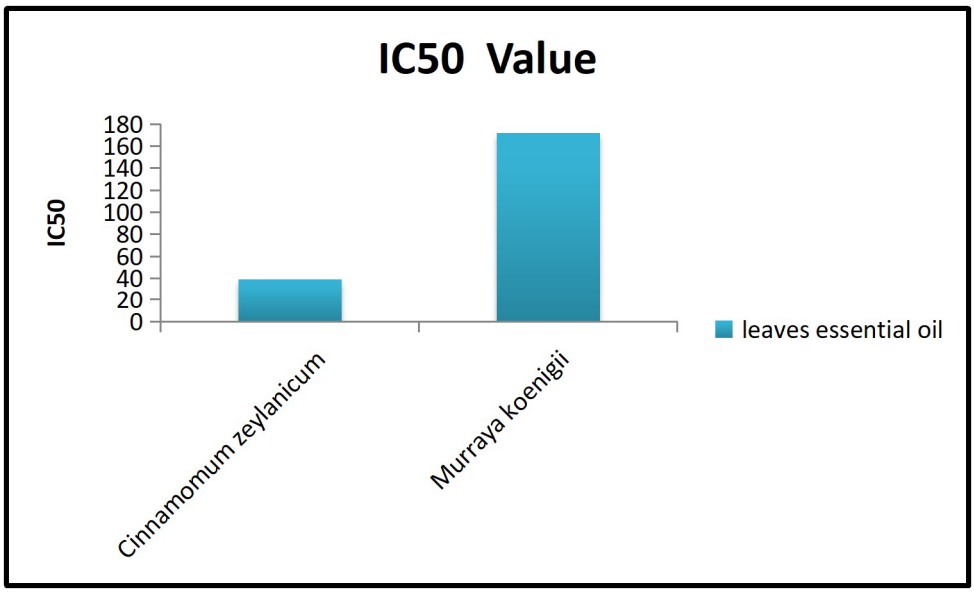


Figure 4.3: IC_{50} Value of *C. zeylanicum* and *M. koenigii*

4.4. Mosquito Larvicidal Activity

Given plants' abundance of bioactive compounds, various dangerous insects may be managed by using them as an excellent substitute for synthetic pesticides. Natural insecticides made from plants are primarily made of extracts or essential oils and have fewer harmful effects on people and the environment (Mahanta *et al*., 2017).

In our study, mosquito larvicidal activity was performed using the essential oils extracted from the leaves of *C. zeylanicum* and *M. koenigii*. Four different oil concentrations were prepared and tested against 3rd instar larvae of *Culex quinquefasciatus.* 20 larvae of 3rd instar larvae were selected for bioassay as they grow properly with a healthy build-up and at their **(Table 4.3, 4.7)**. 1st and 2nd instar larvae are two small, and the 4th stage can form as pupae within 24 hrs. The exposure time and oil concentration were shown to be directly associated with larval mortality in the study. After 24 hours **(Table 4.4, 4.8)** and 48 hours **(Table 4.5, 4.9)** of exposure, larval mortality was measured **.**

The LC_{50} value of essential oil extracted from the leaves of *Cinnamomum zeylanicum* against *Culex quinquefasciatus* was 67.31 ppm and 56.29 ppm at 24 hours and 48 hours, respectively, while the LC_{90} value was 149.91ppm and 133.22 at 24 hours and 48 hours respectively **(Table 4.6)**.

The LC_{50} value of essential oil extracted from the leaves of *M. koenigii* against *Culex quinquefasciatus* was 163.11ppm and 111.32ppm at 24 hours and 48 hours, respectively, while the LC_{90} value was 917.05ppm and 496.95ppm at 24 hours and 48 hours respectively **(Table 4.10)**.

It was discovered that the essential oil of leaves of *C. zeylanicum* was found to be better against *Culex quinquefasciatus* larvae as compared to the essential oil of leaves of

M. koenigii. The essential oil of leaves of *C. zeylanicum* was found to be more toxic against *Culex quinquefasciatus* larvae than the essential oil of *M. koenigii.* **(Plate 4.6)**

In the essential oil derived from *C. zeylanicum* leaves, eugenol was a significant constituent and was more active against *C. quinquefasciatus* (Radhika and Kosmulalage, 2005). According to the Radhika and Kosmulalage, 2005 report, it was revealed that eugenol was the principal constituent in the leaf essential oil of the *C. zeylanicum*, which showed more larvicidal activity. Recently, essential oils have drawn much interest as possible bioactive substances that combat insects.

Mosquitoes react differently to essential oils, and it has been shown that different mosquito developmental stages might have varying sensitivity levels to the same chemical. Insects can absorb essential oil through their skin, inhale it, or digest

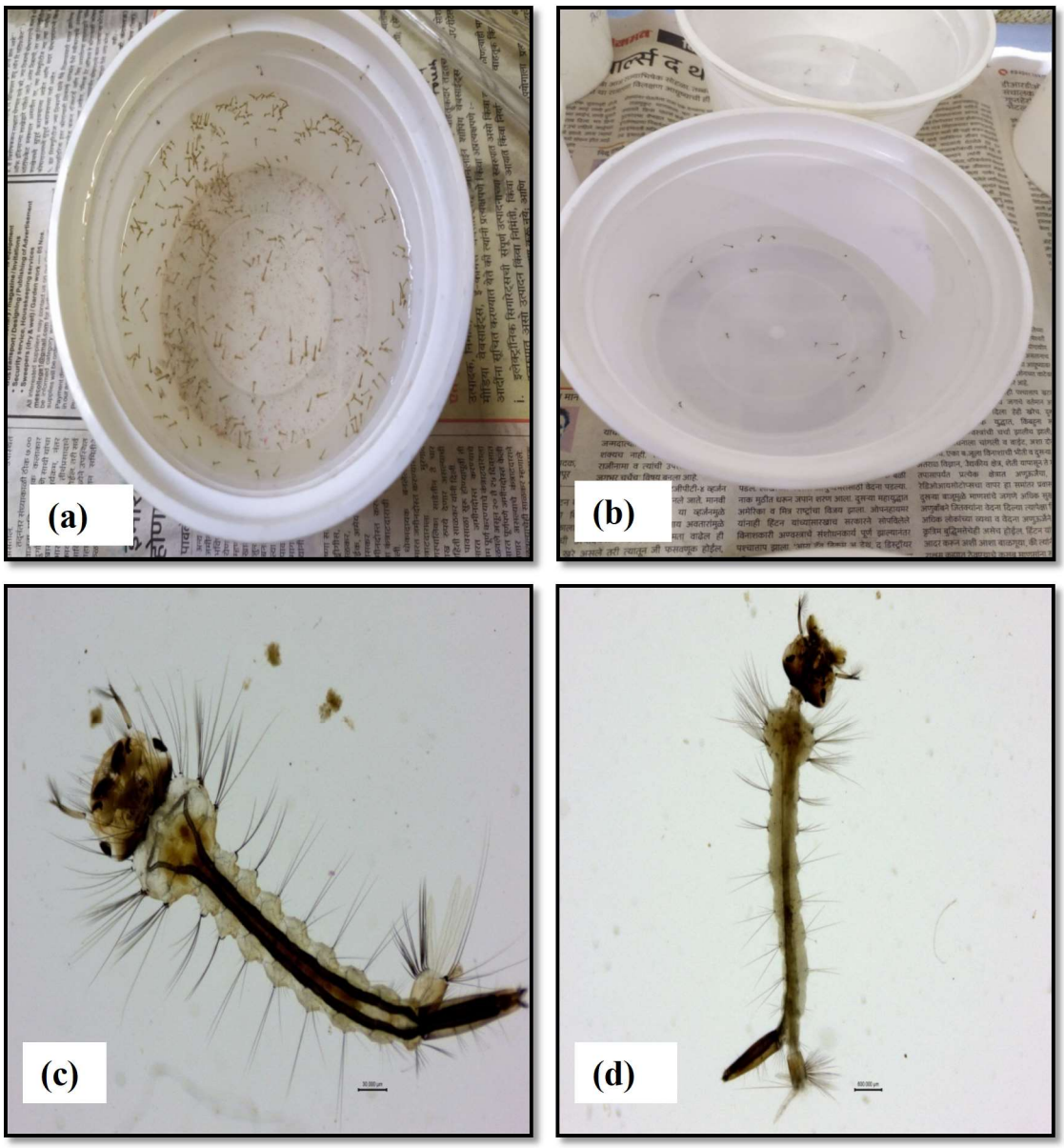


PLATE 4.6 : Larvicidal activity (a) Bowl containing 3rd instar stage larvae , (b) Larvae in different concentration of essential oil , (c) 3rd instar *Culex quinquefasciatus* (untreated) (4X), (d) 3rd instar *Culex quinquefasciatus* (Treated) (4X)

Table 4.3: Effect of leaf essential oil of *Cinnamomum zeylanicum* against 3rd instar *Culex quinquefasciatus* larvae

Name of the sample	*Cinnamomum zeylanicum*
Number of larvae exposed	20
Volume of water	100ml
Replicates	4

Table 4.4: Larvicidal activityof leaf essential oil of *Cinnamomum zeylznicum* against 3rd instar of *Culex quinquefaciatus* larvae after 24 hours.

Sr. No.	**Dose (ppm)**	**No. of dead larvae**				**% Mortality**
		R1	**R2**	**R3**	**R4**	
1	**50**	6	8	7	6	33.75
2	**100**	17	18	16	15	82.5
3	**150**	18	18	19	18	91.25
4	**200**	20	20	20	20	100
5	**Control**	1	2	0	0	3.75

Table 4.5: Larvicidal activityof leaf essential oil of *Cinnamomum zeylznicum* against 3rd instar of *Culex quinquefaciatus* larvae after 48 hours.

Sr. No	**Dose(ppm)**	**No. of dead larvae**				**% Mortality**
		R1	**R2**	**R3**	**R4**	
1	**50**	8	10	10	7	43.75
2	**100**	18	19	17	16	87.5
3	**150**	19	19	20	20	97.5
4	**200**	20	20	20	20	100
5	**Control**	1	2	0	0	3.75

Table 4.6: LC_{50} and LC_{90} values of leaf essential oil of *C. zeylznicum* against 3rd instar larvae of Culex quinquefasciatus after 24 hours and 48 hours of exposure.

Mosquito species	Exposure period	LC50(ppm) (LCL-UCL)	LC90(ppm) (LCL-UCL)	Regresion equation	Slope(±SE)	X^2
Culex quinquefasciatus	**24 hrs**	67.31 (49.9-90.78)	149.91 (111.15-202.19)	Y=3.6937x-1.749	3.694±0.066	0.937
	48hrs	56.29 (40.35-78.53)	133.22 (95.49-185.85)	Y=3.441x-1.0284	3.44±0.074	0.944

Table 4.7: Effect of leaf essential oil of *Murraya koenigii* against 3rd instar *Culex quinquefasciatus* larvae

Name of the sample	*Murraya koenigii*
Number of larvae exposed	20
Volume of water	100ml
Replicates	4

Table 4.8 : Larvicidal activityof leaf essential oil of *Murraya koenigii* against 3rd instar of *Culex quinquefaciatus* larvae after 24 hours.

Sr. No	**Dose (ppm)**	**No. of dead larvae**				**% Mortality**
		R1	**R2**	**R3**	**R4**	
1	**50**	6	7	4	6	28.75
2	**100**	5	11	5	8	36.25
3	**150**	7	9	7	9	40
4	**200**	7	11	11	10	48.75
5	**300**	17	17	17	18	86.25
6	**Control**	1	2	0	0	3.75

Table 4.9: Larvicidal activity of leaf essential oil of *Murraya koenigii* against 3rd instar of *Culex quinquefaciatus* larvae after 48 hours.

Sr. No	**Dose (ppm)**	**No. of dead larvae**				**% Mortality**
		R1	**R2**	**R3**	**R4**	
1	**50**	8	9	6	7	37.5
2	**100**	9	11	8	10	47.5
3	**150**	8	12	8	11	48.75
4	**200**	9	12	12	12	56.25
5	**300**	20	20	20	20	100
6	**Control**	1	2	0	0	3.75

Table 4.10 : LC_{50} and LC_{90} values of leaf essential oil of *M.koenigii* against 3rd instar larvae of *Culex quinquefasciatus* after 24 hours and 48 hours of exposure.

Mosqquito species	Exposure period	LC50 (ppm) (LCL-UCL)	LC90 (ppm) (LCL-UCL)	Regression equation	Slope (±SE)	X^2
Culex quinquefasciatus	24 hrs	163.11 (102.43-259.59)	917.05 (576.21-1459.51)	Y=1.796x+.0387	1.796±0.103	0.208
	48 hrs	111.32 (76.78-161.41)	496.95 (342.75-720.53)	Y=2.3224x+0.28	2.322±0.082	0.070

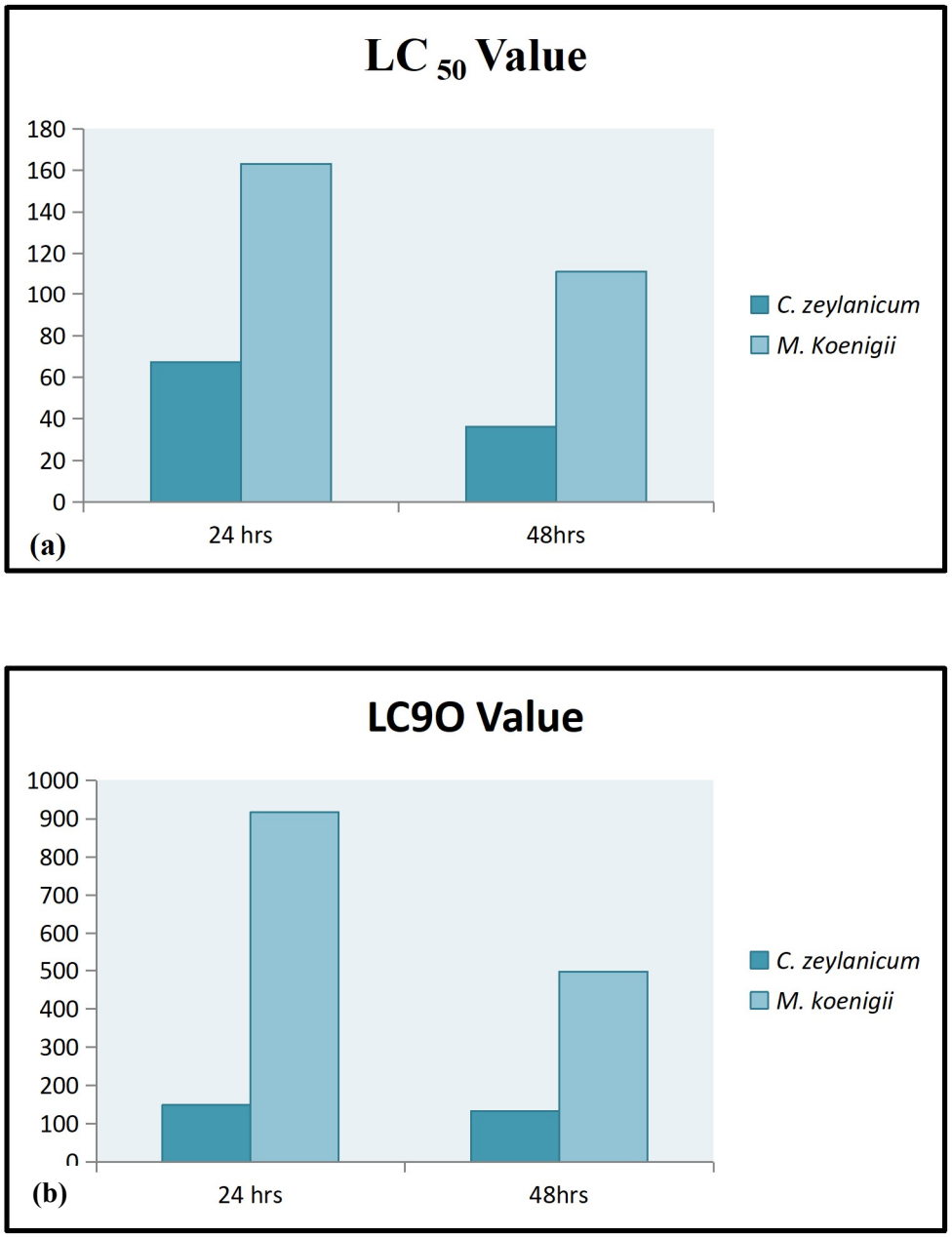


Figure 4.4: Mosquito larvicidal activity (a) LC_{50} value , (b) LC_{90} value

CHAPTER 5: CONCLUSIONS

Cinnamomum zeylanicum, belonging to the family Lauraceae, and *Murraya koenigii*, belonging to the family Rutaceae were studied for their Anatomical, Antioxidant activity, and Larvicidal activity. The leaf essential oil extracted from both plants was used for the study. The essential oil was extracted from the fresh leaves of both plants.

According to their anatomical characterization, different anatomical features were found in *C. zeylanicum* and *M. koenigii*. Oil glands were present on the surface of leaves, stems, and petioles. Stone cells and oxalate crystals were observed in the stem anatomy. Varied vascular bundles are seen in the stems and leaves of *C. zeylanicum*. Plants with well-developed xylem and phloem tissues have adequate water and nutrient transportation systems. Trichomes, or hairs on the stem, may aid in preventing water loss and serve as a defense against herbivores. *M. koenigii* additionally has distinct vascular bundles that are a feature of the leaf stem. Like cinnamon, the plant's phloem and xylem tissues are fully formed and aid in transporting water and nutrients. One may observe glandular structures, which could suggest specific roles like the release of aromatic chemicals.

Leaves, a valuable and adaptable component of the cinnamon tree, have a wide range of applications across several sectors and may have health advantages. Antioxidants in *C. zeylanicum* leaves can protect against oxidative stress and promote general health. Because of its high antioxidant activity, *Cinnamomum zeylanicum* leaf essential oil is a powerful antioxidant source. On the other hand, *Murraya koenigii* leaf essential oil has comparatively less antioxidant activity, indicating that it might not be as valuable as other sources of antioxidants.

Because they contain phenolic and other active components, both essential oils have antioxidant properties. There may be differences in the two plants' components and their concentrations, which could result in differences in the strength and range of their antioxidant activity.

The use of essential oils, which are safer for the environment and target-specific compared to synthetic products, has gained popularity over the past 20 years as a means of preventing illnesses from spreading by mosquitoes. The essential oil extracted from the *Cinnamomum zeylanicum* and *Murraya koenigii* leaves were effective against larvae of *Culex quinquefasciatus*. *C. zeylanicum* oil has maximum effectiveness as larvicidal activity. Physiological and morphological differences were shown to be linked to the variable responses of the same species of mosquito to the essential oils from different plants at different phases of their development. The primary chemicals that make up essential oils are often one of the key components in charge of the specific insecticidal activity of essential oils (Das *et al*., 2015).

REFERENCES

Abeysinghe, P. D., & Scharaschkin, T. (2019). Taxonomic value of petiole anatomy in the genus Cinnamomum (Lauraceae) found in Sri Lanka. *Ruhuna Journal of Science*, *10*(1).

Abeysinghe, T., Kumara, K.A.H., Kaushalya, K.A.D., Chandrika, U.G., Alwis, D.D.D.H., 2021. Phytochemical screening, total polyphenol, flavonoid content, in vitro antioxidant and antibacterial activities of Sri Lankan varieties of *Murraya koenigii* and *Micromelum minutum* leaves. Heliyon 7 (7), e07449.

Ajay, S., Rahul, S., Sumit, G., Paras, M., Mishra, A., & Gaurav, A. (2011). Comprehensive review: *Murraya koenigii* Linn. *Asian Journal of Pharmaceuiticals Life Sci*, *2231*, 4423.

Akanksha R., Chitra P., Geeta T., and Kiran P. (2017). A review of the aroma profile of Cinnamomum species in North and North East India. *World Journal of Pharmaceutical Research.* Volume 6, Issue 11, 200-221. ISSN: 2277-7105.

Al-Safa H. Mohamed, Wafaa Ahmed, Einas Elshatoury, Magdy M.M. (2016). Leaf Anatomy, Chemical Composition as Well as Essential Oils and their Antibacterial Activity of Some Lauraceous Taxa. Taeckholmia 36: 77-101. eISSN: 2357-044X.

Andoğan, B. C., Baydar, H., Kaya, S., Demirci, M., Özbaşar, D., & Mumcu, E. (2002). Antimicrobial activity and chemical composition of some essential oils. *Archives of pharmacal research*, *25*, 860-864.

Andrade, M. A., dasGraças Cardoso, M., Batista, L. R., Mallet, A. C., & Machado, S. M. (2012). Essential oils of *Cinnamomum zeylanicum*, *Cymbopogon nardus* and *Zingiber officinale*: composition, antioxidant and antibacterial activities. *Revista Ciência Agronômica*, *43*(2), 399.

Arivoli, S., Raveen, R., & Samuel, T. (2015). Larvicidal activity of *Murraya koenigii (L.)* Spreng (Rutaceae) hexane leaf extract isolated fractions against *Aedes aegypti* Linnaeus, *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). *Journal of Mosquito Research*, *5*.

Athira, A., & Pushpalatha, E. (2021). Essential oils of a few aromatic plants and their potential as knockdown, repellent and adulticidal agent to the filarial vector, *Culex quinquefasciatus* say (Diptera: Culicidae).

Bakker, M. E., Gerritsen, A. F., & Van Der Schaaf, P. J. (1992). Leaf anatomy of Cinnamomum Schaeffer (Lauraceae) with special reference to oil and mucilage cells. *Blumea: Biodiversity, Evolution and Biogeography of Plants*, *37*(1), 1-30.

Bhandari, P. R. (2012). Curry leaf (*Murraya koenigii*) or cure leaf: review of its curative properties. *Journal of Medical Nutrition and Nutraceuticals*, *1*(2), 92.

Chandrul, K. K., & Singh, B. (2016). Botanical and physico-chemical standardization of aerial parts of Murraya koenigii. *Pharmacophore*, *7*(3), 176-186.

Cheng, S. S., Chung, M. J., Chen, Y. J., & Chang, S. T. (2011). Antipathogenic activities and chemical composition of *Cinnamomum osmophloeum* and *Cinnamomum zeylanicum* leaf essential oils. *Journal of Wood Chemistry and Technology*, *31*(1), 73-87.

Chowdhury, J. U., Bhuiyan, M. N. I., & Yusuf, M. (2008). Chemical composition of the leaf essential oils of *Murraya koenigii* (L.) Spreng and *Murraya paniculata* (L.) Jack. ||| *Bangladesh Journal of Pharmacology*|||, *3*(2), 59-63.

Das, N. G., Goswami, D., & Rabha, B. (2007). Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *Journal of Vector Borne Diseases*, *44*(2), 145.

de Gonçalves, R. A., Pinheiro, A. B., de Oliveira, M. A., do Nascimento, R. T., Rosalem, P. F., Garcia, V. L., & Martins, A. R. (2018). Anatomical characters and chemical profile of leaves of three species in Lauraceae family. *Revista Brasileira de Farmacognosia*, *28*, 1-8.

De Groot, A. C., & Schmidt, E. (2016). Essential oils, part III: chemical composition. *Dermatitis*, *27*(4), 161-169.

Eslahi, H., Fahimi, N., & Sardarian, A. R. (2017). Chemical composition of essential oils. *Essential oils in food processing: chemistry, safety and applications*, 119-171.

Gogoi, R., Sarma, N., Loying, R., Pandey, S. K., Begum, T., & Lal, M. (2021). A comparative analysis of bark and leaf essential oil and their chemical composition, antioxidant, anti-inflammatory, antimicrobial activities and genotoxicity of North East Indian *Cinnamomum zeylanicum* Blume. *The Natural Products Journal*, *11*(1), 74-84.

Govindarajan, N., Ravichandran, L., Sundharamoorthy, S., Chelladurai, P. K., Pandey, A., & Ilavarasan, R. (2022). Comparative morpho-anatomical studies to authenticate and differentiate the stem barks of *Cinnamomum verum* J. Presl and *Cinnamomum cassia* (L.) J. Presl and its market scenario. *Journal of Drug Research in Ayurvedic Sciences*, *7*(3), 185-191.

Gupta, S., Paarakh, P. M., & Gavani, U. (2009). Antioxidant activity of *Murraya koenigii* linn leaves. *Pharmacologyonline*, *1*, 474-478.

Handral, H. K., Jha, P. K., & Shruthi, S. D. (2010). Pharmacognostic and phytochemical studies on the leaves of *Murraya koenigii* L Spreng. *Pharmacophore*, *1*(3), 231-238.

Handral, H. K., Pandith, A., & Shruthi, S. D. (2012). A review on *Murraya koenigii*: multipotential medicinal plant. *Asian Journal of pharmaceutical and clinical research*, *5*(4), 5-14.

Handral, H., Sl, H. O. T. I., & Sd, D. S. (2012). In vitro evaluation of antimicrobial activities of crude extracts from *Murraya koenigii* against pathogenic bacteria. *International Journal Pharmaceutical Science*, *4*, 74-6.

Hari, N., Priya, C., Praseetha, K., Laie, A., & Krishnapriya, S. (2006)A monogram study on anatomical characteristics in *Cinnamomum verum* J. Presl.

Harish M, Anup P, Shiruthi SD. (2012) A review on *Murraya koenigii* multipotential medicinal plant. Asian Journal. Pharmaceutical and clinical Research,; 5(4): 5-14.

Hathurusinghe, B. M., Pushpakumara, D. G., & Bandaranayake, P. C. (2023). Macroscopic and microscopic study on floral biology and pollination of *Cinnamomum verum* Blume (Sri Lankan). *Plos One*, *18*(2), e0271938.

Imen, D., Nadia, S., Souhaila, D., Imed, C., Fatma, G., Assia, L., & Ahmed, L. (2023). The Effect of Drying Condition on Citrus sinensis (Osbek) Peel Waste Essential Oil's Composition, Antioxidant, Antibacterial and Tyrosinase Inhibition Activities. *Waste and Biomass Valorization*, 1-14.

Iqbal, Z., Mehmood, H. K., Hussain, M., Mehmood, M. H. R., & Choudhry, M. N. (2017). Antioxidant activity of essential oil from the leaves and stems of *murraya koenigii*. *World Journal of Pharmaceutical Research*, *6*(7), 267-273.

Ismail, N. A., Roslan, J., Ramli, H., & Sy Mohamad, S. F. (2014). Study of essential oil from malaysian curry leaves. In *International Conference on Biological, Chemical and Environmental Sciences (BCES-2014)* (pp. 1-4).

Jain, M., Gilhotra, R., Singh, R. P., & Mittal, J. (2017). Curry leaf (*Murraya koenigii*): A spice with medicinal property. *MOJ Biol Med*, *2*(3), 236-256.

Jayaprakasha, G. K., & Rao, L. J. M. (2011). Chemistry, biogenesis, and biological activities of *Cinnamomum zeylanicum. Critical reviews in food science and nutrition*, *51*(6), 547-562.

Kale NV, More GS. Bioochemical, antimicrobial and organoleptic analysis of curry leaf. Plant Science Feed, 2014; 4: 6-9.

Kostermans, A. J. G. H. (1957). Lauraceae. *Reinwardtia*, *4*(2), 193-256.

Kovendan, K., Arivoli, S., Maheshwaran, R., Baskar, K., & Vincent, S. (2012). Larvicidal efficacy of *Sphaeranthus indicus*, *Cleistanthus collinus* and *Murraya koenigii* leaf extracts against filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitology Research*, *111*, 1025-1035.

MacLeod, A. J., & Pieris, N. M. (1982). Analysis of the volatile essential oils of *Murraya koenigii* and *Pandanus latifolius*. *Phytochemistry*, *21*(7), 1653-1657.

Mathew, S., & Abraham, T. E. (2006). Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. *Food chemistry*, *94*(4), 520-528.

Narayana, S. K. K., Gopi, D. K., Rubeena, M., & Parameswaran, S. R. (2019). Macro- micro- morphological diagnosis of leaves of two species of *Cinnamomum (C. sulphuratum* and *C. verum*) used as resource of bay leaf. *Ayu*, *40*(3), 196.

Narayanankutty, A., Kunnath, K., Alfarhan, A., Rajagopal, R., & Ramesh, V. (2021). Chemical composition of *Cinnamomum verum* leaf and flower essential oils and analysis of their antibacterial, insecticidal, and larvicidal properties. *Molecules*, *26*(20), 6303.

Nishan, M., & Subramanian, P. (2015). *Murraya koenigii* (curry leave)-A review on its potential. *Int. J. PharmTech Res*, *7*(4), 566-572.

Ohri, S., Singh, G., & Kaur, A. (2023). Larvicidal activity of *Murraya koenigii* against *Aedes* mosquito.

P.J. Raharivelomanana, G.P. Terrom, J.P. Bianchini and P. Coulanges, Study of the antimicrobial action of various essential oils extracted from Malagasy plants, II: Lauraceac. Arch. Inst. Pasteur Madagascar, 56(1), 261-271 (1989).

Prajapati, V., Tripathi, A. K., Aggarwal, K. K., & Khanuja, S. P. S. (2005). Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*. *Bioresource technology*, *96*(16), 1749-1757.

R. Samarasekera, W.A.S. Wijendra and K.S. Kalhari, Antifungal properties of essential oils of Cinnamon (*Cinnamomum zeylanicum*) aganist dermatophytes. Paper No.E2/ 246, Proceedings of the 57th Annual Session, Sri Lanka Association for Advancement of Science, Colombo, Sri Lanka (2001).

Rajendran, M. P., Pallaiyan, B. B., & Selvaraj, N. (2014). Chemical composition, antibacterial and antioxidant profile of essential oil from *Murraya koenigii* (L.) leaves. *Avicenna journal of phytomedicine*, *4*(3), 200.

RAJNIKANT, S. K., & CHATTREE, A. (2015). Antioxidant and antifungal potential of *murraya koenigii* leaves extracts (crude) and essential oil. *Chemical Science*, *4*(1), 222-226.

Ramzan, S., Shaheen, S., Hussain, K., Khan, M. A., Sohail, J., & Khan, F. (2019). Significance of leaf morphoanatomical markers for the authentication of adultered drugs sold in herbal markets of district Lahore, Pakistan. *Microscopy Research and Technique*, *82*(11), 1911-1921.

Rao, B. R., Rajput, D. K., & Mallavarapu, G. R. (2011). Chemical diversity in curry leaf (*Murraya koenigii*) essential oils. *Food Chemistry*, *126*(3), 989-994.

Ravindran, P. N., Shylaja, M., Babu, K. N., & Krishnamoorthy, B. (2003). Botany and crop improvement of cinnamon and cassia. In *Cinnamon and cassia* (pp. 30-95). CRC Press.

Reeta, M., Ravindra, S., Sharma, T., Sumit, N., & Kotecha, M. (2015). Macro and Microscopic study of meetha neem (*Murraya koenigii* linn). *World Journal of Pharmaceutical Research*, *4*(09).

Ribeiro, P. R. E., Montero, I. F., Saravia, S. A. M., Ferraz, V. P., Santos, R. A., MarcÃa, J. A., & Linhares, B. M. (2020). Chemical composition and antioxidant activity in the essential oil of *Cinnamomum zeylanicum* Nees with medicinal interest. *Journal of Medicinal Plants Research*, *14*(7), 326-330.

Romano, C. A., de Oliveira Neto, J. R., da Cunha, L. C., dos Santos, A. H., & de Paula, J. R. (2024). Essential oil-based nanoemulsion of *Murraya koenigii* is an efficient larvicidal against *Aedes aegypti* under field conditions. *Industrial Crops and Products*, *208*, 117836.

Saini, S. C., & Reddy, G. B. S. (2015). A review on curry leaves (*Murraya koenigii*): Versatile multi-potential medicinal plant. *Am J Phytomed Clin Ther*, *3*(04), 363-8.

Samarasekera, R., Kalhari, K. S., & Weerasinghe, I. S. (2005). Mosquitocidal activity of leaf and bark essential oils of Ceylon *Cinnamomum zeylanicum*. *Journal of Essential Oil Research*, *17*(3), 301-303.

Samuel T, Jayakumar M, William SJ. *Culex mosquito*: An overview. In: Defeating the public enemy, the mosquito: A real challenge, William SJ. (Ed), Loyola Publications, Loyola College, Chennai, Tamil Nadu, India. 2007, 95-116.

Schmidt, E., Jirovetz, L., Buchbauer, G., Eller, G. A., Stoilova, I., Krastanov, A. & Geissler, M. (2006). Composition and antioxidant activities of the essential oil of cinnamon (*Cinnamomum zeylanicum* Blume) leaves from Sri Lanka. *Journal of essential oil bearing plants*, *9*(2), 170-182.

Seenivasan, P., Tennyson, S., Jayakumar, M., & Prabu, A. (2019). Larvicidal activity of cinnamaldehyde against the filarial vector *Culex quinquefasciatus*. *RJLBPCS*, *5*(3), 601-608.

Shivkanya, J., Shilpa, P., Sangita, K., & Neeraj, F. (2009). Pharmacognostical studies and antibacterial activity of the leaves of *Murraya koenigii*. *Pharmacognosy Journal*, *1*(3).

Singh, R. I. T. I. K. A., Lawrence, R. E. E. N. A., Lawrence, K., Agarwal, B., Gupta, R. K., & Dar, S. (2015). Antioxidant and Antibacterial Activity of *Syzygium aromaticum*, *Zingiber officinale* and *Cinnamomum zeylanicum* Essential Oils. *Chemical Science Transactions*, *4*(1), 239-245.

Singh, S., More, P. K., & Mohan, S. M. (2014). Curry leaves (*Murraya koenigii* Linn. Sprengal)-a mircale plant. *Indian Journal of Scientific Research*, *4*(1), 46-52.

Soundappan, K., Bajaj, R., Vaidya, B. N., & Joshee, N. (2018). Anatomical and biochemical investigations on medicinal tree *Murraya koenigii* Spreng. *Journal of Biotech Research*, *9*, 70-78.

Sulaiman, S. A. (2013). *Extraction of essential oil from Cinnamomum zeylanicum by various methods as a perfume oil* (Doctoral dissertation, UMP).

Teixeira, B., Marques, A., Ramos, C., Neng, N. R., Nogueira, J. M., Saraiva, J. A., & Nunes, M. L. (2013). Chemical composition and antibacterial and antioxidant properties of commercial essential oils. *Industrial crops and products*, *43*, 587-595.

Tripathi, Y. C., Anjum, N., & Rana, A. (2018). Chemical composition and in vitro antifungal and antioxidant activities of essential oil from *Murraya koenigii* (L.) Spreng. Leaves. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *8*(65), 6-13.

Wang, R., Wang, R., & Yang, B. (2009). Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. *Innovative Food Science & Emerging Technologies*, *10*(2), 289-292.

Weerasekera, A. C., Samarasinghe, K., de Zoysa, H. K. S., Bamunuarachchige, T. C., & Waisundara, V. Y. (2021). *Cinnamomum zeylanicum*: morphology, antioxidant properties and bioactive compounds. *Antioxidants-Benefits, Sources, Mechanisms of Action*.

Wong Y. C., Ahmad-Mudzaqqir, M. Y., Wan-Nurdiyana W. A. (2014). Extraction of essential oil from Cinnamon (*Cinnamomum zeylanicum*). Orient J Chem., 30, 37 – 47

Zahin, M., Aqil, F., Husain, F. M., & Ahmad, I. (2013). Antioxidant capacity and antimutagenic potential of *Murraya koenigii*. *BioMed Research International*, *2013*.