Anatomical Characterization and Antioxidant Potential of Leaf Essential Oils Isolated from *Magnolia champaca* and *Syzygium aromaticum* for their antifungal activity

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Date: April 2024

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I hereby declare that the data presented in this Dissertation report entitled, "**Anatomical characterization and antioxidant potential of leaf essential oils isolated from *Magnolia champaca* and *Syzygium aromaticum* for their antifungal 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 dissertation.

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Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report "**Anatomical characterization and antioxidant potential of leaf essential oils isolated from *Magnolia champaca* and *Syzygium aromaticum* for their antifungal activity**" is a bonafide work carried out by **Ms. Preeti D'Souza** under my supervision in partial fulfilment of the requirements for the award of the degree of **M.Sc.** in the Discipline Botany at the School of Biological Sciences and Biotechnology, Goa University.

Signature and Name of Supervising Teacher
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PREFACE

An essential component of botanical research has long been the study of plant anatomy, which offers priceless insights into the complex structures that support plant function and adaptability. In light of this, the current study examines the morphological features of the leaves, petioles, and stems of two different plant species: *Syzygium aromaticum* and *Magnolia champaca*. A deep curiosity drives this project to understand the distinctive morphological characteristics of these plants, which have drawn much interest due to their wide range of pharmaceutical and industrial uses.

Before we go into the details of our study, it is essential to recognize the abundance of information previously published about the biological traits of *Syzygium aromaticum* and *Magnolia champaca,* as well as plant anatomy. Many studies have clarified the anatomical adaptations of different plant species to their physiological needs and ecological niches, illuminating the wide range of structural traits that plants need to survive in their specific settings.

Even so, there is still a significant knowledge gap on the anatomical structures of *Syzygium aromaticum* and *Magnolia champaca*, especially about their leaves, petioles, and stems. Although earlier research has shed light on these plants' general morphology and therapeutic qualities, a thorough analysis of their anatomical characteristics, essential oil output, and bioactivity profiles still needs to be improved.

By studying the leaves, petioles, and stems of *Magnolia champaca* and *Syzygium aromaticum*, the current study aims to close this anatomical gap. We seek to understand the complex anatomical adaptations of these plants and their possible implications for

industrial and pharmaceutical use by utilizing cutting-edge imaging techniques and experimental methods.

This study is critical because it can improve our understanding of plant anatomy, the chemistry of essential oils, and bioactivity. These discoveries have real-world applications for a variety of sectors. To aid in creating novel botanical products, pharmaceutical formulations, and natural cures, this study will clarify the anatomical adaptations of *Magnolia champaca* and *Syzygium aromaticum* and evaluate their bioactivity profiles. Moreover, the knowledge gathered from this study could help guide conservation initiatives and sustainable farming methods that protect these priceless botanical resources for coming generations.

Also *Syzygium aromaticum* and *Magnolia champaca* leaves have been historically overlooked for their fragrant flowers and bark, although they are rich in untapped essential oil potential. This study looks into these overlooked value leaves' chemical structure and medicinal qualities to find hidden gems. This research promises benefits for industries and communities by utilizing the fragrant essence of leaves and conducting bioassays, expanding our knowledge of Botany and opening up new options for sustainable agriculture and product development. It is also helpful for different industrial purposes.

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Ms. Preeti D'Souza

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ABBREVIATIONS

ENTITY	ABBREVIATION
1,1- diphenyl-2-picrylhydrazyl	DPPH
Absorbance of control	A_0
Absorbance of sample	A_1
Degree Celsius	◦C
Dimethyl sulfoxide	DMSO
Essential oils	EOs
Gas Chromatography-Mass Spectrometry	GC-MS
Lethal concentration killing 50%	IC_{50}
microgram	µg
microlitre	µl
miligram	mg
millilitre	ml
millimetre	mm
nanometer	nm
Percent	%
Potato Dextrose Agar	PDA
Room temperature	RT
Transverse section	TS
Weight in volume	w/v
Zone of Inhibition	ZOI

ABSTRACT

The study was undertaken to understand the anatomical structures of two aromatic plant species leaf, petiole and stem to identify distinctive characteristics, including extraction of the essential oil using hydro-distillation and to evaluate the antioxidant and anti-fungal properties of essential oils. *Magnolia champaca* has typical vascular bundles and trichomes. *Syzygium aromaticum* has specialized idioblast oil cells, crystals, and v-shaped vascular bundle arrangement. The essential oil yield was 1.91% for *Syzygium aromaticum* compared to 0.25% for *Magnolia champaca*. *Syzygium aromaticum* has superior antioxidant and antifungal properties, indicating its potential for various industrial applications. Further investigation of bioactive compounds could increase their utility across different sectors.

Keywords: Essential oil, hydro-distillation, antioxidant, antifungal.

CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

Medicinal and aromatic plants, also referred to as herbs and spices, have a long history of use dating back to around 5000 BC (Christaki, 2012). These natural substances have been valued for their preservative and medicinal properties and their ability to enhance the taste and scent of food. Different parts of the plant are fragrant and possess various bioactivities, which can be helpful for human beings. These plants have a strong aroma and contain volatile organic compounds that can be extracted by different methods and are known as essential oils. These secondary metabolites protect plants against microbes, fungi, viruses, and insects by inhibiting, lowering, or decreasing their growth (Sahoo *et al*., 2022).

1.1.1. ESSENTIAL OILS

Essential oils (EOs) may also attract pollinators to promote pollen and seeds' dispersion and prevent entry of other undesirable insects. Thus, EOs can play a role in mediating the interactions of plants with the environment. EOs are liquid, volatile, limpid, and coloured and are soluble in lipids and organic solvents with lower density than water. They can be present in different plant organs, such as buds, flowers, leave, seeds, twigs, stems, flowers, fruits, roots, wood, or bark, but are generally stored by the plant in secretory cells, cavities, canals, glandular trichomes or epidermic cells (Nazzaro *et al*., 2013). The oil typically carries the name of the plants, where they are determined.

Essential oils possess various bioactivities, i.e., antimicrobial, anti-inflammatory, antioxidant, etc., which make them valuable for commercial products in the pharmaceutical, food, and beverage industries (Sahoo *et al*., 2022). It can also be used in

aromatherapy; it affects humans' moods as they enter through the olfactory system and affects the nervous system, thus improving mood and relaxing or energizing (Boughendjioua, 2018). These EOs also have cosmetic properties and can be used in skincare and hair care products. It can be used in household products for cleaning and antiseptic uses. These oils can be inhaled, massaged on the body, added to the bath or shower, or sprayed. The yield and quality of essential oils are greatly influenced by genetic makeup, agronomic practices, plant age, climate, soil type, and composition (Sahoo *et al.*, 2022).

The present comparative study is done between two aromatic plants for their variation in the yield and chemical composition of leaf essential oil isolated from *Magnolia champaca* and *Syzygium aromaticum*, belonging to the family Magnoliaceae and Myrtaceae, respectively.

1.1.2. FAMILY: MAGNOLIACEAE

It is one of the primitive families of Angiosperms. It comprises evergreen and deciduous trees and shrubs with approximately 240 species, widely distributed in Southern and Eastern Asian tropical, sub-tropical, and temperate zones. Several genera like *Aromadendron, Michelia, Angelitia, Paramichelia, Sampacca,* and *Taulama* had been placed in the subfamily Magnoliodeae.

However, based on DNA work, morphological consideration and nomenclatural changes have merged these genera into a single genus, *Magnolia*. The name of this genus was given in honor of French botanist Pierre Magnol by the famous Carl Linnaeus in 1737 (Sharma *et al.*, 2023).

These family members, magnolias, are distinguished by their elegant, fragrant blooms with many petals grouped in whorls. The Magnoliaceae family has long been used in medicine; bark, leaves, and flowers are occasionally used in herbal medicines.

1.1.2.1. *Magnolia champaca* (L.) Baill. ex-Pierre (Ramyashree & Hemalatha, 2020)

Classification:

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Magnoliales

Family : Magnoliaceae

Genus : *Magnolia*

Species : *champaca*

It is commonly known as Champak/Swarna, Champa/Golden Champa, and is a tall and magnificent evergreen tree belonging to the Magnoliaceae family. It is cultivated in gardens and near temples and is well known for its fragrant flowers **(Plate 1.1, 1.2)**.

1.1.2.2. Origin and Distribution

It is highly distributed in the Sub-Himalayan region, West Bengal, Myanmar, and South India, apart from China. It is renowned for its perfumery and is envisaged as the Joy perfume tree. “Joy,” the second best-selling perfume in the world, is derived in part from the essential oil of *champaca* flowers (Rajshree and Ranjana, 2016).

1.1.2.3. Botanical Description

It is a tree growing up to 30 meters in height, with grey bark and lenticellate. Stem aerial, erect, woody. Leaves simple, alternate, spiral, 10 to 25 cm lamina, lanceolate to elliptic-lanceolate, acuminate apex, acute base. The margin is slightly undulate, glabrous, strongly and articulately nerved. Petiole is 1 to 3 cm in length and stout. Flowers are solitary, axillary, large, yellow to orange, bracteate with short pedicel, complete, actinomorphic, and fragrant. Perianth usually has 15 to 20 tepals, in whorls of 3 each; either all three whorls are petalloid, or sometimes the outermost tepals become sepaloid. Fruit is follicle, warty, about 2 to 3 cm in length, arranged as spikes, dehiscing dorsally (Ramyashree and Hemalatha, 2020).

1.1.2.4. Importance and Uses

This plant is traditionally well-known for curing fever, colic, leprosy, coughs, rheumatism, and remedies for various disorders. According to the Indian system of medicine, the root bark of *M. champaca* is used as a purgative and emmenagogue, and different disorders like abscesses, inflammation, constipation, amenorrhoea, and dysmenorrhoea are used. The bark is used as a febrifuge. The stem bark is reported as astringent, febrifuge, diuretic, stimulant, and expectorant. At the same time, flowers treat chronic gastritis, fever, strangury, cough, bronchitis, and cardiac debilitus. Fruits are bitter, astringent, acrid, refrigerant, hemostatic, digestive, carminative, depurative, digestive, anthelmintic, diuretic, expectorant, cardiotonic, stimulant, stomachic, and antipyretic (Vivek *et al*., 2011). The volatile oil of leaves contains several compounds, mainly benzyl acetate, linalool, and isoeugenol, and has been used as a starting material in perfumes (Lago *et al*., 2009).

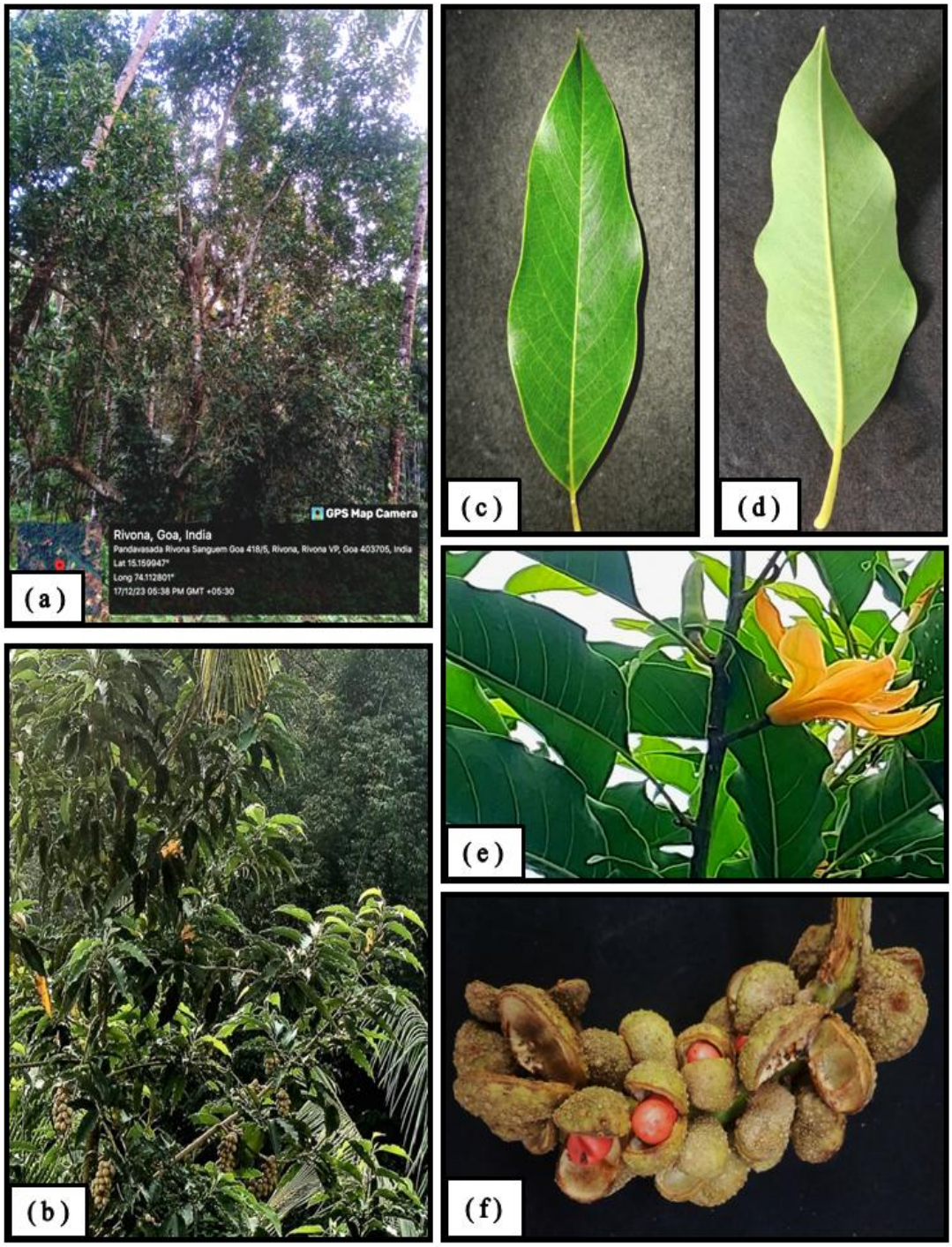


Plate 1.1: ***Magnolia champaca*** **(a)** Habitat, **(b)** Habit along with fruits, **(c)** Leaf (adaxial side), **(d)** Leaf (abaxial side), **(e)** Twig with the flower, **(f)** Fruits along with seeds.

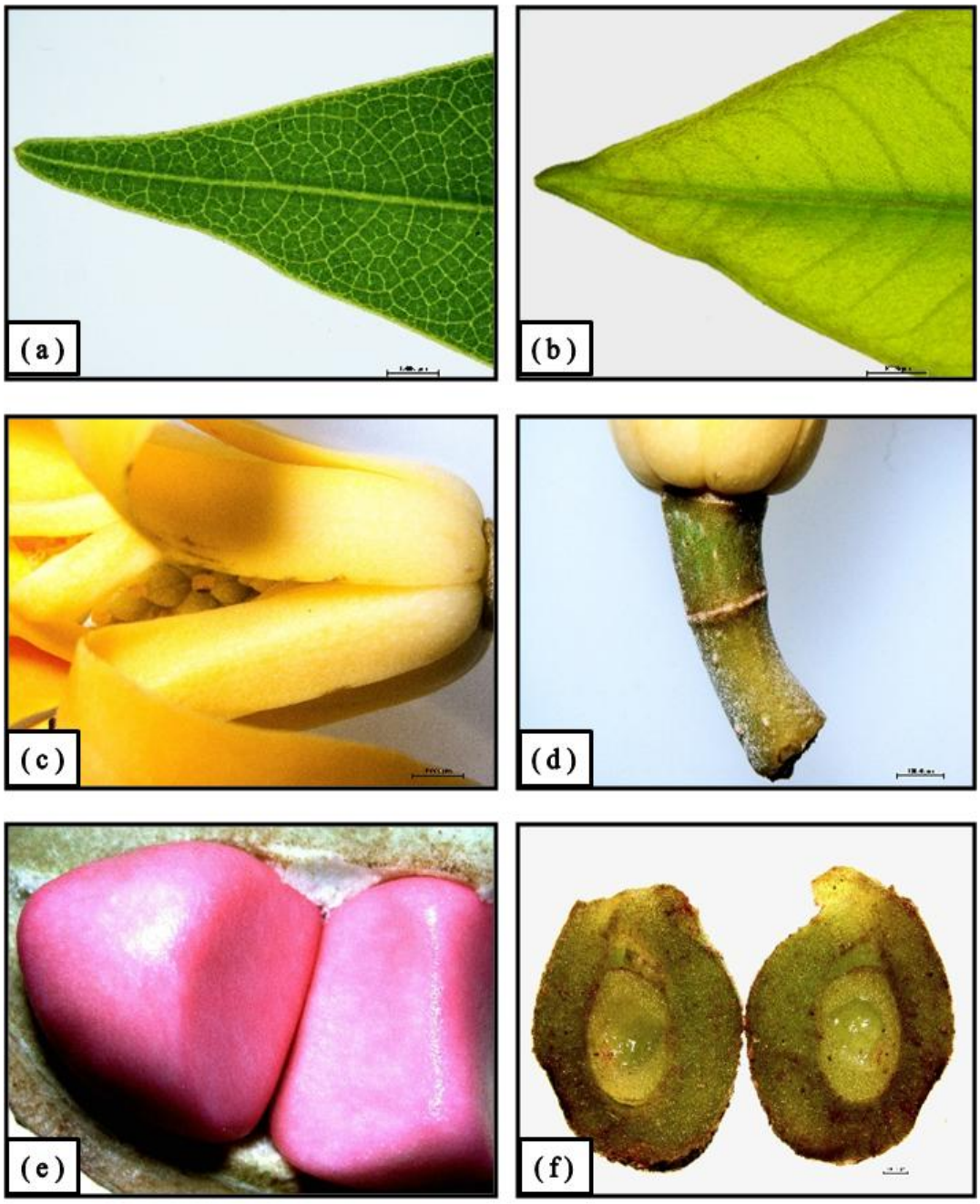


Plate 1.2: Macroscopic images of ***M. champaca.*** **(a)** Mature leaf apex, **(b)** Young leaf apex, **(c)** Closer view of the flower, **(d)** Pedicel of flower, **(e)** Mature fruit with seeds, **(f)** Young fruit cut open.

Essential oils are used in aroma therapy and the perfumery industry and are obtained from flower parts. *M. champaca* reported maximum pharmacological activities like anti-microbial, anti-pyretic, anti-inflammatory, antioxidant, insecticidal, anti-uretic, carminative, antidiabetic, etc (Ramyashree and Hemalatha, 2020).

1.1.3. FAMILY: MYRTACEAE

The Myrtaceae, or the myrtle family, comprises at least 140 genera and some 3800 to 5650 species. Many vital trees and shrubs belong to Myrtaceae. Four genera of interest produce edible fruits: *Psidium, Eugenia, Syzygium, and Feijoa. Syzygium* has about 500 species, most of which originated and are growing mainly in southeast Asia. The important species are *S. jambos, S. malaccense, S. suborbiculare, S. paniculatum, S. acqueum, S. cordatum, S. cumini, S. forte, S. aromaticum* etc (Mitra *et al*., 2012).

1.1.3.1. ***Syzygium aromaticum*** **(L.) Merr. & L.M. Perry (**Kaur and Kaushal, 2019)

Classification:

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Myrtales
Family	: Myrtaceae
Genus	: *Syzygium*
Species	: *aromaticum*

It is commonly called clove and is the second most important and most valuable spice in world trade. Clove requires damp tropical and sub-tropical environments for growth. It is

cultivated in Indonesia, Sri Lanka, India, Tanzania, Malaysia, Madagascar, and Pakistan **(Plate 1.3, 1.4)**.

1.1.3.2. Origin and Distribution

Clove is commonly used in cultivation and is indigenous to North Maluku Islands in Indonesia. Major cultivator countries of clove are Pemba, Zanzibar, Indonesia, and Madagascar, and some of the wild clove varieties are found in Bacan, Ternate, Motir, Tidore, Makian, and Western parts of Irian Jaya. India has become the second largest consumer of clove after Indonesia (Board, 2010). The clove tree can live up to 100 years and above. The tree prefers to grow in well-drained soil with sufficient soil moisture. Clove trees require heavy sunlight with high atmospheric temperature (25 to 35°C), well-distributed rainfall above 150 cm, and high humidity above 70% (Danthu *et al*., 2014). The crop cannot withstand waterlogged conditions. In India, clove grows well in the deep black, loamy soil of humid tropics and successfully grows in the red soils of the midlands of Kerala and the hilly terrain of Western Ghats in Karnataka and Tamil Nadu (Byng, 2016).

1.1.3.3. Botanical Description

Clove is an aromatic spice tree. The term clove is taken from the French words ‘clove’ and ‘cloud,’ which means ‘nail.’ Clove is conical myrtle, a medium-sized tree with a straight trunk that grows up to 10 to 12 m in height. The branches are semi-erect, greyish in colour, and dense. Leaves are large, oblong to elliptic, simple, obovate opposite, glabrous, and possess plenty of oil glands on the lower surface. The tree begins flowering in about seven years and continues for 80 years or more.

Flowers are small, and crimson are hermaphrodite (bisexual) and are borne at the terminal ends of small branches. Each peduncle carries 3 to 4 stalked flowers, and the

inflorescence length remains 4 to 5 cm. Initially, flower buds are pale yellow with a glossy appearance and turn green to bright red at maturity. These are 1-2 cm long with cylindrical thick ovary consisting of four fleshy sepals. Buds are divided into elongated stems and a globose bulbous head, stimulating nails. Commercially, cloves used are air-dried unopened flower buds, 2.5 cm in length and 1.25 cm wide. Fruit matures nine months after flowering, and the red ovary gradually turns reddish purple. The fruit contains nearly one or two seeds, known as the 'mother of clove.' The cultivated trees are rarely allowed to reach the fruit stage. These are harvested when they develop dark red ellipsoid berries (Kaur and Kaushal, 2019).

1.1.3.4. Importance and Uses

Cloves have many uses ranging from culinary to medicine. Clove is a valuable kitchen spice for studding onions, tomatoes, salads, herbal teas, and soups. It is also used to flavour meat products, cookies, chewing gums, spiced fruits, pickles, chocolates, soft drinks, puddings, sandwiches, pastries, and candies. Volatile oil imparts essence to perfumes, soaps, toothpaste, and pharmaceuticals. In Indonesia, a mixture of clove and tobacco in a ratio of 1:2 is used to make a particular cigarette, "Kretek." Clove possesses antibacterial potential and is used in various types of mouthwash, dental creams, throat sprays, and toothpaste to kill pathogens. It is also used to relieve sore gums. Clove oil has anti-inflammatory properties due to the presence of flavonoids. Pure clove oil is used in aromatherapy for arthritis and rheumatism. A paste of clove powder and honey is used to cure skin conditions. A paste of water and clove powder boosts the healing process of bites and cuts. Clove treats digestive disorders, including loose motion, flatulence, nausea, and dyspepsia. Clove oil improves the body's defense system and helps to fight against invading microbes (Hussain *et al.,* 2017).

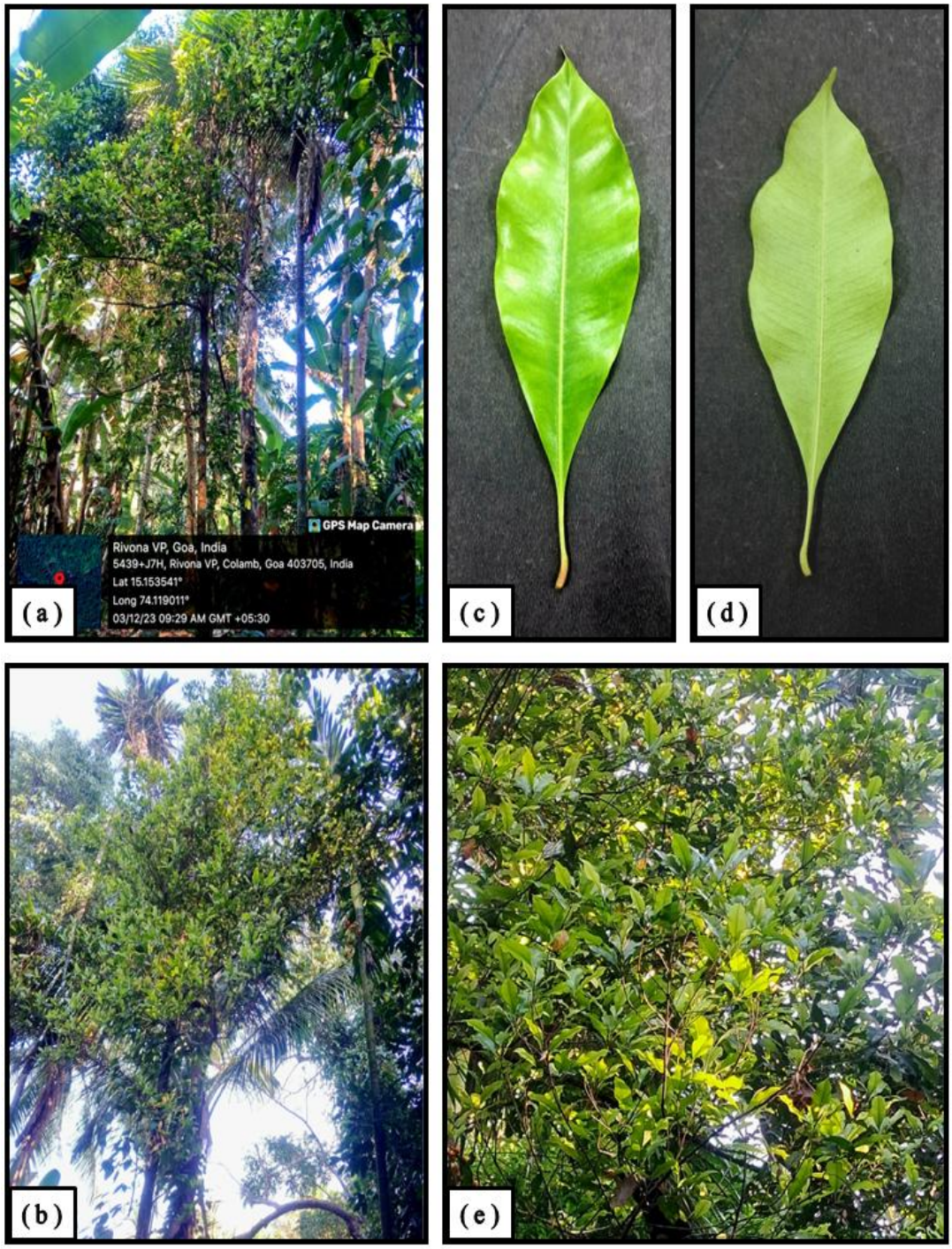


Plate 1.3: ***Syzygium aromaticum*** **(a)** Habitat, **(b)** Habit, **(c)** Leaf (adaxial side), **(d)** Leaf (abaxial side), **(e)** Twig along with leaves.

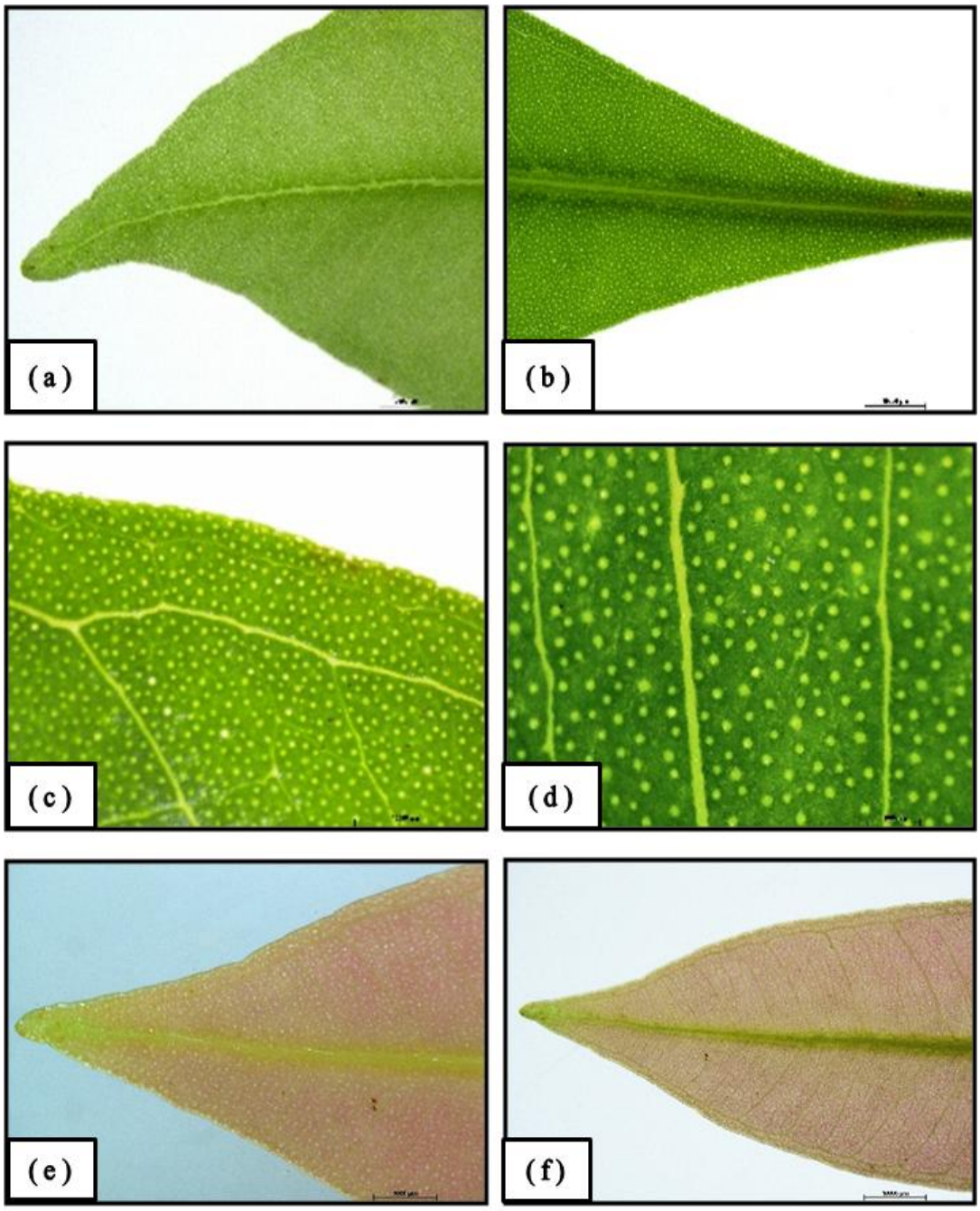


Plate 1.4: Macroscopic images of *S. aromaticum*. **(a)** Mature leaf apex, **(b)** Mature leaf base, **(c)** & **(d)** Leaf showing oil glands, **(e)** & **(f)** Young leaf showing oil glands.

1.1.4. ANATOMY

In identifying a plant species, anatomical characteristics of vegetative organs such as leaves are needed to complete taxonomic data and increase group understanding within taxa (Chatri *et al*., 2020). It helps to investigate the relationship between aroma production and a plant's secretory structures. Through light microscopy, oil cells and secretory cavities distributed near the adaxial and abaxial epidermal layers large in size, up to 60 μm length, were found in *S. aromaticum*. Other leaf anatomical characteristics, such as the shape of the petiole and midrib, the pattern of vascular bundle, palisade, and spongy mesophyll, and the presence or absence of brachysclereids and crystals, are also known (Zaman *et al*., 2018).

In a taxonomic treatment by Figlar & Nooteboom (2004) based on similar morphological and molecular data, it was proposed that the Magnolioideae is treated as a monotypic subfamily with the single genus *Magnolia*. Most of the species of *Michelia* are transferred to the genus *Magnolia*. Hence, it is essential to know the anatomical and morphological features of the plants to put them into different taxa. Also, the leaves have been considered critical characters for the easy identification of the plants at the juvenile stage. The similarities in seedling morphology rightly support the placement of *Michelia* under the genus *Magnolia*, as treated recently. These taxa face various threats, mainly due to exploitation for their commercial potential as timber-yielding and horticultural and medicinal plants. Consequently, conservation programs for these plants can be planned through their identification at juvenile stages in natural sites (Bose *et al*., 2017).

1.1.5. ANTIOXIDANT

Today, there is an increasing interest in antioxidants, mainly to prevent the known harmful effects of free radicals in human metabolism and their deterioration during the processing and storage of fatty foods. In both cases, natural-source antioxidants are preferred over synthetic antioxidants. So, there has been a parallel increase in the use of assays to estimate antioxidant efficacy in human metabolism and food systems.

Today, many bioanalytical methods measure the antioxidant effect. The 1,1-diphenyl-2-picrylhydrazil (DPPH) removing assay is the most putative, popular, and commonly used method to determine antioxidant ability (Gulcin and Alwasel, 2023). When a DPPH solution is mixed with a solution of a substance capable of donating a hydrogen atom, this violet colour disappears, resulting in the reduced form of the DPPH radical (DPPH-H). The wider band is responsible for the deep violet colour of the DPPH solution.

Hydrazine (DPPH-H) formation induces the disappearance of the visible band as the colour of the solution changes from violet to pale yellow due to radical reduction by hydrogen atom transfer from antioxidants, which are H donors. The colour intensity of this reaction, known as the "DPPH test," and can be quickly recorded by UV-vis spectroscopy (Yapıcı *et al*., 2021)

Lipid peroxidation and microbial contamination in foods result in food deterioration and shelf-life reduction and lead to disease and economic losses. The food industry faces enormous pressure from food deterioration caused by the two factors above (Ye *et al*., 2013). Adding antioxidants is an effective means of storing food to slow down the oxidation of food or even deter corruption. Synthetic antioxidants are restricted because of their carcinogenicity (Kimmel *et al*., 1986). Thus, there has been

increasing interest in finding natural, effective, and safe antioxidants since they can protect the human body from free radicals and retard the progress of many chronic diseases (Nandita and Rajini, 2004).

Spices used in different types of food to improve flavour since ancient times are well known for their antioxidant properties. It was reported that extracts obtained from spices had antioxidant activities (Telci *et al*., 2009).

1.1.6. ANTIFUNGAL ACTIVITY

Since ancient times, folk medicine and agro-food science have benefitted from using plant derivatives, such as essential oils, to combat diseases and preserve food. In Nature, essential oils play a fundamental role in protecting the plant from biotic and abiotic attacks to which it may be subjected (Nazzaro *et al*., 2017).

There is an increasing demand to reduce the use of chemicals as antimicrobial agents in nutrition and to combat various infections due to increasingly aggressive and increasingly endogenous microorganisms resistant to synthetic antimicrobials. In this direction, substances derived from plants, such as hydro-alcoholic extracts or essential oils, can play a fundamental role. The versatility of such substances is enormous; the same plant can provide a pool of substances with a vast spectrum of action due to their different chemical structure.

Plant EOs are usually complex mixtures of natural compounds, both polar and non-polar. Well-known for their antiseptic and medicinal properties (analgesic, sedative, anti-inflammatory, spasmolytic, local anesthetic, anti-carcinogenic), they are also used in embalmment and, due to their antimicrobial and antioxidant activity as natural additives in foods and food products (Altay *et al*., 2019).

Eukaryotic organisms cause fungal infections and it is more difficult to ascertain their presence and apply the appropriate treatment compared to bacterial infections. The fungi's cell wall may be considered the prime target for selectively toxic antifungal agents because of its chitin structure, which is absent in human cells. Chemical treatments are largely effective, but resistant strains and intrinsically resistant species can be developed.

The onset and severity of the fungal infection depends on the inoculum charge, the host's immunological state, and resistance. EOs can represent one of the most promising natural products for fungal inhibition (Elshafie *et al*., 2015).

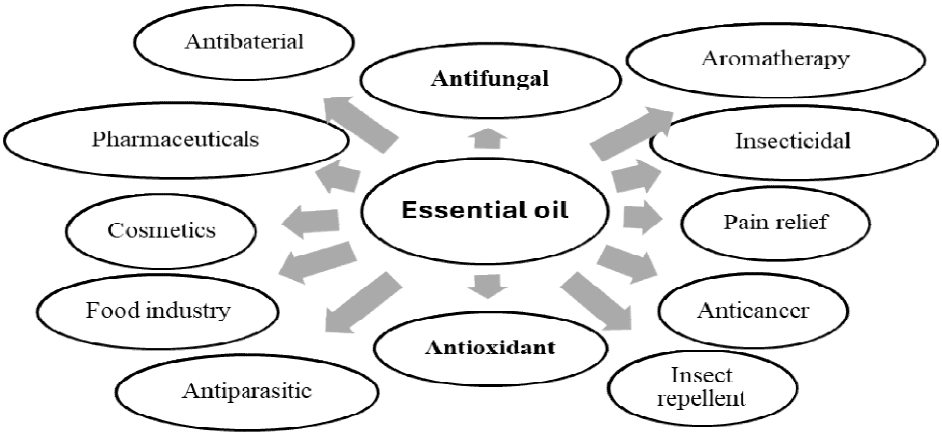


Figure 1.1: Application of essential oils.

1.2. AIM AND OBJECTIVES

AIM: To study the anatomical characterization and antioxidant potential of leaf essential oils isolated from *Magnolia champaca* and *Syzygium aromaticum* for their antifungal activity.

OBJECTIVES: *M. champaca* belongs to the family Magnoliaceae. It is commonly known as Swarna Champa or Kanak Champa. The plant is traditionally used against several diseases, such as fever, colic, leprosy, cough, rheumatism, and inflammatory

conditions. Essential oil from *S. aromaticum* is known for its antimicrobial activity against several pathogenic bacteria. Cloves have high bioactive compounds with crucial uses in the perfume and medicine industries. "Exploring the Antimicrobial and Antioxidant Potential of Traditional Medicine: Proposing Further Investigation into the Pharmacologically Active Natural Products of the Plant."

Moreover, both these aromatic plant species possess medicinal properties. However, people tend to give importance only to the flowers of *M. champaca* for their fragrance, neglecting the potential benefits of its leaves. Similarly, there needs to be more awareness regarding the medicinal uses of *S. aromaticum'*s leaves, resulting in their diminished importance. It is crucial to properly inform individuals on the bioactivities of these plants' leaves, including their antioxidant and antifungal properties, to utilize their medicinal value fully. It has attracted many industrialists and researchers to screen plants to study the biological activities of their oils, both in phytochemical, pharmacological, and therapeutic aspects.

Specific Objectives :

1. Collection of *Magnolia champaca* and *Syzygium aromaticum* for anatomical characterization.
2. Extraction and isolation of essential oil from leaves of *Magnolia champaca* and *Syzygium aromaticum* using hydro-distillation method.
3. Determination of antioxidant potential of leaf essential oil from selected plant species.
4. Evaluation of the antifungal activity of leaf essential oil from selected plant species.

1.3. HYPOTHESIS/ RESEARCH QUESTION

This study hypothesizes that the anatomical structures of leaves, petioles, and stems in *Magnolia champaca* and *Syzygium aromaticum* will exhibit distinctive features. It is anticipated that *Magnolia champaca* will display typical vascular bundles and trichomes. At the same time, *Syzygium aromaticum* will reveal specialized idioblast oil cells, crystals, and a V-shaped arrangement of vascular bundles. Furthermore, it is hypothesized that *Syzygium aromaticum* will yield a higher percentage of essential oil than *Magnolia champaca* and demonstrate superior antioxidant and antifungal activity.

1.4. SCOPE

The scope of this investigation encompasses the comprehensive analysis of anatomical structures in leaves, petioles, and stems of *Magnolia champaca* and *Syzygium aromaticum*. The study aims to elucidate the distinct morphological features present in each species and explore their potential implications for its functionality. Furthermore, the investigation extends to the quantitative assessment of essential oil yield and qualitative evaluation of antioxidant and antifungal properties. The findings of this study have implications for understanding the botanical characteristics and industrial applications of *Magnolia champaca* and *Syzygium aromaticum*, contributing to advancements in botanical research and product development in various sectors.

1. The EOs can be used to treat fungal infections.
2. It can be used as a food preservative as it has antioxidant activity.
3. *S. aromaticum* EO can be used in aromatherapy to manage stress or anxiety.
4. *S. aromaticum* EO can also be antibacterial as it shows a solid antifungal property.

5. *Magnolia champaca* EO can be diluted in a carrier oil and used for massage therapy. Its relaxing properties can help alleviate muscle tension and promote a sense of well-being.
6. The EOs can also be used in the pharmaceutical industry to prepare antifungal tablets.
7. It can be used in cosmetic products as it has antifungal and antioxidant properties.
8. EOs oil can be mixed with other valuable oils and can be used to prepare pain relief oils.

CHAPTER 2: LITERATURE REVIEW

The relevant literature on the present study has been reviewed to understand the different parameters of the study that were used to achieve the mentioned objectives.

2.1. ANATOMY

Anatomical studies carried out on various *Magnolia* species

A comparative study of the anatomical structure of the leaf of *the Magnolia soulangeana* hybrid plant (*Magnolia denudata* × *Magnolia liliiflora*) in sunny and shadow conditions of the Tashkent Botanical Garden was carried out and a different combination of xeromorphic and mesomorphic features was determined, which ensures adaptation to habitat conditions. Mesomorphic features like epidermal cells are more sinuous; large, few epidermal cells with thin outer walls; large cell spongy parenchyma; non-submerged stomata; large vessels in conductive bundles predominate in sunny and shadow habitats. Xeromorphic signs like small, numerous epidermal cells with thick outer walls, small cell spongy parenchyma, high palisade index, and small vessels in conductive bundles predominate in sunny and shadow habitats. In sunny and shadow conditions, mesomorphic characters are more prevalent in *Magnolia soulangeana* than xeromorphic ones, which indicates more adaptability to mesophytic habitat conditions (Duschanova, 2021).

Futorna *et al*. (2020) did a quantitative anatomical analysis of the leaves of three species of the *Magnolia* genus: *Magnolia kobus* Sarg, *Magnolia obovata*, Thunb *Magnolia denudata* Desr growing on the territory of Academician A.V. Fomin Botanical Garden of Kiev Taras Shevchenko National University. They found that in the first stages of onto morphogenesis, *M. kobus* is characterized by a less specialized type of

mesophyll (homogeneous spongy type), unlike *M. obovata* and *M. denudate*, which are characterized by the dorsoventral type. The least variable anatomical feature of the leaves of the studied species is their thickness (CV = 2%). Other anatomical features are characterized by average values of the variation coefficients (15-31%). Based on the correlation matrix using the "maximum correlation path method," this paper constructed the correlation pleiades of leaf blade anatomical features of the studied plants. Using the results of correlation analysis, they determined groups of features that exhibit the interconnected manifestation in ontogenesis by genetic, physiological, and biochemical reasoning of the three studied species of the *Magnolia* genus. Only in *M. obovata* are most of the studied anatomical features characterized by high correlation coefficients in the first stages of morphogenesis. The plants *M. cobus* and *M. denudate* have more vital consistency between the features of epidermal tissue. They assume those species suffer from a lack of moisture and high temperatures in the first stages of morphogenesis.

The qualitative and quantitative wood anatomical characteristics of four *Magnolia* species, namely *M. cathcartii*, *M. champaca*, *M. doltsopa*, *and M. lanuginosa,* carried out by Sharma *et al*. (2023) from forests of North Sikkim, NE India. They found that the study showed a homogeneous structure among *Magnolia* species. There were some distinct anatomical features like both multiseriate and biseriate rays in *M. cathcartii*, intervessel pits scalariform arranged in 2-3 rows in *M. champaca*, biseriate rays, scanty paratracheal parenchyma, intervessel pits rounded, alternate, biseriate rays in *M. doltsopa* and distended rays near the marginal parenchyma in *M. lanuginosa* which can be used to identify individual species of *Magnolia*. Among species, there was also significant variation in quantitative anatomical characteristics (no. of rays, vessel frequency, fibre length). They concluded that qualitative and quantitative anatomical features are essential for identifying *Magnolia* species.

Anatomical studies carried out on *Syzygium* species

Zaman *et al*. (2018) conducted anatomical and histochemical studies on leaves of *Syzygium aromaticum* and *Clausena excavata*. The study was conducted in order to investigate the relationship between aroma production and a plant's secretory structures. Leaves from the two tropical aromatic plants were sampled from the Institute of Bioscience (IBS) Conservatory Park and transversely sectioned through lamina, midrib, and petiole with a sliding microtome for anatomical investigation. Through light microscopy, oil cells and secretory cavities were distributed near the adaxial and abaxial epidermal layers, large in size, up to 60 µm length. Other leaf anatomical characteristics, such as the shape of the petiole and midrib, the pattern of vascular bundle, palisade, and spongy mesophyll, and the presence or absence of brachy sclereids and crystals, were also observed. This study also aimed to investigate the leaf's secretory structures responsible for plants' aroma production and to detect the presence of terpenes and essential oil in secretory structures histochemically.

Chatri *et al*. (2020) determined the cross-sectional characteristics of the leaves and stomata of three plants of *Syzygium*. The plants were *Syzygium aromatic*um (L.) Merr. & Perry, *Syzygium malaccense* (L.) Merr. & Perry, and *Syzygium polyanthum* (Wight.) Walp. The results of this study indicate that the three plants have secretory cavities that produce oil glands. Furthermore, it was found to have an anomocytic stomata type on *S. aromaticum* (L.) Merr. & Perry and *S. malaccense* (L.) Merr. & Perri, while *S. polyanthum* (Wight.) Walp. Has a parasitic stomata type. The spread of stomata is only found at the abaxial surface of the leaf (hypostomatic), which is the length of stomata in the *S. aromaticum* (L.) Merr. & Perry was 60.20 ± 6.99 µm2, in *S. malaccense* (L.) Merr. & Perry was 108.29 ± 21.17 µm2, and *S. polyanthum* (Wight.) Walp. Was 53.17 ± 9.32 µm2.

Soh *et al*. (2011) examined the leaf anatomy of 81 species of *Syzygium*, and general generic and subgeneric descriptions of *Syzygium* leaf anatomy were given. Four stomatal types (anisocytic, anomocytic, cyclo-staurocytic, and paracytic) that occur exclusively or in combinations are recognized. The presence or absence of adaxial phloem partition differentiates two major vascular systems. Phylogenetic inferences were conducted using maximum parsimony and Bayesian methods for separate and combined analyses of DNA (ETS and ITS) sequences and morphological (leaf anatomy and macromorphology) data. Results of the combined analyses gave slightly higher support values for clade than analyses based on DNA sequences. The subgenera Perikion, Sequestratum, and *Syzygium* are strongly supported, but the subgenus *Acmena* is moderately supported in the Bayesian but not in the parsimony analysis of combined data sets. The relationships among subgenera still need to be fully resolved. All the leaf anatomical characters examined are homoplastic. They found no unique leaf anatomical characters, allowing the four *Syzygium* subgenera to be delimited. However, combinations of non-unique leaf anatomical characters, including stomatal types, crystal types, frequency, and midrib vascular system (adaxial phloem partition), are diagnostic for subgeneric groups.

2.2. Isolation and Analysis of Essential Oil

Sahoo *et al*. (2022) investigated the variation in the yield and chemical composition of leaf essential oil isolated from 52 accessions of *M. champaca*. Through hydrodistillation, essential oil yield ranged from 0.06% to 0.31% (v/w) on a fresh weight basis. GC-MS analysis identified a total of 65 phytoconstituents. Sesquiterpene hydrocarbons constituted a significant fraction, followed by sesquiterpene alcohols. The essential oils were rich in β-elemene, γ-muurolene, and β-caryophyllene. Chemometrics analyses such as PCA, PLS-DA, sPLS-DA, and cluster analyses such as hierarchical

clustering, i.e., dendrogram and partitional clustering, i.e., K-means classified the essential oils of *M. champaca* populations into three different chemotypes: chemotype I (β-element), chemotype II (γ-microlens) and chemotype III (β-caryophyllene).

Ma *et al*. (2012) investigated the volatile oils from the leaves of the three species *Michelia coriace*a H. T. Chang et B. L. Chen, *Michelia opipara* H. T. Chang et B. L. Chen and *Michelia champaca* Linn. were extracted with SDE, and their chemical components were analyzed by Gas Chromatography-Mass (GC-MS). They found a total of 20 compounds were identified from *Michelia coriacea* H. T. Chang et B. L. Chen, whose main components are alpha-Farnesene, beta-Maaliene, germacrene B and Valencene; a total of 36 compounds were identified from *Michelia opipara* Chang et B. L. Chen, whose main components are Nerolidol, alpha-Pinene, beta-Linalool and 2,6-Octadienal, 3,7-dimethyl-3, 7; a total of 19 compounds were identified from *Michelia champaca* Linn., whose main components are Germacrene B, beta-Linalool, Ocimene, Caryophyllene, Eucalyptol, β-elemene, and Benzylcarbinyl isobutytate.

Bhuiyan *et al*. (2010) analyzed essential oil obtained from fresh leaves and dry buds of *Syzigium caryophyllatum*, which were analyzed using gas chromatography-mass spectrometry (GC-MS). Thirty-eight components were identified in the leaf oil. The main components were eugenol (74.3%), eucalyptol (5.8%), caryophyllene (3.85%) and α-cadinol (2.43%). 31 components were identified in bud oil, with the main components being eugenol (49.7%), caryophyllene (18.9%), benzene,1-ethyl-3-nitro (11.1%), and benzoic acid,3-(1-methyl ethyl) (8.9%). The clove oil from Bangladesh was comparable in terms of its eugenol content. They suggested that clove can be grown as an economically viable crop in Bangladesh.

Boughendjioua (2018) investigated gas chromatography-mass spectrometry (GC/MS) separation of compounds from clove essential oil (*Syzygium aromaticum*). The results showed that the essential oil mainly contained about eugenol (80.00 %) followed by eugenyl acetate (5.01 %) and β-caryophyllene (2.9 %) and stated that in Algeria (North Africa) clove is widely used by traditional practitioners in phytotherapy for these different therapeutic properties, even if this plant is not native to North Africa.

Hamad *et al*. (2017) analyzed essential oils from the leaves of two *Syzygium* species commonly used as spices in Indonesia (*S. polyanthum* and *S. aromaticum*) by gas chromatography/mass spectrometry (GC/MS). The major constituents of essential oil of *S. polyanthum* were cis-4-decanal, 1-decyl aldehyde, and capryl aldehyde. In contrast, the major constituents of essential oil of *S. aromaticum* were p-eugenol and β-caryophyllene; beta-caryophyllene, α-humulene, α-farnesene, and caryophyllene oxide were detected in both essential oils of *S. polyanthum* and *S. aromaticum*.

2.3. Antioxidant Activity

Hasan *et al*. (2020) carried out a study to determine the antioxidant properties of the methanolic extract of *Magnolia champaca* stem bark and its different fractions. Antioxidant activity was assessed using total antioxidant capacity, ferric reducing power, DPPH, hydroxyl, and hydrogen peroxide scavenging assay. Among the different fractions, chloroform fraction (CHF) and ethyl acetate fraction (EAF) showed the highest antioxidant activity. In contrast, aqueous fraction (AQF) showed the lowest activity in DPPH radical scavenging assay with IC50 of 12.12, 22.41, and 55.16 µg/ml, respectively. The study revealed that the different fractions of stem bark of *M. champaca*, especially CHF and EAF, possess antioxidant properties.

Sinha *et al*. (2017) studied the antioxidant activity of *M. champaca* L. leaves with three extracts, i.e., ethanol, methanol, and aqueous. They found that the ethanol extract of *M. champaca* L. has a potent antioxidant ability of 49.14% at 150 µg/ml concentration in DPPH, and the inhibition concentration 50% value of ethanol extract was found to be lowest at 5.41 µg/ml as compared to standard 4.59 µg/ml. A good correlation was also found between the concentration of extract and percentage inhibition with values r2 = 0.998. In the reducing power assay, the percentage inhibition of ethanol extract was 39.12% at 150 µg/ml concentration, and a good correlation was found between the concentration of extract and percentage inhibition with value r2 = 0.967.

Mashkor, (2015) studied the antioxidant capacity of *Syzygium aromaticum* from the different parts of clove, including their stem and fruits, determined by total phenol content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH). The selected parts of the clove plant varied significantly. The results showed that the highest antioxidant activity through TPC and TFC was observed in fruit (247.61 and 141.70 mg/100 dry weight) followed by stem (209.48 and 126.50 mg/100 g), respectively. On the other hand, fruits exhibited a significantly higher scavenging effect compared to stem samples. FRAP and DPPH showed that fruit samples had the highest antioxidant content (437.29 mg TE/100 g dry weight and 87.50%, respectively). The antioxidant activities of different parts of clove extracts indicate that the consumption of the whole fruit and stem supplies the essential quantities of numerous necessary nutrients for the human diet, which includes vitamins high phenolic compounds content (TPC, TFC) and antioxidant activity (FRAP, DPPH). In brief, when all the parameters measured were considered, antioxidants were highly remarkable in the sequence of clove fruits > clove stem.

2.4. Antifungal Activity

Kumar *et al*. (2011) carried out the antimicrobial activities of various extracts of *Michelia champaca* Linn. The flowers' methanolic, ethanolic and aqueous extracts showed antimicrobial activities on *Staphylococcus aureus* and *Bacillus subtilis*, which are gram-positive bacteria. These extracts showed detectable antimicrobial activity on *Escherichia coli* and *Pseudomonas aeruginosa* (except methanolic extract), which are Gram-negative bacteria, and antimicrobial activity against a *Candida albicans* fungus. These results could suggest that some of the studied extracts are effective against Gram-positive bacteria, Gram-negative bacteria (except methanolic extract) and fungi. The study suggests that *M. champaca* flower extract possesses antimicrobial solid activities.

Khairan and Septiya (2021) studied the white champaca (*Magnolia alba*) plant for its antimicrobial activity. The study was to investigate the antibacterial activities of n-hexane, ethyl acetate, and methanolic *Magnolia alba* flower extracts on *Staphylococcus epidermidis* and *Staphylococcus aureus*. The Kirby-Bauer diffusion method determined the antibacterial activity of the *Magnolia alba* flower extracts. The antibacterial activity of the n-hexane, ethyl acetate, and methanolic *Magnolia alba* flower extracts was determined at four different concentrations of 5, 10, 20, and 50%. Results indicated that n-hexane extract had no activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*. Meanwhile, ethyl acetate and methanolic extracts had antibacterial activities against *Staphylococcus epidermidis* and *Staphylococcus aureus*. The diameter zones of inhibition exhibited by the ethyl acetate extract against *Staphylococcus epidermidis* and *Staphylococcus aureus* ranged between 10.45 – 21.03 mm and 10.26 – 26.13 mm, respectively. The diameter zones of inhibition exhibited by the methanolic extract against *Staphylococcus epidermidis* ranged between 11.96 – 18.01 mm, and against *Staphylococcus aureus* ranged between 7.23 – 13.9 mm. In conclusion, the ethyl acetate

Magnolia alba flower extracts gave higher antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Bajpai (2012) evaluated the in vitro and in vivo antifungal efficacy of essential oil and extracts derived from the flower and leaves of *Magnolia liliflora*, against plant pathogenic fungi. The oil and various leaf extracts such as hexane, chloroform, ethyl acetate and methanol revealed promising antifungal effects against *Botrytis cinerea* KACC 40573, *Colletotrichum capsici* KACC 40978, *Fusarium oxysporum* KACC 41083, *Fusarium solani* KACC 41092, *Phytophthora capsici* KACC 40157, *Rhizoctonia solani* KACC 40111 and *Sclerotinia sclerotiorum* KACC 41065 as radial growth inhibition percentages of 38 to 65.6% and 7.6 to 57.3, respectively along with their respective MIC and MFC values ranging from (125 to 500 and 125 to 100 µg mL-1) and (500 to 4,000 and 500 to 8,000 µg mL-1). The oil substantially affected the spore germination of all tested plant pathogens and the concentration and time-dependent kinetic inhibition of *P. capsici* KACC40157. Also, the oil displayed a potent in vivo antifungal effect against one of the selected plant pathogens, *P. capsici* KACC 40157, on greenhouse-grown pepper plants. It was found that the flower oil and leaf extracts of *M. liliflora* could be used as natural alternatives to synthetic fungicides to control the in vitro and in vivo growth of certain important plant pathogenic fungi.

Hamad *et al*. (2017) compared the essential oils of the leaves of two *Syzygium* species commonly used as spices in Indonesia (*Syzygium polyanthum* and *S. aromaticum*) and were evaluated for their minimum inhibitory concentration (MIC) against five food-borne microorganisms. The essential oils of both plants were obtained by the hydro-distillation method. Both essential oils strongly inhibited *Bacillus subtilis* growth. Essential oil of *S. aromaticum* showed more potent inhibitory activity against

Staphylococcus aureus, *Salmonella typhimurium,* and *Vibrio cholera* than that of *S. polyanthum*. Both essential oils did not inhibit the growth of *Escherichia coli*.

Sukohar *et al*. (2022) evaluated the antimicrobial activity of clove leaf essential oil against *Candida albicans* and *Streptococcus mutans*. The disc diffusion method determined the antimicrobial efficiency of *Syzygium aromaticum* L. leaf essential oil against *Candida albicans* and *Streptococcus mutans*. Clove leaf essential oil exhibited antimicrobial activity against pathogenic isolates. *Candida albicans* were recorded at 0.5%, having inhibition zones of 33.3±0.28 mm, 1% 34±0.00mm, and 1.5% 35±0.28mm. While against gram-positive bacteria, *Streptococcus mutans* at 0.5% presented an inhibition zone of 19.95±1.76mm, 1% of 20.5±2.12mm, 1.5% of 22.1±1.55mm. The study indicates that the essential oil of *Syzygium aromaticum* L leaves can be considered a potential antimicrobial agent.

Pinto *et al*. (2009) studied the antifungal activity of clove essential oil (EO) obtained commercially from *Syzygium aromaticum* and analyzed by GC and GCMS. The EO analyzed showed a high content of eugenol (85.3 %). MICs, determined according to Clinical and Laboratory Standards Institute protocols, and minimum fungicidal concentration were used to evaluate the antifungal activity of the clove oil and its main component, eugenol, against *Candida*, *Aspergillus*, and dermatophyte clinical and American Type Culture Collection strains. The EO and eugenol showed inhibitory activity against all the tested strains. Flow cytometry and inhibition of ergosterol synthesis studies were performed to clarify its mechanism of action on yeasts and filamentous fungi. Propidium iodide rapidly penetrated most yeast cells when the cells were treated with concentrations just over the MICs, meaning that the fungicidal effect resulted from an extensive cell membrane lesion. Clove oil and eugenol also caused a considerable reduction in the quantity of ergosterol, a specific fungal cell membrane

component. Germ tube formation by *Candida albicans* was entirely or almost completely inhibited by oil and eugenol concentrations below the MIC values. It was found that clove oil and eugenol have considerable antifungal activity against clinically relevant fungi, including fluconazole-resistant strains, deserving further investigation for clinical application in the treatment of fungal infections.

Khan *et al*. (2011) screened specific plant essential oils and active compounds for antifungal activity and their in vitro interaction with fluconazole against drug-resistant pathogenic fungi. The methods included disc diffusion, broth microdilution, time-kill methods, and checkerboard microtiter tests. Oil compositions are evaluated by gas chromatography-mass spectrometry (GC-MS) analysis. The fungal strains resisted at least two drugs (fluconazole and itraconazole). Ten of the 21 essential oils or active compounds tested showed promising antifungal activity. GC-MS analysis revealed significant active compounds in the essential oils used. Cinnamaldehyde showed the most promising antifungal activity and killing potency against *Aspergillus fumigatus* MTCC2550 and *Trichophyton rubrum* IOA-9. Cinnamaldehyde showed the most substantial synergy with fluconazole against *A. fumigatus* and *T. rubrum* by reducing the minimum inhibitory concentration of fluconazole up to 8-fold. Zones of lysis of the cell wall and cell membrane appeared to be where cinnamaldehyde acted on fungi. This study highlights the broad-spectrum antifungal activity of essential oils and active compounds and their synergy with fluconazole against drug-resistant fungi.

CHAPTER 3: MATERIALS AND METHODS

3.1. Collection of plant samples

The fresh leaf samples of both the plants (*S. aromaticum* and *M. champaca*) were collected from their natural habitat of Sanguem taluka in mid-October **(Table 3.1)**. The mature and healthy leaves and stems were collected and fruits were collected in separate zip-lock polythene bags and brought to the laboratory for anatomical and also for extraction of essential oil. Plants were authenticated using Flora.

Table 3.1: Plant collection site.

Sr. No.	Plant Name	Collection site and location
1	*Magnolia champaca* (L.) Baill.ex Pierre	Rivona Sanguem, Goa 15.1599 47° N 74.1128 01° E
2	*Syzygium aromaticum* (L.) Merr. & L.M. Perry	Rivona, Colamb, Goa 15.1535 41° N 74.1190 11° E

3.2. ANATOMICAL STUDIES (Zaman *et al.*, 2018)

Various parts of both plants were collected for anatomical studies. Freehand sections were taken from fresh leaf (middle portion of the leaf with midrib), petiole (center of the petiole), and stem (center of the stem). Sections were stained with 0.1% safranin for 2-3 minutes, rinsed with distilled water, mounted on a slide with 10% glycerine, and examined under bright field Nikon Eclipse E200 microscope under 4X, 10X, 40X. The desirable portions were photographed using a digital compact camera attached to the microscope, and the images were captured using TC-capture software.

The following procedure was adopted for the preparation of the stain.

Safranin: 0.1% w/v Safranin was prepared by dissolving 0.1g of safranin in 100 mL of distilled water.

3.3. ISOLATION OF ESSENTIAL OIL (Anu *et al.*, 2015)

Fresh leaves of both aromatic plants were cut separately into small pieces with Secateur. 100g of each plant sample and 1000mL of distilled water were subjected to hydro-distillation using a Clevenger-type apparatus with 5L capacity for 3 hours at 70°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 amber vials at 4°C until further analysis **(Plate 3.1)**.

Essential oil yield was calculated using the formula:

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

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

The plant extract's antioxidant capacity depends upon the extract's composition and the conditions of the test system (Tagad *et al*., 2018). The antioxidant studies were carried out on the essential oils of the leaves of *Magnolia champaca* (L.) and *Syzygium aromaticum* (L.) using a 2,2-diphenyl-1-picrylhydrazyle (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

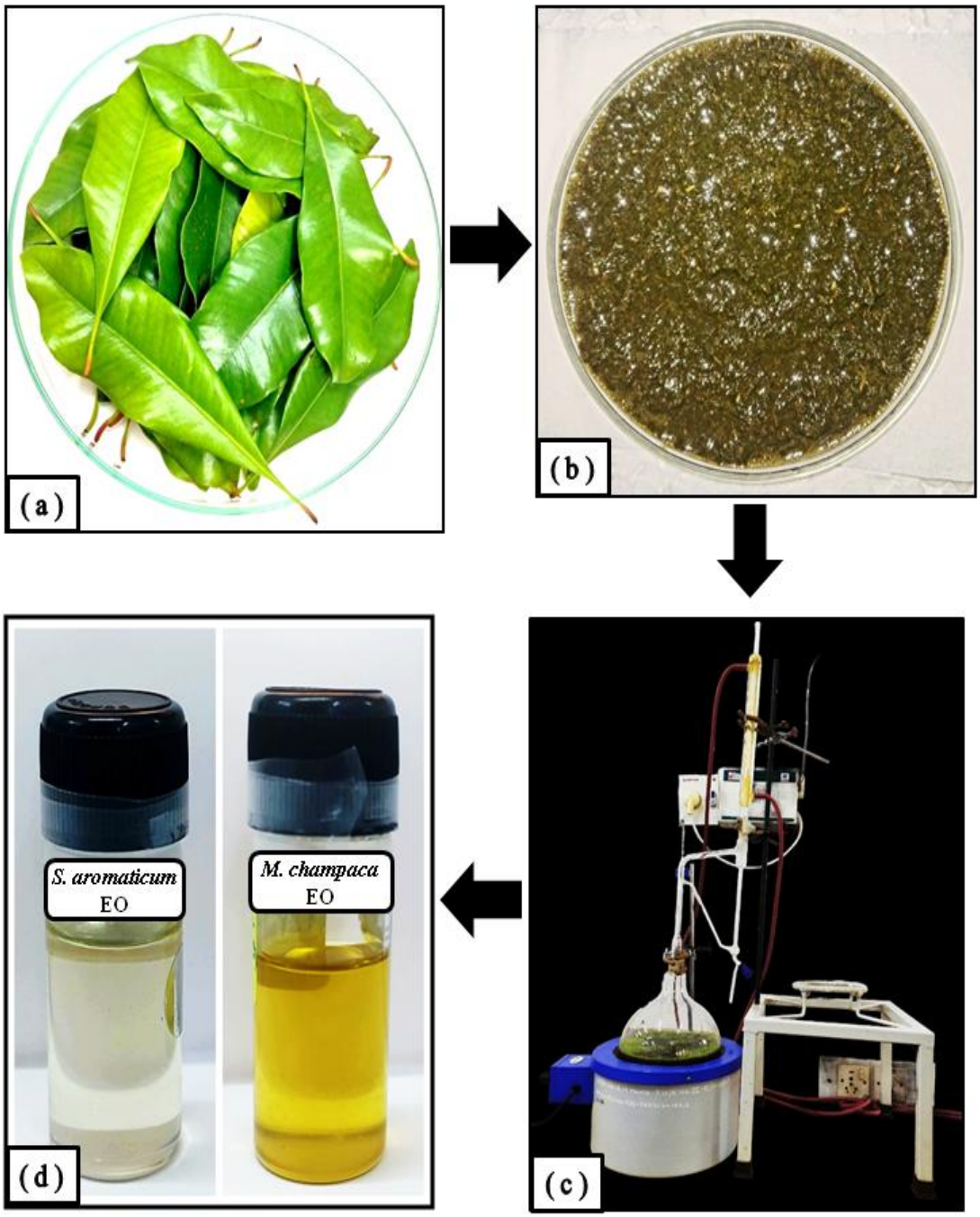


Plate 3.1: Process of essential oil extraction from *M. champaca* and *S. aromaticum* leaves. (a) Fresh leaves, (b) Paste of the leaves, (c) Hydro-distillation method, (d) Extracted essential oils.

prepared by adding 10mL of stock solution to 45mL of ethanol, which was used for the experiment.

b. Preparation of test solution:

The oil stock solution was prepared using ethanol in a 1:1 ratio. Using this, serial dilutions were performed to prepare various concentrations (12:5µg/ml., 25 µg/ml, 50 µg/mL, 100 µg/ml, and 200 µg/ml) of ethanol.

c. Preparation of L-ascorbic acid solution:

10mg of ascorbic acid was dissolved in 10 mL distilled water. Serial dilution was carried out to prepare various concentrations (12.5µg/ml, 25µg/ml, 50µg/mL, 100µg/ml, and 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 distilled water and incubated in the dark for 30 minutes at room temperature. Ethanol was used as a Blank. The absorbance was measured at 517nm.

The following equation calculated the percent inhibition.

$\% \text{ Inhibition} = (A_0-A_1)/A_0 \times 100$

Where A_0 is the absorbance of the control

A_1 is the absorbance of the sample

The antioxidant assay was performed in triplicate, and the mean value was expressed.

IC_{50} value was calculated by plotting percent (%) inhibition against the sample's concentration (µg/mL).

3.5. ANTIFUNGAL ACTIVITY (Mailafia *et al*., 2017; Elias *et al.,* 2019; González *et al*., 2021; Ibrahim *et al*., 2015).

An anti-fungal study used the essential oils extracted from *M. champaca* and *S. aromaticum* leaves on *Aspergillus* species.

3.5.1. Collection of Samples:

Infected *Allium cepa* L. (Onion) was collected from the local market and brought to the laboratory in clean polythene bags.

3.5.2. Sterilization of glassware

Moist Heat Sterilization: Clean and dry petri plates, measuring cylinders, test tubes, and other required glassware were washed and wrapped separately in paper and sterilized by autoclaving at 15 lbs/ 121^{o} for 20 mins.

3.5.3. Preparation of media:

Around 9.75g of PDA (Potato Dextrose Agar) media dissolved in 250 ml of distilled water. The media was digested in the microwave by gentle heating and constant stirring, autoclaved at 15 lbs pressure for 20 minutes, cooled at 45^{o}C, and then poured into sterilized Petri plates under aseptic condition under the Laminar Air Flow.

3.5.4. Method of Isolation:

With the sterilized inoculation loop, a small portion of the infected onion peel was streaked on the PDA plate and incubated for 48 hours in the dark. The fungal colonies

developed after 48 hours were purified by repeated subculturing on sterile PDA plates under aseptic conditions.

3.5.5. Identification of Fungal strain:

The wet mount was prepared using a fresh fungal culture of 48 hours using lactophenol cotton blue as the mounting medium. The fungal strains were picked up from pure colonies and placed on a clean slide containing a drop of mounting medium. It was teased using clean needles, and a cover slip was placed. The slide was observed under 10x and 40x magnification. The structural characteristics of spores, such as mycelium and sporulating structures, were noted. The fungal culture was tentatively identified by the Microbiology Department, SBSB, Goa University **(Plate 3.2)**.

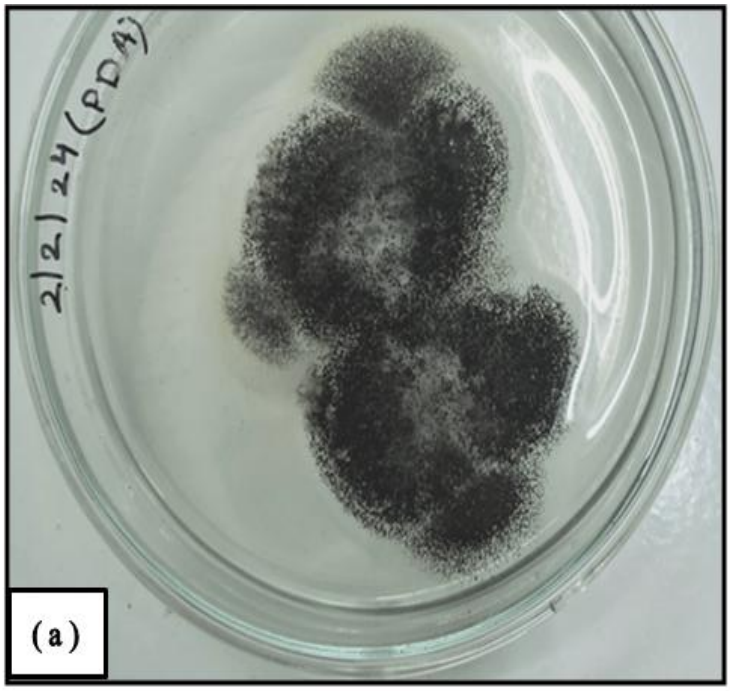
3.5.6. Preparation of Fungal spore suspension:

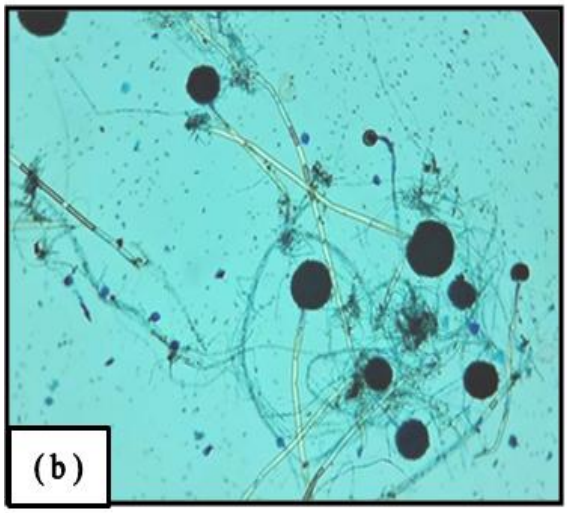
In laminar airflow, the slants were prepared by inoculating the pure culture and incubating for 48 hours at room temperature. The fungal suspension was prepared by adding sterile saline to the slants, scrapping the spores, collecting the spore suspension, filtering it through muslin cloth, then adding a drop of tween 20 solvent to it to wet the spores.

The antifungal study was carried out by the following method:

3.5.7. Agar Well Diffusion Method:

a. Spreading of fungal suspension: (100µl) of fungal spore suspension was uniformly spread plated on PDA plates using a sterile glass spreader under aseptic conditions. The plates were kept standing for 1 hour.





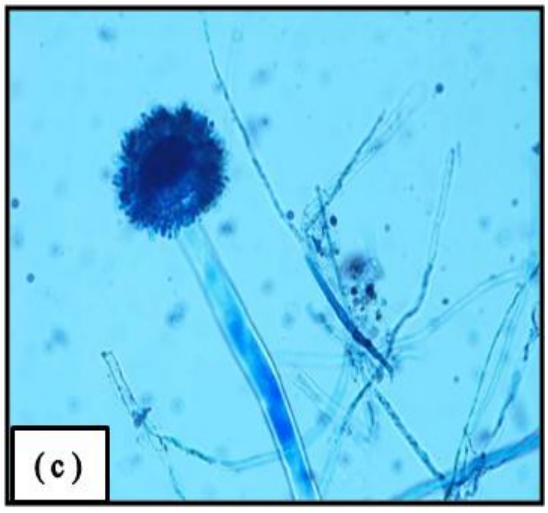


Plate 3.2: Isolation and tentative identification of fungus.
(a) Subcultured plate with fungus, **(b) & (c)** Microscopic images of the wet mount.

b. Preparation of Wells:

Wells were made with the help of a sterile cork borer (10mm diameter) at the center of the plate.

3.5.8. Addition of oil samples into the Wells:

a. The stock solution: 1:1 ratio of the oil: DMSO (Dimethyl Sulfoxide) was prepared and used. The essential oil from the stock solution was used in different volumes (100, 200, 300, 400µl) and was added to each well of different Petri plates using micropipettes.

b. Positive control: The antifungal agent (Fluconazole tablet) was powdered under sterile conditions, and 33mg of it was dissolved in 1mL of DMSO, i.e. (33mg/mL) was used as positive control.

c. Negative control: DMSO solvent was used as the negative control.

Later, the plates were incubated at RT for 48 hours. After the incubation period, antifungal activity was determined by measuring the zone of inhibition around each well and measured (in mm).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 ANATOMICAL STUDIES

4.1.1. *Magnolia champaca*

4.1.1.1. Leaf anatomy

The transverse section of the leaf has a single layer of epidermis covered in a thin layer of cuticle. On both sides of the leaf, there are a lot of uniseriate covering trichomes. The characteristics of lower epidermal cells were identical to those of upper ones, but they had many stomatal apparatus dispersed throughout them. The cells of the upper epidermis had irregular morphologies with curved walls. The leaf's midrib displayed a characteristic plano-convex outline and a dorsiventral structure in the basal and middle regions. The midrib region of the leaf was discovered to be covered in clusters of collenchymatous cells that covered the vascular bundles. A heart-shaped pattern of vascular bundles occupies the center region of the midrib. The lamina showed a single layer of epidermis on the adaxial and abaxial side cells. On the adaxial side, there were four to six layers of spongy mesophyll cells, which had an oval form, and five to seven layers of palisade mesophyll cells on the abaxial side. It had paracytic stomata on the abaxial leaf surface **(Plate 4.1).**

4.1.1.2. Petiole anatomy

Several essential structures are visible in the transverse section of the petiole. The central vascular bundle is at its center and comprises the xylem and phloem tissues. The ground tissue encircles the vascular bundle and also contains parenchyma cells. The outermost epidermis has non-glandular trichomes **(Plate 4.2: a, b, f).**

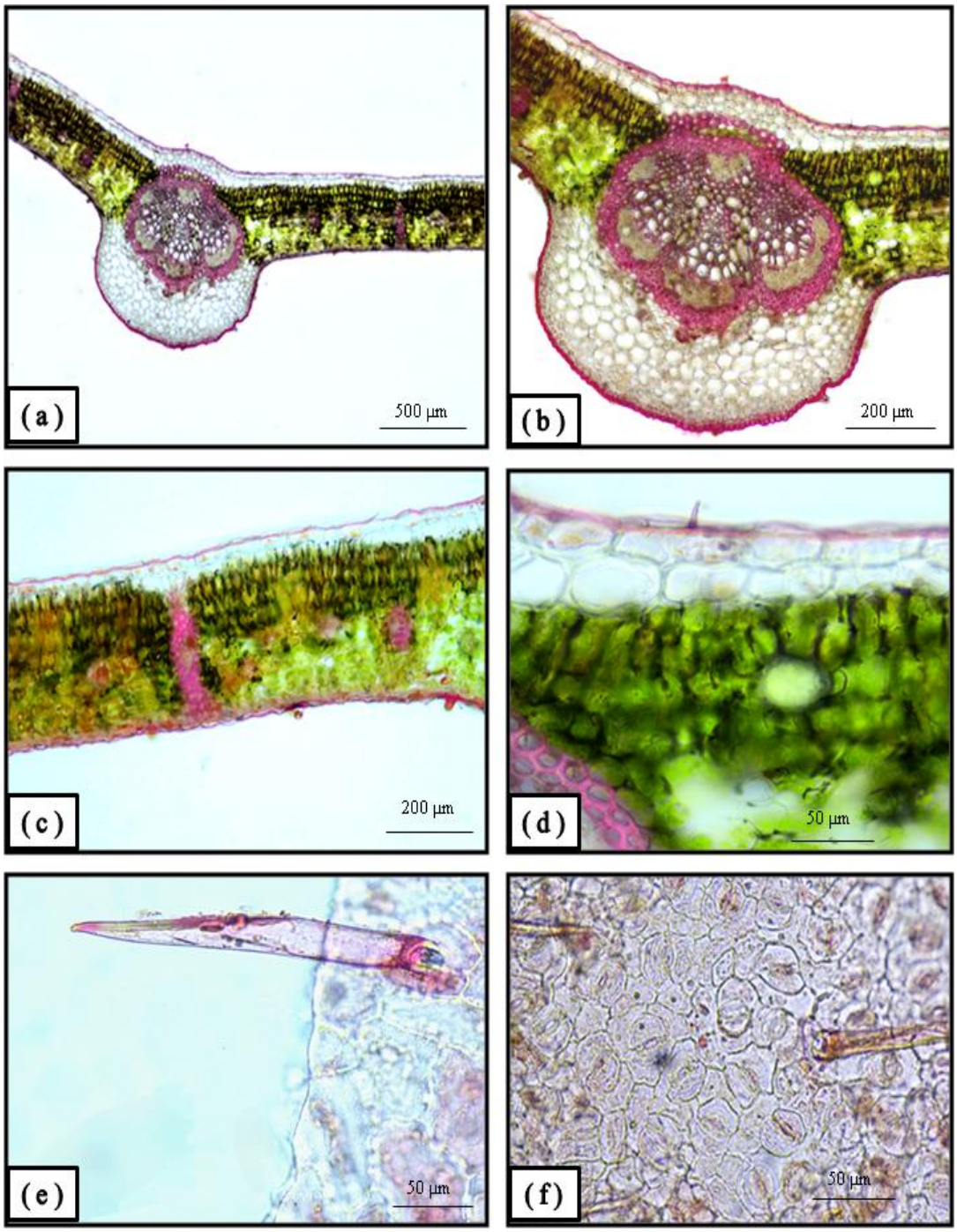


Plate 4.1: Anatomy of *M. champaca* leaf. (a) Overview of leaf (4X), **(b)** T.S of leaf with ground tissue; vascular bundle; adaxial and abaxial epidermis (10X), **(c)** T.S of lamina with mesophyll tissue (10X), **(d)** Epidermis having non glandular trichome (40X), **(e)** Unicellular trichome arising from epidermal layer (40X), **(f)** Abaxial epidermis with paracytic stomata (40X).

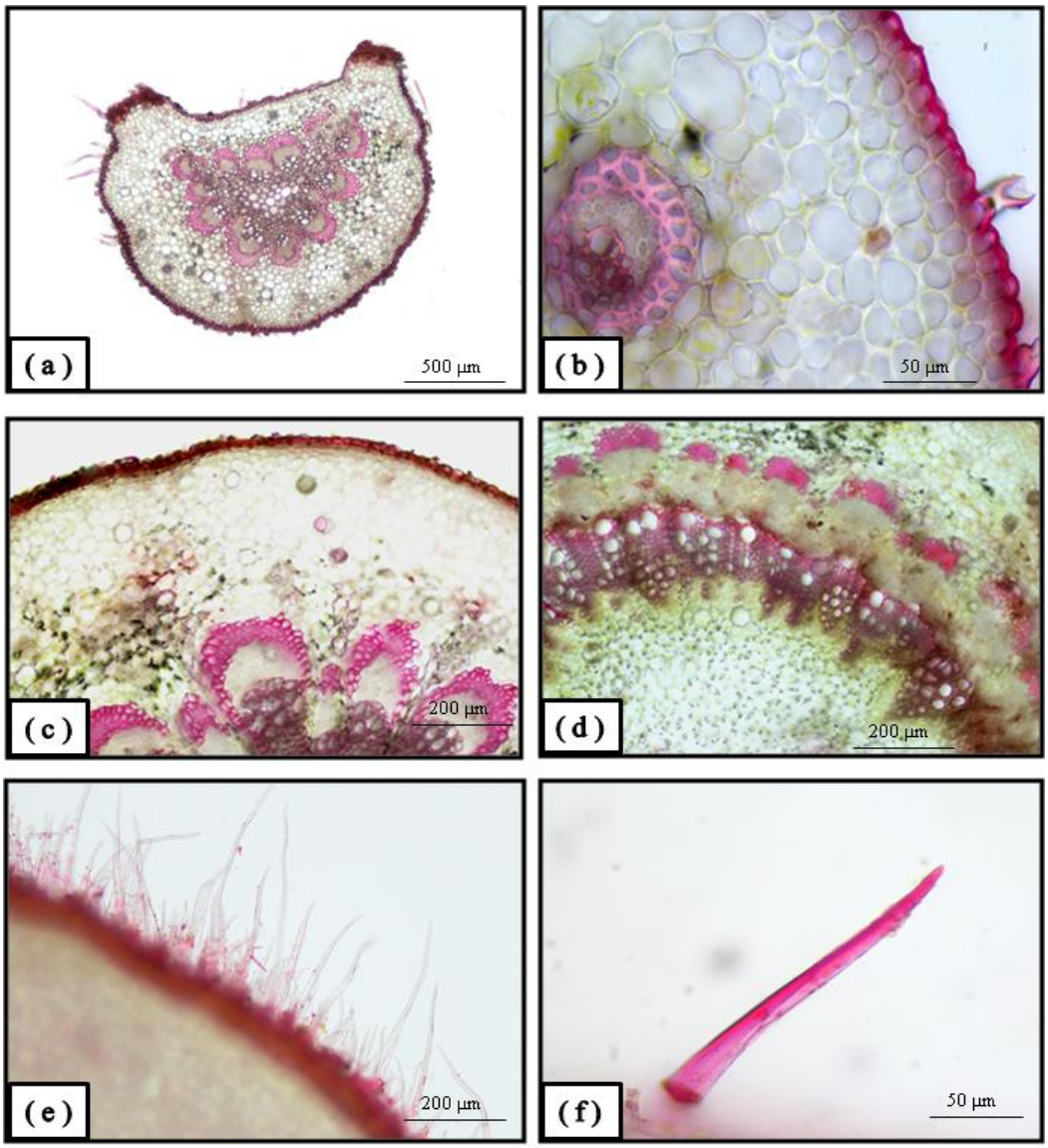


Plate 4.2: Anatomy of *M. champaca* petiole and stem. (a) Overview of the petiole (4X), **(b)** T.S of petiole showing epidermal layer and vascular bundle (40X), **(c)** T.S of stem showing epidermal layer and cortex (10X), **(d)** Pith surrounded by vascular tissue (10X), **(e)** Section showing non glandular trichomes arising from the epidermal layer (10X), **(f)** Non glandular unicellular trichome (40X).

4.1.1.3. Stem anatomy

Several distinguishing characteristics of a *Magnolia champaca* stem are visible when being cut transversely. A big, noticeable pith made of parenchyma cells is in the middle. Vascular bundles are arranged in a ring that encircles the pith in a distinctive arrangement. Both phloem and xylem tissue are present in these bundles. The vascular bundles are often distributed or grouped in a circle inside the stem. The ground tissue region known as the cortex encircles the vascular bundles. Furthermore, the epidermis is the outermost layer. Understanding the stem's transverse section may assist one in better understanding *Magnolia champaca*'s internal structure and other plant species' necessary adaptations **(Plate 4.2: c, d, e)**.

Geetha *et al*. (2011) found similar results and concluded that the leaf consists of a layer of epidermis covered with thin cuticles, and the trichomes are abundantly found on both sides. Karthikeyan *et al*. (2016) reported that *M. champaca* leaf powder contains oil granules and round, thin-walled cells packed with dense, globular structures that appear to contain oil. The mesophyll tissue of the leaf contains the cells containing these oil bodies. Several factors, like geographical origin and temperature, may interfere with the variation of specific characteristics.

4.1.2. *Syzygium aromaticum*

4.1.2.1. Leaf anatomy

The transverse section of the leaf showed an arc-shaped abaxial surface and a straight adaxial surface. The ground tissue near the adaxial and abaxial surfaces had secretory cavities. The vascular tissue of *S. aromaticum* is V-shaped and open, with an intraxylary phloem. There were sclerenchyma cells throughout the vascular bundle. There were both

solitary rhombus and druse crystals in parenchyma cells. The adaxial epidermis is thicker than the abaxial epidermis in a transverse section of the lamina. There were secretory cavities between the spongy mesophylls and the palisade. The parenchyma cells had druse and single crystals **(Plate 4.3)**.

4.1.2.2. Petiole anatomy

The petiole outlines in transverse sections are U-shaped and extend outward on the adaxial surface. Secretory cavities were abundant in the ground tissue close to the epidermis. The petiole's overall vascular system was crescent-shaped and open, with an intraxylary phloem. It was found that the parenchyma cells were composed of more than ten layers. Parenchyma cells contained idioblast tannin, which had a black colour. Druse and solitary crystals were also visible **(Plate 4.4: a, b, c, d)**.

4.1.2.3. Stem anatomy

A thin or thick cuticle layer covers the uniseriate epidermis, followed by a multilayered cortex full of large secreting cavities and taniniferous cells. The vascular tissue is a continuous bicollateral cylinder. The pith presents the center of the stem, which comprises parenchymatous storage cells with roughly large intercellular spaces ranging from isodiametric to polyhedral thin layered cells. Druse crystals are present and abundant in cells **(Plate 4.4: e, f)**.

Zaman *et al*. (2018) found similar results, and they also reported about the branchysclereids, Idioblast mucilage cells, idioblast tannin cells, solitary cuboid shape, and druse crystals found in the leaf and the petiole sections. Taha *et al*. (2012) concluded that *Syzygium aromaticum* has secretory cavities that produce oil glands and was seen as an anomocytic stomata type.

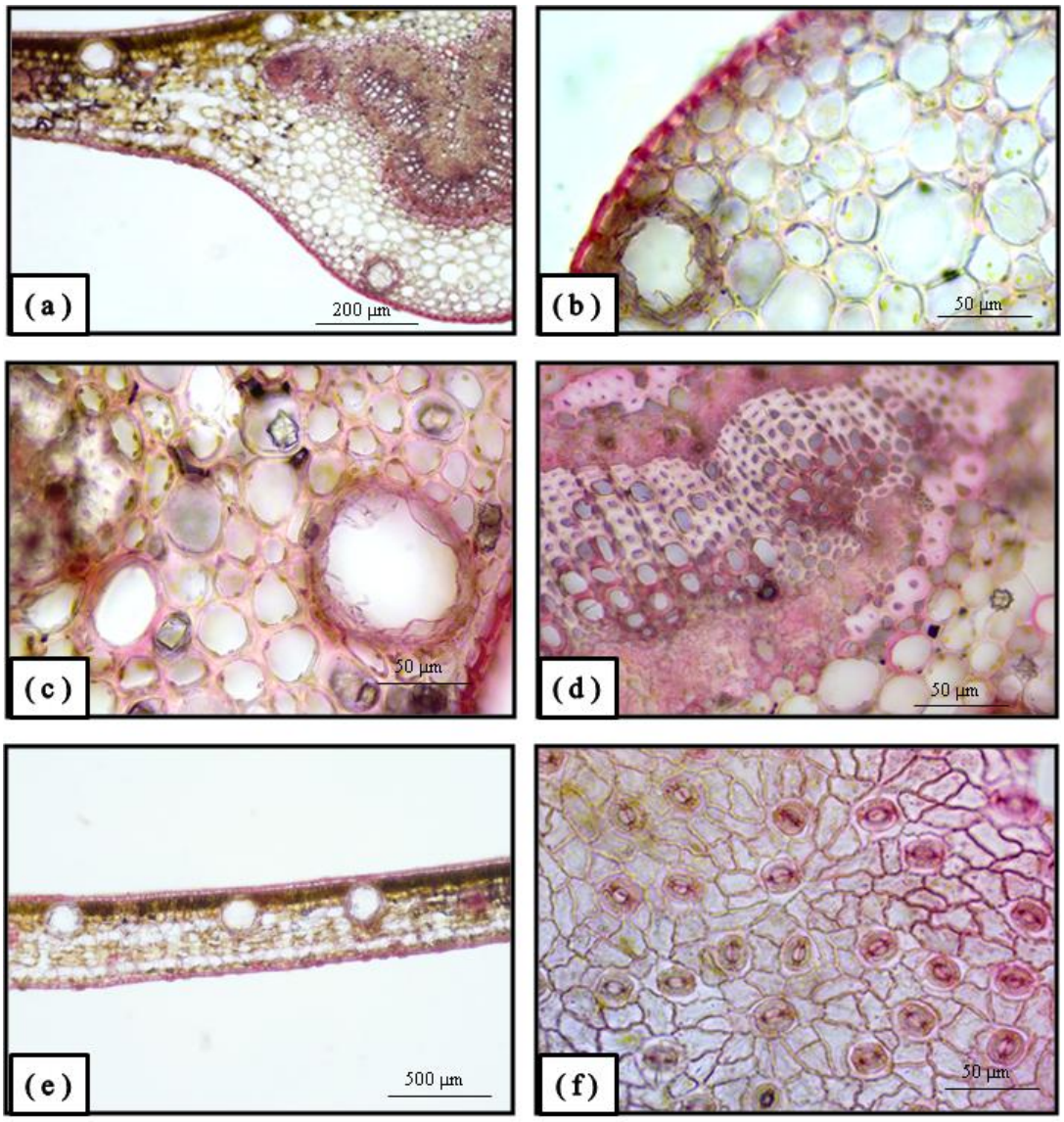


Plate 4.3: Anatomy of *S. aromaticum* leaf. (a) T.S of the leaf through midrib and lamina showing vascular bundle; open vascular tissue (V-shaped) with intraxylary phloem (10X), **(b)** Idioblast oil cell (io) in the ground tissue near the epidermis (40X), **(c)** Druse crystals and single rhombus crystal near the epidermis (40X), **(d)** T.S showing druse crystals (40X), **(e)** T.S of lamina showing idioblast oil cells (io) in parenchymatous cells (4X), **(f)** Abaxial epidermis showing anomocytic stomata (40X).

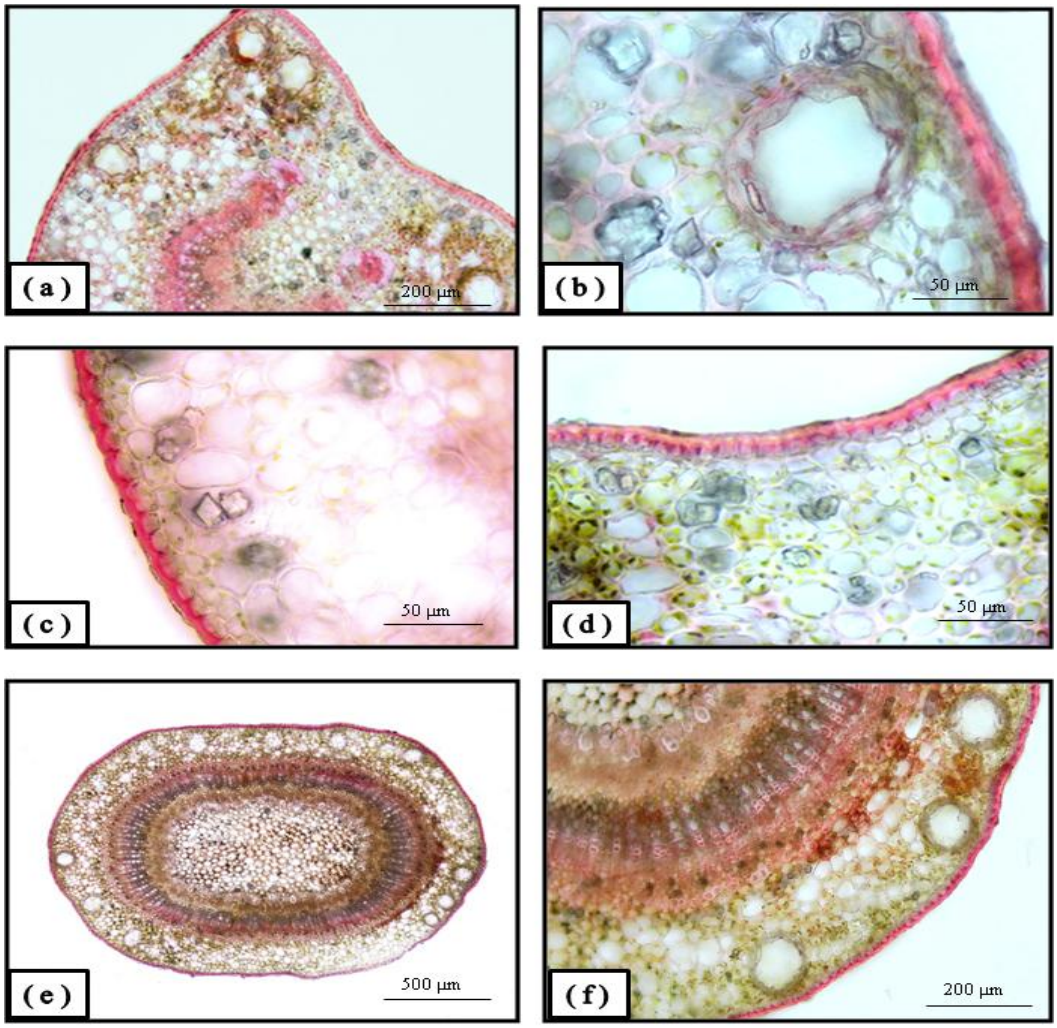


Plate 4.4: Anatomy of *S. aromaticum* petiole and stem. (a) T.S of petiole having open type vascular bundle with presence of intraxylary phloem (10X), **(b)** Idioblast oil cell surrounded by druse crystals (40X), **(c)** Rhombus crystal and druse crystal in parenchyma cells (40X), **(d)** Idioblast tannin cell with druse and solitary crystals in parenchyma cells (40X), **(e)** Overview of stem (4X), **(f)** Idioblast oil cells near the epidermis and presence of druse crystals (10X).

4.2. ESSENTIAL OIL EXTRACTION

Using a Clevenger hydro-distillation apparatus, the essential oil of fresh *M. champaca* and *S. aromaticum* leaves was extracted **(Plate 3.1: d)**. *M. champaca* yielded 0.25% of its essential oil, whereas *S. aromaticum* yielded 1.91%. The essential oil extracted from *M. champaca* leaves had a strong and uncharacteristic smell and was pale yellow. The essential oil extracted from *S. aromaticum* leaves smelled warm, spicy, and robust, and it was colourless.

Similar results were reported by (Sahoo *et al*., 2022) showed that the essential oil of *M. champaca* leaf has a strong scent and a wide range of medicinal uses. However, the % yield varied in the 0.06 ± 0.003% range. On the other hand, the leaf essential oil of *S. aromaticum* possesses antibacterial activity, which enable it to clean surfaces and purify the air effectively. Its leaf essential oil was colourless to pale yellow with medium to watery viscosity, miscible with 96% ethanol, methylene chloride, toluene and oils fat (Boughendjioua, 2018) found that essential oil mainly consists of eugenol (80.00%). Through their fragrant attributes and therapeutic effects, both oils enhance everyday routines and environments with distinctive characteristics, ultimately resulting in holistic well-being. The geographical origin might influence the essential oil yield, environmental factors, genetic makeup, plant age, climate, soil type (Lago *et al*., 2009).

4.3. ANTIOXIDANT ACTIVITY

The antioxidant studies of the essential oils extracted from *M. champaca* and *S. aromaticum* leaves were carried out using the 1,1- diphenyl-2-picrylhydrazyl (DPPH) method. This method measures the decrease in the absorption of the DPPH solution after adding an antioxidant at 517nm. Ascorbic acid (10mg/mL distilled water) was used as a positive control **(Plate 4.5: a).**

In this case, when the essential oil concentration increased, the absorbance decreased as the antioxidant reacted with the DPPH to reduce it to DPPH-H. This demonstrated the antioxidant component in the EO's capacity to scavenge hydrogen by donating energy.

The study's results showed the antioxidant activity of the essential oils found in the leaves of both plants. With an IC_{50} value of 41.86µg/mL, the EOs from *S. aromaticum* leaves showed the highest free radical scavenging activity. In contrast, the EOs from *M. champaca* leaves had the lowest antioxidant activity, with an IC_{50} value of 217.76µg/mL. When comparing the extract's capacity to scavenge radicals, L-ascorbic acid was utilized as a standard, and its IC_{50} value was 43.17µg/mL. The concentration at which a chemical has half of its maximal inhibitory effect is represented by the IC_{50} value. Therefore, the efficiency of an antagonist in blocking a specific biochemical process is defined by the IC_{50} value **(Plate 4.5: b, c)**.

Table 4.1: DPPH free radical scavenging assay: % Scavenging activity of DPPH by L-Ascorbic acid and the essential oils extracted from the leaves of *M. champaca* and *S. aromaticum.*

Sr. No.	**Concentration (µg/mL)**	**L-Ascorbic acid (µg/ml)**	**Leaves Essential oil (µg/mL)**	
			M. champaca	*S. aromaticum*
1	12.5	11.98± 0.008	2.48± 0.002	14.34± 0.005
2	25	48.82± 0.004	5.68± 0.010	36.43± 0.005
3	50	69.68± 0.007	16.26± 0.03	77.51± 0.008
4	100	85.83± 0.005	23.07± 0.004	93.79± 0.005
5	200	95.19± 0.002	45.62± 0.010	97.67± 0.004

Several studies have explored the antioxidant activity of *M. champaca* extract. For example, a paper by Sinha *et al.* (2017) investigated the antioxidant and anti-inflammatory properties of *M. champaca* extract and reported significant scavenging activity against free radicals. Another study by Hasan *et al*. (2020) assessed the antioxidant potential of *M. champaca* extract and its phenolic constituents. The findings suggested that *M. champaca* extract exhibited notable antioxidant activity attributed to its phenolic compounds, supporting the results of the current study.

Clove essential oil has been extensively studied for its antioxidant properties. Research by Mashkor (2015) investigated the antioxidant of clove essential oil and found it possesses potent antioxidant potential due to its high phenolic content, particularly eugenol. Similarly, a review by Haro-González *et al.* (2021) highlighted the antioxidant and therapeutic potential of clove essential oil in various diseases, including cardiovascular disorders and cancer. The review emphasized the role of eugenol as a critical antioxidant component in clove essential oil.

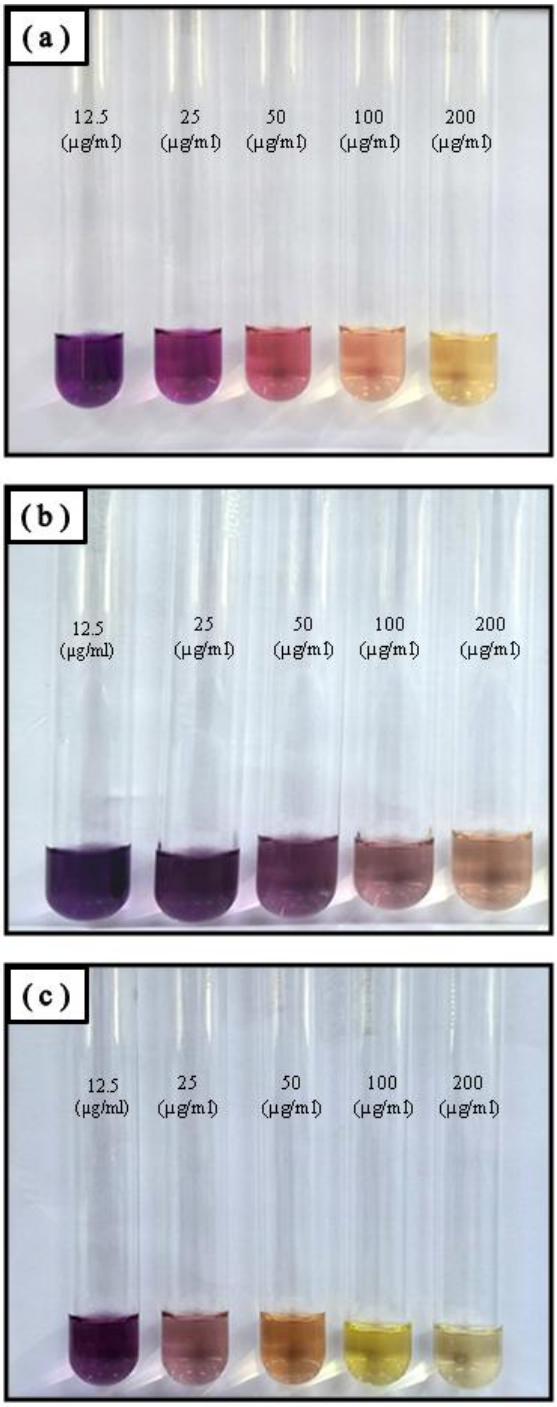


Plate 4.5: Antioxidant studies using DPPH method. (a) L-Ascorbic acid as standard **(b)** *M. champaca*, **(c)** *S. aromaticum.*

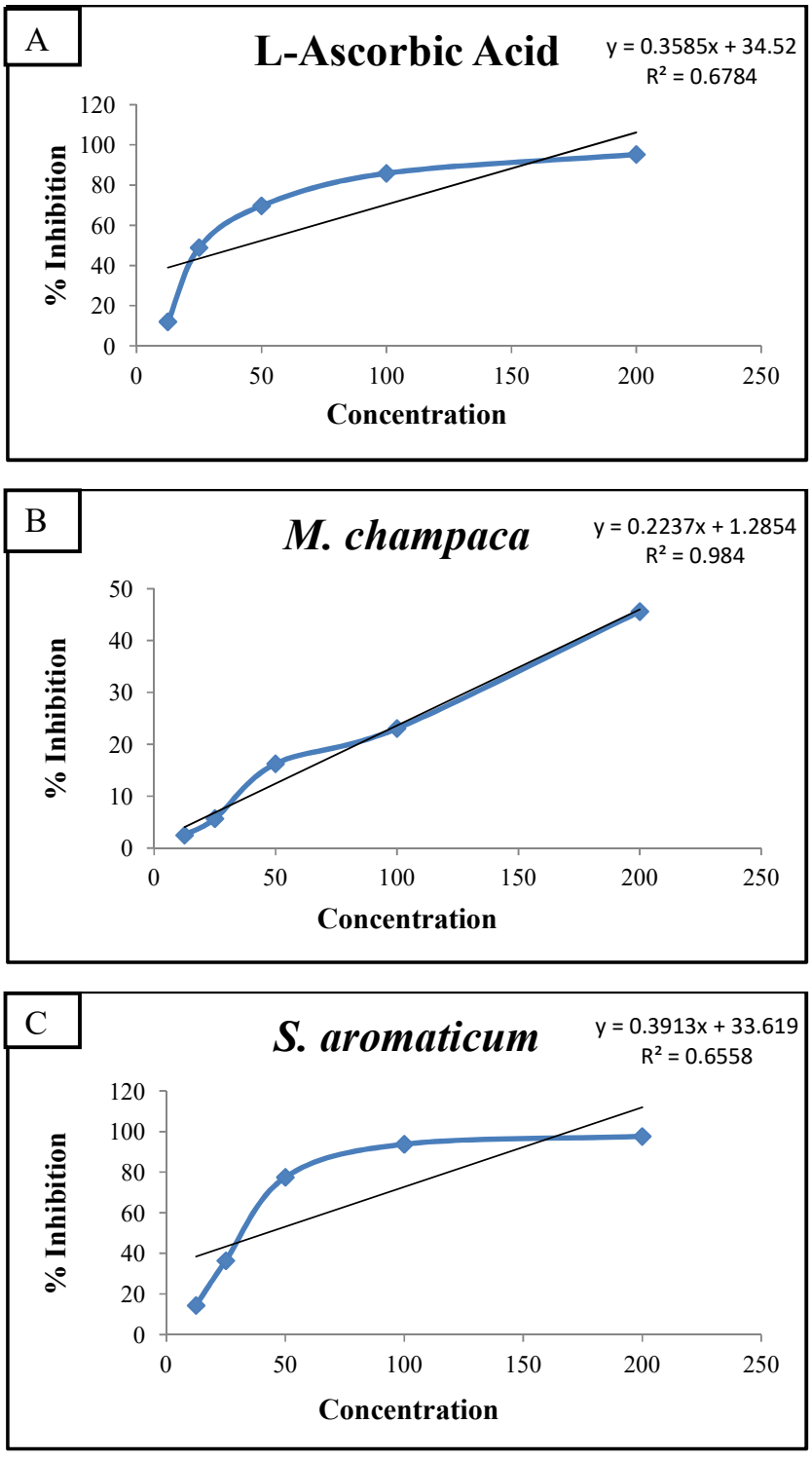


Figure 4.1: DPPH radical activity of (A) L-Ascorbic acid, (B) *M. champaca* , (C) *S. aromaticum*.

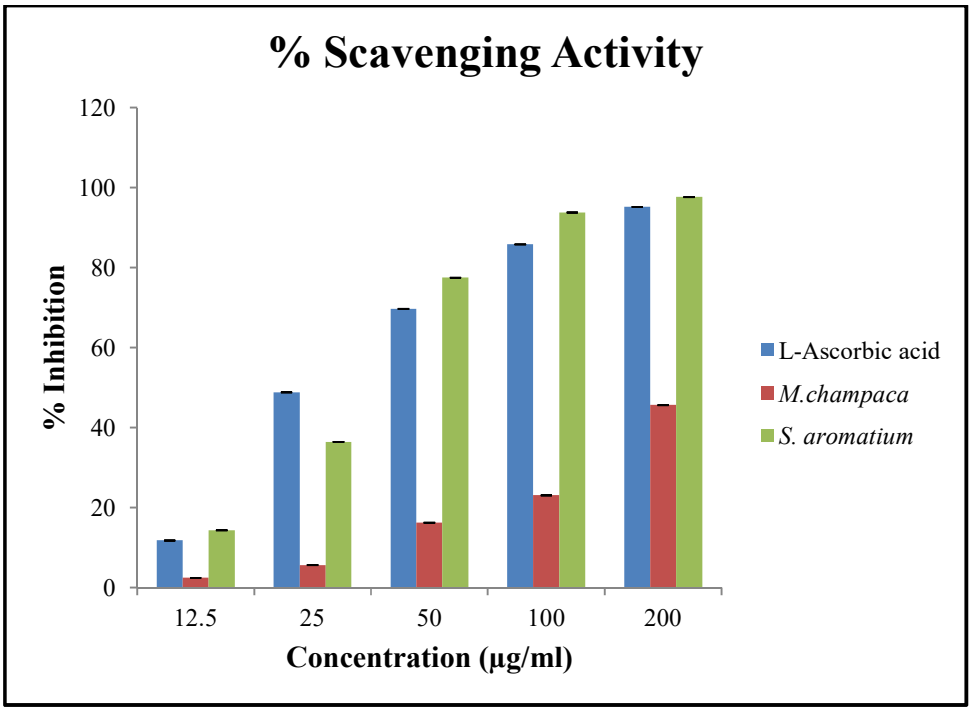


Figure 4.2: Percentage Scavenging activity of L-Ascorbic acid with *M. champaca* and *S. aromaticum* leaf essential oils.

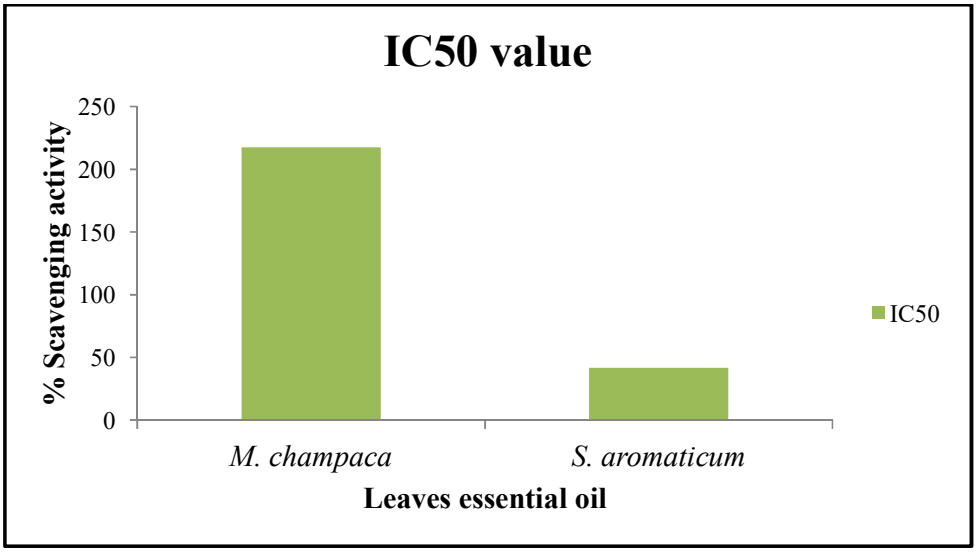


Figure 4.3: IC 50 value of *M. champaca* and *S. aromaticum* leaf essential oils.

4.4. ANTIFUNGAL ACTIVITY

Using the well-established Agar well diffusion method, the antifungal potential of essential oils extracted from the leaves of two different plant species, *M. champaca,* and *S. aromaticum*, was rigorously evaluated in the current study against the tentatively identified *Aspergillus* species. This technique included using a cork borer to create a single well in each plate within the potato dextrose agar (PDA) medium by spreading a fungal suspension onto the surface of the medium. The extracted essential oils were then carefully diluted in a 1:1 ratio with dimethyl sulfoxide (DMSO) to ensure appropriate solubility and different volumes (between 100 and 400 µl) were applied to each well.

The results of this study showed that there was a significant difference in the two essential oils' antifungal effectiveness. However, at all evaluated volumes (100µl, 200µl, 300µl, and 400µl), the essential oil extracted from *S. aromaticum* leaves showed extreme antifungal activity against the tested *Aspergillus* species. On the other hand, the essential oil that was isolated from the leaves of *M. champaca* showed a more complex response, showing little antifungal activity at lower concentrations and a significant increase in inhibitory effects only at the highest tested volume (400 µl) shown in **(Table 4.2, 4.3).**

Carefully carried out in triplicate, the antifungal activity assessment produced accurate and uniform results. The data obtained was carefully analyzed to record the mean values of the inhibition zones seen for every essential oil concentration. Additionally, clear and significant inhibition zones were visible on the positive control plates (antifungal agent), confirming the efficacy of the experimental setup. However, no inhibition zones were seen on the negative control plates (i.e., using DMSO).

The finding that the essential oil extracted from *S. aromaticum* leaves had more antifungal activity than the positive control was notable, indicating that it has significant potential as a natural antifungal agent. These findings highlight the potential use of *S. aromaticum* leaves essential oil as a substitute for synthetic antifungal medications, possibly providing a more secure, long-lasting, and ecologically friendly way to fight fungus. Moreover, more research is necessary to clarify the underlying mechanisms and maximize the therapeutic potential of *M. champaca* leaf essential oil's limited antifungal activity at larger doses **(Plate 4.6)**.

Table 4.2: Antifungal activity of *M. champaca* leaves essential oil against *Aspergillus* species.

Sr. No.	Volumes in µl	*M. champaca* leaves essential oil Zone of Inhibition in mm
1	100	0
2	200	0
3	300	0
4	400	3 ± 1
5	Positive control	5 ± 0

Table 4.3: Antifungal activity of *Syzygium aromaticum* leaves essential oil against *Aspergillus* species.

Sr. No.	Volumes in µl	*S. aromaticum* leaves essential oil Zone of Inhibition in mm
1	100	9 ± 1
2	200	12 ± 0
3	300	13.3 ± 0.57
4	400	15.7 ± 0.57
5	Positive control	5 ± 0

A study by Manhas and Dahiya (2017) investigated the antimicrobial potential of *M. champaca* essential oil against various pathogenic microorganisms, including bacteria and fungi. The findings revealed significant inhibitory effects of *M. champaca* essential oil against both Gram-positive and Gram-negative bacteria and fungal strains. These results corroborate the antimicrobial activity observed in the current study, albeit with variations in the specific microorganisms tested.

Numerous studies have extensively documented the antimicrobial properties of clove essential oil, particularly its potent activity against bacteria, fungi, and other microorganisms. For example, a review by Sharma *et al*. (2017) summarized the antimicrobial efficacy of clove essential oil against a wide range of pathogens, including multidrug-resistant strains. The antimicrobial activity of clove essential oil is primarily attributed to its high eugenol content, which disrupts microbial cell membranes and inhibits microbial growth.

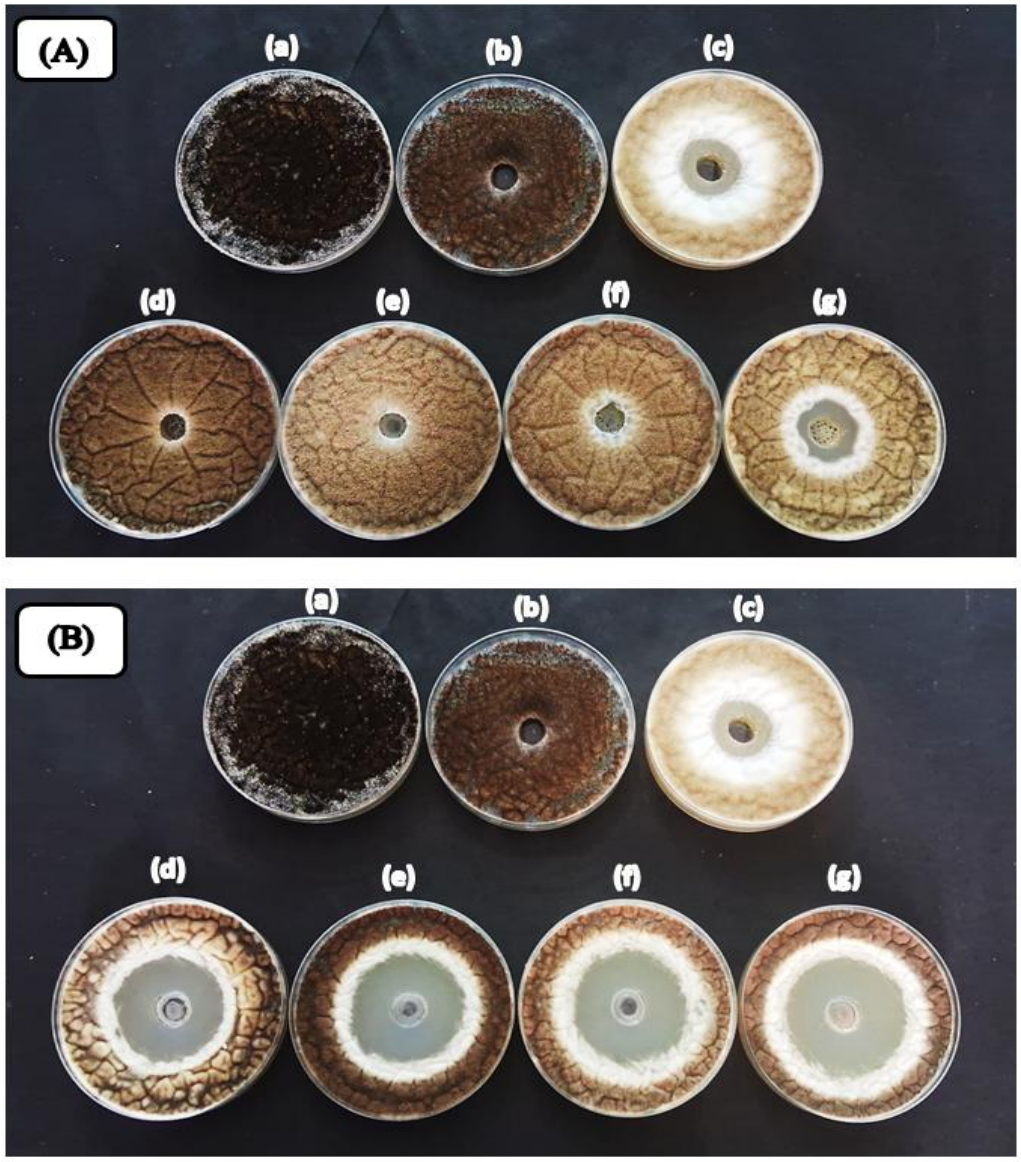


Plate 4.6: Image showing inhibition zone of antifungal activity of essential oils extracted from **(A)** *M. champaca*, **(B)** *S. aromaticum* (1:1 ratio with DMSO) of different volumes against *Aspergillus* species **(a)** Control, **(b)** Negative control, **(c)** Positive control, **(d)** 100µl **(e)** 200µl **(f)** 300µl **(g)** 400µl.

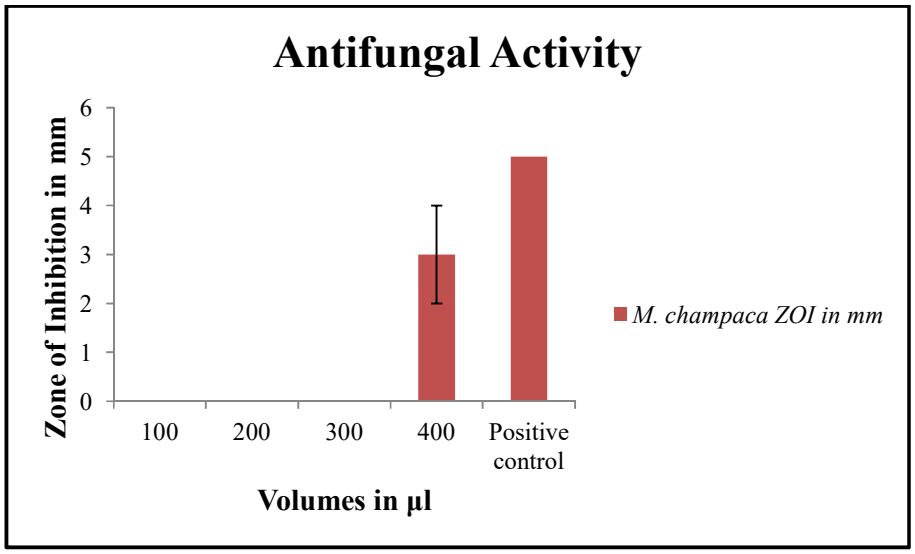


Figure 4.4: Antifungal activity of *M. champaca* leaves essential oil against *Aspergillus* species.

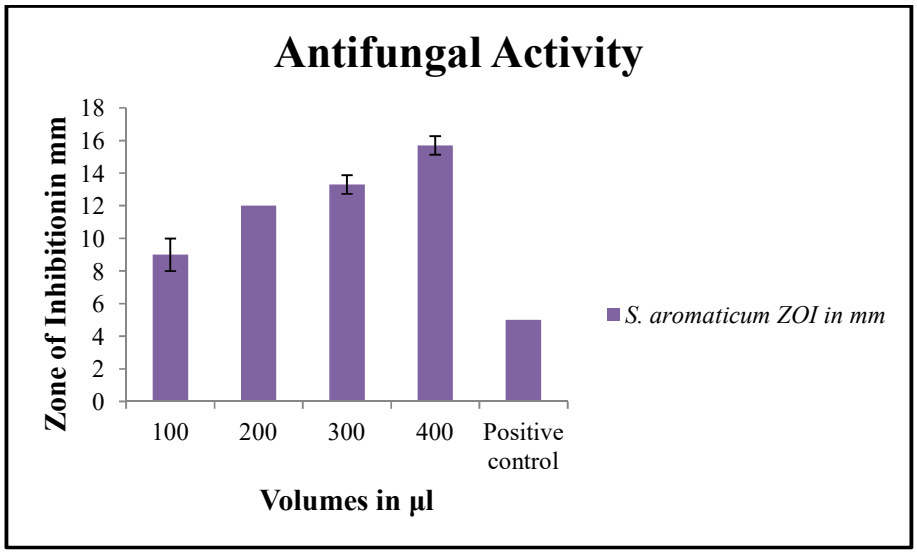


Figure 4.5: Antifungal activity of *S. aromaticum* leaves essential oil against *Aspergillus* species.

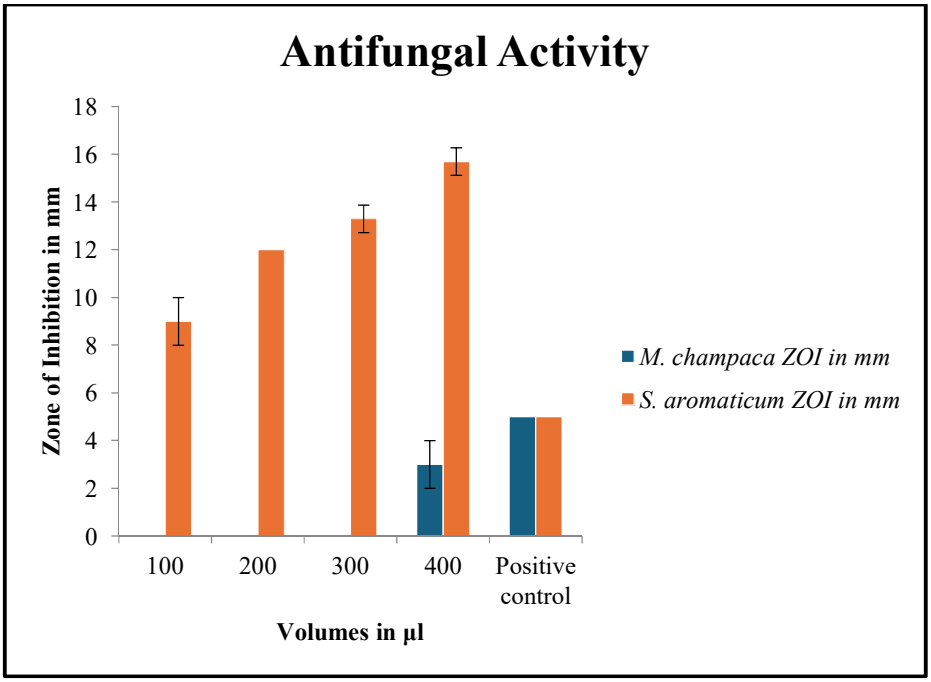


Figure 4.6: Comparison graph of antifungal activity of *M. champaca*, *S. aromaticum* leaf essential oils against *Aspergillus* species.

CHAPTER 5: CONCLUSIONS

The anatomical studies of *Syzygium aromaticum* and *Magnolia champaca* leaves, petioles, and stems revealed striking differences in their structural features. *S. aromaticum* exhibited idioblast oil cells, crystals, and a distinctive v-shaped vascular bundle arrangement. Conversely, *M. champaca* displayed trichomes and more conventional vascular bundle structures. These differences highlight the diverse anatomical adaptations of each plant species to their respective environments and physiological requirements. The idioblast oil cells and crystals in *S. aromaticum* suggest specialized essential oil production and storage mechanisms, potentially contributing to its renowned aromatic properties. On the other hand, the trichomes observed in *M. champaca* may serve various functions, such as protection against herbivores or regulating moisture levels.

The extraction of essential oils from fresh leaves of *M. champaca* and *S. aromaticum* using a Clevenger hydro-distillation apparatus yielded distinct outcomes. *M. champaca* produced a relatively lower yield of 0.25%, while *S. aromaticum* yielded substantially higher at 1.91%. The essential oil from *M. champaca* exhibited a strong, pale yellowish aroma, contrasting with the warm, spicy, and colorless aroma of *S. aromaticum*. These differences in yield and aroma likely stem from variations in their chemical compositions and plant physiology. Further exploration into the specific volatile compounds in each essential oil could elucidate their potential applications in the perfumery, cosmetics, and aromatherapy industries.

The study concluded significant variances in the antioxidant activity of the essential oils isolated from *M. champaca* and *S. aromaticum* leaves. *S. aromaticum* demonstrated high free radical scavenging activity, more significant than the

effectiveness of the standard antioxidant L-ascorbic acid. *M. champaca*, on the other hand, showed less antioxidant activity. This shows that essential oil can be used in food industries and pharmaceuticals, but only after the oil has been purified.

The antifungal study demonstrated that the essential oil from *S. aromaticum* leaves exhibited potent and consistent antifungal activity against tested *Aspergillus* species across all evaluated volumes. Conversely, the essential oil from *M. champaca* displayed a more variable response, showing minimal antifungal activity at lower concentrations and a significant increase in inhibitory effects only at the highest tested volume. These findings underscore the potential of *S. aromaticum* essential oil as a robust antifungal agent while highlighting the need for further research to elucidate the antifungal mechanisms of *M. champaca* essential oil.

The study revealed that both *S. aromaticum* and *M. champaca* essential oils exhibit antioxidant and antifungal activity. However, the essential oil from *S. aromaticum* leaves outperformed *M. champaca* in both assays, indicating its potential for diverse industrial applications. Further exploration of the bioactive compounds in these oils could enhance their utilization in various industries.

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