PHYTOCHEMICAL ANALYSIS AND ANTIFUNGAL PROPERTIES OF THE STEM BARK OF *VATERIA INDICA* Linn.

DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF

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IN BOTANY

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CERTIFICATE

This is to certify that the dissertation entitled "Phytochemical Analysis and Antifungal Properties of The Stem Bark of *Vateria indica* Linn." submitted in partial fulfilment of the requirements for the award of the Degree of Bachelor of Science in Botany is an authentic work carried out by Anjali A.S. (Reg No: AB21BOT010) under the supervision and guidance of Miss. Rishika P.S.

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ABSTRACT

Vateria indica Linn. is a tree species endemic to Western Ghats, India. It belongs to the family Ditperocarpaceae. It has immense therapeutic potential and has been used in Ayurveda, Siddha, and Unani medicines for various treatments. However, major studies have not yet been conducted on the antifungal properties of the tree. The present study is focused on the active principles and antifungal properties of the stem bark of *Vateria indica* against *Candida albicans. C. albicans* causes oral and vaginal candidiases and life-threatening systemic infections in humans. The stem bark of the tree was collected, dried, extracted with ethanol, and analyzed for the active principles. The extract of the bark was found to be rich in carbohydrates, flavonoids, phenolic compounds, tannins, and saponins. The antifungal activity of the extract was screened against *C. albicans*. The soxhlet extract restricted the growth of *C. albicans* at higher concentrations. *V. indica* stem bark extract is a latent antifungal agent against the human pathogen *C. albicans.* This property of the tree can be potentially exploited in the future with the further advance of studies in this field.

CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1. INTRODUCTION

Dipterocarpaceae is a family of tall hardwood tropical trees which are predominantly seen in Southeast Asia. They have two-winged fruits and are a source of valuable timber, aromatic oils and resins. One species of tree in the Dipterocarpaceae family is *Vateria indica*, sometimes known as the white dammar *(Gerry Moore et al., 2015).* A synonym for *Vateria indica* L. is *Vateria malabarica* Blume *(Manivannan et al., 2010).* There are three recognized species in the genus *Vateria*: *V. copallifera* is found in Sri Lanka; *V. indica* and *V. macrocarpa* are found in India.

Kingdom	Plantae
Phylum	Tracheophyta
Class	Mangnoliospida
Order	Malvales
Family	Dipterocarpaceae
Genus	*Vateria* L.
Species	*Vateria indica* L.

The kind is native to India's southern and central Western Ghats Mountain range, which stretches from the Agasthyamalai Hills in the south to southern Maharashtra (*Anurag et al., 2020*). According to *Anurag et al.* (2020), it is mostly seen in the states of Kerala, Tamil Nadu, and Karnataka. According to *Neginhal et al.* (2004), the species is found in evergreen woods in the coastal plains and foothills of Karnataka, usually reaching an elevation of 760 meters or 800 meters on the windward side of the Western Ghats. Trees can be found up to 1200 meters above sea level, though they are more numerous at lower elevations *(Troup et al., 1921).*

The tree can be found beside roadsides, away from forests, as avenue trees *(Neginhal et al., 2004).* Although the species is not native to the Uttara Kannada area of Karnataka,

it was brought to the region approximately 500 years ago by the Sonda kinds, who planted it along the sides of the highways in the cities of Sirsi, Siddapur, and Yellapur *(Neginhal et al., 2004).* It was widely planted in Dakshina Kannada, Kerala's Malabar and Travancore districts as an avenue tree *(Troup et al., 1921). V. indica* is found in regions within its distributional range that have mean annual temperatures of little above 27 °C and 2000–3000 mm of rainfall *(Troup et al., 1921).* There are 118 to 130 rainy days on average per year, with an annual humidity of 77–79% inside the distribution zone *(Troup et al., 1921).* The trees are usually found in forests with a thick layer of humus covering the top *(Troup et al., 1921).* Although the trees can also be found in lowland and plateau areas, they are primarily found in humid, damp forest tracts along well-drained river banks and valleys *(Troup et al., 1921).* At lower elevations, *V. indica*-dominated woods are supported by valleys with thick sandy soil and a high water table *(Yadav et al., 1970)*. There are multiple common names for the species in various languages *(Sasidharan et al., 2004)*.

Languages	Names
Sanskrit	Mandadhupa, Sarja
English	Indian Copal Tree, White Dammer Tree
Marathi	Dhoop, Chandrusa
Kannada	Bileedaamar, Bilagaggala
Telugu	Kahruba Shamai
Tamil	Vellaidaman, Attam
Malayalam	Vellapayin

The trees have cylindrical, straight trunks, reaching heights of up to 40 m, occasionally up to 60 m *(Sasidharan et al., 2004)*. In evergreen forests, it can grow to a large girth, with an individual reaching a girth of up to 5.26 m recorded in Kodagu *(Troup et al., 1921).* The bark is smooth and grey, with green and white spots on the trunk and a cream-coloured bald patch *(Neginhal et al., 2004).* When scarred, it emits a white aromatic resin. The tree has dense foliage in an oval or dome-shaped canopy *(Sasidharan et al., 2004)*. Young twigs are almost cylindrical and have stellate or star-shaped hairs *(Sasidharan et al., 2004)*. The leaves, simple, alternate, and spirally arranged around the twigs, are leathery, about 8–27 x 4.5–10 cm in size, glabrous, elliptic-oblong, with a short tip, rounded base, and entire margin *(Sasidharan et al.,*

2004). Young leaves are dark red or maroon, changing to rose-red and green as the leaf matures *(Sasidharan et al., 2004)*. The petioles are 2 to 3.5 cm long, swollen at the apex, and almost glabrous, with narrow lateral stipules that are deciduous *(Sasidharan et al., 2004)*. Leaf venation comprises 13 to 20 pairs of secondary nerves with closely parallel tertiary nerves at right angles to the secondary nerves *(Sasidharan et al., 2004)*. Inflorescences appear in axillary panicles densely clothed with stellate hairs *(Sasidharan et al., 2004)*. The flowers are white and fragrant, about 2 cm in diameter, with 5 petals, about 40–50 stamens, and yellow, columnar-shaped anthers that protrude beyond the anthers *(Ashton et al., 1988)*. The fruit is a 3-valved capsule, brown, oblong, or ovoid, about 6.4 x 3.8 cm in size *(Ashton et al., 1988)*. The base of the fruit has persistent remnants of the calyx with five sepals bent back *(Joshi et al., 1980)*. The ovary is 3-celled, with 2 ovules in each cell, but the fruit typically produces a single seed with large cotyledons *(Sasidharan et al., 2004)*. The average weight of the ripe fruit is 72.6 g; the fruit has a thick and hard pericarp and bulky uterus weighing about 13.2 g *(Sinu et al., 2016)*.

The resin of the *V. indica* L. is known therapeutically in Indian traditional systems of medicine, the Ayurveda and the Siddha *(Shantha et al., 2011)*. In Ayurveda, it is known as Sarja rasa, and in Siddha, Vellai Kungiliyam *(Shantha et al., 2011)*. Studies have shown that the resin has resveratrol with anti-tumour properties *(Shantha et al., 2011)*. Kungiliya parpam (KP) is a Siddha preparation made from this resin that is effective in treating urinary disorders *(Shantha et al., 2011)*. No physicochemical studies evaluating traditional preparation methods have been conducted *(Shantha et al., 2011)*. A study was therefore undertaken to conduct a preliminary physicochemical evaluation of Sarja rasa and Kungiliya parpam samples and compare the changes with the working hypothesis that traditional methods of preparation can reconstitute and fortify the phytocompounds present in the resin to confer various medicinal properties *(Shantha et al., 2011)*. The physicochemical constituents of two samples (Sarja rasa and Kungiliya parpam) were evaluated by the solvent extraction method using the Soxhlet apparatus *(Shantha et al., 2011)*. Benzene, petroleum ether, chloroform, and ethyl alcohol extracts of both samples were estimated and compared *(Shantha et al., 2011)*. Solvent extraction of both samples revealed that there was a decrease in wt.%. of the benzene extract with an increase in percentage w/w of all other solvent extracts in KP *(Shantha et al., 2011)*. This provides preliminary hints about the phytochemical

mechanisms involved in traditional preparation methods *(Shantha et al., 2011)*. The study demonstrates the reconstitution of phytocompounds by traditional Kungiliya parpam preparation methods using Sarja rasa, which may contribute to the unique medicinal properties of KP *(Shantha et al., 2011)*. Advanced pharmacological and HPLC studies could help provide a modern scientific basis for traditional medicinal methods *(Shantha et al., 2011)*. Another piece of traditional knowledge related to the resin extracted from this tree is that its powdered form is used in temple rituals such as mudiyattu and theeyattu. Cotton torches are an inevitable part of these rituals during the Purappadus as well as during the battle. Dried and powdered pinewood resin ('Thelli') is thrown into the flame of a cotton torch, which becomes very inflated and adds to the solemnity of the scene. In addition, it has proven medicinal value as the emitted smoke purifies and freshens the atmosphere and destroys harmful elements that cause infectious diseases.

V. indica is an indigenous medicinal plant species native to the Western Ghats *(K. R. H. et al., 2010)*. The tree is used commercially mainly for the production of plywood *(Warrier et al., 2020)*. It is also an important source of oleoresin in India *(Shiva et al., 1998)*. *V. indica* kernels from India yield about 22% fat by solvent extraction *(Shiva et al., 1998)*. The fruits of *V. indica* contain 25% tannin *(Shiva et al., 1998)*. The seeds are edible *(Shiva et al., 1998)*. The leaves and bark are mainly used for tanning and medicinal purposes *(Shiva et al., 1998)*. The bark, resin, and leaves of the plant are used in Ayurvedic, Siddha, Unani, and folk medicine to treat leprosy, eczema, rheumatism, diarrhoea, and ulcers *(K. R. H. et al., 2010)*. A study of the stem for phytonutrients revealed the existence of carbohydrates, tannins, phenols, and flavonoids in the aqueous and ethanolic extracts *(Bugade et al., 2022)*. Petroleum ethers extracted from the stem of the tree showed the occurrence of phytosterols *(Bugade et al., 2022)*. Leaves and roots showed the presence of bergenin and hopeophenol. The bark consists of bioactive components such as oligostabinoids and monoterpenes *(Bugade et al., 2022)*. The bark of the *V. indica* stem also consists of various polyphenols, resveratrol derivatives, and bergenin *(Mishima et al., 2003)*. The resin is a complex mixture of several triterpenes, hydrocarbons, ketones, alcohols, and small amounts of sesquiterpenes *(Bugade et al., 2022)*. The plant showed anti-inflammatory effects, anti-tumour effects, anthelmintic effects, antiulcer effects, antimicrobial effects, anti-cancer effects, antioxidant properties, antimutagenic properties, and so on *(Bugade et al., 2022)*.

In vitro analysis of an ethanol extract from the bark of a tree trunk showed anticancer activity against mouse sarcoma 180 cells *(Mishima et al., 2003).* The ethanolic extract of the bark of *V. indica* has a high content of phenols and flavonoids. This property makes it a good source of antioxidant and antimutagenic substances *(N. et al., 2012).* The seeds are used to prepare *V. indica* L. seed butter, commonly called Malabar Tallow. It is an edible fat extracted from the falling seeds, which are abundantly available in June and July *(Rajmohan et al., 2017).* The crude ethanolic extract of *V. indica* showed notable paresis and death of caterpillars at a concentration of 50 mg/ml *(JK et al., 2018).* Acetone, ethanol, and petroleum ether extracts of *V. indica* bark showed active cytotoxicity in cancer cells, making it a potent anticancer agent *(JK et al., 2018).* An aqueous extract of the stem bark of *V. indica* L. showed anti-obesity properties in test organisms fed a high-fat diet, making it a potential herbal therapeutic agent for obesity *(Alva et al., 2018).* Ethanol extract of *V. indica* bark was useful in neuroprotection and memory improvement in amnesic mice *(Alshabi et al., 2020).* ZnOVI nanostructures induced by an aqueous extract of *V. indica* fruit with potential anticancer and photocatalytic activities have excellent cytotoxicity against human triple-negative breast cancer cells *(D'Souza et al., 2021).* Biodiesel blends produced from *V. indica* showed dominant combustion properties. Emissions of unburnt hydrocarbons, CO, and smoke were lower, while nitrogen oxides were higher than for neat diesel, enabling its use as a biofuel *(Gowda et al., 2021).* The resin of *V. indica* is chiefly used as a fragrance material *(Nandkishore et al, 2023)*. It was found to be useful to be a tonic, carminative, and expectorant. *V. indica resin* is also used for the treatment of gouty arthritis *(Kayani et al, 2014).* The resin from *V. indica Linn.* possesses resveratrols having anti-tumour properties *(Venkateshwarlu. et al, 2011).* The resin also has an anti-microbial activity that was found to be used in treating urinary tract and gastrointestinal disorders *(Venkateshwarlu. et al, 2011)*. The aqueous extract of *V. indica* resin impeded the heat-induced albumin denaturation, and proteinase activity and stabilized the Red Blood cell membrane *(Kavitha et al., 2017).* The GC-MS result of *V. indica* resin possessed the sesquiterpenoid compound which has an anti-inflammatory activity *(Kavitha et al, 2017).* The resin is employed in the medicament for ailments such as chronic bronchitis and throat troubles and for treatment of cough, asthma, leprosy, skin eruptions, crack infection, wounds, ulcers, etc *(JK et al, 2018).* The resin has been used traditionally in India for centuries for curing haemorrhoids *(Ahad et al., 2020).* The resin is also used in several antiseptic and anti-inflammatory

ointments *(Nandkishore et al., 2023). V. indica* resin-based ointment was found to be more effective in reducing the radiation (RT) induced burns caused during radiation therapy in cancer treatment *(Mirza et al., 2023). V. indica* have shown inhibitory effects for bacteria staphylococcus aureus and many more. Studies state that the plant shows antimicrobial activity due to the presence of flavonoids in the extract *(Suresh et al., 2022).* The previous studies point to the wide range of medicinal effects of the extracts of different parts of *V. indica.* The stem bark of the tree has immense potential as a therapeutic agent. Therefore, in this study, we explore more into the antimicrobial effect of the stem-bark extract of *V. indica*.

1.2. OBJECTIVES

- To conduct the phytochemical analysis of the stem bark of *Vateria indica* Linn.
- To identify the phytochemicals in the stem bark of *Vateria indica* Linn.
- To analyze the antifungal activity of *V. indica* stem bark extract against *Candida albicans.*

CHAPTER 2

REVIEW OF LITERATURE

2.1. *VATERIA INDICA*

2.1.1. HABIT AND MORPHOLOGY

Vateria indica L. is a large tree that originated in India. It grows in the swamps and forests of the middle of the Western Ghats, but is sparsely populated *(Kavitha et al.,2017).* This species is mainly found in Karnataka, Kerala, Tamil Nadu and Maharashtra. *(JK et al.,2018).* A slow-growing tree species found in emerging species or canopy in low and medium altitude evergreen forests, with a smooth, grey bark with green and white stems and the trunk is grey, green, and white. The plant has dense foliage and fragrant white flowers *(Alva et al.,2018)*

Vateria indica is a large magnificent evergreen resinous tree growing up to 25m tall *(Agro-techniques of selected medicinal plants, 2022).* The bark is smooth, about 1cm thick, whitish grey blotched with green, bitter, and acrid in taste, peeling off into round flakes *(Agro-techniques of selected medicinal plants, 2022)*. Blaze is dull brown and wood is white and hard. Young branchlets are drooping, with minute stellate trichomes *(Agro-techniques of selected medicinal plants, 2022)*. Leaves are alternate, elliptic, oblong, 10-25cm X 5-10cm in size, heart-shaped or rounded, apex acuminate, margin entire, and leathery in appearance. Stipules are prominent *(Agro-techniques of selected medicinal plants, 2022)*. Flowers are bisexual. The flowers are white with a slight fragrance and are arranged in panicles. Panicles are robust, multi-branched up to 15cm long, and drooping. Fruits of *Vateria indica* are fleshy capsules, pale brown, and hard when in dry condition *(Agro-techniques of selected medicinal plants, 2022).*

Vateria indica wood has been used for making tea chests, partitions, packing and cordite cases, coffins, boxes, planking, posts, floorings, ceilings, and cabinets, besides bobbins and shuttles in the textile industry, oars for sea-going vessels, and match-splints *(Joshi et al., 1980).* Large amounts of *Vateria indica* timber were shipped from the Malabar region to Bombay to be sold as "Malabar White Pine" (Vellapiney), with around 6200 tons of timber used per annum in the late 1960's *(Venkatesh et al.,2010).* The wood,

after preservative treatment was also used for railway sleepers *(Joshi et al.,1980).*

Languages	Names
Sanskrit	Mandadhupa, Sarja
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Tamil	Vellaidaman, Attam
Malayalam	Vellapayin

2.1.2. USES OF THE PLANT

The resin of *Vateria indica*, extracted by scratching the tree's bark, is called white dammar, also known as "Malabar fallow", "Dhupa fat", "Indian Copal", or "piney resin" *(Dymock et al.,1890).* It is used as incense in India, for incense sticks, and to manufacture candles and soaps *(Anurag et al.,2020).* From dried kernels, a fat called "piney tallow" was extracted, which was used to adulterate ghee, making candles and soaps, to treat chronic rheumatism, and for sizing cotton yarn in place of animal tallow *(Joshi et al.,1980).* The resin mixed with coconut oil makes an excellent varnish resembling copal *(Joshi et al.,1980).* The bark, resin, and leaves are used in Ayurvedic, Siddha, Unani, and folk medicine for the treatment of leprosy, eczema, rheumatism, diarrhoea, and ulcers *(Selvaraj et al.,2014).* Fine shavings of resin are administered internally to check diarrhoea *(Joshi et al.,1980). Vateria indica* oil, produced from the seeds, is refined to yield a fat used in confectionery and cosmetics.

The resin from the tree *Vateria indica* L. is therapeutically known in Indian traditional systems of medicine the Ayurveda and the Siddha *(Shantha et al.,2011).* It is known as Sarja rasa in Ayurveda and Vellai Kungiliyam in Siddha *(Shantha et al.,2011).* Studies have shown that the resin possesses resveratrols having anti-tumor properties *(Shantha et al.,2011)* Kungiliya parpam (KP) is a Siddha preparation made from this resin that is effective in the management of urinary tract disorders *(Shantha et al.,2011).* There have been no physicochemical studies evaluating traditional methods of preparation *(Shantha et al.,2011).*

Hence a study was undertaken to do a preliminary physico-chemical evaluation of samples Sarja rasa and Kungiliya parpam and compare the changes with a working hypothesis that, Traditional methods of preparation might reconstitute and fortify the Phyto-compounds present in the resin to bestow various healing properties *(Shantha et al.,2011).* Physicochemical constituents of the two samples (Sarja rasa and Kungiliya parpam) were evaluated using the solvent extraction method using the Soxhlet apparatus *(Shantha et al.,2011).* Benzene, petroleum ether, chloroform, and ethyl alcohol extracts of both samples were estimated and compared *(Shantha et al,2011).* Solvent extraction of both samples revealed that there was a decrease in %w/w of benzene extract with an increase in percentage weight/weight (%w/w) values of all other solvent extracts in KP *(Shantha et al.,2011).* This provides a preliminary hint toward the phytochemical mechanism involved in the traditional method of preparation (*Shantha et al.,2011).* The study demonstrates the reconstitution of phytocompounds with traditional methods of preparation of Kungiliya parpam using Sarja rasa which, might contribute to the unique medicinal properties of KP *(Shantha et al.,2011)*. Advanced pharmacological and HPLC studies might help in eliciting a modern scientific basis for traditional methods of medicinal preparations *(Shantha et al.,2011).* Another traditional knowledge related to the resin extracted from this tree is that its powder form is used in temple rituals like mudiyattu and theeyattu. Cotton torches are an unavoidable part of these rituals during the Purappadus as well as the battle. The dried and powdered resin of pine wood (Thelli') is thrown onto the flame of the cotton torch which inflates highly and adds to the seriousness of the scene.. Further, this has proven medical value as the smoke emitted cleans and refreshes the atmosphere and destroys harmful elements that cause contagious diseases.

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2.2. PHYTOCHEMICALS

Phytochemicals are bioactive compounds produced by plants as a result of primary and Secondary metabolism. Plants produce phytochemicals to protect themselves from the environment and predators. Phytochemicals are found in many plants which include vegetables, fruits, grains, nuts, and more. Vegetables and fruits that are brightly coloured present higher concentrations of phytochemicals. Phytochrmicals are also natural antioxidants. Some important phytochemicals found in plants are alkaloids, flavonoids, saponins, terpenoids, phenolic compounds, polyphenols, triterpenoids, and so on.

2.2.1. METHODS OF PHYTOCHEMICAL ANALYSIS

The commonly used methods for phytochemical analysis are Chromatography, spectroscopy, biological assay, and other extraction techniques such as hot extraction or cold extraction. In chromatography, various bioactive compounds are isolated using chromatographic techniques. The most commonly used methods are column, paper, and thin layer chromatography methods because of their convenience and various availability of stationary phases such as silica gel, polyamide, cellulose, and alumina gel for separating the compounds. Spectroscopy is used for studying the structure of molecules by interaction with electromagnetic radiation. For this, UV spectroscopy, mass spectroscopy, IR spectroscopy, MNR spectroscopy, etc. are used commonly. Biological assay helps to understand the biological activity of phytochemicals, for their anti-cancerous, antimicrobial, or antifungal properties. It involves evaluating its effect on living organisms or biological systems. Extraction techniques are used to extract phytochemical compounds from plant materials by dissolving them in a suitable solvent. The most commonly used extraction methods are maceration, Soxhlet extraction, supercritical fluid, and enzyme-assisted extraction.

2.3. MICROORGANISMS

A microorganism is an organism of microscopic size, which may exist in its single-celled form or as a colony of cells. The possible existence of unseen microbial life was suspected from ancient times, such as in Jain scriptures from the 6th century BC India. The scientific study of microorganisms began with their observation under a microscope in the 1670s by Anton Van Leeuwenhoek. In the 1850s Louis Pasteur found that microorganisms caused food spoilage debunking the theory of spontaneous generation.

The life of microbes is very diverse. Microbes are classified into three main domains – Archaea, Bacteria, and Eukaryota. Many multicellular organisms are microscopic, namely microbes, some fungi, and some algae, but these are generally not considered as microorganisms. Microbes have different habitats and live everywhere from the poles to the equator, deserts, geysers, rocks, and the deep sea. Some microbes are adapted to extreme conditions such as very hot or very cold conditions, high pressure, high radiations, etc.

2.3.1. EFFECT OF MICROBIAL LIFE ON EARTH

Microbes are omnipresent in the biosphere and their presence invariable affects the environment in which they grow. The effects of the microbes on the environment in which they grow can be beneficial or harmful or inapparent about human measure or observation. The most significant effect of microbes on earth is their ability to recycle the primary elements that make up all living systems, especially carbon, oxygen, and nitrogen *(Gupta et al. 2016).*

Microbes are important in human culture and health in many ways, serving to ferment foods, treat sewage, and produce fuel, enzymes other bioactive compounds. Microbes are essential tools in biology as model organisms and have been put to use in biological warfare and bioterrorism. They are vital components of fertile soil. The pathogens responsible for many infectious diseases are microbes.

2.3.2. BENEFICIAL EFFECTS OF MICROBES

Microorganisms have provided many economically valuable products and processes used every day by all citizens. With the advent of modern biotechnological tools and processes, potential applications of microorganisms are expected to increase significantly. Exploration of microbial diversity will not only be a voyage into Earth's biosphere that will lead to the discovery of new and unusual organisms, but also a means of discovering new products and technologies for agriculture, bioremediation, medicine, pharmaceutical industries, and biotechnology.

The importance of microorganisms in agriculture is enormous and extends beyond geochemical cycles. Indeed, most of the fertility of soil is derived from microbial mineralization and in production of nitrogen for plant growth. These processes extend to lichen and cyanobacterial-dominated soils which occupy a larger surface area on Earth than in tropical rain forests. Mycorrhizal fungi form important rhizosphere associations with almost all plants. Such associations are essential for optimum growth and permit some plants to grow in areas they could not otherwise colonize. Recent advances in agriculture stem from breakthroughs in the genetic engineering of plants; one of the most dramatic examples is that of the bacterium *Agrobacterium tumefaciens*. Normally the causative of crown gall disease in plants, this bacterium has been used to transfer favorable properties into an agriculturally important plant species, thereby providing a mechanism for introducing genes that provide resistance to plant diseases, insects, or pesticides into plants. Microorganisms are important in recycling waste materials. Sewage (wastewater) treatment and the breakdown of garbage in landfills occur because of microorganisms. These microorganisms do this “for free” because, in most cases, they derive energy from the process.

Microorganisms are at the core of biotechnology. Many antibiotics and anti-tumour agents are derived from microorganisms, including penicillin, streptomycin, and chloramphenicol. The emergence of multiple antibiotic-resistant pathogenic bacteria has necessitated the search for new antibiotics. Because there are so many types of microorganisms, they produce many unique products currently useful in biotechnology *(The Microbial World: Foundation of the Biosphere, 1996).*

2.3.3. DETRIMENTAL EFFECTS OF MICROBES

Microbes can cause infectious diseases in other living organisms. There are strong pieces of evidence that microbes may contribute to many non-infectious chronic diseases such as some forms of cancer and coronary heart diseases. Different diseases are caused by different microorganisms. Microbes cause food poisoning. Microbial pollution is a serious case of a food safety issue because it can lead to a wide range of foodborne *diseases (Bintsis, 2018).* When microbes grow on food it soon begins to smell bad, look slimy, change colour, taste awful or even get a furry coating and is inedible. The growth of microbes in food leads to a change in the chemical composition of the food due to their actions. Pathogenic bacteria may be introduced to both animal and non-animal food products during any stage of packaging or storage which may lead to food contamination *(Bintsis, 2018).* Apart from bacteria, viruses, fungi, and protozoa may also contaminate food *(Bintsis, 2018).* Certain microbes cause diseases and are called pathogens. Microbes that cause diseases in plants are called plant pathogens and those that cause diseases in animals are called animal pathogens. These microbes enter the bodies of living organisms invading them and multiplying inside their bodies disrupting and causing damage to the vital functions and systems thus causing diseases. Pathogenic bacteria and fungi induce many kinds of symptoms in plants they infect. They cause leaf spots and blights, soft rots of fruits, roots and storage organs, wilts, overgrowths, scabs, and cankers *[George N. Agrios, Plant Pathology 5th edition, 2005]*. In animals, many species of bacteria, fungi, protozoa, and viruses are observed to be pathogenic. They cause wound infections, soft tissue infections, splenic dysfunction, brucellosis, rabies, cholera, pneumonia, tuberculosis, typhoid

Microbes also cause the degradation of metals. This phenomenon is called microbial corrosion, bacterial corrosion, bio-corrosion, microbiologically influenced corrosion, or microbially induced corrosion. This is a type of corrosion brought about by the activities and presence of microbes such as bacteria, fungi, and algae that colonize metal and non-metal surfaces. This process of degradation chiefly acts on metalloids, metals, and rock-based matter. This is a serious and costly problem that affects various industries and infrastructures.

2.3.4. ANTIMICROBIAL AGENTS

Antimicrobial agents are cornerstones of modern medicine, playing a vital role in preventing and treating infectious diseases caused by microorganisms like bacteria, fungi, viruses, and parasites *(Tortora et al, 2018).* These agents work by either killing the microbes directly or inhibiting their growth, allowing the body's immune system to eliminate the infection *(Lewis et al, 2012).* Their impact is undeniable preventing countless infections. There are several main categories of antimicrobial agents, each targeting a specific type of microorganism.

- Antibiotics- These are natural or synthetic substances that primarily target bacteria. Some examples are erythromycin, penicillin, and amoxicillin *(Tortora et al, 2018).*

- Antivirals- Antivirals are antimicrobial agents against viruses. They prevent the replication of viruses in the host cells thus halting the spread of viral infections. Examples: Acyclovir for Herpes and Lamivudine for HIV *(J.D. et al, 2007).*

- Antifungals- They protect the organisms from fungal infections. They usually work by disrupting the fungal cell membrane and inhibiting the synthesis of essential fungal components. Examples: Fluconazole and clotrimazole *(Ghannoum et al, 1999).*

- Antiparasitic- Antiparasitic prevents the parasitic infections caused by worms and protozoa. Some examples are metronidazole and ivermectin *(Tortora et al, 2018).*

Each class of antimicrobial agent works through distinct mechanisms. Antibiotics usually work by disrupting cell wall synthesis, protein production, or DNA replication in bacteria. Antiseptics and disinfectants often damage the microbial cell membrane or denature proteins, leading to cell death. Antifungals can target fungal cell wall synthesis or disrupt the essential fungal metabolic processes *(Ghannoum et al, 1999).* Antivirals can inhibit viral attachment to host cells, prevent viral replication, or block the release of newly formed viruses *(J.D. et al, 2007).* Antiparasitic may affect the parasite's

nervous system, energy production, or muscle function, ultimately leading to the parasite's death or expulsion.

2.3.5. FUNGI

Fungi belong to eukaryotes and, therefore, are more closely related to plants and animals than bacteria or archaea *(Gupta et al, 2016).* The fungal cell consists of a membrane-bound nucleus with chromosomes containing the genetic material, e.g., DNA, membrane-bound organelles, e.g., mitochondria, and a cell wall composed of glucans and chitin *(Gupta et al, 2016).* Fungi are heterotrophic organisms. They derive their food from nonliving organic sources, e.g., saprophytic fungi, which feed on dead or decaying organic materials. Few fungi also exist as unicellular organisms, e.g., yeast, which grows through cylindrical threadlike structures (2–10 cm in diameter) known as hyphae *(Gupta et al, 2016).* These hyphae may be either septate, e.g., compartmentalized through cross walls, or non-septate *(Gupta et al, 2016).* The hypha is a main part of fungus and constitutes a mycelium. Finely branched mycelium occupies a large surface area in the soil and produces a range of enzymes acting on soil organic matter to produce nutrients and energy required for fungal growth. Fungi can reproduce both sexually, e.g., through spores, and asexually, e.g., through budding or binary fission *(Gupta et al, 2016).* Fungi are highly diverse and play a wide range of roles in their surrounding environment such as decomposers, mutualists, endophytes of plants, pathogens, and predators *(Gupta et al, 2016)* Fungal hyphae are the basic components of soil food webs since they constitute a food source for soil biota, whereas fungal sporocarps provide food for larger animals *(Gupta et al, 2016).* Still, despite their ecological significance, fungi can also have a mischievous impact on mortal health, food security, and structure. One of the most common ways fungi affect humans is through the corruption of food. Molds, a type of fungus, readily grow on fruits, vegetables, and grains, rendering them indigestible and potentially dangerous. Fungal growth not only reduces the aesthetic appeal and nutritive value of food but can also produce mycotoxins, a class of poisonous secondary metabolites *(Bennet et al, 2003).* Mycotoxin impurity can lead to a range of health problems, from gastrointestinal torture to liver damage and indeed death *(IFSH).* A particularly ruinous illustration is the Great Irish Potato shortage *(1845- 1849),* caused by the oomycete fungus, *Phytophthora*

infestans, which wiped out a significant portion of the potato crop, leading to wide starvation and death *(WHO).* Beyond food corruption, fungi are responsible for a multitude of factory conditions. Fungal pathogens can infect the colourful corridor of a factory, causing root spoilage, hanging, scars, and mildew. These conditions significantly reduce crop yields and profitable affairs for growers. For case, fungal conditions are estimated to beget global agrarian losses of around 20 annually *(FAO, 2019).* likewise, some fungi can directly beget conditions in humans and creatures. These fungal infections, known as mycoses, can range from mild skin conditions like athlete's bottom and ringworm to life-changing ails similar to invasive aspergillosis and cryptococcosis *(Mayo Foundations, 2022).* individualities with weakened vulnerable systems are particularly susceptible to fungal infections. also, exposure to fungal spores in damp and inadequately voiced surroundings can spark antipathetic responses and respiratory problems like asthma *(NCBI, 2016).*

2.3.6. PLANT-BASED ANTIFUNGAL AGENTS

Numerous plants exhibit antifungal properties. The Myrtaceae family boasts *Eugenia uniflora* (pitanga) and *Psidium guajava* (guava), whose leaves contain compounds like eugenol and gallic acid with potent antifungal activity *(D'Souza et al.,2017)*. Guava has antifungal properties against *Candida albicans* a common fungal pathogen, which makes it a promising agent for the treatment of candidiasis *(El-Ghayyouby et al.,2014). Curcuma longa* (turmeric), a member of the Zingiberaceae family, possesses curcumin, a curcuminoid with broad-spectrum antifungal effects *(Aggarwal et al.,2009)*. Beyond these well-known examples, research has identified antifungal potential in a wider range of plants, including *Piptadenia colubrina* (paroba), *Schinus terebinthifolius* (Brazilian pepper tree), and many more *(D'Souza et al.,2017)*. The antifungal activity of plants arises from a complex interplay of various bioactive compounds. Essential oils, extracted from plants like *Cinnamomum zeylanicum* (cinnamon), *Syzygium aromaticum* (clove), and *Mentha arvensis* (peppermint), rich in terpenes and phenolics, are a major source of antifungal properties *(Singh et al.,2011).* Studies have shown that cinnamon, clove, and tea tree oil exhibit significant antifungal activity against a wide range of fungal pathogens *(Singh et al.,2010).* Allicin, the active compound in garlic,

also demonstrates antifungal properties *(Suleria et al.,2008)*. Additionally, plant extracts containing alkaloids, flavonoids, and other secondary metabolites have been shown to inhibit fungal growth and spore germination *(Bhalodia et Shukla, 2011)*. Plant essential oils, volatile aromatic compounds extracted from various plant parts, are another source of natural antifungal agents. Tea tree oil (*Melaleuca alternifolia*) oil possesses potent antifungal activity against Candida albicans, a common fungal pathogen *(Suleria et al.,2008)*. Similarly, thyme oil (Thymus vulgaris) exhibits antifungal properties against various dermatophytes which are fungi that cause skin infections *(Bhalodia et Shukla, 2011)*. Plant-based antifungal agents offer several advantages over conventional medications. They are often considered to be safer with fewer side effects *(Bhalodia et Shukla, 2011)*. A review by *Singh et al, 2011* highlights the potential of medicinal plants as a source of safe and effective antifungal agents. Additionally, plant-based anti-fungal agents can be more readily available and affordable in resource-limited settings.

CHAPTER 3

MATERIALS AND METHODS

3.1. Collection of Plant Material

The plant material was collected from South Chittoor, Ernakulam, Kerala on 1st January 2024. It was identified by assessing the morphological characters and the identity was confirmed as *V. indica*.

Fig 3.1: *Vateria indica* - Habit

3.2. Preparation of Plant Extract

The collected plant material, stem bark, was cleaned and chopped into small pieces, air-dried, and finely ground using a grinder. 20g of the plant material was extracted using 200ml of ethanol in Soxhlet apparatus for 1 hour 15 minutes at 50-degree Celsius temperature. The solvent was collected and evaporated by boiling in a water bath. The residue was taken and stored in a glass bottle till use.

Fig 3.2: *Vateria indica* – dried stem bark

Fig 3.3: Extraction using Soxhlet apparatus

3.3. Phytochemical Analysis

3.3.1. Preparation of stock solution of the extract

The stock solution for phytochemical analysis of the extract was prepared by mixing 3g of the extract in 10 ml of ethanol. After dissolving it was made up to 30 ml.

3.3.2. Qualitative analysis for primary metabolites

3.3.2.1. Test for Carbohydrates

A) Fehling's Test

2mL of the extract was taken from the stock solution and transferred into a test tube. To this 1mL of each Fehling's reagent A and Fehling's reagent B was added. The mixture was then heated for a few minutes. The resulting orange-to-red precipitate indicates the presence of carbohydrates.

B) Molisch's Test

1mL of the extract was measured out from the stock solution and transferred into a test tube. To this, two drops of Molisch's reagent were added and mixed well. Then 1mL of concentrated Sulphuric acid was added along the sides of the test tube without shaking. At the junction of the two liquids, a violet-coloured ring appears indicating the presence of carbohydrates.

3.3.2.2. Test for Proteins

A) Preparation of 40% NaOH

Initially, 40% NaOH solution was prepared by dissolving 4 grams of anhydrous Sodium Hydroxide in 10mL of distilled water. This was transferred to a glass beaker.

B) Preparation of CUSO4 solution

CuSO4 solution was prepared by dissolving 1 gram of Copper Sulphate crystals in 10mL of distilled water. This was transferred to another beaker for further use.

C) Biuret Test

2mL of sample was taken from the stock solution and transferred into a test tube. To this, a few drops of the CuSO4 solution was added followed by the addition of 40% NaOH. It was shaken well. The mixture turns into a purple colour.

D) Xanthoproteic Test

1mL of the sample was measured out of the stock solution and transferred into a test tube. To this a few drops of concentrated Nitric acid were added, shaken well, and cooled to room temperature. The solution turns into a yellow colour. On addition of 40% NaOH, the colour changes from yellow to bright orange which indicates the presence of proteins.

3.3.3. Qualitative Analysis for Secondary Metabolites

3.3.3.1. Test for Alkaloids

A) Meyer's Reagent Test *(Ciulci, 1994)*

1 ml of extract was taken in a test tube (the solvent used is distilled water), and 2 to 3 drops of Meyer's reagent were added. A white precipitate with Mayer's reagent indicated the presence of alkaloids.

B) Dragendorff's reagent *(Ciulci, 1994)*

1 ml of extract was taken in a test tube (the solvent used is distilled water), and 2 to 3 drops of Dragendorff's reagent was added. An orange-red precipitate or turbidity with Dragendorff's o indicated the presence of alkaloids.

3.3.3.2. Test for Flavonoids

A) Alkaline reagent test

2mL of the stock solution was taken in the test tube (the solvent used is distilled water). To these 2 to 3 drops of Sodium Hydroxide solution was added. Initially, a deep yellow colour appeared but gradually became colourless with the addition of dilute HCL indicating the presence of flavonoids.

3.3.3.3. Test for Saponins

A) Froth Formation Test

1 gram of the powdered sample was taken in a test tube. To this 10 ml of distilled water was added. The test tube was then vigorously shaken. The formation of bubbles or persistent foam indicates the presence of saponins.

3.3.3.4. Test for Terpenoids *(Harborne, 1973)*

5 ml of the extract (the solvent used is distilled water) was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish-brown colouration of the inference showed the presence of terpenoids.

3.3.3.5. Test for Triterpenoids *(Harborne, 1984)*

The extracts (the solvent used is distilled water) were treated with chloroform with a few drops of concentrated sulphuric acid, shaken well, and allowed to stand by for some time. The formation of a yellow-colored lower layer indicated the presence of triterpenoids.

3.3.3.6. Test for phenolic compounds

A) Ferric Chloride Test *(Mace, 1963)*

The extract was dissolved in distilled water and a few drops of neutral 5% ferric chloride solution was added. Phenolic compounds were indicated with the presence of a dark green colour.

3.3.3.7. Test for Tannins

A) Braymer's test

A portion of the extract was mixed with 2 mL of distilled water followed by the addition of a few drops of 10% ferric chloride solution. The formation of bluish-black or greenish-black colouration was taken as positive for the presence of tannins.

B) Preparation of 1N NaOH

To make 1N solution of NaOH 40g of Sodium Hydroxide was made up to 1000mL by dissolving in distilled water.

C) Alkaline Reagent Test

2mL of 1N NaOH was taken in a test tube. To this 2 mL of plant extract was added. The appearance of yellow to red colour revealed the existence of tannins.

3.4. Antifungal Activity Test

3.4.1. Preparation of Concentrations

The extract was collected in a clean glass cylinder and later transferred into a Petri plate for evaporation. After the evaporation of ethanol, the residue was scrapped out and stored in a clean container in the refrigerator for further use.

3.4.1.1. Preparation of 2% Solution

For the preparation of a 2% solution of the extract, 0.1 gm of the extract was measured and taken in a 25 mL beaker. It was dissolved in 2mL of distilled water and made up to 5mL. The concentration of this solution is 2%.

3.4.1.2. Preparation of 10% Solution

For the preparation of a 10% solution of the extract, 0.5gm of the substance was measured using a weighing machine and transferred into a 25 ml beaker. This was then mixed with 2 mL of distilled water and made up to 5 mL. The concentration of this solution is 5%.

Fig 3.4: Preparation of 10% and 2% solution from the extract

4.4.2. SDA plate preparation

6.5 g of SDA (Sabouraud Dextrose Agar) is dissolved in 100ml distilled water. Boil for 1 minute to completely dissolve the components. Sterilize by autoclaving at 121°C for 15 minutes. The mixture is poured into the Petri plate and waited to solidify the medium.

3.4.3. Plate Inoculation

The inoculation of Candida albicans was conducted within a biohazard cabinet. A sterile cotton swab was used to pick the colonies from the SDA plate and then streaked over the entire surface of the SDA agar plate in four different directions. The agar plate was then left undisturbed for 5-10 minutes before the test samples were applied. Wells were prepared and a volume of 100 μL of the test samples was added into the wells. Water was used as the negative control, followed by 1mg/mL of fluconazole as the standard antifungal agent (positive control). The plate samples were subsequently incubated at 28°C for 48 hours before the inhibition zone around each sample disc was examined. The diameter (mm) of the inhibition zone was measured to indicate the presence of antifungal activity for each sample in comparison to the positive control. The test was done in duplicate.

CHAPTER 4

RESULT AND DISCUSSION

4.1. Phytochemical Analysis

4.1.1. Qualitative Analysis of Primary Metabolites

4.1.1.1. Test for Carbohydrates

A) Fehling's Test

On heating with Fehling's A and Fehling's B reagents, the sample produced a reddish-orange precipitate. The presence of carbohydrates was identified.

Fig 4.1: Felhing's test for carbohydrates

B) Molisch's Test

The addition of concentrated Sulphuric acid along the sides of the test tube without shaking resulted in the formation of a violet-coloured ring at the junction of the 2 liquids. The presence of carbohydrates was confirmed.

Fig 4.2: Formation of a violet-coloured ring in Molisch's test for carbohydrates

4.1.1.2. Test for Proteins

A) Biuret Test

There was no colour change observed after the mixing of the reagents with the sample which indicated proteins were absent in the tested sample.

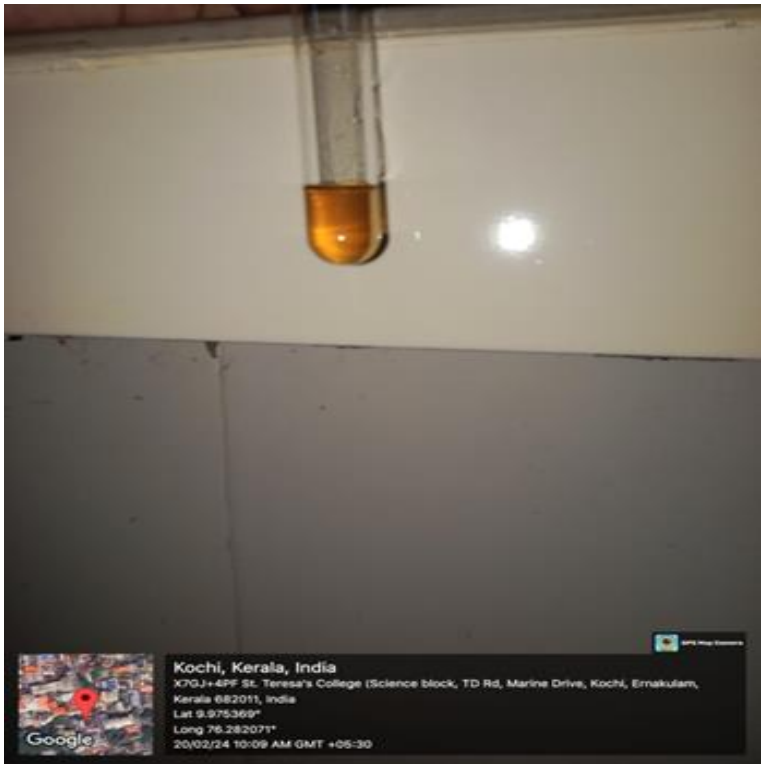

Fig 4.3: Biuret Test for Proteins

B) Xanthoproteic Test

No colour change was observed after the addition of 40% NaOH solution. Hence proteins are absent in the tested sample.

Fig 4.4: Xanthoproteic test for proteins

4.1.2. Qualitative Analysis for Secondary Metabolites

4.1.2.1. Test for Alkaloids

A) Meyer's Reagent Test

In Meyer’s test, no white precipitate was formed which indicated the absence of alkaloids.

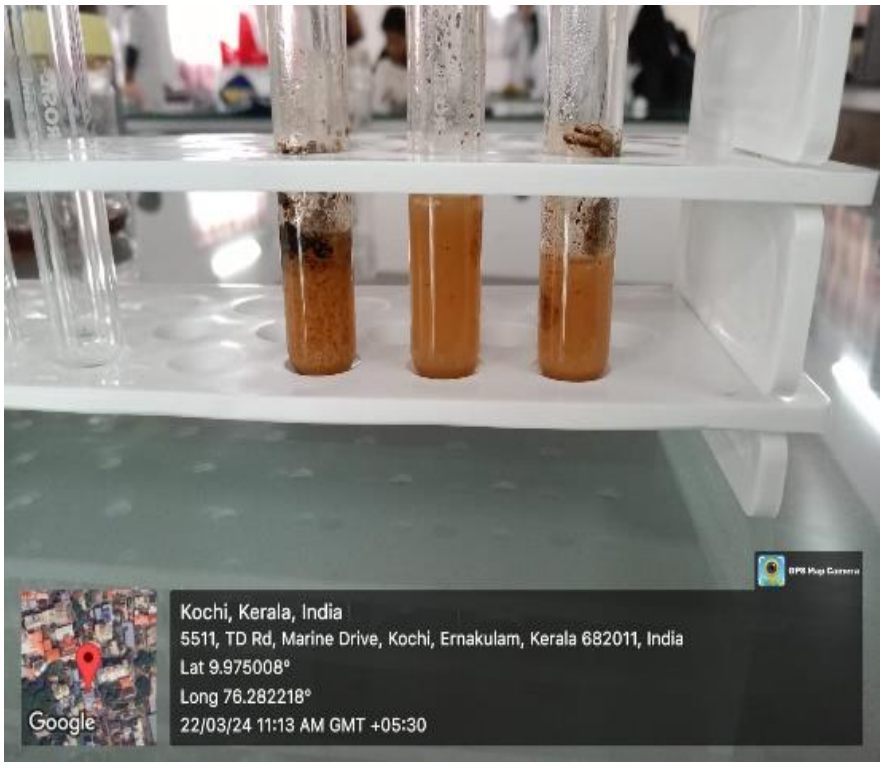

Fig 4.5: Meyer’s test for alkaloids

B) Dragendorff's Reagent Test

In Dragendorff's test, the sample became turbid on reaction with Dragendorff's reagent which indicated the presence of alkaloids.

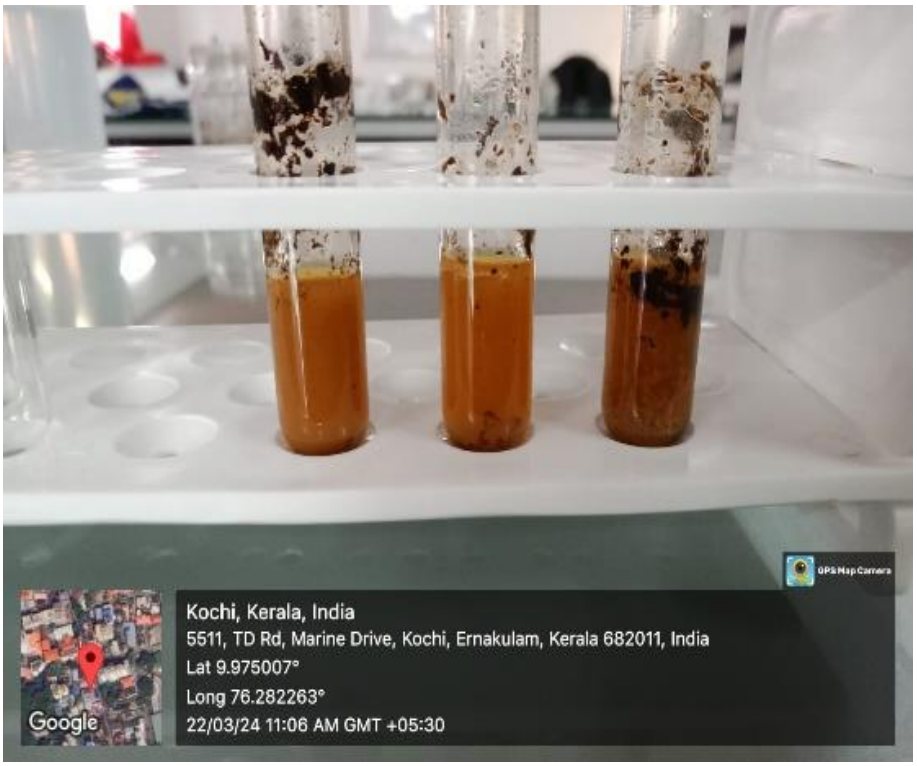

Fig 4.6: Formation of turbidity in Dragendorff's test for alkaloids

4.1.2.2. Test for Flavonoids

A) Alkaline Reagent Test

Initially on the addition of Sodium Hydroxide solution a deep yellow colour appeared. It gradually became colourless on drop-by-drop addition of dilute HCl. The presence of flavonoids was confirmed.

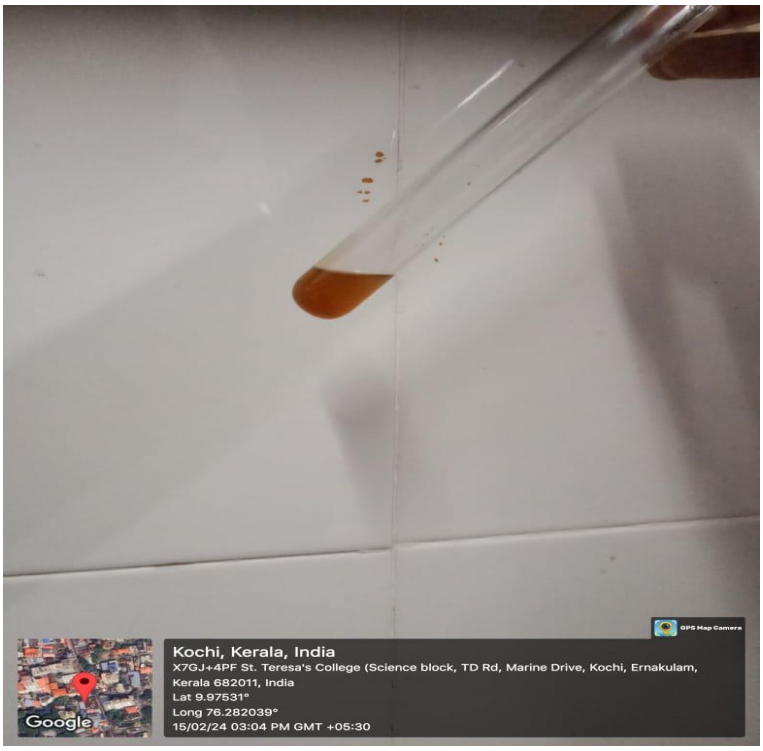

Fig 4.7: Formation of deep yellow colour on the addition of NaOH in alkaline reagent

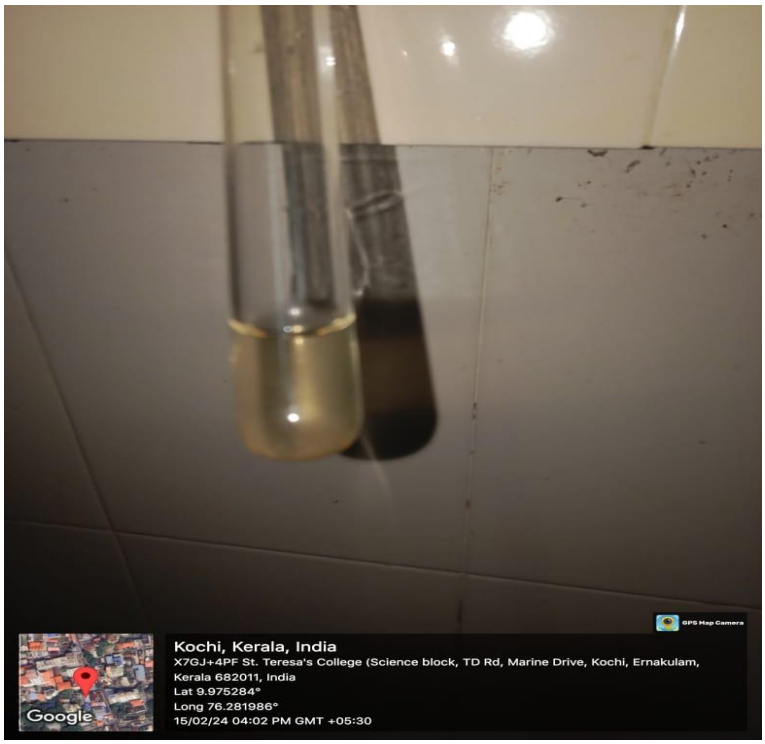

Fig 4.8: Formation colourless solution on addition of dil. HCl

4.1.2.3. Test for Saponins

A) Froth Formation Test

Persistent foam was produced on the vigorous shaking of the sample with distilled water. The presence of saponins was confirmed.

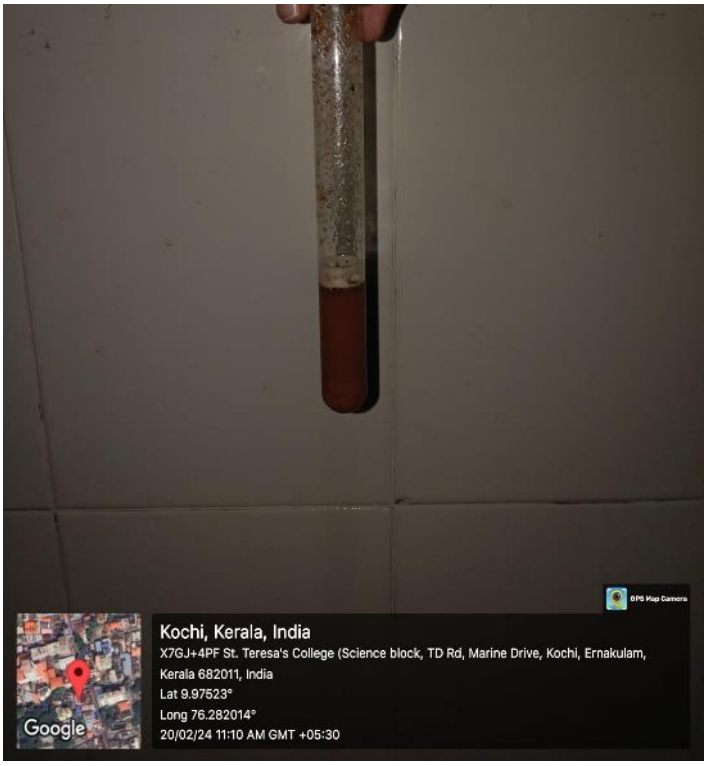

Fig 4.9: Persistent foam–froth formation test

4.1.2.4. Test for Terpenoids

There was no reddish-brown colouration. This indicated the absence of terpenoids.

Fig 4.10: Test for terpenoids

4.1.2.5. Test for Triterpenoids

There was no yellow-coloured layer formation which indicated the absence of triterpenoids.

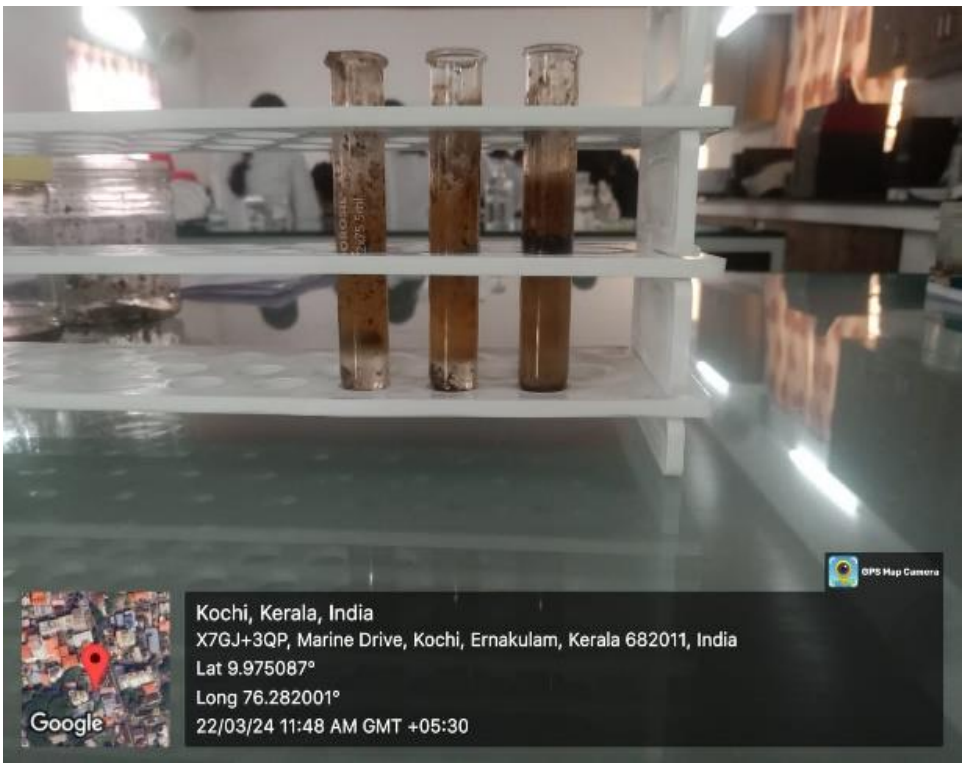

Fig 4.11: Test for triterpenoids

4.1.2.6. Test for Phenolic compounds

A) Neutral ferric Chloride Test

A dark green color was formed on the addition of a 5% neutral ferric chloride solution which indicated the presence of phenolic compounds in the sample.

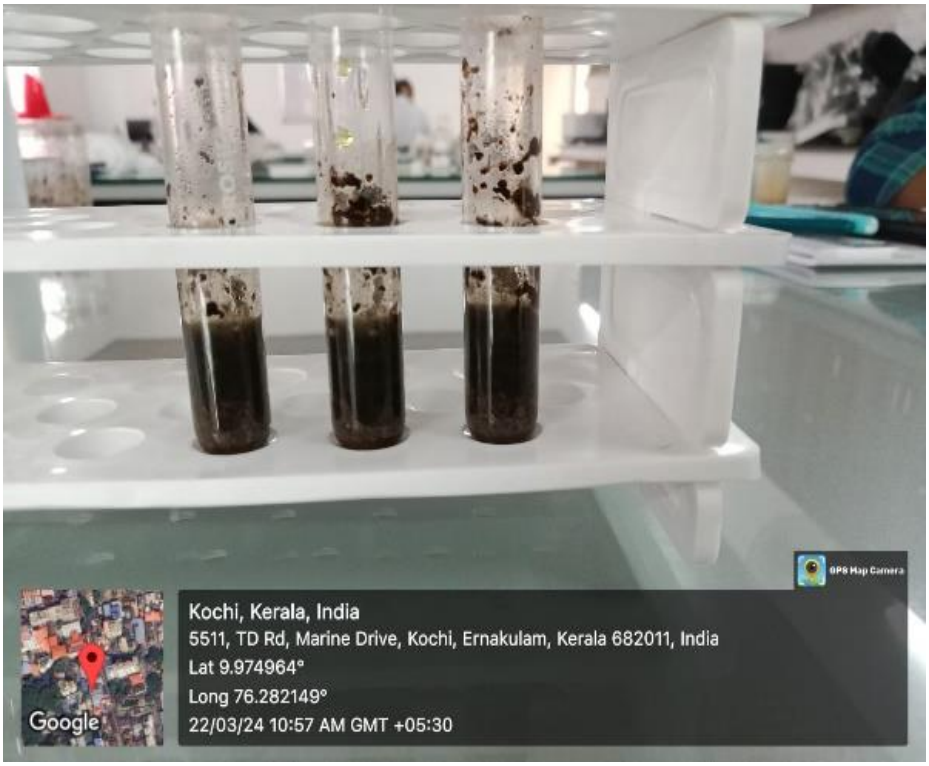

Fig 4.12: Neutral ferric chloride test for phenolic compounds

4.1.2.7. Test for Tannins

A) Braymer’s Test

A bluish-black colour was formed on the addition of 10% ferric chloride solution which indicated the presence of tannins.

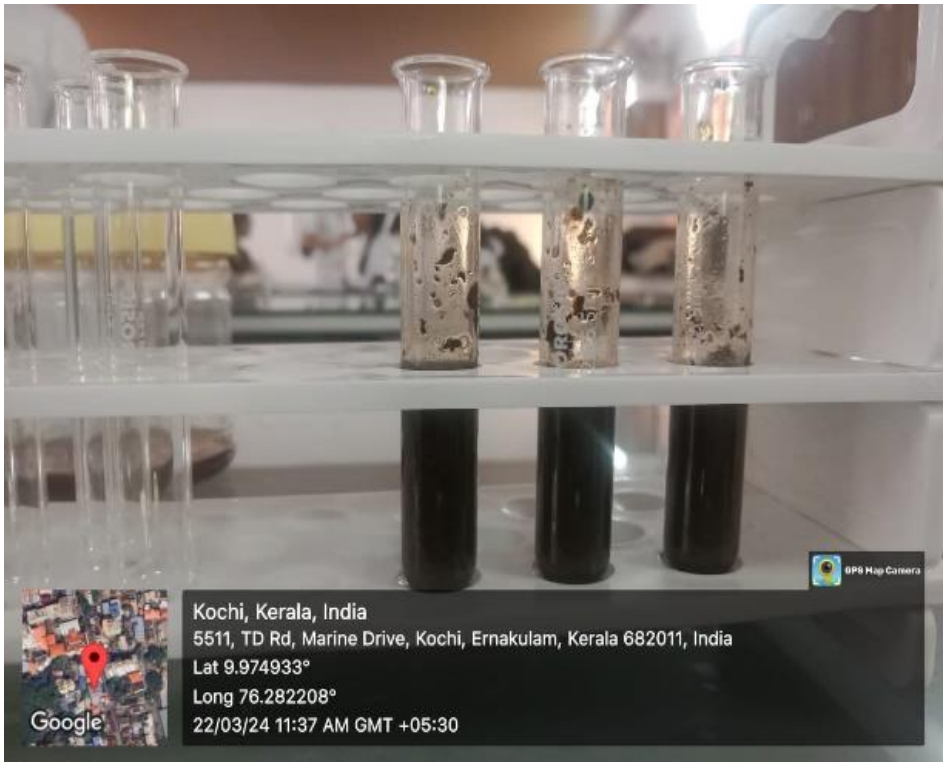

Fig 4.13: Bluish-black colour formation in Braymer's test for tannins

B) Alkaline Reagent Test

A deep yellow colour was formed with the addition of 1N NaOH solution. The presence of tannins was confirmed.

Fig 4.14: Deep yellow colour in Alkaline reagent test for tannins

	Phytochemicals analyzed	**Test**	**T_1**	**T_2**	**T_3**	**Average**
1.	Carbohydrate	Fehling's test	+++	+++	+++	+++
		Molich's test	+++	+++	+++	+++
2.	Protein	Biuret test	-	-	-	-
		Xanthoproteic test	-	-	-	-
3.	Alkaloids	Mayer's reagent test	-	-	-	-
		Dragendorff 's test	++	++	++	++
4.	Flavonoids	Alkaline reagent test	+++	+++	+++	+++
5.	Saponins	Froth formation test	+++	+++	+++	+++
6.	Terpenoids	Triterpenoids test	-	-	-	-
7.	Phenolic compounds	Neutral ferric chloride test	+++	+++	+++	+++
8.	Tannins	Braymer's test	+++	+++	+++	+++
		Alkaline reagent test	++	++	++	++

Table 1: Results of phytochemical analysis of the stem bark of *V. indica*

4.2. Antifungal Activity Test

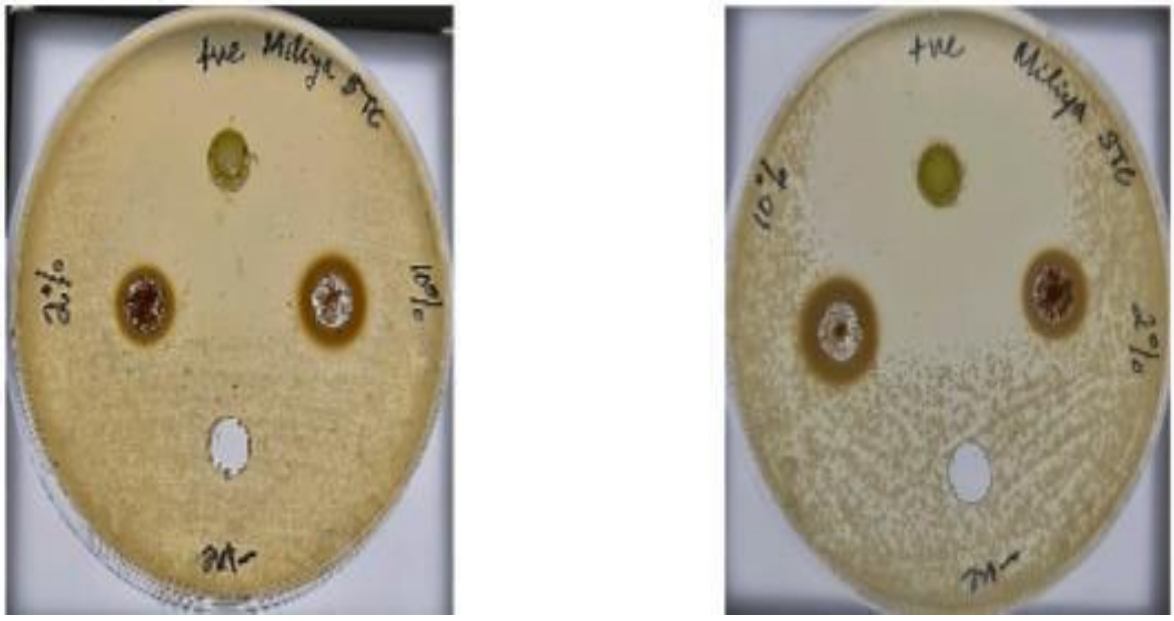

Fig 4.15: Antifungal activity of the test samples against *Candida albicans*

Sample	**Zone of inhibition (mm)**
Positive control	34±1
Negative control	0
Test sample 2%	0
Test sample 10%	5±2

Table 2: Results showing the analysis of antimicrobial activity

4.3.. Discussion

V. indica is an endemic plant species found in the Western Ghats region of India. It is well-known among the local people for its various uses in traditional and ethnic medicines. The plant is commonly known as White Dammar *(Gerry Moore et al., 2015)* and every part of it, including the wood, bark, leaves, fruits, and resin, is useful for different purposes. The wood is used for furniture *(Warrier et al., 2020)*, while the bark, leaves, fruits, and resin have numerous therapeutic properties *(Shiva et al., 1998; Mishima et al., 2003; K.R.H. et al., 2018; Kayani et al., 2014; Alva et al., 2018; Alshabi et al., 2020; Bugade et al., 2022)*. The seeds of the plant are also edible *(Shiva et al., 1998)*. The bark and leaf extract of the plant have been found to possess antimicrobial

properties against bacteria, fungi, protozoans, and worms *(J.K. et al., 2018)*. Moreover, several endophytic fungi reside within the plant, including *Coniothyrium* species, *Acremonium, Aspergillus, Colletotrichum,* and *Penicillium (Ruma et., 2011).* Research on *V. indica* suggests that it has anti-inflammatory, anti-tumour, and wound-healing properties *(J.K. et al., 2018*; *Bugade et al., 2022)*. The plant is also used in Ayurvedic formulations to treat various skin diseases associated with fungal infections like ringworm or dermatophytosis *(Vidhya, 2018; J.K. et al., 2018)*. While there have been several studies on the therapeutic effects of *V. indica,* its antifungal properties have not been extensively investigated. A study conducted to evaluate the antimicrobial properties of the aqueous extract of the leaves of *V. indica* showed no fungicidal effect against six strains of fungi *(Shrisha et al., 2011)* and hence the present research can be considered the first direct study on the antifungal properties of the stem bark of *V. indica.* The ethanolic extract of the stem bark showed positive antifungal properties against *C. albicans*. The plant bark also contains phytochemicals like alkaloids, flavonoids, saponins, phenolic compounds, and tannins. Plant extracts possess low toxicity and minimal environmental pollution. It causes only a few or no harm to the non-target organism (*Opara and Wokocha, 2008).* This shows its potential as a source for developing a plant-based natural fungicide.

Conclusion

The phytochemical analysis of the stem bark of *V. indica* gave complimentary results for Fehling's test, Molisch's test, alkaline reagent test for flavonoids, froth formation test, neutral ferric chloride test, and alkaline reagent test for tannins. These results indicate the presence of carbohydrates, flavonoids, saponins, phenolic compounds, and tannins respectively. There were no desired results for the Biuret Test xanthoproteic test and test for triterpinoids which designated the absence of proteins, terpinoids and triterpinoids in the sample analysed. In the test for antifungal exertion of the sample, no activity against the fungi was noted at 2% concentration. A veritably small antifungal activity was displayed at 10% concentration against *Candida albicans* compared to the positive control. Hence, it was concluded that the ethanolic extract of *Vateria indica* stem possesses antifungal activity against *Candida sp.* at higher concentrations. With further progress in the research in this area, *V. indica* can serve as a potential natural antifungal agent.

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