Latex of *Ficus auriculata* (Lour.): A Green alternative to synthetic antimicrobials

Dissertation submitted in partial fulfilment of
the requirements for the award of Degree of Master of Science in
BOTANY

By

NAME: SIYAN MARIA SHAJI

Reg. No.: AM23BOT010

THE FEAR OF THE LORD IS THE BEGINNING OF WISDOM

ST. TERESA'S COLLEGE (AUTONOMOUS) ESTD 1925

DEPARTMENT OF BOTANY AND CENTRE FOR RESEARCH

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This is to certify that the dissertation entitled **"Latex of *Ficus auriculata* (Lour.): A Green alternative to synthetic antimicrobials"** is an authentic record of work carried out by Ms. **Siyan Maria Shaji** (Reg. No. AM23BOT010) under my supervision and guidance in the partial fulfilment of the requirement of the degree of M.Sc. Degree of Mahatma Gandhi University Kottayam. I further certify that no part of this work embodied in this project has been submitted for the award of any degree or diploma.

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DECLARATION

I hereby declare that the project entitled "**Latex of *Ficus auriculata* (Lour.): A Green alternative to synthetic antimicrobials**" submitted to Mahatma Gandhi University, Kottayam, in partial fulfilment of the requirement for the Degree of Master of Science in Botany is an original project done by me under the supervision and guidance of Dr. Tintu Jose Manicketh, Assistant Professor, Department of Botany, St. Teresa's College (Autonomous), Ernakulam.

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SIYAN MARIA SHAJI

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CHAPTER 1

INTRODUCTION

Plants have been a source of inspiration for new medicine molecules in the past because plant-based medications have increased human health and well-being to a very great extent (Iwu et al., 1999). According to Abdallah, E. M. (2011) the largest known biochemical and pharmacological resources on the planet are located in plants. These living reservoirs can create an infinite quantity of biological molecules. Several research has been conducted on the antibacterial properties of medicinal plants, and on the basis of ethno-botanical information, they have shown promising effectiveness against multi-drug resistant pathogens the current antibiotics failed to eradicate.

Plant latex is a sap produced in the tissue known as laticifer. They are secreted from a wound point in plant tissues shortly after injury as part of their defence system (Marisetti et al., 2025). Latex has been found in trees, shrubs, and herbs; it can be found in the roots, fruits, and leaves of these plants. Nonetheless, green tissues like stems and leaves are where it is more commonly found (Freitas et al., 2024). The laticifers can be identified as one row of specialised cells that spread out across the plant's body and grow alongside it. Laticifers have the ability to both expand and anastomose in order to move through plant tissue and form a network resembling a web. The tubular framework that surrounds each tissue is particularly well-developed in leaves. As a result, latex flows more quickly via leaf and petioles but can still trickle through roots, fruits, or trunks (Ramos et al., 2020). Although the latex found in laticifers is typically milky or white, it can occasionally be yellow, orange, red, brown, or even colourless (Abarca et al., 2019).

Latex is produced by approximately 20,000 angiosperm plant species belonging to more than 40 families and makes up 8.9% of the angiosperm plants. This figure goes up to 35,000 species when conifers and resin exuding plants are included (Konno et al., 2011). Family members of the Euphorbiaceae (Spurge), Apocynaceae (Dogbane), Moraceae (Mulberry), Papaveraceae (Poppy), and Asclepiadaceae (Milkweed) occur in a wide variety of habitats and are reported to synthesize latex as a defence mechanism (Molik et al., 2025). Rubber, cardenolides, alkaloids, terpenoids, and other proteins and enzymes like glucosidases, chitinases, and proteases are some of the numerous substances and proteins present in latex (Konno et al., 2011).

Marisetti et al., (2025) reported that the chemical composition of latex is complex and includes a wide range of phytochemicals, such as terpenoids (sesquiterpenes, diterpenes, and triterpenes), alkaloids (indole, steroid, terpenoid, isoquinoline, iboga, and phenanthroindolizidine moieties), carbohydrates, fatty acids, furanocoumarins, glycosides (cardenolides), mucilages, organic acids, phenolics, resins, saponins, sterols, chitinases, glucosidases, hevein, lipases, oxidases, papain, peptidases, peroxidases (POD), plasmin, proteases, and phosphatase thrombin are some of the enzymes derived from plants that produce latex. Different plant species have different quantities of these phytoconstituents.

Latex has a wide range of uses, including as wound healing, herbivore protection, and microbial infection prevention. These specific metabolites were responsible for latex's cytotoxic, insect-repellent, anthelmintic, antibacterial, and antifungal properties (Abarca et al., 2019).

Latex-secreting plants have evolved to release antimicrobial peptides (AMPs) as a defence against microbial invasions. When the plants are injured, they release latex, which has antimicrobial qualities and keeps the wound from getting infected. The latex's antimicrobial proteins 6 (AMPs) work by rupturing the invading microorganisms' cell membranes, preventing colonisation and infection. The competitive biological settings, such as deserts and tropical rainforests, where various plants generate latex, have probably fuelled the evolution of extremely powerful and varied AMPs (Molik et al., 2025).

The Euphorbiaceae is a varied flowering plant family with more than 300 genera and five sub-families and 7,500 species. It is mostly found in the temperate and tropical regions. Several species in the family are used as medicine to treat gonorrhoea, warts, headache, paralysis, stomach ache, and other skin conditions. A number of Euphorbiaceae tribe members, such as the rubber tree (*Hevea brasiliensis* Muell. Arg.), the castor oil plant (*Ricinus communis* L.), the pencil tree (*Euphorbia tirucalli* L.), and the poinsettia (*E. pulcherrima* Willd. Ex Klotzsch), are all major latex yielders. Although these plants' latex may be poisonous and skin-irritating, it also contains beneficial compounds with biological activity that protects against microbial invasion, such as antibacterial activity. Additionally, analgesic, anti-inflammatory, and anticancer properties have been discovered for members of the spurge family. By using latex, which shields them from illness, herbivores, environmental stress, and competition from other plants, plants can thrive in a variety of unfavourable settings. The use of latex by plants allows them to thrive in a variety of harsh conditions by shielding them from diseases, herbivores, environmental stress, and other environmental competition (Molik et al., 2025).

There are over 400 genus and 5,000 species in the Apocynaceae family, which includes lianas, shrubs, trees, and herbs, vines and perennial stem succulents. They can be found across the entire the globe, including the tropical and subtropical areas of Africa, America and Asia, while some are found in temperate regions as well. Apocynaceae plants are a significant source of latex. The latex contains large amounts of proteins, AMPs, sterols, alkaloids, glycosides, tannins, and cardenolides. These substances have a variety of biological properties. Alkaloids are helpful in the treatment of infectious diseases and pain because of their analgesic, anti-inflammatory, and antibacterial properties. Due to their anticancer properties, cardenolides are employed to create therapeutic anticancer medications. Glycosides, sterols, and tannins with strong antioxidant qualities offer defence against oxidative stress-related illnesses (Molik et al., 2025).

Featuring 37 genera and more than 1,000 species, the Moraceae family is commonly known as figs or mulberry. Deciduous or evergreen trees and shrubs are native to tropical and subtropical regions. Almost every plant with milky sap belongs to this family. The latex of Ficus species, such as the common fig, contains bioactive peptides and enzymes that have been proven to have antimicrobial, anti-inflammatory, and wound-healing qualities. Numerous phytochemicals have been identified in member species, including phenolic acids, flavonoids, ascorbic acid, and triterpenoids. Because of their hepatoprotective and anticancer properties, triterpenoids are used to treat liver diseases and cancer.

Strong anti-inflammatory and antioxidant qualities are possessed by flavonoids and phenolic acids. They can be used to treat illnesses linked to oxidative stress, such as neurological and cardiovascular disorders. Additionally, a variety of bacterial and fungal illnesses have been successfully treated by their antibacterial action. In addition to providing defence from herbivorous animals, latex in this family aids in wound healing and prevents infections at the site of injury (Molik et al., 2025).

With over 40 genus and 770 species, the Papaveraceae family is primarily found in temperate climates. The latex of Papaver somniferum, which contains alkaloids including morphine, codeine, and thebaine, is well known. *Chelidonium majus* latex contains thiol protease inhibitors, or cystatins, which have antiviral and antibacterial properties. The alkaloids in this family's latex provide a powerful resistance against illness and herbivores (Molik et al., 2025).

With about 300 genus and 2,800 species, the Asclepiadaceae family is widely distributed, especially in North America and Africa. While monarch butterflies have been found to be drawn to certain milkweeds, the latex of the milkweed plant contains cardenolides (harmful steroid), alkaloids, and enzymes that effectively guard against diseases and herbivores because their larvae are designed to consume the toxic latex. Milkweed latex poisons many animals, protecting them from herbivory and preventing microbiological illness (Molik et al., 2025).

One of the largest plant families in the world, the Asteraceae family has 1,620 genera and over 23,000 species, of which over 1,800 produce latex. They are present practically everywhere. AMPs and sesquiterpene lactones, which are found in latex-producing plants like dandelions and lettuce, have a wide range of biological uses. Sesquiterpene lactones are helpful in the treatment of inflammatory illnesses and cancer because of their anti-inflammatory properties. By rupturing phospholipid membranes of the microorganism's cell, they prevent bacterial attack. The sticky latex found in the Asteraceae plants gives plants a chemical barrier against microbes and herbivores, enabling them to thrive in a variety of settings (Molik et al., 2025).

Tropical South and Central America comprise the majority of the Caricaceae family. Papaya plant latex contains proteolytic enzymes such as papain and chymopapain, which have shown wound-healing activity. The use of these enzymes and other antimicrobial compounds in traditional medicine to heal infections and promote wound healing has been established. Papaya latex defends the plant against disease by functioning as an antibacterial and herbivore barrier (Molik et al., 2025).

The family Convolvulaceae featuring about 1,900 species and 55 genera, which is widely distributed over the globe but most dominated in tropical and subtropical regions. Although less studied compared to other families with latex-producing abilities, the latex secreted by the members contains alkaloids and antibacterial chemicals. The latex tends to repel herbivores mainly, while it could also serve as a defence to prevent infection from getting near (Molik et al., 2025).

Singh et al., (2011) reported that in Moraceae family, *Ficus* is the most important genus for its numbers, medicinal and ecological use. In the sub-tropical and tropical regions of the world, the *Ficus* genus comprises approximately 800 species of trees, shrubs, and epiphytes and is thus one of the largest angiosperm genera. It contains both deciduous and evergreen free-standing trees, stranglers, climbers, creepers, small shrubs, lithophytes, and rheophytes and is one of the most diverse plant genera in habit of growth.

The abundance of various bioactive phytochemical compounds in the roots, stem, bark, leaves, fruit, latex, and pulp of *Ficus* plants like polyphenols, phenolic acids, triterpenoids, flavonoids, anthocyanins, carotenoids, glycosides, polysaccharides, reducing compounds, and vitamins C, E, and K make them medicinally relevant. Due to their considerable antioxidant capacity with regard to metal chelating, metal reducing, lipid reducing, and free radical scavenging, the majority of these phytochemical compounds could be helpful in alleviating oxidative stress within biological systems. Due to the rich phytochemical contents and potent antioxidant activity, these plants have a wide range of biological activities, such as antibacterial, antidiabetic, anti-obesity, hepatoprotective, cardioprotective, renal-protective, and anticancer activity. Various diseases, like diabetes, stomach aches, piles, skin diseases, inflammation, and cancer, have been found to be cured very effectively (Nawaz et al., 2020).

Even though latex from *Ficus* species has various medical advantages, only a few scientific research studies have been conducted on the topic. The main reason for this is that latex is a complicated mixture of unstable chemicals that solidify quickly, making extraction and analysis challenging. Variation in latex composition and the presence of harmful or irritating compounds further hampered studies based on the surroundings. As a result, compared to other plant components like leaves and bark, latex has received less research.
The development of tolerance in almost all human-infecting bacteria significantly reduces the effectiveness of antimicrobials, a medication that can save lives.
Infection by resistant microorganisms increases morbidity and mortality (Collignon et al., 2016). The conflict between humans and harmful bacteria never ends. Humans create new medications to combat the illness, while microorganisms create new strategies to fortify themselves and prolong their lives. However, plants can create novel, natural antimicrobials more quickly than manufactured medications (Abdallah, 2011). The current study focuses on identifying and using the phytochemical compounds extracted from latex of *Ficus auriculata* (Lour.) and their applications as natural antimicrobials.

1.1 SIGNIFICANCE OF PRESENT STUDY

Ficus auriculata (Lour.) member of family Moraceae is a latex producing plants. Due to its enormous leaves, it is also referred as the Elephant Ear Fig Tree. The plant is used in folk medicine to cure a various human disease and possess high ethnomedical importance. The plant is commonly spotted in locality of Ernakulam. Large quantity of raw materials can be obtained from the plant. In the past few years, there is an increase in the number of antibiotics resistant pathogens. Plant based extracts abundant in secondary metabolites has gained attention due to their large spectrum bioactivities with reduced side effects, making it necessary to evaluate the potential of the *Ficus auriculata* (Lour.). The present study would help to explore the hidden potential of the latex of *Ficus auriculata* (Lour.).

- The study will provide knowledge on the benefits of *Ficus auriculata* latex.
- The study contributes to various applications of *Ficus auriculata* (Lour.).

1.2 BACKGROUND OF THE STUDY

The antimicrobial potential of aqueous and ethanol latex extracts of *Ficus auriculata* (Lour.) were identified. The study found that both extracts exhibited antibacterial activity against *Staphylococcus aureus* (gram-positive bacteria) and *Pseudomonas aeruginosa* (gram-negative bacteria), but showed no antifungal activity against *Candida albicans* and *Aspergillus niger* fungal strains. The preliminary phytochemical screening confirmed the presence of terpenoids, glycosides, phytosterols, and flavonoids, supporting the hypothesis that these bioactive compounds are likely responsible for the antibacterial effects. This study aligns with earlier findings on the antimicrobial properties of various extracts from *Ficus auriculata* bark, leaves and fruits. Tamta et al., (2021) reported that the leaves, bark and fruits extract possess antioxidant, antibacterial and anti-inflammatory activity.

Similarly, Bertoletti et al., (2018) revealed strong antioxidant and antimicrobial activities as well as potential herbicidal of young and adult leaves. The bark stem extracts of *Ficus auriculata* possessed moderate to weak antibacterial activity but strong antioxidant activity Gaire et al., (2011).

This research provides a scientific basis for the traditional use of *Ficus religiosa* and supports its potential application in developing herbal antibacterial agents. The absence of antifungal activity also highlight s the selectivity of the plant's bioactive compounds and suggests the need for further investigation into their mechanisms of action.

1.3 OBJECTIVE

The objectives of the current study are:

- To study the antibacterial, antifungal properties of *Ficus auriculata* (Lour.) latex.
- To identify the phytochemical compounds present in the latex of *Ficus auriculata* (Lour.).

CHAPTER 2
REVIEW OF LITERATURE

Harborne (1973) introduced standardised procedures for the extraction, identification, and analysis of phytochemicals from plants. Trease and Evans (1989) provided a basis for the evaluation of the therapeutic potential of both traditional and modern pharmacognosy by presenting significant methods for the phytochemical screening and classification of plant-derived chemicals. Sofowora (1993) evaluated the role of medicinal plants in African traditional medicine by highlighting the phytochemical properties and therapeutic potential of the plants. The book also explains how to evaluate the safety and efficacy of herbal remedies.

The potential of phytochemicals as new antibacterial agents was studied by Iwu et al., (1999), who also emphasizing the importance of these compounds in drug discovery and their effectiveness against resistant diseases. Hudzicki (2009) provided a detailed protocol on the Kirby-Bauer disc diffusion technique, a standardized technique used to assess bacterium's susceptibility to antibiotics. It includes preparation, interpretation, and quality control procedures. According to Abdallah (2011), plants constitute useful alternative sources of antimicrobials as their rich in bioactive compounds. The research supports the employment of medicinal herbs in combating infection that is antibiotic-resistant. The methanolic extract may be a natural therapeutic agent because it demonstrated strong antioxidant and moderate antibacterial activity against *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli*.

Gaire *et al.*, (2011) isolated phytochemicals such as alkaloids, flavonoids, tannins, and glycosides from the stem bark of *Ficus auriculata*.

The defence mechanism found in plant latex along with other exudates, which are made up of a variety of compounds and proteins that thwart attacks by pathogens and herbivores, are described by Konno (2011). The study highlights the critical role these compounds play in protecting plants. Singh et al. (2011) evaluated the pharmacological characteristics, phytochemical constituents, and traditional medicinal uses of *Ficus religiosa*.

The study is on the plant's bioactive components and how these can be used therapeutically to treat a range of illnesses. Salem et al. (2013) assessed the phytochemical makeup and antimicrobial activity of a few *Ficus* species, observing that bioactive compounds such flavonoids, tannins, and phenolics were responsible for the plant's ability to combat bacteria and fungi.

Salem et al. (2014) synthesised silver nanoparticles (AgNPs) from *Ficus sycomorus* leaf extracts and latex. Both Gram-positive as well as Gram-negative bacteria were significantly inhibited by the resultant AgNPs, indicating that they may be used as natural antimicrobials. *Ficus religiosa* latex extracts demonstrated significant antibacterial activity against a range of bacterial and fungal infections in the study conducted by Tulasi et al. (2015). This supports the idea that it could be used as a natural antibacterial agent. Collignon et al. (2016) examined the World Health Organization's most recent ranking of antibiotics according to their significance for human health, which was designed to direct risk management tactics to lessen antimicrobial resistance (AMR) brought on by the production of food animals. According to the research, in order to maintain the effectiveness of critical antimicrobials for human health, their usage in food animals should be minimised.

Bertoletti et al. (2018) used leaf extracts of Ficus auriculata to perform its phytotoxic, antimicrobial, and antioxidant activities. According to the study, the extracts demonstrated strong antioxidant qualities in addition to strong antibacterial activity against a variety of bacterial strains. Additionally, the extracts showed phytotoxicity, suggesting possible uses in agriculture and medicine. Abarca et al. (2019) examined the ecological and chemical significance of plant latex, emphasising its role as a defence mechanism and a source of bioactive compounds. The study emphasises how latex's complex chemical composition and ecological functions make it a material with great potential for drug development and pest management.

Ficus carica, Ficus sycomorus, and Euphorbia tirucalli latex extracts were evaluated by Abdel-Aty et al. (2019), who found a high phytochemical content with notable cytotoxic and antioxidant properties.The outcomes validated their potential for application in medication and therapy. The ethanolic and aqueous extracts of Calotropis gigantea root and latex exhibited potent antibacterial activity, according to Kori and Alawa (2014). Using phytochemical screening, alkaloid, flavonoids, and glycosides were found. According to Upadhyay's (2015) research, fruit latexes from 10 laticiferous plants shown broad-spectrum antimicrobial action against fungi and bacteria, bolstering their inherent antibacterial compositions.

Mishra and Parida (2020) assessed the therapeutic potential of several plant latex types, emphasising their diverse pharmacological activity, high bioactive content, and exceptional medicinal potential in drug discovery. Nawaz et al. (2020) assessed the phytochemical makeup, antioxidant capability, and therapeutic importance of the majority of Ficus species.

The research identifies significant bioactive compounds to substantiate the plant's antibacterial, anti-inflammatory, and wound-healing properties, such as flavonoids, phenolics, tannins, and alkaloids. It further establishes position of *Ficus* in natural medicine and drug research through its traditional and modern therapeutic potential.

Prastiyanto *et al.,* (2020) performed antibacterial activity, on *Jatropha sp*. latex and detected the presence of phytochemicals that are active against to multi-drugs resistant pathogens. It indicates the potential for developing natural antibacterial agents with it. Ramos *et al.,* (2020) studied on latex, produced by laticifers, acts as a chemical and physical barrier to exclude infections and herbivores. It focuses on the significance of latex in plant defense strategies and its intricate composition, which consists of proteins and secondary metabolites. According to Murugesu et al., (2021) both *Ficus religiosa* and *Ficus benghalensis* contain a wide range of phytochemicals such as terpenoids, flavonoids and tannins possessing strong anti-inflammatory, anti-microbial, antidiabetic, and antioxidant activities. The review highlights both their modern therapeutic promise and ancient use.

Tamta et al., (2021) examined the pharmacological characteristics, phytochemical elements, and folk uses of *Ficus auriculata*, focusing on the plant's richness of bioactive compounds like flavonoids, tannins, and saponins. The anti-inflammatory, hepatoprotective, antibacterial, and antioxidant activities of the plant are noted in the study as possible remedies for various diseases. Mohammad and Alzweiri (2022) identified the latex of *Ficus carica* is high in enzymes, flavonoids, and phenolics, exhibiting complex pharmacological activities such as antimicrobial, anticancer, and anti-inflammatory activities. The results emphasize its therapeutic potential but requires additional clinical trials.

Hegazy et al., (2023) investigated the composition and biological activity of *Ficus carica* latex, they found a diverse assortment of bioactive compounds, including terpenoids, flavonoids, phenolics, and enzymes like chitinases and proteases. The study identified the potential of the latex in therapeutic usage as well as in various industries by pointing to its diverse pharmacological activities, such as antibacterial, anticancer, antioxidant, anti-inflammatory, and antiparasitic activities.

Amri *et al.*, (2024) examined the anatomical characters of *Ficus auriculata* to evaluate its taxonomic rank in the family Moraceae. Characterized specific leaf and stem features justified its classification and enhance understanding of its evolutionary relationships. Antibacterial activity of *Ficus alba* and *Ficus carica* latex was evaluated by Aslan (2024) against a variety of bacterial and fungal infections was. As per the study, both types of latex contained antibacterial properties, but *F. carica* latex contained the highest activity *Candida albicans* (15.3 mm) and *Escherichia coli* (13.3 mm) exhibited highest inhibition than *Acinetobacter baumannii* (8.3 mm). These results demonstrate the potential of natural antibacterial agent activity of fig latex, and especially against drug-resistant organisms.

Bhatt et al*.,* (2024) determined *Ficus auriculata* (Timla) as an unexploited fruit and more nutritious than *Ficus carica* (Anjeer) because it contains more protein, magnesium, potassium, and iron. The study encourages more consumption and research by pointing out its ability to cure nutrient deficiencies and promote health. Freitas et al*.,* (2024) highlighted laticifers as significant plant defense complexes that deter pathogens and herbivores through the emission of latex containing rich bioactive chemicals. The paper emphasized their composition and chemical profile across species.

Marisetti et al*.,* (2025) studied on the composition, production and potential medicinal applications of natural latex, discussing its application in industry, agriculture and medicine and describing its antibacterial and anti-inflammatory properties. Molik *et al.,* (2025) identified that a diverse array of antimicrobial peptides, proteins, and enzymes with great potential for drug discovery are found in latex of medicinal plants. Based on the research, latex is a natural source of bioactive compounds that can be employed to combat resistant infections and develop new treatments.

CHAPTER 3

MATERIALS AND METHOD

3.1 PLANT MATERIAL

Systematic position

Kingdom	: Plantae
Division	: Angiospermae
Class	: Dicotyledons
Subclass	: Monochlamydeae
Order	: Urticales
Family	: Moraceae
Genus	: *Ficus*
Species	: *auriculata*

Fig. 1: Habit of *Ficus auriculata* (Lour.)

The Moraceae family comprises 37 genera and approximately 1100 tropical tree species. Moraceae latex is milky or occasionally watery, making it different from other families. *Ficus* is the most popular of the 37 genera in the Moraceae family due to its benefits. It is considered to be one of the largest flowering plants in the temperate tropical and semi-tropical areas. *Ficus auriculata* (Lour.), an ethnomedicinal plant, is used in traditional folk medicine to cure a variety of human diseases (Amri et al., 2024). *Ficus auriculata* (Lour.) plants are also referred to as "Roxburg Fig" or "Elephant ear fig" (Bhatt et al 2024). The shrub is perennial, with coarse, greyish-brown bark and aerial roots, growing to a height of 4 to 10 meters (Fig.1). The leaves of the young plant are red, while those of the mature plant are dark green. The leaves are simple, oval or ovate, measuring 5–12.5 cm in width and 5-8 cm in length. The stipules measure 1.5–2 cm long, and the petioles 1.2–5 cm long. The fruit is 3-5 cm in diameter, pear-shaped, and has embedded achenes with 8-12 longitudinal ridges. The fruit darkens to red when mature (Amri et al., 2024).

3.2 COLLECTION OF PLANT LATEX

The latex of *Ficus auriculata* (Lour.) or Elephant ear fig tree was collected out from the sites of St. Teresa's College junction (Lat 9.975827°) and CUSAT (Lat 10.045761°) of Ernakulam district, Kerala. The tree surface was cleaned with distilled water and incision were made on the bark using a sharp knife (Fig. 2). The latex was harvested in liquid form from stem and branches (Fig. 3). Fresh latex was stored in sterilized vials at 4°C to avoid coagulation.

Fig.2: Latex from the bark of *F. auriculata* (Lour.)

Fig.3: Collection of latex from the bark incision

3.3 PREPARATION OF LATEX EXTRACT

Latex is a complex emulsion consisting of microparticles of polymers in aqueous solution. Obtaining latex for extraction is itself a bit laborious process. Prior to the extraction the area was sterilized with 70% ethanol and the glassware was kept in hot air oven to prevent contamination. First 10ml of crude latex was diluted with distilled water in the ratio 1:1. This mixture was then subjected to centrifugation at 5000 RPM in the laboratory for 20 minutes (Fig.4 and 5). The supernatant (sample A) obtained was filtered from the pellet (Kori and Alawa, 2024) and stored in a sterile test tube. The supernatant was then combined with 70% ethanol in 1:1.5 proportion. This composition was centrifuged for 10 minutes at 3000 RPM. The supernatant (Sample B) was collected and both the samples were stored in a sterilized reagent bottle at 4°C (Fig. 6).

Fig.4: Omega C8C Centrifuge

Fig.5: Latex centrifugation

Fig.6: Sample A (right) and Sample B (left)

3.4 PHYTOCHEMICAL ANALYSIS

The phytochemical analysis was carried out on both the samples using standard procedure described by Sofowora, 1993; Trease and Evans, 1989; Harborne, 1973. The presence of tannins, flavonoids, phenol, phytosterols, glycosides, alkaloids, glycosides, coumarins and terpenoids were carried out.

3.4.1 Test for Tannins

A) Preparation of 5% Ferric chloride

5% $FeCl_3$ solution was prepared by dissolving 0.25grams of ferric chloride in 100 ml of distilled water. This was transferred to a glass beaker.

B) Ferric Chloride Test for Tannins

To 2 ml of test solution add few drops of 5% ferric chloride solution. The formation of blue-green colour indicated the presence of tannins.

3.4.2 Test for Flavonoids

A) Preparation of 2% Sodium hydroxide

To make 2% solution of NaOH, 0.5 g of sodium hydroxide was mixed with 100 ml distilled water.

B) Sodium hydroxide test

2 ml of 2% Sodium hydroxide was added to the test solution; a concentrated yellow colour was produced which decolourises after addition of 2 drops of HCl acid, indicated the presence of flavonoids.

3.4.3 Test for Phenol

Sample extract was mixed with iodine in 1:1 ratio. Formation of red colour indicated the presence of phenols.

3.4.4 Test for Phytosterol

To 2 ml extract add few drops of concentrated sulphuric acid. A red colour was formed in the lower layer of the mixture, indicating the presence of phytosterol.

3.4.5 Test for Glycosides (Liebermann's test)

Aqueous extract was mixed with each of 2 ml of glacial acetic acid and drops of ferric chloride. The mixture was cooled in ice. Carefully concentrated sulphuric acid was added. A brown ring was observed which indicated the presence of steroidal nucleus, i.e., glycine portion of glycoside.

3.4.6 Test for Alkaloids

2 ml extract was treated with 3 ml of 2% picric acid and observed for the formation of orange colour which indicates the presence of alkaloids.

3.4.7 Test for Coumarins

3 ml of 10 % sodium hydroxide was added to 2 ml aqueous plant extract and yellow colour was observed in positive results.

3.4.8 Test for Terpenoids

2ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water bath and then boiled with 3 ml of H_2SO_4 concentrated. A reddish-brown colour formed which showed the entity of terpenoids.

3.5 TEST FOR ANTIMICROBIAL ACTIVITY

The antimicrobial analysis was carried out on both the samples using standard procedure described by Hudzicki (2009).

3.5.1 Preparation of inoculum

Using a sterile inoculating loop, touch the isolated colonies of the organism to be tested. Suspended the organism in 2 ml of sterile saline. The turbidity of this suspension was adjusted to a 0.5 McFarland standard by adding more culture broth if the suspension is too light or diluting with sterile saline if the suspension is too heavy.

3.5.2 Preparation of Muller Hinton agar plates

The Muller Hinton agar media was prepared by dissolving 3.8 g MHA powder in 100 ml distilled water. The medium was sterilized at 121°C in 15 psi pressure (autoclaving). The prepared media was poured onto the sterilised plates and allowed to solidify.

3.5.3 Preparation of Sabouraud Dextrose Agar plates

6.5 g of Sabouraud Dextrose Agar was dissolved in 100 ml distilled water. Boil for one minute to completely dissolve the ingredients. Sterilize the medium by autoclaving at 121°C for 15 minutes. Once the mixture was poured into the petri plate, the medium was left to harden.

3.5.4 Plate Inoculation

MHA plates for bacterial strains and SDA plates were used to inoculate fungal strains. A sterile swab was dipped into the inoculum tube. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The dried surface of MH agar plate was inoculated with *Staphylococcus aureus, Pseudomonas aeruginosa* by spreading the swab over the entire agar surface rotate the plate approximately each time to ensure an even distribution of the inoculum. Similarly, the SDA plates were inoculated with *Aspergillus niger* and *Candida albicans.* The swabs were discarded into an appropriate container. The plates were left undisturbed in room temperature for 10-15 minutes, for the surface of the agar plate to dry before proceeding to the next step.

3.5.5 Well diffusion assay

The wells of 7 mm were cut in the inoculated plate using sterile hollow rod and the rods were sterilised by cleaning them with a sterile alcohol and allowing them to air dry or by showing them directly to the flame. The samples solution of 100µl were carefully dispensed into the well using pipette. 100µl of amoxicillin (1mg/ml) solution was taken as positive control and were carefully pipetted into the well followed by ethanol (negative control) was into another well. After placing the sample, the lid was replaced to minimize exposure of the agar surface to room air. The plates are then incubated at 37°C in an incubator for 18 to 24 hours. The test was done in triplicates.

CHAPTER 4

RESULTS

4.1 PHYTOCHEMICAL ANALYSIS

The latex extract of *Ficus auriculata* (Lour.) was qualitatively analysed to identify the phytochemical compounds present in the given samples A (aqueous extract) and B (ethanolic extract). Experiments were conducted to detect the presence of secondary metabolites like tannins, flavonoids, phenol, phytosterols, glycosides, alkaloids, glycosides, coumarins and terpenoids.

4.1.1 Test for Tannins

The presence of tannins in both the sample were analysed by adding few drops of 5% ferric chloride solution into 2ml test solution. The development of blue –green colour is used as the qualitative indicator.

- **Sample A**
  Upon addition of ferric chloride showed no blue-green colour, indicating the absence of tannins.
- **Sample B**
  Similarly, in sample B no blue-green colour was observed, which indicates the absence of tannins.

Fig.7: Test for Tannins (Sample A)

Fig. 8: Test for Tannins (Sample B)

4.4.2 Test for Flavonoids

The detection of flavonoid was done by adding 2 ml of 2% sodium hydroxide to the test solution, concentrated yellow colour was produced, followed by addition of 2 drops of HCl acid. A colour change occurs which was denoted the presence of flavonoids.

- **Sample A**

  When NaOH was added to the sample no yellow colour was produced, indicated the absence of flavonoids.

- **Sample B**

  Addition of NaOH to the sample generated a concentrated yellow colour, which get decolourised after the addition of 2 drops of HCl acid. This indicates the presence of flavonoids.

Fig. 9: Test for Flavonoids (Sample A)

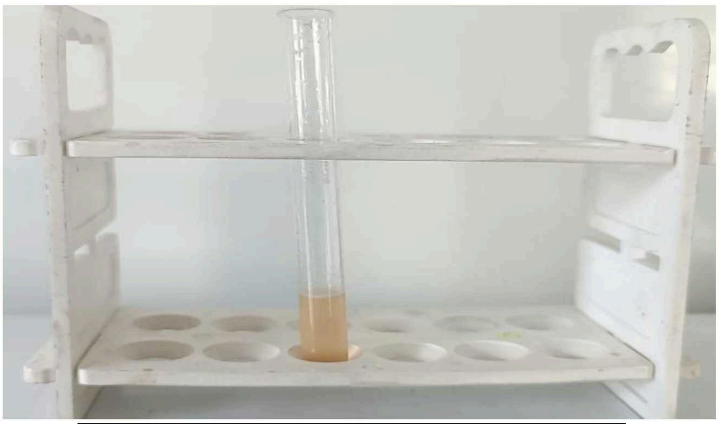

Fig.10: Test for Flavonoids (Sample B)

4.4.3 Test for Phenol

Sample extract was mixed with iodine solution in the ratio 1:1. The formation of red colour determined the presence of phenols.

- **Sample A**

  When the extract was combines with iodine solution in equal volume no colour change occurred. This denotes the absence of phenol.

- **Sample B**

  No red colour was observed during the reaction, indicated the lack of phenol in the sample.

Fig. 11: Test for Phenol (Sample A)

Fig. 12: Test for Phenol (Sample B)

4.4.4 Test for Phytosterol

Phytosterol was detected by adding a few drops of concentrated sulphuric acid into 2ml extract which resulted in formation of red colour in the lower layer of the mixture.

- **Sample A**

  Red colour was formed on the lower layer of the mixture on the addition of concentrated sulphuric acid to the sample confirming the presence of phytosterol.
- **Sample B**

  During the test, presence of phytosterol was observed as the extract produced a red colour in the lower layer of the solution.

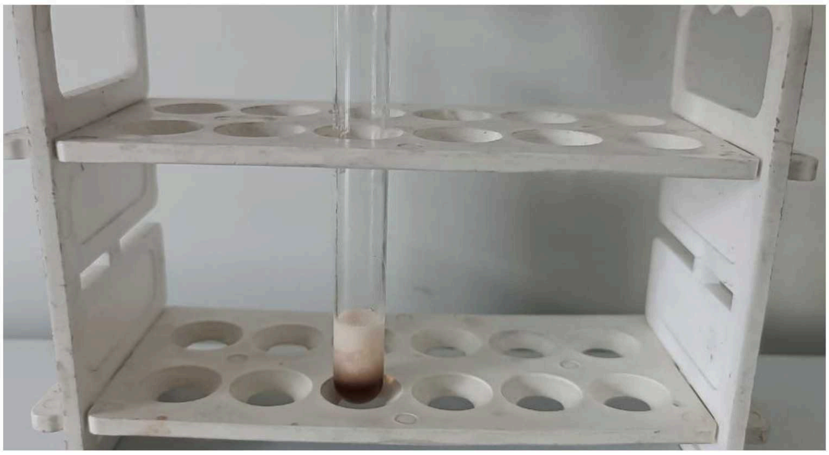

Fig.13: Test for Phytosterol (Sample A)

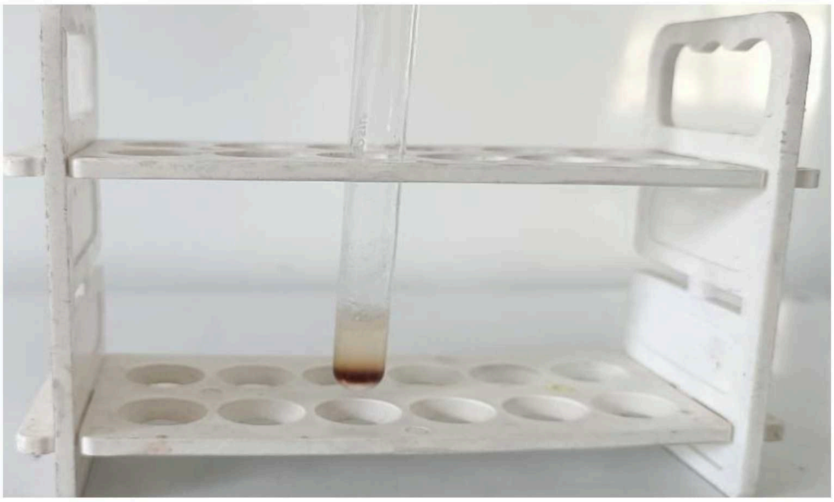

Fig.14: Test for Phytosterol (Sample B)

4.4.5 Test for Glycosides (Liebermann's test)

Extract was mixed with each of 2 ml of glacial acetic acid and few drops of ferric chloride, the mixture was cooled in ice. Concentrated sulphuric acid was added carefully. A brown ring was observed which indicated the presence of glycine portion of glycoside.

- **Sample A**

  A brown ring was formed when the extract was subjected to testing. This confirmed the presence of glycosides in the sample.

- **Sample B**

  No ring was formed during the analysis, which denotes the absence of glycosides.

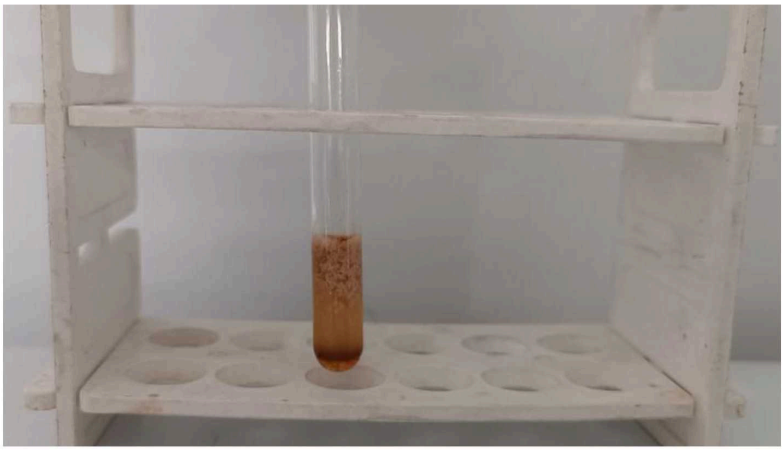

Fig.15: Test for Glycosides (Sample A)

Fig.16: Test for Glycosides (Sample B)

4.4.6 Test for Alkaloids

2 ml extract was treated with 3 ml of 2% picric acid and formation of orange colour was used to indicate the presence of alkaloids.

- **Sample A**

  No colour change was observed which indicated the absence of alkaloids.

- **Sample B**

  During analysis the extract failed to form orange colour, reporting the absence of alkaloids.

Fig. 17: Test for Alkaloids (Sample A)

Fig.18: Test for Alkaloids (Sample B)

4.4.7 Test for Coumarins

3 ml of 10 % sodium hydroxide was added to 2 ml plant extract and yellow colour was used as the indicator.

- **Sample A**

  No yellow colour was observed during the test, this denotes absence of coumarins.
- **Sample B**

  When extract was mixed with NaOH no colour change occurred, reporting the absence of coumarins.

Fig.19: Test for Coumarins (Sample A)

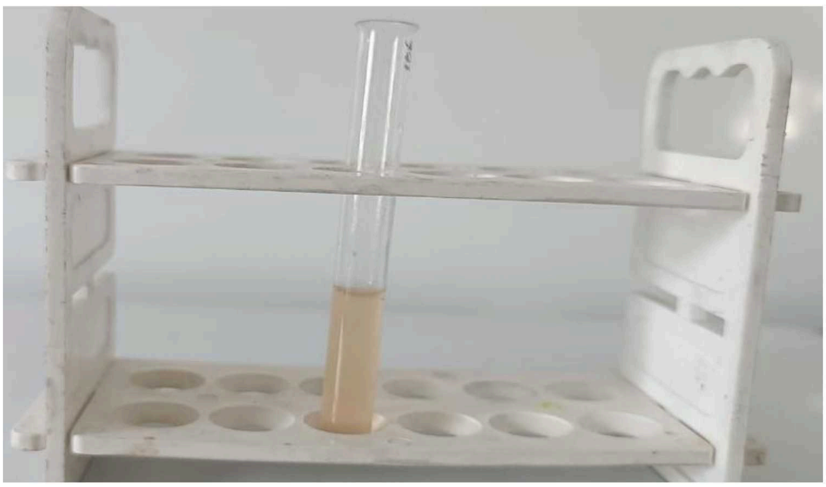

Fig. 20: Test for Coumarins (Sample B)

4.4.8 Test for Terpenoids

2 ml of chloroform was added to 5 ml plant extract and evaporated on the water bath, later boiled with 3 ml of concentrated H_2SO_4. A reddish-brown colour formed indicates the entity of terpenoids.

- **Sample A**

  Red-brown colour was formed when concentrated sulphuric acid was added to the mixture confirming the presence of terpenoids.

- **Sample B**

  During the test, presence of terpenoids was observed as the extract produced a red colour after adding concentrated H_2SO_4.

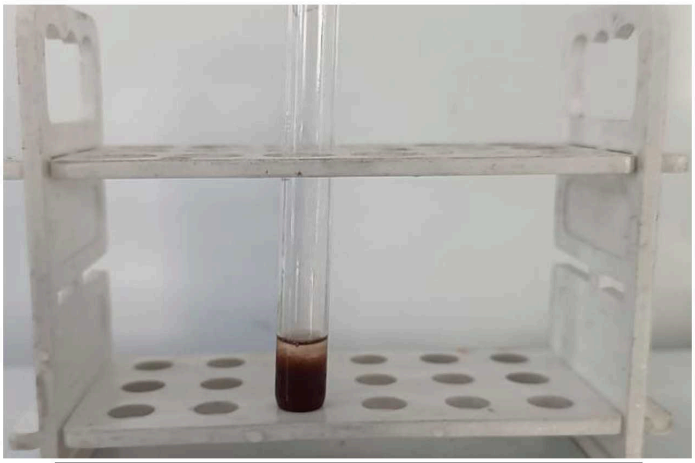

Fig. 21: Test for Terpenoids (Sample A)

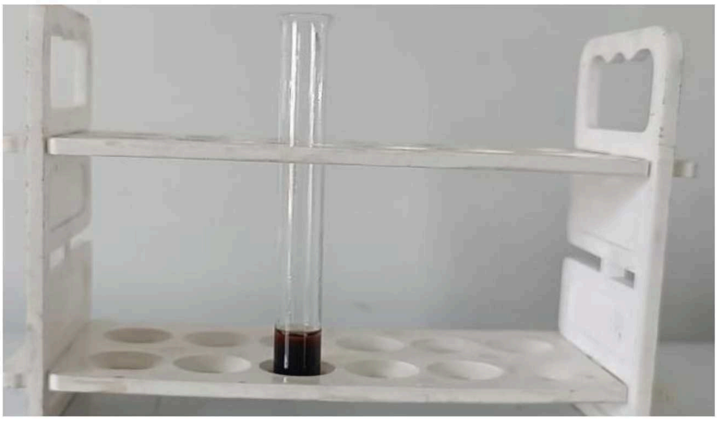

Fig. 22: Test for Terpenoids (Sample B)

The latex extract obtained using different solvent was screened for the presence of various bioactive phytochemical compounds. The aqueous latex extracts of *Ficus auriculata* (Lour.) was found rich in and phytosterol, glycosides and terpenoids. The ethanolic extract showed positive results for phytosterol, terpenoids and flavonoid. Both the latex extracts produced negative results for alkaloids, tannins, coumarins and phenol (Table 1).

Table 1. Phytochemical Analysis of *Ficus auriculata* (Lour.) Latex Extracts

Sl. No.	Constituents	Aqueous extract	Ethanolic extract
1	Tannins	-	-
2	Flavonoids	-	+
3	Phenol	-	-
4	Phytosterol	+	+
5	Glycosides	+	-
6	Coumarins	-	-
7	Terpenoids	+	+
8	Alkaloids	-	-

(+) indicates present (-) indicates absent

4.2 ANTIMICROBIAL ACTIVITY OF DIFFERENT LATEX EXTRACTS

Antimicrobial activity of different solvent extract of *F. auriculata* (Lour.) is shown in table 2 that the aqueous extracts of latex contribute satisfactory inhibitory actions against bacterial microbes ranging from 12 mm to 15 mm diameter inhibitory zones. The ethanolic extracts only exhibited inhibition against the bacteria *Staphylococcus aureus* only. The ethanolic and aqueous extracts of latex showed zero inhibition to all the fungal strains. The aqueous extract of latex has maximum zone of inhibition against the *Staphylococcus aureus* the common gram-positive pathogenic microorganism when compared to *Pseudomonas aeruginosa.*

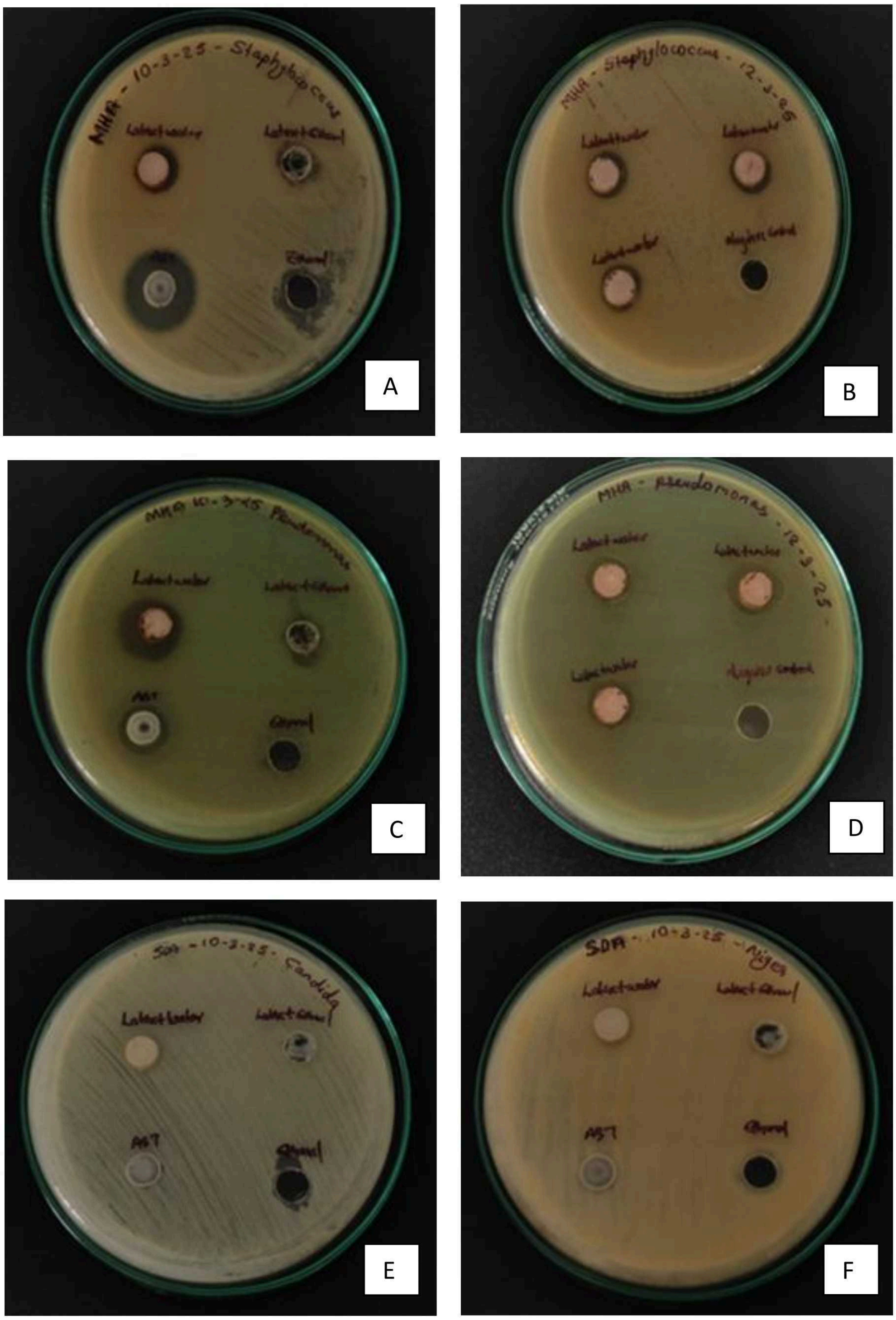

Fig. 23: **Antimicrobial Activity**; Plate (A- B): Antibacterial activity of Sample A and B against *S. aureus* and *P. aeruginosa,* Plate (C- D): Antibacterial activity of Sample A against *S. aureus* and *P. aeruginosa,* Plate (E-F): Antifungal activity of Sample A and B against *C. albicans* and *A. niger*

Table 2: Antimicrobial Activity of *Ficus auriculata* (Lour.) Latex Extracts

Sl. No.	Extracts	Bacterial Strains Zone of Inhibition (in mm)		Fungal Strains Zone of Inhibition (in mm)	
		Staphylococcus aureus	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Candida albicans*
1	Aqueous extract	13	15	NIZ	NIZ
		13	12	NIZ	NIZ
		13	13	NIZ	NIZ
2	Ethanolic extract	12	NIZ	NIZ	NIZ
		12	NIZ	NIZ	NIZ
		12	NIZ	NIZ	NIZ

NIZ- No Inhibition Zone

CHAPTER 5
DISCUSSION

The purpose of the study is to evaluate the antibacterial, antifungal and phytochemical properties of *Ficus auriculata* (Lour.) latex, providing new insights into its potential as a natural antimicrobial agent. The results were correlated with the similar studies on other *Ficus* species and other plant species like *Calotropis, Euphorbia*, *Hevea* etc, highlighting key differences and similarities in antimicrobial and phytochemical compositions.

In the present study, the extracts showed antibacterial actions due to presence of compounds like phytosterol, flavonoids, glycosides and terpenoid showing its therapeutic potentials. Similar results were also observed with the study of the latex of *Ficus carica* (Mohammad and Alzweiri 2022).

Flavonoids, phenolic acids, tannins, and alkaloids are particularly rich in these plants. Similar to these findings, a similar study from the latex of *Ficus auriculata* can also be used as a natural antimicrobial due to presence of compounds like glycosides, terpenoids, flavonoids etc enabling its application in medicine and pharmaceuticals (Murugesu et al., 2021).

In the current study the both the latex extract exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The phytochemical analysis detected the presence of flavonoid in ethanolic extract along with phytosterol and terpenoid. The aqueous extract also tested positive for phytosterol, glycosides and terpenoid making them a potential antibacterial agent. Similar findings were also revealed by the study of Prastiyanto et al., (2020).

Hegazy et al., (2023) reported that *F. cairica* latex is rich in various chemical constituents like proteins, antioxidants, terpenoids, sterols, flavonoids, coumarins, phenolic acids and volatile compounds which exhibited various biological activities, such as antibacterial, antifungal, antivirals, anticancer activities etc. These findings compared to the present research suggests Ficus auriculata latex has a selective antimicrobial outline, either due to differences in compound concentration or the absence of enzyme-based activity, the selective in its antibacterial action indicates its use as a source of targeted plant antibacterial agents.

Nawaz et al., (2020) detected various bioactive phytochemical compounds in the roots, stem, bark, leaves, fruit, latex, and pulp of *Ficus* plants like polyphenols, phenolic acids, triterpenoids, flavonoids, glycosides and many more make them medicinally relevant. Due to its rich phytochemical contents and potent, these plants have a wide range of biological activities, such as antibacterial, antidiabetic, anti-obesity, hepatoprotective, cardioprotective, renal-protective, and anticancer activity making them a useful in various treatments. Unlike the earlier study, latex of *Ficus auriculata* exhibited selective antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* but displayed no antifungal activity against *Candida albicans* and *Aspergillus niger*. In both studies flavonoids and terpenoids act as antimicrobial agents, however this selective activity of the latex sample shows more focused antibacterial potential using it to investigating plant-derived antibacterial agents with low antifungal activity and using them in wound healing and agriculture.

In the current study, the latex extract revealed the presence of phytochemical compounds like terpenoids, glycosides, phytosterol and flavonoids which contributed to the antibacterial activity against *Staphylococcus aureus and Pseudomonas aeruginosa.* Similar outcomes were observed in the study of Salem et al., (2013) who explored the antimicrobial properties and phytochemical composition of various *Ficus* species including *Ficus benghalensis, Ficus sycomorus, Ficus benjamina, Ficus religiosa, Ficus auriculata, Ficus racemosa, Ficus retusa,* *Ficus microcarpa, Ficus carica, Ficus lyrata, Ficus deltoidea, Ficus elastica, Ficus nitida, Ficus polita, Ficus tsiela, Ficus exasperata, Ficus capensis, and Ficus palmata*. The phytochemical analysis detected the presence of flavonoids, tannins, phenolic chemicals, saponins, steroids, terpenoids, alkaloids, glycosides, carbohydrates, proteins, essential oils, resins, and latex enzymes like serine proteases. The extracts were tested against a broad range of microorganisms, including bacteria like *Salmonella typhi, Citrobacter freundii, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris,* viruses like HSV-1, ECV-11, Adenovirus; and fungi *Candida albicans, Aspergillus niger, Microsporum canis, and many other organisms.* The alcoholic extracts of leaves and fruits from *Ficus auriculata* showed high antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa*. The antimicrobial activities were dose-dependent. Flavonoids, phenolic acids, tannins, saponins, and terpenoids were some of the phytochemicals identified in *F. auriculata.*

Abdel-Aty et al., (2019) evaluated the phytochemical profiles, antioxidant capacities, and cytotoxic effects of latex extracts obtained from *Ficus carica, Ficus sycomorus, and Euphorbia tirucalli* to identify potential therapeutic agents using Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC). Phenolic compounds, flavonoids, tannins, saponins, steroids, terpenoids, alkaloids (in minor quantities), glycosides and coumarins (traces) were detected contributing to their strong antioxidant and cytotoxic activities. Comparing the results with present study points out the presence of similar phytochemicals and selective bioactivity of the latex which can be applied in eliminating pathogenic bacteria without harming beneficial fungi.

Tulasi et al., (2015) estimated the antimicrobial activity of *Ficus religiosa* latex extract. Phytochemicals like Flavonoids, phenolic acids, tannins, saponins, alkaloids, glycosides, terpenoids, and steroids were identified. The aqueous extract had the highest antibacterial activity against *E. coli* at 100 mg/mL dosage, with an inhibition zone measuring 17 mm.

aqueous and n-butanol extracts were found to possess significant antibacterial activity compared to other extracts. The n-butanol extract inhibited *Candida albicans* more, while the aqueous and chloroform extracts exhibited moderate inhibitions and the effects was dose-dependent. Similarly, the present study showed antibacterial activity with 12-15mm ZOI (50% v/v) against *Staphylococcus aureus and Pseudomonas aeruginosa* on aqueous and ethanolic extracts, but no antifungal activity. The presence of flavonoids, phytosterols, glycosides, terpenoids and its concentration may impart to the antimicrobial potential observed.

Aslan (2024) evaluated the antimicrobial activity of *Ficus alba* and *Ficus carica* latex against selected bacterial and fungal pathogens. Both latexes exhibited significant antimicrobial activity, but *Ficus carica* exhibit broader and more potent effects. The microbial action was found to be concentration-dependent. The extracts were tested against various bacteria and the fungus *Candida albicans*. Phytochemical screening revealed the presence of flavonoids, tannins saponins, alkaloids, terpenoids, and phenolic compounds, which are known for their antimicrobial efficacy. Both latex samples showed effective antimicrobial activity, supporting their potential use as natural alternatives to synthetic antimicrobial agents. Although in the previous study both antifungal and antibacterial actions of extracts was reported but the latex of *Ficus auriculata* showed only antibacterial action and no antifungal activity. This may be related to microbial strains, extraction methods, concentration of extracts or the absence of certain bioactive compounds. This specificity of the latex is valuable for developing plant-based antibacterial alternatives with minimal ecological damage.

Mishra and Parida (2020) explored the varied therapeutic applications of plant latex emphasizing its capability in antimicrobial treatments, highlighting plant latex as a rich source of bioactive compounds, including, flavonoids, terpenoids, phenolic compounds, and enzymes like proteases and peroxidases. These phytochemicals contribute to the latex's antimicrobial properties, making it effective against a range of pathogens. The antimicrobial efficacy of plant latex from *Ficus carica*, *F. elastica*, *Calotropis gigantea, C. procera, Euphorbia tirucalli, E. hirta* and many more showed antibacterial and antifungal properties. The antimicrobial action is attributed by the presence of compounds that disrupt microbial cell walls and inhibit essential enzymatic functions, developing them as new therapeutic agents. In contrast, the current research having similar phytochemicals exhibited only antibacterial activity, this property of latex helps to minimize microbial imbalance and resistance can be used to discover next generation natural therapeutical agents.

Upadhyay (2015) assessed the antimicrobial activity of fruit latexes from ten laticiferous plants, namely *Ficus benghalensis, Calotropis procera, and Carica papaya*. Latex extracts contain potent antibacterial activity against bacteria like *Staphylococcus aureus, Escherichia coli, Bacillus cereus, and Klebsiella pneumoniae* and antifungal activity against *Candida albicans*. Phytochemical screening indicated the occurrence of proteases, chitinases, flavonoids, alkaloids, and phenolic compounds that are responsible for the antimicrobial activity. Whereas in the current study, latex possess antibacterial activity and inactive against fungi. This project can be used for developing antimicrobial agent for setting bacterial control, wound healing formulation.

Bertoletti et al. (2018) evaluated the antioxidant, antimicrobial, and phytotoxic activities of *Ficus auriculata* leaf extracts prepared using various extraction methods, including combinations of water, ethanol, ultrasound, and cellulase treatment. The extracts, especially those obtained using the water/ethanol/ultrasound method (M2), showed the highest total phenolic content and strongest antioxidant activity. Phytochemical screening confirmed the presence of phenolic compounds, flavonoids, and other antioxidant-related metabolites. Antimicrobial testing revealed that the extracts effectively inhibited the growth of *Escherichia coli, Salmonella enteritidis*, *Staphylococcus aureus* and *Listeria monocytogenes*. In the present research, the latex extracts demonstrated antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using terpenoids, glycosides, phytosterol and flavonoids. Particularly no antifungal activity was observed in the latex, which could suggest that the bioactive compounds present are more effective against bacteria than fungi or due to its concentration indicating the pharmacological potential of *Ficus auriculata* (Lour.) against bacterial infections.

CHAPTER 6
CONCLUSION

The current study investigated that the latex of *Ficus auriculata* (Lour.) as a potential antibacterial agent, particularly against Gram-positive bacteria. Phytochemical investigation revealed the presence of flavonoids, phytosterol, terpenoids and glycosides, which are the contributors to its antibacterial potential. This result indicates that the latex of *Ficus auriculata* (Lour.) may be a potential source of natural antibacterial compounds with medicinal, pharmaceutical, and biotechnological applications. Although the latex lack antifungal activity, its phytochemical richness offers valuable opportunities for further research. Future studies will be mainly focusing on isolating and characterizing the individual active compounds, optimizing extraction techniques, and enhancing its efficacy through formulation improvements. The therapeutic potential of latex could be increased and enhanced by screening it against a greater range of bacterial and fungal species and evaluating the synergistic interactions with standard antibiotics. The potential of *Ficus auriculata* (Lour.) latex as a natural antibacterial agent, its effectiveness against *Staphylococcus aureus* and *Pseudomonas aeruginosa* suggests applications in various industries like medicine, pharmaceutical, agricultural, cosmetic and textile as a sustainable alternative to synthetic antimicrobials.

CHAPTER 7

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