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Antimicrobial and Phytochemical Investigation of Elaeocarpus Tectorius Leaf and Bark Extracts

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Plants generate a diverse array of phytochemical constituents, which are secondary metabolites that find application in the pharmaceutical industry, either directly or indirectly. Throughout history, humans have adeptly harnessed different parts of plants or their extracts to combat a wide range of ailments, such as bacterial infections. In the current investigation, chloroform, aqueous, acetone, ethyl acetate, and methanol extracts of Elaeocarpus tectorius leaf and bark were evaluated for their antimicrobial properties using the well-diffusion method against 4 bacterial strains: Streptococcus mutans, Pseudomonas fluroscens, Staphylococcus aureus, and Bacillus subtilis. Both leaf and bark demonstrated satisfactory activity, with the leaf displaying the highest level of activity. The methanol, chloroform, and aqueous extracts showed the highest activity, followed by the ethyl acetate extracts. Less or no activity was observed in the acetone extracts. The extracts underwent screening to identify their phytochemical components through standard protocols. They were found to comprise alkaloids, flavonoids, glycosides, cardiac glycosides, tannins, phenols, steroids, quinones, proteins, carbohydrates, and saponins. The antibacterial activity of these extracts may be attributed to the presence of flavonoids, steroids, saponins, or tannins. Additional studies are necessary in order to identify the specific active components of Elaeocarpus tectorius.

1. Introduction

The field of phytochemical analysis is increasingly significant due to the global trend towards natural and plant-based remedies. As people become more aware of the limitations and side effects of synthetic drugs, there is a parallel rise in the exploration of traditional plants for their medicinal properties. Phytochemicals can exhibit a wide range of effects, from anti-inflammatory to anti-cancer properties. Through rigorous scientific research, the active constituents in these plants can be isolated, studied, and tested, paving the way for the development of new therapies. Moreover, this analysis can help ensure the quality and safety of herbal products available on the market. By understanding the chemical makeup of these plants, researchers can better assess their efficacy and potential interactions with other medications. The integration of this knowledge into modern medicine not only supports the development of new treatments but also promotes a more holistic approach to healthcare, emphasizing the value of natural resources in therapeutic practices. These plants have been used for centuries in traditional medicine to treat various ailments, and understanding their chemical makeup is crucial for validating their therapeutic properties. Phytochemicals are naturally occurring compounds that contribute to the color, flavor, and disease resistance of plants. They are classified into various categories, including alkaloids, flavonoids, tannins, and terpenes, each with unique biological activities. By analyzing these compounds, researchers can determine their potential health benefits and how they might work against diseases. For instance, some phytochemicals are known to have antioxidant properties, which help protect the body from damage caused by free radicals. Other compounds may have anti-inflammatory or antimicrobial effects, making them valuable in the development of new medicines. The process of phytochemical analysis often involves several techniques, such as chromatography and spectroscopy, to separate and identify these compounds in plant samples. As science continues to explore the rich diversity of medicinal plants, phytochemical analysis plays a key role in discovering new drug candidates and enhancing our understanding of plant-based therapies, which can lead to more effective treatments in modern medicine.

Medicinal plants have been used for centuries in traditional medicine to treat various ailments, and one of their significant benefits is their antimicrobial activity. This means that certain plants possess natural compounds capable of inhibiting the growth of harmful microorganisms like bacteria, viruses, and fungi. As the world faces rising issues with antibiotic resistance, researchers are increasingly turning to nature for alternatives. Many medicinal plants are rich in bioactive compounds such as flavonoids, alkaloids, and essential oils, which have been scientifically proven to exhibit antimicrobial properties. For instance, plants like garlic, ginger, and turmeric have been noted not only for their health benefits but also for their ability to fight infections effectively. These natural remedies offer a promising solution to enhance human health without the side effects often associated with synthetic medications. The study of these plants is crucial, as they might hold the key to developing new, effective treatments for infectious diseases. Additionally, utilizing medicinal plants can promote sustainable practices in healthcare, connecting communities with their traditional knowledge and natural resources while ensuring a harmonious balance between nature and human health. As interest in herbal medicine grows, so does the potential for integrating these powerful plants into modern medicinal practices.

The potential of medicinal plants to serve as antimicrobial agents is an encouraging prospect for human health and well-being. With antibiotic resistance on the rise, it is more important than ever to seek out sustainable and effective alternatives. Nature has provided humanity with a wealth of plants that have evolved unique defenses against microbial threats, which can be leveraged for modern treatments. This ongoing research aims to systematically examine various plant species and their components to understand how they function at a molecular level. Additionally, the significance of cultivating and preserving traditional knowledge of these plants cannot be overstated. Indigenous communities often hold invaluable insights into the use of local flora as medicine, and integrating this wisdom with scientific research can lead to more comprehensive and effective health solutions. By drawing connections between traditional practices and contemporary science, we might be able to create new therapies that are not only effective but also rooted in sustainable practices. Ultimately, the objective is to contribute to a future where herbal remedies complement modern medicine, ensuring that we have a diverse arsenal to combat microbial diseases.

2. Methodology

Plant description

Plants in the Elaeocarpus genus are mostly distinguished by their evergreen trees or shrubs nature. Some may also be epiphytes or lianes, while a few may momentarily turn deciduous. The leaves are arranged alternately, in a simple form (strictly compound with only one leaflet), with a swelling where the petiole meets the lamina. They often have toothed edges, prominent veins, and tend to turn red before falling. The flowers are commonly arranged in a raceme; they are generally bisexual and contain four or five sepals and petals, as well as numerous stamens. The petals usually feature intricately divided, linear lobes. The fruit, usually found in shades of blue or occasionally black, is an oval to spherical drupe with a textured endocarp. The Elaeocarpus tectorius species is naturally found from the Indian Subcontinent to Indo-China. It is a tree that primarily thrives in the lush tropical biome. (Figure 1)

Figure 1: Elaeocarpus tectorius

Phytochemical analysis

Phytochemical analysis is a vital process utilized to identify and quantify the various bioactive compounds present in plant extracts. The procedure typically begins with the collection of fresh plant material, which is then thoroughly washed to remove any contaminants. The cleaned plant sample is air-dried or oven-dried at low temperatures to preserve its active components. Once dried, the sample is ground into a fine powder to increase the surface area for extraction. The powdered plant material is then subjected to extraction using solvents such as ethanol, methanol, or water, with the choice of solvent depending on the specific phytochemicals of interest. The Soxhlet extraction process can involve techniques such as maceration, Soxhlet extraction, or ultrasonic extraction to efficiently draw out the desired compounds. After the extraction, the resulting solution is filtered to remove solid residues, yielding a clear plant extract. The results of such analysis can provide valuable insights into the potential medicinal properties of the plant, contributing to the fields of pharmacology, nutrition, and herbal medicine. List of phytochemical tests performed are Carbohydrate, Tannin, Saponin, Flavonoid, Alkaloid, Quinone, Glycoside, Cardiac Glycoside, Terpenoid, Phenol and Protein Tests by following standard methodologies established by Harborne (1973) and Sofowora (1993) (1,2). (Figure 2-4)

Figure 2: Dried stem and leaves of Elaeocarpus tectorius

Figure 3: Dried stem and leaves powders of Elaeocarpus tectorius

Figure 4: stem and leaves extracts of Elaeocarpus tectorius.

Antimicrobial activity

The procedure for evaluating antimicrobial activity using plant extracts involves several systematic steps aimed at determining the effectiveness of these natural substances against various microorganisms. Initially, plant materials, such as leaves, stems, or roots, are collected, thoroughly washed to remove any contaminants, and then dried. Once adequately dried, the materials are crushed into a fine powder to increase the surface area for extraction. The powdered plant material was performed for Soxhlet extraction with different solvents, like methanol or ethanol, in a sterile container for a defined period, typically 24 to 48 hours for bacteria and 3-5 days for fungi. This process allows the active compounds to leach into the solvent, resulting in crude extract rich in phytochemicals. After the extraction period, the mixture is filtered to separate the liquid extract from the solid plant remnants. The final extract can then be concentrated if necessary, usually through evaporation. Subsequently, antimicrobial activity is assessed using various methods such as the disc diffusion method or broth dilution method, where the extract is subjected to exposure against specific bacterial or fungal strains. The agar cup procedure is a widely used method to test the effectiveness of plant extracts against various microorganisms like bacteria and fungi. This process begins with preparing an agar plate, which serves as the growth medium for the microorganisms. Small wells, or cups, are then made in the agar using a specialized tool. After this, the plant extract, which may come from leaves, stems, or roots, is introduced into these wells. The agar plate is then inoculated with the target microorganisms, allowing them to spread and grow across the surface. The effectiveness is measured by observing zones of inhibition or determining the minimum inhibitory concentration, thus providing insights into the antimicrobial potential of the plant extracts being studied.

3. Results and Discussion

In the current era, plant and herb resources are plentiful, yet they are rapidly declining because of the continuous progress of civilization (3). Numerous studies have focused on isolating plant chemicals, yet only a limited number of screening programs have been launched for crude plant materials. It is widely acknowledged that the medicinal value of plants is attributed to the bioactive phytocomponents they contain (4).

In the present study Elaeocarpus tectorius leaf and bark extracts i.e., chloroform, aqueous, acetone, Ethyl acetate and methanol was evaluated for phytochemical and antibacterial analysis. The initial analysis of the phytochemical profile indicates that the different extracts of the formulation contain a diverse mix of essential phytochemicals, such as alkaloids, flavonoids, glycosides, cardiac glycosides, tannins, phenols, steroids, quinones, proteins, carbohydrates, and saponins as shown in Table 1 and figure 5. Medicinal plants and herbs contain essential phytochemicals, a diverse range of primary and secondary plant metabolites. These compounds are behind various biological activities like anti-hyperglycemic, anti-inflammatory, anti-diabetic, and anti-microbial effects [5,6]. All the extracts confirm the presence of alkaloids, glycosides, cardiac glycosides, tannins, phenols and carbohydrates and all the extracts confirm the absence of flavonoids and steroids. All bark extracts have been verified to contain quinones whereas quinones are absent in leaf extracts. All the extracts confirm the presence of proteins except acetone and methanol extracts of bark. All the extracts confirm the presence of saponins except Ethyl acetate extract of bark and methanol extract of leaf.

Table1: Phytochemical analysis of Elaeocarpus tectorius.

S. NO	Phytochemical test	*Bark extract* ACETONE	*Bark extract METHNOL*	*Bark extract* ETHYL ACETATE	*Bark extract CHLORO FORM*	*Leaf extract* ACETONE	*Leaf extract METHNOL*	*Leaf extract* ETHYL ACETATE	*Leaf extract CHLORO FORM*
1.	Alkaloids	Present	Present	Present	Present	Present	Present	Present	Present
2.	Flavanoids	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3.	Glycosides	Present	Present	Present	Present	Present	Present	Present	Present
4.	Cardiac glycosides	Present	Present	Present	Present	Present	Present	Present	Present
5.	Tannins	Present	Present	Present	Present	Present	Present	Present	Present
6.	Phenols	Present	Present	Present	Present	Present	Present	Present	Present
7.	Steroids	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
8.	Quniones	Present	Present	Present	Present	Absent	Absent	Absent	Absent
9.	Proteins	Absent	Absent	Present	Present	Present	Present	Present	Present
10.	Carbohydrates	Present	Present	Present	Present	Present	Present	Present	Present
11.	Saponins	Present	Present	Absent	Present	Present	Absent	Present	Present

Figure 5: Phytochemical analysis of Elaeocarpus tectorius

All of these phytochemicals were identified in the plant that was examined. The analysis of phytochemicals is essential to confirm the biomarker compounds so as to assess and ensure the quality of plant materials. In a different research, the screening of phytochemicals in the fruits of E requires careful examination. In the study by Tectorius, it was noted that all these phytochemicals were found in the fruit powder. These phytochemicals are known to offer various health benefits, underscoring the significance of this plant species due to the presence of biologically crucial secondary metabolites. The quantification of total phenolics, flavonoids, tannins, and terpenoids was then performed. Plant phenolic compounds, like flavonoids and tannins, are recognized for their potent antioxidants. Their ability to scavenge free radicals plays a crucial role in combating oxidative stress-related conditions such as cancer and neurodegenerative diseases. They also demonstrate beneficial properties such as antimicrobial, anti-inflammatory, and cardioprotective effects. Terpenoids are highly valuable secondary metabolites due to their anticancer, antimicrobial, anti-inflammatory, hypoglycemic, and antioxidant properties. The plant extracts contained significant amounts of these crucial phytochemicals. Other plants from the genus Elaeocarpus were also discovered to have a significant amount of phenolic compounds, emphasizing their biological significance. Antibiotic resistance poses a significant health threat to the community. There is a risk of losing the effectiveness of common antibiotics in a short period due to the rapid development of microbial resistance against antibiotics. This scenario calls for the screening of efficient, secure, and affordable antimicrobial compounds from medicinal plants [7].

Leaf and stem showed adequate activity among both leaf showed the highest activity. Highest activity was observed methanol, chloroform and aqueous extracts followed by ethyl acetate less or no activity was observed in acetone extracts. Highest activity was observed in Bacillus subtilis both leaf and stem extracts followed by Staphylococcus aureus, Streptococcus mutans, and Pseudomonas fluorescens respectively at 10mg/l concentration given in table 2(Figure 6-10).

Table 2: Antibacterial analysis of Elaeocarpus tectorius

Sample	***Streptococcus mutans***			***Staphylococcus aureus***			**Bacillus subtilis**			**Pseudomonas fluorescens**		
	10Mg	5Mg	2.5Mg	10Mg	5Mg	2.5Mg	10Mg	5Mg	2.5Mg	10Mg	5Mg	2.5Mg
MB	15	13	11	24	22	20	18	13	10	19	16	13
ML	20	18	17	18	8	7	26	24	20	18	14	11
CB	17	12	10	14	13	8	18	10	8	16	14	12
CL	24	22	18	24	23	18	26	20	19	20	19	16
EB	15	14	10	15	13	10	16	15	9	16	14	13
EL	17	14	12	24	20	19	20	19	18	19	16	13
Aq. B	18	14	13	15	10	9	16	15	7	22	18	13
Aq. L	14	14	14	18	14	12	26	20	18	19	18	16
AB	14	10	10	16	12	10	14	12	13	16	13	11
AL	--	--	--	22	19	16	--	--	--	--	--	--

MB- Methanol bark, ML – METHANOL LEAF, CB- CHLOROFORM BARK, CL-CHLOROFORM LEAF,EB- ETHYLACETATE BARK, EL- ETHYLACEATE LEAF, AQ.B-AQUEOUS BARK, AQ.L- AQUEOUS LEAF, HB- ACETONE BARK, HL-ACETONE LEAF.

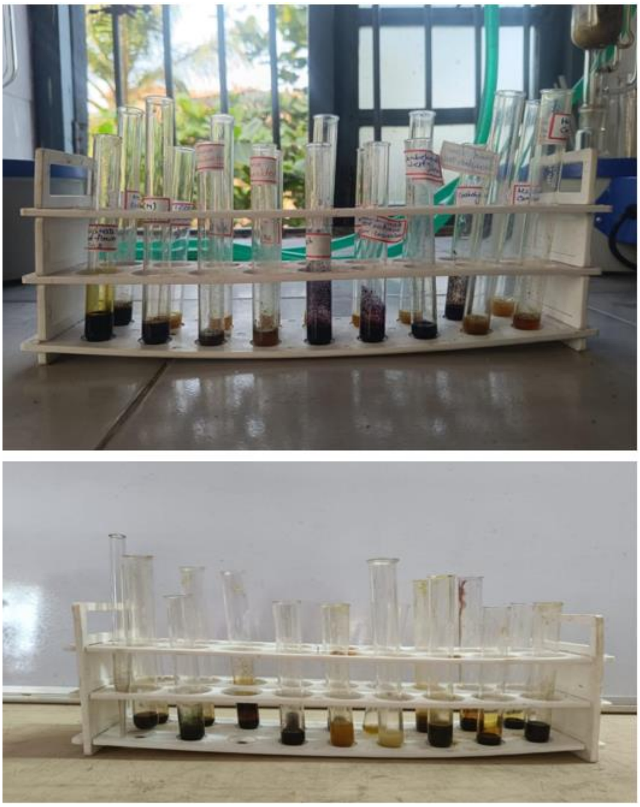

Figure 6: Antibacterial activity of Elaeocarpus tectorius methanol extract

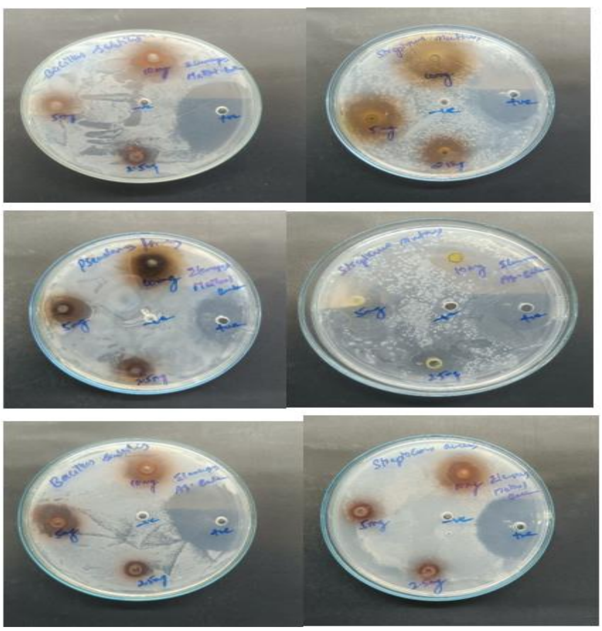

Figure 7: Antibacterial activity of Elaeocarpus tectorius chloroform extract

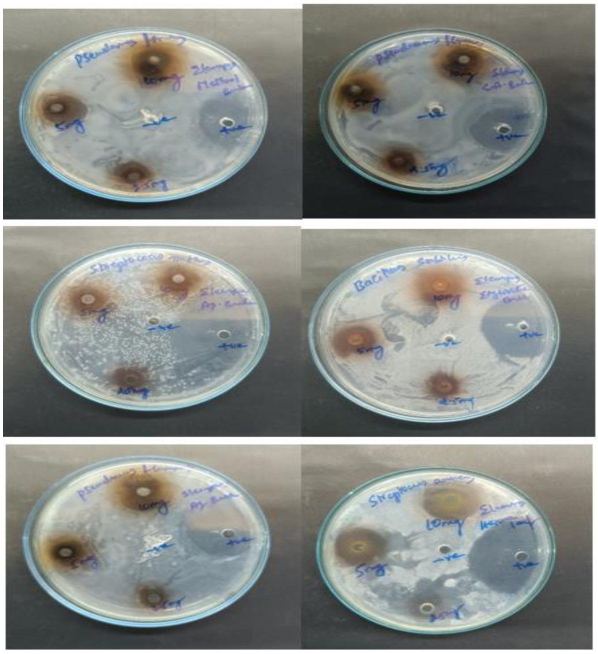

Figure 8: Antibacterial activity of Elaeocarpus tectorius Ethyl acetate extract

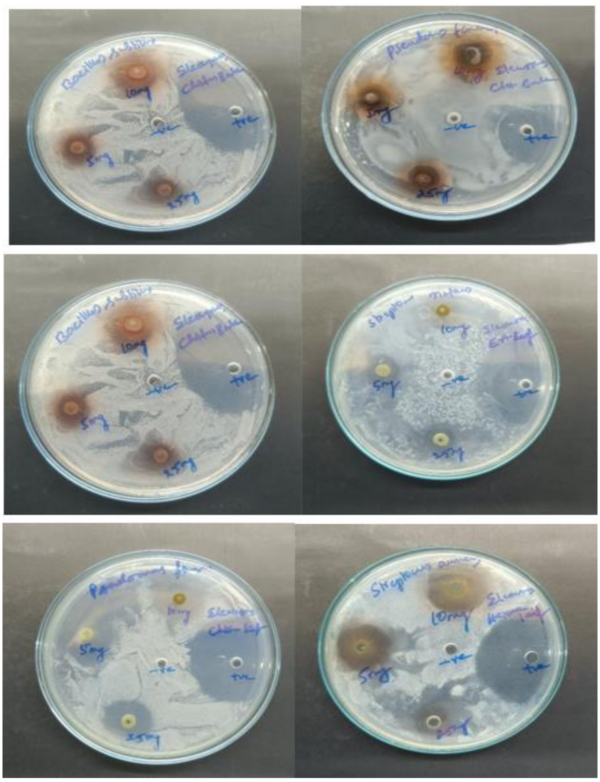

Figure 9: Antibacterial activity of Elaeocarpus tectorius Aqueous extract

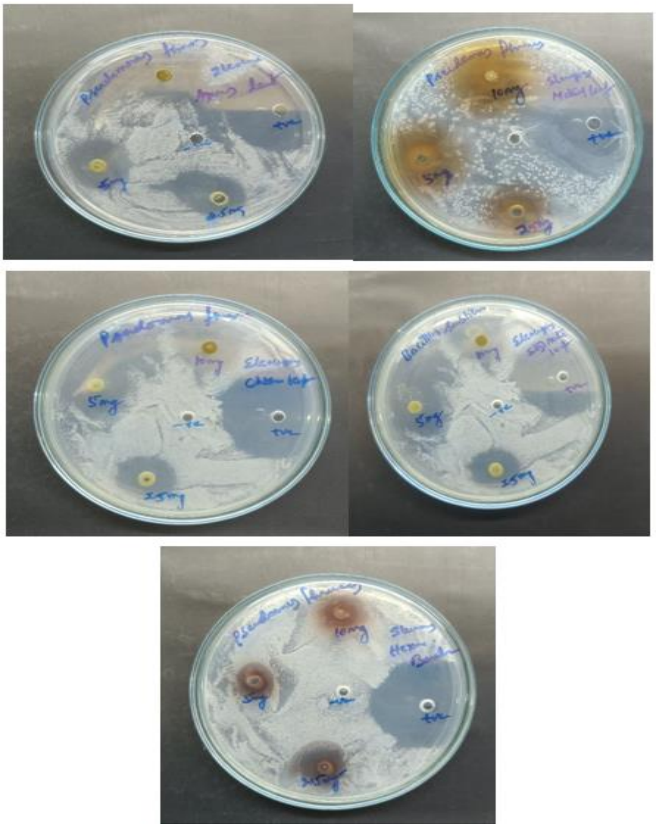

Figure 10: Antibacterial activity of Elaeocarpus tectorius Acetone extract

Bacillus subtilis (26 mm) exhibited the largest zone of inhibition in the methanolic leaf extract of Elaeocarpus tectorius, followed by Streptococcus mutans (20 mm) and Pseudomonas fluroscens and Staphylococus aureus (18 mm). The bark extract showed maximum zone of inhibition in Staphylococus aureus (24 mm) followed by Pseudomonas fluroscens (19 mm), Bacillus subtilis (18 mm) and Streptococcus mutans (15 mm).

Bacillus subtilis (26 mm) exhibited the largest zone of inhibition in the chloroform leaf extract of Elaeocarpus tectorius, followed by Staphylococus aureus and Streptococcus mutans (24 mm), and Pseudomonas fluroscens (50 mm). Staphylococus aureus (14 mm), Pseudomonas fluroscens (16 mm), Streptococcus mutans (17 mm), and Bacillus subtilis (18 mm), exhibited the maximum zone of inhibition in the bark extract.

Staphylococus aureus (24 mm) demonstrated the largest zone of inhibition in the ethyl acetate leaf extract of Elaeocarpus tectorius followed by Bacillus subtilis (20 mm), Pseudomonas fluroscens (19 mm) and Streptococcus mutans (17 mm). Gram negative bacterial i.e., Bacillus subtilis and Pseudomonas fluroscens (16 mm) showed largest zone of inhibition in the ethyl acetate bark extract followed by gram positive bacterial i.e., Staphylococus aureus and Streptococcus mutans (15 mm).

Bacillus subtilis (26 mm) displayed the largest zone of inhibition in the acetone leaf extract of

Elaeocarpus tectorius, followed by Pseudomonas fluroscens (19 mm), Staphylococus aureus (18 mm), and Streptococcus mutans (14 mm). The bark extract exhibited the largest zone of inhibition in Pseudomonas fluroscens (22 mm), Streptococcus mutans (18 mm), Bacillus subtilis (16 mm), and Staphylococus aureus (15 mm).

The zone of inhibition was observed only in Staphylococus aureus (22 mm) in the aqueous leaf extract of Elaeocarpus tectorius. Pseudomonas fluroscens, and Staphylococus aureus (16 mm) displayed the largest zone of inhibition in the acetone bark extract of Elaeocarpus tectorius, followed by Streptococcus mutans, Bacillus subtilis all had lower zones of inhibition in the bark extract (14 mm).

Under oxidative stress conditions, the free radicals generated as by-products in living organisms through the metabolic process play a significant role in the development of pathophysiological disorders such as cancer, genotoxic diseases, diabetes mellitus, premature aging, and stress-related illnesses. The challenges posed by multidrug-resistant (MDR) microbial pathogens in treating diseases continue to persist, despite advancements in the field of human healthcare. Numerous synthetic chemical substances are currently utilized as medication to combat disorders caused by free radicals and diseases from pathogenic microbes. However, many of these synthetic drugs lack specificity and are unable to fully alleviate the associated diseases or disorders. The growing number of failures and side effects linked to antibiotics has led to the necessity of seeking out novel and potent medications. Over the last twenty years, there has been a growing interest in exploring new broad spectrum medications derived from plants as substitutes for synthetic drugs. The global recognition of herbal medicine, achieved by examining active compounds in plant extracts, offers a promising avenue for discovering new antibiotic prototypes. Contrary to synthetic drugs with severe side effects like neurotoxicity, nephrotoxicity, and hepatotoxicity, researchers are focusing on natural antioxidants and antimicrobial agents derived from plants to create safer medications. The alkaloids present in plants are utilized in medicine as anesthetic agents (8-12). Saponins, discovered in plants, have been recognized as the source of the stimulating and revitalizing effects observed in Chinese and Japanese medicinal herbs, as detailed by Alinnor in 2008 (13). The results of this research indicate that the phytochemical compounds identified could be the active ingredients contributing to the effectiveness of the leaves of the plants under investigation. Certain compounds have been confirmed to be associated with antimicrobial activity. This link was highlighted in a study (14). Hence, it is reasonable to deduce that plant extracts could be a valuable resource for producing pharmaceuticals used to combat different microbial infections (15). The antibacterial activity of the leaf extract and chitosan-alginate microcapsules was examined through the agar well diffusion method. The extracts and microcapsules demonstrated excellent inhibitory potential against all tested microorganisms, such as Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, and Bacillus cereus. In terms of plant growth, the highest was seen with cereus, while the lowest was noticed with P. aeruginosa. The correlation between phenolic and tannin content and the antibacterial properties of plants has previously been documented. This study further validates this correlation as the leaf extract was discovered to have elevated levels of phenols and tannins. This could be a result of their interaction with the microbial enzyme system, nucleic acid, and cellular membrane. This study has convincingly demonstrated the impact of microencapsulation on the biological functions of the leaf extract from Elaeocarpus tectorius.

4. Conclusion

The aforementioned studies suggest that traditional plants could serve as novel reservoirs of antimicrobials, housing resilient, biologically potent compounds. These findings lay a solid groundwork for integrating plant-based remedies into contemporary medical practices. Local ethno-medical preparations and plant-based prescriptions should be scientifically assessed and appropriately disseminated. Furthermore, expanding knowledge on traditional botanical preparations of medicinal plants can pave the way for further exploration in pharmacology, phytochemistry, ethnobotany, and other biological actions crucial for drug discovery.

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