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A STUDY ON ANTIMICROBIAL ACTIVITY OF *PARMOTREMA PERLATUM* EXTRACT AGAINST SOME HUMAN PATHOGENIC BACTERIA AND FUNGUS

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ABSTRACT:
Parmotrema perlatum, commonly known as black stone flower, kallu hoovu, or kalpasi, is a lichen species used as a spice in India and traditionally recognized for its medicinal properties. It is considered more effective than other spices due to its strong antimicrobial and antifungal activities. The antifungal properties of *Parmotrema perlatum* extracts can help prevent fungal growth in stored foods, reducing spoilage and extending shelf life. A study was conducted to evaluate the antimicrobial potential of *Parmotrema perlatum* against various bacterial and fungal pathogens. Methanol and ethanol were used to extract the bioactive components. The antimicrobial efficacy was assessed by measuring the zone of inhibition (ZOI) using the disc diffusion method and determining the minimum inhibitory concentration (MIC). The study revealed a significant correlation between the concentration of the extract and the ZOI. Tested bacteria included Escherichia coli, Pseudomonas species, and Bacillus subtilis. The findings suggest that *Parmotrema perlatum* contains potent antimicrobial compounds, which can be further explored for developing new lead molecules to combat pathogenic microorganisms. Given the rise in drug resistance and reduced efficacy of existing antibacterial and antifungal drugs, identifying novel molecules with unique mechanisms of action has become crucial.
Keywords:

Keywords: - *Parmotrema perlatum*, Antimicrobial, Zone of Inhibition, Minimum Inhibitory Concentration.

INTRODUCTION :

Parmotrema perlatum, also known as black stone flower or kalpasi, is lichen species widely used as a spice in India (Daswani, S. et al., 2021). Lichens are composite organisms formed through a symbiotic association between fungi and algae or cyanobacteria (or both) (Hoda, S. et al., 2015). The genus *Parmotrema* is characterized by large, leaf-like thalli with broad lobes, an extensive erhizinate marginal zone on the underside, pored epicortex, thick-walled, hyaline ellipsoid ascospores, sublageniform or filiform conidia, and the presence or absence of marginal cilia (Hoda, S. et al., 2015). The genera *Rimelia, Canomaculina,* and Concamerella, previously classified separately, have been synonymized with *Parmotrema*, a conclusion confirmed by morphological studies conducted by Louwhoff and Crisp (Jayalal, U. et al., 2013). Lichens are commonly used as bioindicators to monitor environmental changes, including air pollution, forest health, and climate change (Stelate, A. et al., 2022). Additionally, lichens and their metabolites exhibit a variety of biological activities, including antimicrobial, antiviral, antiprotozoal, enzyme inhibition, insecticidal, antitermite, cytotoxic, antioxidant, wound healing, analgesic, and anti-inflammatory properties (Plaza, C.M. et al., 2018). Several natural compounds isolated from lichens, such as usnic acid, lobaric acid, atranorin, protolichesterinic acid, and salazinic acid, have demonstrated strong antibiotic activity against Gram-positive bacteria and are effective against pathogenic dermatophyte fungi (Kerboua, M. et al., 2022). This study aimed to evaluate the in-vitro antimicrobial activity of *Parmotrema perlatum* against common bacterial and fungal pathogens. The sensitivity of the pathogens to *Parmotrema perlatum* was assessed using the disc diffusion method to calculate the zone of inhibition (ZOI) and the micro-broth dilution

method to determine the minimum inhibitory concentration (MIC) using micro-broth dilution method (Hoda, S et al., 2015).

REVIEW OF LITERATURE :

Ms. K. Neelima , Asst. Prof. Dr. G. Sony , Dr. Y. Sabitha et al (2022) *Parmotrema perlatum* is recognized for its unique and diverse biologically active compounds, which contribute to its natural antioxidant, antimicrobial, and anticoagulant properties. As natural antibiotics, its metabolites exhibit a broad spectrum of biological activities, including antifungal, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative, and cytotoxic effects, making it a promising candidate for drug development. Similarly, both *Parmotrema perlatum* and *Vitex negundo* are nutrient-rich and abundant in phytochemicals, showcasing strong potential as food supplements. This study concludes that the compounds derived from *Parmotrema perlatum* and *Vitex negundo* could serve as lead compounds for developing potent anti-inflammatory drugs, offering potential treatments for various conditions such as cancer, neurological disorders, aging, and inflammation.

P. R. Dwarakanath . K. Abinaya · K. Nagasathya · S. Meenakumari · Subash C. B. Gopinath . Pachaiappan Raman et al (2022) A study was conducted on the extraction of secondary metabolites from the foliose lichen *Parmotrema perlatum*, focusing on their antioxidant and antibacterial properties. Secondary metabolites were extracted using hexane, chloroform, and methanol, and analyzed through gas chromatography-mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC–MS/MS). The bioactive compounds identified from *Parmotrema perlatum* demonstrated various biological activities. The antibacterial efficacy of the extracts was tested against one Gram-positive and two Gram-negative bacterial strains, with the methanol extract exhibiting the highest antibacterial activity.

Simran Daswani and Wasim Raja et al (2021) A study was conducted to assess the antibacterial properties of the methanolic extract of Parmotrema perlatum. The research demonstrated its effectiveness against various bacterial strains, with different concentrations of the extract producing varying zones of inhibition. This highlights the potential of Parmotrema perlatum as a natural remedy, as herbal medicines have consistently proven beneficial worldwide. Being readily available, this extract serves as an important and essential component in the treatment of various infections and diseases

K. Varalakshmi Devi , E. Bhargav , G. Swaruparani and M. Vijaya Jyothi et al (2021) *Ceiba pentandra and Parmotrema perlatum lichen are two remarkable spices renowned for their therapeutic properties. Both are widely recognized for their use as appetizers. Decoctions prepared from their powders are traditionally used to alleviate conditions such as cough, anorexia, and helminthiasis. This study aims to identify the phytochemical compounds present in the methanolic and n-hexane extracts of Ceiba pentandra and Parmotrema perlatum through phytochemical analysis. These spices not only enhance the* flavor *of food but also provide the body with bioactive therapeutic compounds*

Shanu Hoda and Pooja Vijayaraghavan et al (2015) The antimicrobial potential of *Parmotrema perlatum* was evaluated against various bacterial and fungal pathogens. Hexane was utilized to extract the bioactive compounds from *Parmotrema perlatum.* The study concluded that the lichen extract demonstrated notable antibacterial activity compared to its antifungal effects.

Ritika Chauhan and Jayanti Abraham et al (2013) A study was conducted to examine the antimicrobial properties of lichen species belonging to the *Parmotrema* genus. The findings

revealed that methanol extracts from the tested *Parmotrema* species exhibited significant antimicrobial activity. This suggests that the active compounds present in these extracts could serve as natural antimicrobial agents against pathogens. The study concludes that these active components have the potential to be utilized in developing new drugs to combat pathogenic microorganisms.

RESULT :

The analysis of the tested samples revealed their ability to inhibit membrane lysis. *Parmotrema perlatum* and *Vitex negundo* provided the highest level of protection at concentrated inhibition. The results indicate that all the samples effectively protected erythrocyte membranes from damage. Phytochemicals are plant-based compounds that do not provide nutrition but have varying degrees of disease-preventive properties. They serve as valuable sources for raw materials used in both traditional and modern medicine. Phytochemicals can exert their health-protective effects in various ways (Ms. K. Neelima et al., 2022).

The hexane extract of *Parmotrema perlatum* showed a predominant composition of benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester (62.2%), and phosphinous chloride (1.737%). The chloroform extract primarily consisted of benzoic acid (92.47%) and 1,4-benzenediol, 2,5-dimethyl (4.518%). The methanolic extract contained orcinol (63.26%), with atraric acid (21.38%) derived from a compound called atranorin. The extracts also revealed the presence of pigments like phosphinous chloride and p-xylenolphthalein. Additional compounds identified in smaller quantities included succinic acid, oleic acid, methyl stearate, and tetracosane. Other compounds, such as octadecane, 3-ethyl-5-(2-ethylbutyl)-, eicosane, and 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, were found in both the hexane and methanolic extracts. GC–MS analysis helps in identifying potential new drug molecules, including those with antimicrobial and antioxidant properties (P. R. Dwarakanath et al., 2022).

The antibacterial activity of a 50% methanolic extract of *Parmotrema perlatum* against *Pseudomonas aeruginosa* is most effective at 100% concentration after 24 hours, with a 20.00 mm zone of inhibition. A similar zone is observed at 50% concentration after 24 hours. In contrast, the 75% concentration shows a mild effect with a 12.00 mm zone of inhibition. For *Staphylococcus aureus,* both 75% and 100% concentrations exhibit the strongest effect, each resulting in a 20.00 mm zone of inhibition, while the 50% concentration shows a mild effect with a 7.00 mm zone. In the case of *Klebsiella pneumoniae,* the 100% concentration is most effective, producing a 15.00 mm zone of inhibition, while the 25% concentration shows a mild effect with a 9.00 mm zone. Lastly, for Proteus mirabilis, the 100% concentration demonstrates the best effect, with a 15.00 mm zone of inhibition, while the 25% concentration shows a mild effect with an 8.00 mm zone (Daswani, S et al., 2021).

We got the result the Alkaloids, terpenoids, flavonoids, saponins, tannins, steroids, phosphates and sulphates were identified as major constituents in methanolic extracts of *Parmotrema perlatum* whereas carbohydrates, and proteins were found to be absent in both extracts of Parmotrema perlatum. Methanolic extract of Ceiba pentandra contains alkaloids, tannins, saponins and flavonoids, carbohydrates, phosphates and sulphates as the constituents whereas n-hexane extract has given the positive result for alkaloids, saponins, tannins and carbohydrates. Steroids were absent in both Ceiba pentandra extracts (Devi, K. V et al., 2021).

The antimicrobial activity of the hexane extract of the lichen *Parmotrema perlatum* was assessed by observing the presence or absence of inhibition zones and measuring their diameters. The results demonstrated strong efficacy of the extract

against bacterial pathogens. *Bacillus subtilis* exhibited the largest inhibition zone of 21.2 mm, while *Pseudomonas species* showed the smallest zone at 18.4 mm. Fungal pathogens were also tested for zone of inhibition (ZOI) using the disc diffusion method. *Aspergillus fumigatus* displayed the highest inhibition with a 14 mm zone. The minimum inhibitory concentration (MIC) was determined using a micro broth dilution method. The MIC for the extract was found to be 0.312 µg/ml for *Bacillus subtilis* and 0.625 µg/ml for *Escherichia coli.* For fungal pathogens, the MIC was higher, with A. fumigatus showing an MIC of 1.25 mg/ml and other fungal species demonstrating inhibition at 2.5 mg/ml (Hoda, S et al., 2015).

The antibacterial activity of *Parmotrema* species of lichen was demonstrated by both aqueous and methanol extracts. These extracts inhibited all the tested bacterial species, except for *Salmonella species,* which showed a minimal zone of inhibition. The antifungal activity was observed against five fungal pathogens, with *Scedosporium* species being resistant. Aqueous extracts did not show any antibacterial or antifungal effects. Among the methanol extracts, the weakest antibacterial activity was observed against Salmonella sp. (12 mm) and *Aspergillus terreus* JAS1 (16 mm), while the strongest activity was against Staphylococcus aureus (26 mm), Pseudomonas aeruginosa (24 mm), *Ganoderma species* JAS4 (21 mm), and *Fusarium species* (21 mm) at a concentration of 50 mg/ml. The antimicrobial activity of methanolic extracts of *Parmotrema* species at varying concentrations (25 µl, 50 µl, 75 µl, and 100 µl) is depicted graphically (Chauhan, R. et al., 2013).

DISCUSSION :

The results indicate that *Parmotrema perlatum* demonstrates significant antimicrobial activity against various bacteria and fungi. Methanolic, ethyl acetate, and acetone extracts of *Parmotrema perlatum* showed notable antibacterial effects against *Staphylococcus aureus* when tested using the Kirby-Bauer disc diffusion and Mueller-Hinton agar plate methods. Our review confirms the antibacterial activity of a 50% methanolic extract of *Parmotrema perlatum* against several bacteria, including *Pseudomonas* aeruginosa, *Klebsiella pneumoniae, Proteus mirabilis,* and Staphylococcus *aureus*. Different concentrations of the extract resulted in varying sizes of inhibition zones. This research aims to explore the antibacterial potential of *Parmotrema perlatum,* as herbal medicine has long been recognized for its benefits, offering opportunities to discover new medicinal plants for treating various diseases (Daswani, S. et al., 2021). Our findings suggest that the tested lichen extract has more significant antibacterial properties compared to antifungal activity, which is consistent with other studies. For instance, Gulluce et al. reported that the methanol extract of the lichen *Parmeli saxatilis* exhibited stronger antibacterial than antifungal properties. Similar results were noted in another review, where extracts of *Parmeli perlata* were obtained using hot and cold extraction methods with various solvents. Bacteria tend to be more susceptible than fungi due to differences in their cell wall structure and permeability. Gram-positive bacteria have cell walls composed of peptidoglycans and teichoic acids, while Gram-negative bacteria feature peptidoglycans, lipopolysaccharides, and lipoproteins. Fungal cell walls, consisting of chitin and glucan, are less permeable. This review also suggests that bioactive compounds derived from hexane extracts of P. *perlatum* can serve as natural antimicrobial agents and may be used to develop new drugs targeting pathogens (Hoda, S. et al., 2015). According to Devi et al., when comparing the compound libraries of Ceiba pentandra and Parmotrema *perlatum* based on retention times and peak areas, Parmotrema perlatum contains notable amounts of terpenes and sesquiterpenes

such as Carvone, Copaene, Cubebene, Viridifloral, and Rishitin. Other compounds include Sambucol (an immune-stimulating anthocyanin), Longidione (a tricyclic hydrocarbon), *Platambin* (a naphthalene derivative), and Stigmastan 3,5-diene (a phytosterol). In contrast, *Ceiba pentandra* extracts contain saturated dicarboxylic acids like azelaic and suberic acids, along with limonene and limonene-6-ol, which are known for their therapeutic properties. Methanolic extracts of both plants showed stronger antimicrobial activity against resistant microorganisms than n-hexane extracts (Devi, K. V. et al., 2021). According to Ritika Chauhan et al., the methanol extracts of *Parmotrema* species demonstrated both strong antibacterial and antifungal properties. These extracts inhibited all tested microorganisms, confirming lichens as abundant sources of natural antimicrobial agents and biologically active compounds (Chauhan, R. et al., 2013).

CONCLUSION :

The conclusion of the study emphasizes the significant antimicrobial activity of *Parmotrema perlatum.* The results highlight its potential in fighting bacterial infections, as demonstrated by its effectiveness at a 100% concentration against various bacteria. This suggests that *Parmotrema perlatum* could be utilized as a natural antimicrobial agent for the development of new drugs to combat pathogens. Additionally, the plant's rich nutrient and phytochemical content, along with *Vitex negundo,* suggest their potential as food supplements. The review also explores the possibility of using the compounds from these plants as lead agents for developing anti-inflammatory drugs, which could aid in treating conditions like cancer, neurological disorders, aging, and inflammation. Overall, these plants, through their therapeutic properties, offer both medicinal and nutritional benefits.

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