Phytochemical Analysis and Antimicrobial Activity of Edible Lichen

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ABSTRACT
Lichens are composite organisms consisting of a symbiotic association of a fungus (the mycobiont) with a photosynthetic partner (the phytobiont), usually either a green alga or cyanobacterium. The morphology, physiology and biochemistry of lichens are very different from those of the isolated fungus and alga in culture. Lichens occur in some of the most extreme environments on the Earth and may be useful to scientists in many commercial applications. Antibacterial, antifungal and phytochemical analysis of edible lichen, (Platismatia glauca) was studied in this work.

Keywords: Edible Lichen, Platismatia glauca, secondary metabolites, antimicrobial

1. INTRODUCTION
Lichens are two different organisms living together in a symbiotic relationship of algal and fungal cells. The major partners in this symbiotic relationship are fungal-mycobiont and algae- phytobiont [1,2]. It was reported that about 300 genera and 25000 species lichens are recognized worldwide. They can produce variety of unique secondary metabolites under living conditions [3]. Lichens are slow growing [4], they adapt to extreme conditions such as temperatures, high concentrations of salinity, air pollution and highly nitrified environments [5]. India represents about 10 % of lichens in world lichen population [6]. Lichens produce a wide range of organic compounds that can be divided into primary and secondary metabolites [7]. The present study focuses the antimicrobial activity of Platismatia glauca methanol extract against human pathogens.

2. MATERIALS AND METHODS
Thalli of Lichens purchased from local herbal market. It is mainly used as edible spice. Lichen was authenticated by Dr. S. Chandra Mohan, Shanmuga Centre for Medicinal Plants Research, Thanjavur, Tamil Nadu.

Figure 1: Kalpasi (Platismatia glauca)

2.1. Preparation of the extracts : Collected lichen was cleaned of extraneous material, dried at room temperature and ground into a coarse powder. Ten gm of this coarse powder macerated with three solvents separately namely diethyl ether (non polar), acetone (mid polar) and methanol (highly polar). The mixture were poured in bottles and kept inside the incubator for a period of 72 hours at room
temperature. The respective infusions were filtered with Whatman No.1 filter paper. The filtrates were evaporated to get crude diethyl ether, acetone and methanol extract respectively. These crude extracts were redissolved in respective solvents and the antibacterial activity was carried.

2.2. Bacterial strains: Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis and Klebsiella pneumonia and fungal strains: Aspergillus niger and Aspergillus flavus were used.

2.3. Assay of Antibacterial Activity: Antibacterial activity was determined by disc diffusion method as described by Bauer et al., 1966 [8]. By inoculating a loopful of strain in Mueller Hinton broth separately and incubated at 37°C on a rotary shaker for 12 hrs. Then 0.1 ml of fresh inoculum (containing around 1 - 2 × 10^6 CFU/ml as per McFarland standards) was spread onto the surface of sterile Mueller Hinton agar plates using a sterilized spreader. Then discs impregnated with compounds (1mg/disc) and solvent controls were placed on plates with the help of a sterilized forceps and the plates were incubated aerobically at 37°C. Similarly a negative solvent control (of 1µl) was spread onto the surface of sterile Mueller Hinton agar plates separately and incubated at 37°C on a rotary shaker for 12 hrs. Then 0.1 ml of fresh inoculum was determined by disc diffusion method as described by Bauer et al., 1966 [8] . Potato Dextrose Agar (PDA) was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was added 10ml/L tartaric acid (10%) act as antibacterial agents and poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swabs. The various solvents extract prepared discs individually were placed on the each petriplates and also placed control and standard (Amphotericin B (100 Units/Discs)) discs. The plates were incubated at 28°C for 72 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

2.5. Preliminary phytochemical analysis of lichen extracts: All the three extracts of the selected were subjected to preliminary phytochemical analysis for detection of secondary metabolites by the procedure of Rashmi et al., 2014 [5].

3. RESULTS AND DISCUSSION

Preliminary phytochemical analysis of the lichen showing the presence of steroids, phenolic compounds, reducing sugars, flavonoids, glycosides, saponins, alkaloids and tannins. Preliminary phytochemical analysis of the lichen showed in Table 1.

Table 1: Preliminary phytochemical analysis of Lichen

| Extracts       | Steroids | Phenolic | Reducing Sugar | Flavonoids | Glycosides | Saponin | Alkaloids | Quinones | Tannins |
|----------------|----------|----------|----------------|------------|------------|---------|-----------|----------|---------|
| Diethyl ether  | +        | +        | +              | -          | +          | +       | -         | +        |         |
| Methanol       | +        | +        | +              | +          | +          | +       | +         | +        |         |
| Acetone        | -        | +        | +              | +          | +          | +       | +         | +        |         |

The antibacterial effect of methanol lichen extract was tested against human pathogens including Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis and Klebsiella pneumonia. The antifungal effect of methanol extract was tested against Aspergillus niger and Aspergillus flavus. Platismatia glauca showed maximum activity against Bacillus subtilis with the inhibition of 31mm followed by Escherichia coli with 26mm. The zone of inhibition was found to be 19mm for Aspergillus flavus and 16mm Aspergillus niger (Table 2 and Fig. 2).

2.4. Assay of Antifungal Activity: Antifungal activity test was carried out following the modification of the method originally described by Bauer et al., 1966 [8]. Potato Dextrose Agar (PDA) was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was added 10ml/L tartaric acid (10%) act as antibacterial agents and poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swabs. The various solvents extract prepared discs individually were placed on the each petriplates and also placed control and standard (Amphotericin B (100 Units/Discs)) discs. The plates were incubated at 28°C for 72 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

Table 2: Assay of Antimicrobial Activity

| Sample | Microorganism Name | Zone of Inhibition (mm in diameter) |
|--------|--------------------|-----------------------------------|
|        | Control | Standard*  | Sample |
| 1      | Escherichia coli   | -             | 15          | 26 |
| 2      | Klebsiella pneumonia | -          | 18          | 21 |
| 3      | Staphylococcus aureus | -         | 22          | 23 |
| 4      | Bacillus subtilis   | -             | 22          | 31 |
| 5      | Aspergillus niger   | -             | 23          | 16 |
| 6      | Aspergillus flavus   | -             | 25          | 19 |

*NTF-Nitrofurantoin (300µg), AP-Amphotericin-B
Lichens, the symbiotic organisms of fungi and algae, are slow-growing organisms [9]. Lichens are of great interest now-a-days because of their unique secondary metabolites. The biological activities of the lichen are due to the presence of secondary compounds produced by the lichen mycobiont. These lichen secondary metabolites are awesome compounds exhibiting excellent bioactivities in terms of antimicrobial, antioxidant, anti-inflammatory and anti-diabetic activities etc [10]. Their secondary metabolites as the “lichen substances,” are amino acid, sugar, quinones, chromones, xanthones, dibenzofuranes, terpenoids, steroids, carotenoids and diphenyl ethers. They have been used by humans for centuries as food, dye and therapeutic traditional medicine. Their efficacy is due to the synthesis of unique secondary compounds, a number of which have important biological roles [11]. Phytochemical investigation of the methanol extract of Platismatia glauca revealed the presence of alkaloids, phenol, saponins, terpenoids, glycosides, and flavonoids which may causing antimicrobial activity against tested human pathogens.

4. CONCLUSION

This study revealed that lichen, Platismatia glauca might be useful as an antibacterial, antifungal agent. It can be useful in treatment of numerous diseases caused by these microorganisms. Recent progress in lichens and lichen-forming fungi was discussed with emphasis on their potential to accelerate commercialization of lichen-based products. Lichens are an untapped source of biological activities of industrial importance and their potential is yet to be fully explored and utilized. Lichen-derived bioactive compounds hold great promise for biopharmaceutical applications as antimicrobial, antioxidant and cytotoxic agents and in the development of new formulations or technologies for the benefit of human life.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5. REFERENCES

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