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INVITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF EDIBLE LICHEN: PARMOTREMA PERLATUM

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ABSTRACT

Lichen is a complex symbiotic relationship arose from algae or cyanobacteria that live together with some fungal species. Some of them are edible and consumed as spice such as *Parmotremaperlatum*. The current study aimed to evaluate *invitro* antioxidant and antimicrobial properties of the ethanol extract of lichen *Parmotremaperlatum.*

Key words: *Lichen, Parmotremaperlatum,* antioxidant, antimicrobial, DPPH, *E.coli*

INTRODUCTION

A lichen is a composite organism that arises from algae or cyanobacteria living among filaments of multiple fungi species in a mutualistic relationship [1,2]. Lichens come in many colors, sizes, and forms. The properties are sometimes plant-like, but lichens are not plants. Lichens may have tiny, leafless branches (fruticose), flat leaf-like structures (foliose), flakes that lie on the surface like peeling paint (crustose), a powder-like appearance (leprose), or other growth forms [3]. Lichens do not have roots that absorb water and nutrients as plants do [4], but like plants, they produce their own nutrition by photosynthesis. When they grow on plants, they do not live as parasites, but instead use the plants as a substrate.Lichens occur from sea level to high alpine elevations, in many environmental conditions, and can grow on almost any surface. Lichens are abundant growing on bark, leaves, mosses, on other lichens, and hanging from branches "living on thin air" (epiphytes) in rain forests and in temperate woodland. They grow on rock, walls, gravestones, roofs, exposed soil surfaces, and in the soil as part of a biological soil crust. Different kinds of lichens have adapted to survive in some of the most extreme environments on Earth: arctic tundra, hot dry deserts, rocky coasts, and toxic slag heaps. They can even live inside solid rock, growing between the grains.It is estimated that 6% of Earth's land surface is covered by lichens [5]. There are about 20,000 known species of lichens. Lichens may be long-lived, with some considered to be among the oldest living things [6].They are among the first living things to grow on fresh rock exposed after an event such as a landslide. The long life-span and slow and regular growth rate of some lichens can be used to date events (lichenometry).

Parmotremaperlatum, commonly known as black stone flower or kalpasi, is a species of lichen used as spice in India. The species occurs throughout the temperate Northern and Southern Hemispheres [7].It is one of the ingredients, used for cooking meats, fish and vegetables. Some of the other names for it include *shaileyam* in Sanskrit, *kalpasi* in Tamil, *dagadphool* in Marathi, *RaathiPootha* in Telugu and *pattharkephool* in Hindi. In this study, *invitro* antioxidant and antibacterial activity of ethanolextract of *Parmotremaperlatum* was evaluated against human pathogenic bacteria. This study was designed to contribute scientific proof for utilization of locally available edible lichen as herbal medicine in comparison to the commercial antibiotics having various side effects.

MATERIALS AND METHODS

Lichen material

Parmotremaperlatum was purchased from a herbal market at,Kulasekharam , Kanyakumari District, Tamil Nadu, India. Lichen was authenticated by Dr. Sanjeeva Nayaka, Scientist Lichenology Laboratory Plant Biodiversity and Conservation Biology Division National Botanical Research Institute (NBRI-CSIR) Rana Pratap Marg, Lucknow - 226001, U.P., India.

Preparation of lichen extract

Dried lichen was mechanically ground and filtered by the refinery to get a fine powder. Fifty grams of this powder was macerated in 1000 ml of ethanol and mixed well. The mixture was poured in a dark well tighten bottle and kept inside the incubator at 40ºC for 3 days, with frequent shaking. Then, the infusion was filtered with Whatman filter paper No.1. The filtrate was left to evaporate inside an incubator for two days to get about 10 grams of dry extract.

Phytochemical evaluation

The ethanol extract was used to qualitatively test the presence of various phytochemical constituents [8]. Phyto chemical constituents such as alkaloids(Dragondroff's test), flavonoids (Sodium hydroxide solution test), phenols (Ferric chloride test), saponins (Frothing test) and triterpenoids (Salkowski Test) were evaluated

Test microorganisms

Bacterial strains: Gram –ve strain *Escherichia coli* (ATCC – 25922) and gram +vestrain, *Staphylococcus aureus* (ATCC – 25923) were used.

Antimicrobial Assay

Disc Preparation: The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C.After the sterilization the moisture discs were dried on hot air oven at 50°C.Then ethanol extract discs and control discs were prepared.

Assay of Antibacterial Activity: Antibacterial activity test was carried out following the modification of the method originally described by Bauer et al., (1966) [9]. Muller Hinton agar was prepared and autoclaved at 15 lbspressure for 20 minutes and cooled to 45ºC. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The extract prepared discs individually were placed on the each petriplates and also placed control and standard (Nitrofurantoin - 300µg) discs. The plates were incubated at 37ºC for24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

***Invitro*antioxidant activity: DPPH radical-scavenging activity:** Different concentrations of lichen extract (20, 40, 60 and 80 µg/ml) was chosen for *in vitro* antioxidant activity. L-Ascorbic acid (20, 40, 60 and 80 µg/ml) was used as the reference standard. DPPH radical-scavenging activity was determined by the method of Shimada, *et al.,* (1992) [10]. Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room

temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{A_C - A_S}{A_C} \right) X\ 100$$

Where A_C=Absorbance of the control and A_S = Absorbance of reaction mixture (in the presence of sample).

Statistical Analysis: The results were presented as mean ± SD. For the calculation of $IC_{50,}$ Linear regression analysis was done using Microsoft Excel.

RESULTS AND DISCUSSION

Phytochemical investigation of the lichen extract of *Parmotremaperlatum* revealed the presence of phytochemical compounds like alkaloids, phenol, saponins, flavonoids and terpenoids.

The antibacterial effect of acetone extract was tested against human pathogens including Staphylococcus *aureus* and *Escherichia coli*. *Parmotremaperlatum*showed maximum activity againstE.coli (21 mm) and *S.aureus* (19 mm), showed in Table 1 and Fig.1.

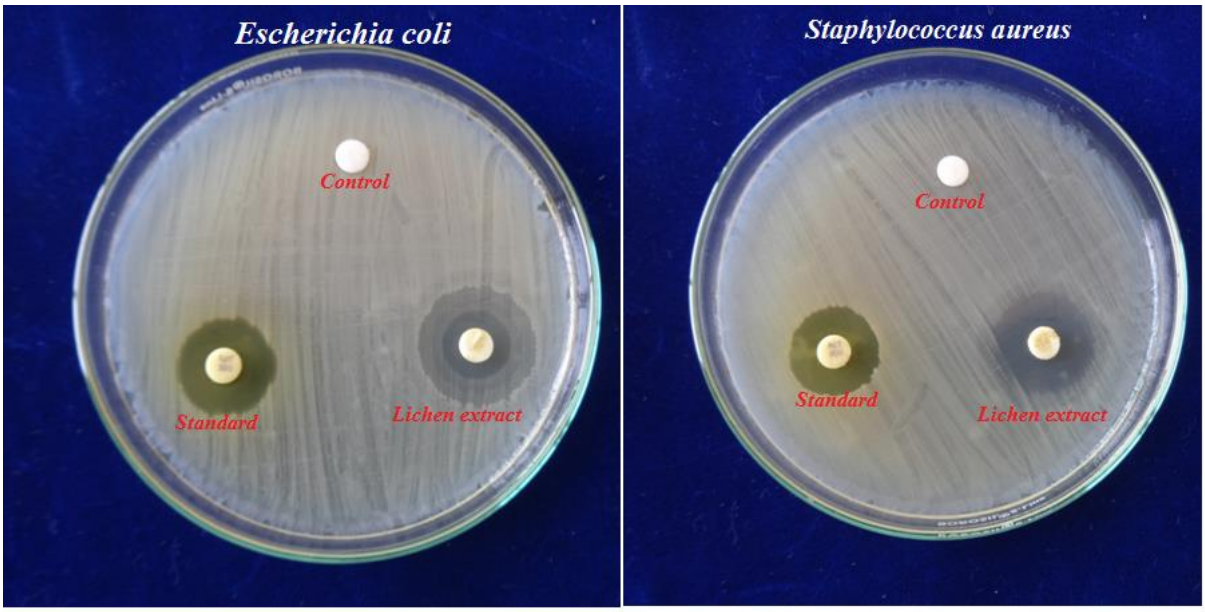

Fig.1: Antibacterial activity of Lichen extract against *E.coli* and *S.aureus*

Table – 1: ASSAY OF ANTBACTERIAL ACTIVITY

S. No.	Bacterial strain	Zone of Inhibition (mm in diameter)		
		Control	Standard*	Lichen extract
1	***Staphylococcus aureus***	-	**16**	**19**
2	***Escherichia coli***	-	**17**	**21**

*Nitrofurantoin (300µg)

Scavenging activity of DPPH radical

Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues and cells [11]. This oxidative stress may be related to many disorders, such as cancer, atherosclerosis, diabetes and liver cirrhosis [12]. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The radical scavenging activity of mushroom extract was tested against the DPPH. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extract. The method of scavenging DPPH free radicals can be used to evaluate the antioxidant activity of specific compounds or extracts in a short time [13]. In **Table 2**, the scavenging activity of the DPPH radical due to its reduction by tested mushroom was illustrated.

Table 2: DPPH Radical scavenging activity of *Parmotremaperlatum* extract at different concentrations

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**IC_{50} (µg/ml)**
Standard (Ascorbic acid)	32.12±0.24	58.64±1.24	78.68±2.24	82.80±4.68	36.36±2.1
***Parmotremaperlatum* extract**	10.82±1.82	18.02±2.12	28.20±2.82	38.94±4.42	49.23±2.79

Values were expressed as Mean ± SD for triplicate

The lichen extract contained antioxidant phytochemicals such as flavonoids, phenolic compounds, terpenoidsetc, which could react rapidly with DPPH radicals, and reduce most DPPH radicals. This result reveals that the extract was a free radical inhibitor or scavenger, acting possibly as primary antioxidants. Antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reaction [14]. The results indicated that ethanol extract of *Parmotremaperlatum* have a potential effect on scavenging free radical. The IC_{50} of *Parmotremaperlatum* and standard were 49.23±2.79 and 34.83±3.45µg/ml, respectively, shown in **Fig.3** and **Fig.4**.

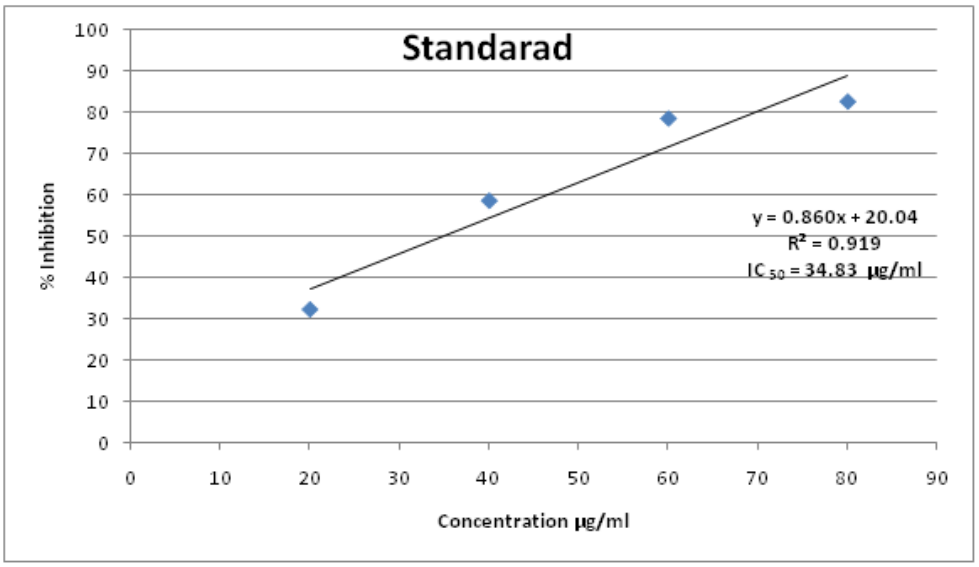

Fig.3: DPPH Radical scavenging activity of Standard (Vitamin C)at different concentrations

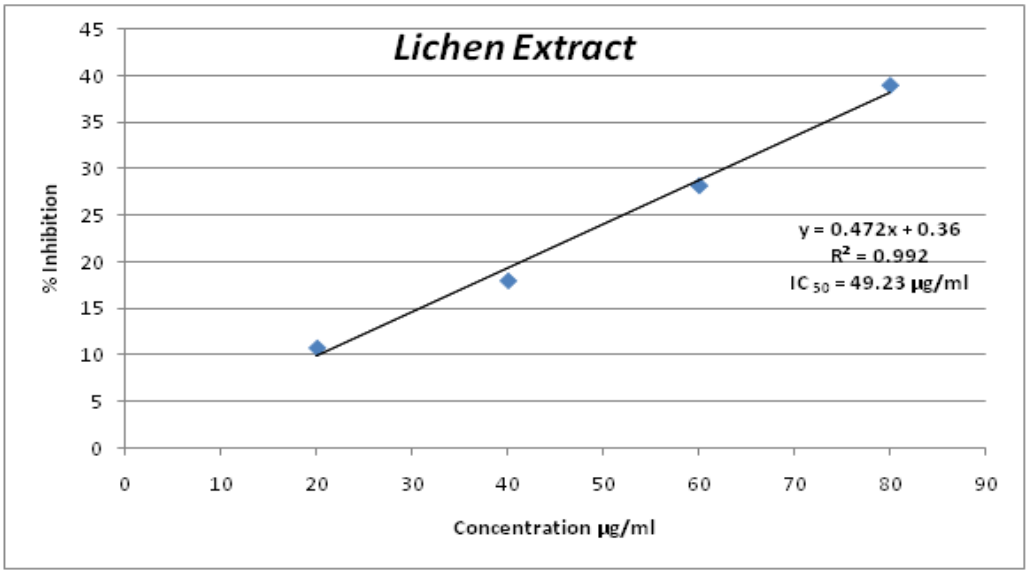

Fig.4: DPPH Radical scavenging activity of *Parmotremaperlatum* extract different concentrations

The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity. A lower IC_{50} meant better radical scavenging activity. Ethanol extract of the tested mushroom showed a good scavenging activity on DPPH radical.

In the current study, the ethanol extract of *Parmotremaperlatum*, lichen widely consumed as a spice showed a remarkable antibacterial activity against tested Gram-positive and Gram-negative bacteria, furthermore, the extract exhibited antioxidant activity. The study also showed high antibacterial activity against the gram-positive *Staphylococcus aureus and gram-negative E.coli*which are pathogens widely associated with urinary tract infections, particularly in women [15]. The flavonoid and phenolic constituents are responsible for antioxidant property of lichens [16-19]. Hence the presence of flavonoid and phenolic constituents of ethanol extract of Parmotremaperlatum from the phytochemical study confirmed that the lichens are very good antioxidant agents.

CONCLUSION

This study revealed that the lichen extract showed highest antibacterial effect on Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus* and *Escherichia coli*. Furthermore, lichen extract *Parmotremaperlatum*, showed higher antioxidant activity, comparable with standard Ascorbic acid. Accordingly, this lichen represents a powerful source of new antimicrobial molecules for drug industries and food preservation. From the obtained results, it is concluded that the lichen *Parmotremaperlatum* possesses good antimicrobial and antioxidant activity and it may be considered as a source of potential antioxidant and antimicrobial agents.

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