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# EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ANALYSIS OF MEDICINALLY IMPORTANT INDIAN LICHEN SPECIES

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## ABSTRACT

Lichen is a unique symbiotic organism that shows the amazing collaboration between fungi and algae in nature. It can survive in some of the harshest settings on Earth and provides important ecological and therapeutic advantages. This study examines the antibacterial and antioxidant properties of the lichen species *Rocella montagnei*, which is widespread to India and has been used medicinally for a long time. We clarify its potential as a source of natural antioxidants and antibacterial agents through careful examination. Using a range of assays, such as disc diffusion and DPPH scavenging assays, we were able to detect strong antioxidant qualities as well as notable inhibitory effects against a variety of pathogens. Our results highlight *Rocella montagnei* medicinal potential and support further research into its use in pharmaceutical and nutraceutical applications.

**KEYWORDS:** Antioxidant, Antimicrobial, Lichen, DPPH, Disc Diffusion assay.

## INTRODUCTION

A unique kind of extracellular specialized metabolites from lichens, which are known as lichen metabolite. In India, lichens are utilized in folklore as medicine and many Indian medicinal practitioners used lichen as an herb during the medieval period (SELVAM et al., 2022). It has been documented that more than 1050 secondary metabolites were found so far and among them, 550 are unique in lichens. Most lichen substances are phenolic compounds,

dibenzofuranes and usnic acids, depsidones, depsones, lactones, quinines and Pulvinic acid derivatives (Pandey et al., 2019). Lichen produces a wide range of secondary metabolites with different biological properties, including antimicrobial, antiviral, antioxidant, antiherpes, insecticidal, allelochemical, and allergenic action, antiproliferative, enzyme inhibition, antitumor, cytotoxicity, etc (Bhattacharyya et al., 2016; Yousuf et al., 2014) The majority of the compounds responsible for these activities originate from the fungal mycobiont (Dixit et al., 2017). In this context, lichens (a mutualistic existence of algae and fungi) considered as a prominent resource for the reason that the secondary metabolites of the lichen functions as defence mechanism against biotic and abiotic stresses (Sastry et al., 2018).

The increased state of oxidative stress has been linked to many chronic diseases, including cancer, diabetes, neurological disorders, and cardiovascular disorders. Based on this, numerous research organizations have led initiatives to evaluate the antioxidant qualities of natural items. These characteristics have either been studied chemically (in vitro) or biologically (in vivo), or both. Lichens have been a significant source of novel antioxidant compounds in this setting (White et al., 2014). Numerous assays have been used to evaluate their antioxidant potential, including DPPH radical scavenging, reducing power, superoxide anion radical scavenging, nitric oxide radical scavenging, and inhibition of lipid peroxidation (Thadhani & Karunaratne 2017). Recently, a systematic study of the pharmacological characteristics of lichen compounds has begun, and recent studies have focused heavily on them (Fernández-Moriano et al., 2016).

The use of lichens in medicine is based on the fact that they contain unique and varied biologically active substances, mainly with antimicrobial actions. The intensive use of antibiotics has selected for antibiotic resistance factors and facilitated the spread of multiply resistant microorganism (Srivastava et al., 2013). E. coli are a widespread bacterial species that comprise a broad variety of strains and can be highly pathogenic and are generally present in healthy intestines.

*E. coli* is also recognized as a major cause of urinary tract infections (UTIs) that can lead to the development of acute cystitis and pyelonephritis (Arbab et al., 2022). Another organism which causes infection *is Klebsiella pneumoniae*. it was first described by Carl Friedlander in 1882 as a bacterium isolated from the lungs of patients who had died from pneumonia. *Klebsiella* species are ubiquitously found in nature including water, soil and animals and cause various infections including pneumonia, UTIs, bloodstream infections and sepsis

(Bengoechea & Sa Pessoa 2019). *Bacillus subtilis* have occasionally been reported in connection with food borne illness. Members of the *B. subtilis* group were reported to produce substances toxic to mammalian cells such as lichenysin A, from B. licheniformis connected to a fatal case also found in strains isolated from vomit and from mastitic milk (Constantin et al., 2009).

Due to having phytochemicals, plants become an important research source. Drugs which are extracted from plants are very effective, easily available and less expensive and they rarely have side effects associated with them. As a result of this union, lichens have a new anatomical, morphological and physiological properties which unlike organisms that they constitute (AYDIN & KINALIOGLU 2013). The lichen species *Roccella montagnei* (family *Roccelleceae*) a fruticose lichen growing luxuriantly along the coastal regions of India (Sastry et al., 2018). This lichen possesses a wide no. of compounds such as Roccellic acid, Orcinol, Lecanoric acid, Montagnetol, Methyl orsellinate, Meso-erythritol, Erythritol, β-carotene and β-sitosterol (Dixit et al., 2017).

As very few studies have been reported so far on phytochemical parameters. Hence, this study was undertaken to evaluate the antimicrobial and antioxidant properties of the ethanolic extracts of *Roccella montagnei.*

**Table 1: Illustration of Lichens secondary metabolites and their biological activity.**

| Lichens | Secondary metabolites | Biological activities | references |
|---|---|---|---|
| *R. montagnei* | Erythrin, Roccellic acid | Anticancer | (Tatipamula et al., 2019) (Kambar et al., 2014). |
| *Ramalina terebrata* | Ramalin | Antioxidant, Antibacterial | (Elkhateeb et al., 2021). |
| *R. hossei* | Usnic acid, Sekikaic | NA | (Kambar et al., 2014). |
| *Roccella phycopsis,* | Phenolic and favonoid contents | Antibacterial activity | (Ben et al., 2021) |
| *Usnia albopunctata* | Protocetraric acid | Antimicrobial drug against pathogenic microbes. | (Elkhateeb et al., 2021). (Nishanth et al., 2015). |
| *Cetraria islandia* | Fumaroprotocetraric and Protocetraric acid. | HIV-1 reverse transcriptase inhibitor | (Elkhateeb, et al., 2021). |
| *Usnea longissima,* *U. articulate* | Usnic acid | detoxication; treat dyspepsia, | (White et al., 2014). (Zhao et al., 2021). |
| *Stereocaulon alpinum* | Lobaric acid | Antibacterial, antifungal, anticancer | (Elkhateeb et al., 2021). |
| *Usnia barbata* | Salazinic acid | UV-B protection effects | (Engel et al., 2007), |

| | | | |
|---|---|---|---|
| | | | (Zugic et al., 2018) |
| *Pseudevemia furfuracea* | Physodic acid, chloroatranorin, atranorin, and olivetoric acid | Antimicrobial | (Elkhateeb, et al., 2021). |

## MATERIAL AND METHODS

### Collection and identification of lichen Samples

Based on the development of medicinally significant lichens The Indian state of Odisha is the source of *Rocella montagnei*. The lichen that was gathered displayed shrubby and fruity branch morphologies, which are characteristic of *R. montagnei* fruticose lichens. Using a chisel and hammer, lichen samples were collected from Odisha's coastal region, and their ecological notes were used to identify the samples from various sites in various habitat types. The specimens that were gathered were examined morphologically. The specimens were taken into the Department of Environmental Microbiology at Babasaheb Bhimrao Ambedkar University in Lucknow, India, and placed on a paper envelope with information on the locality.

### Preparation of lichen crude extract

**Figure 1: Extraction Process by Washing and Filtration.**

Four times, the gathered *Roccella montagnei* (a medicinally significant lichen) samples were thoroughly cleansed under running water. To get rid of undesired microbiological contamination, the samples were cleaned four times with distilled water until no foam was left behind. The first wash was conducted using Tween 80 detergent. The sample was allowed to air dry for three days at a room temperature of 25±5°C. After that, it was crushed using a mortar and pestle to create a fine powder. In a 500 ml flask, 10gm of lichen powder were mixed with 100 mL of a hydro alcoholic solution (50% ethanol, 50% Milli Q Water). The flask was placed in a shaker set at a temperature between 30 and 40˚C for three to five days after being adequately coated in aluminum foil and sealed with a cotton plug. Filter paper marked Watman No. 1 was used to filter the produced solution. Transfer onto a Petri

plate and allow to evaporate the Ethanol and distributed H2O at 25±5°C.Ultimately, the crude extract is collected and kept for future experiments at 4°C.

**Antimicrobial activity of lichen**

The antimicrobial activity of the lichen is tested in vitro against the following gram positive and gram negative bacteria: *Bacillus subtilis, E. coli and Klebsiella pneumoniae* Obtained from Microbial Type Culture Collection, Rhizosphere Laboratory, Babasaheb Bhimrao Ambedkar University, Lucknow, India were used throughout the study.

**Antibacterial activity by Disc-Diffusion assay**

Antibacterial studies were performed using the standard disc diffusion method with some modification (Murray et al., 1995). 10 mg/mL of the lichen extract was dissolved in the (10% DMSO to 80% distilled water) solvent to reach a final concentration. Use 50 µL of bacterial suspension and spread it on a nutrient agar (NA) medium plate. Discs (6 mm diameter) were loaded with 10 µg/mL, 20 µg/mL and 30 µg/mL of extract and placed on the petri plate. Spectinomycin (0.2µg/disc) was used as a positive reference standard to determine sensitivity. The inoculated plates were incubated at 37±5°C for 24 hours. Antimicrobial activity was assessed by measuring the zone of inhibition against each test organism (Gulluce et al., 2006). Each assay in this experiment was repeated four times. 28±0.2 mm.

**Scavenging DPPH assay**

The assay of 1,1-diphenyl-2-picrylhydrazyl (DPPH) is based on the reduction of DPPH. Initially, 0.1 mM DPPH in methanol was prepared and reacted with known concentrations (20, 40, 60, 80 and 100 µl/mL) of lichen samples and standards (ascorbic acid 10, 20, 30, 40 and 50 µl/mL) and incubated for 30 minutes at room temperature in the dark. The absorbance was then measured at 517 nm in a UV-visible spectrometer.

**Figure 2: DPPH Solution.**

**RESULT AND DISCUSSION**

**Screening of Antibacterial activity**

The inhibitory zones were recorded and measured with the help of Hi-Antibiotic Zone Scale. In this study, we investigated the antimicrobial effects of extracts from the lichen *R. montagnei* against *Bacillus Subtilis, Klebsiella pneumonia and E. coli* in vitro. A variety of pathogenic microbial species are susceptible to the antibacterial effects of lichens.

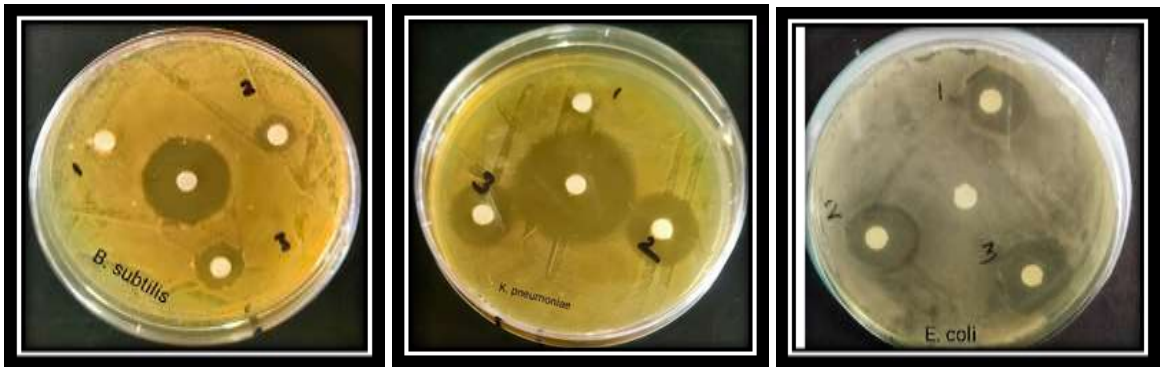



**Figure 3:** ***Bacillus Subtilis, Klebsiella pneumonia, E. coli.***

The extract inhibited all microbial growth at the different concentrations of 10 µg/ml, 20 µg/ml, and 30 µg/ml against the microbes: *Bacillus subtilis* ZOI 16±1mm, *Klebsiella pneumonia* ZOI 20±0.6 mm, *E. coli* ZOI 12.6±8.6. The lichen extract and its components studied in this experiment showed relatively strong antibacterial activity against Klebsiella pneumoniae, and the inhibition zone was 20 ± 0.6 mm. A previous study showed that *Klebsiella pneumonia* has a ZOI 18 ± 0.7 (pandey et al., 2019). *E. coli* and *Bacillus subtilis* exhibits a lower ZOI 12.6±8.6 and 16±1mm as compared to previous research ZOI of 30±1.4 (pandey et al., 2019), 28±0.2 mm (Sastry et al., 2018). The results of antimicrobial activity of extracts are given in Table below.

**Table 2:** ***Zone of inhibition (mm) of extract of R. montagnei against pathogen.***

| S.No. | Bacterial pathogen | Methanol extract | | | Control (Streptomycin) |
|---|---|---|---|---|---|
| | | 10 µL | 20µL | 30µL | |
| 1 | *Bacillus subtilis* | 1 | 8 | 16.67 | 27.7 |
| 2 | *Klebsiella pneumoniae* | 0.6 | 15.6 | 20 | 33 |
| 3 | *E.coli* | 8.6 | 10 | 12.3 | 22 |

**Antioxidant activity of lichen by Scavenging DPPH radical**

The objective of the current investigation, which included extraction and qualitative assessment, was to determine the antioxidant activity of the lichen *R. Montagnei*. Due to the ability of DPPH to trap free radicals, which can be measured by a change in optical absorption, antioxidant activity has been determined. In DPPH radical scavenging assay, the

antioxidant capable substances are able to reduce the stable purple colored DPPH radical to a yellow colored non-radical DPPH form.

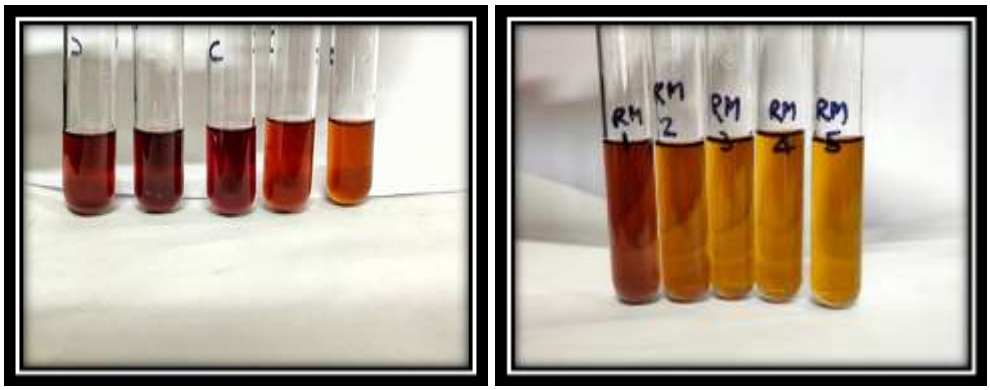
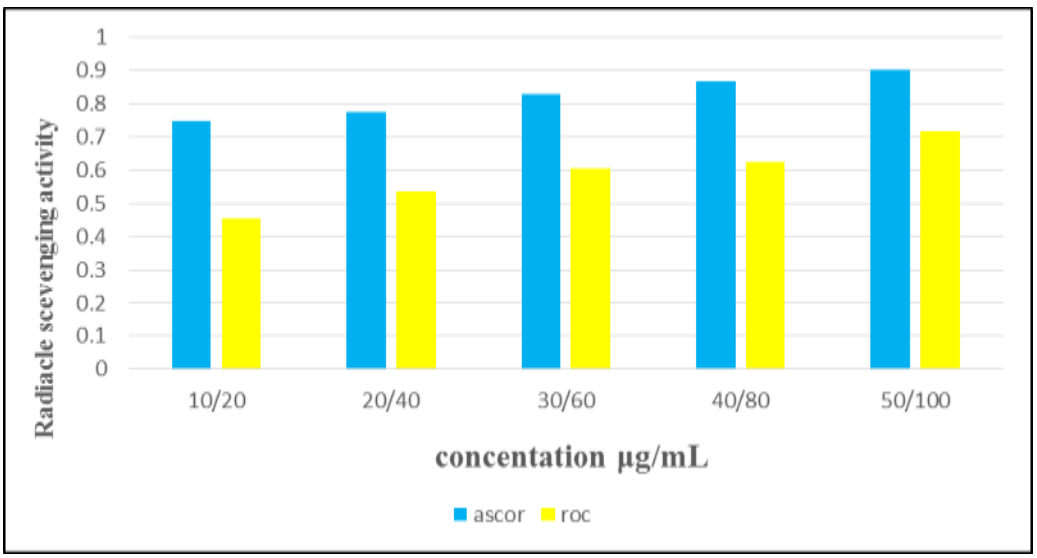



**Figure 4: Result of DPPH assay of *R. montagnei* with respect to Ascorbic acid (Control).**

Graph below displays the tested lichen extracts' DPPH radical scavengers. The lichens' extracts demonstrated strong DPPH radical scavenging abilities. Lichen extracts had different concentration-related scavenging effects, ranging from 0.45 to 0.71.




**Graph 1: *Graphical view of lichens scavenging activity and their conc. (µg/mL).***

## CONCLUSION

Evaluation of the antimicrobial and antioxidant properties of medically important Indian lichen species provides insight into their potential in the medical and pharmaceutical industries. Lichens are special organisms that have been used in medicine for centuries. It can be stated that tested *Roccella montagnei* lichen extracts and their compounds have a strong antioxidant, antimicrobial activity in- vitro. On the basis of these results, lichen appear to be

good antioxidant, antimicrobial agents and also could be of significance in the food and pharmaceutical industry and to control various human, animal and plant diseases.

Antibacterial assays of Indian lichen species revealed their ability to inhibit the growth of various pathogenic bacteria. These include bacteria such as *Klebsiella pneumoniae, Bacillus subtilis* and *Escherichia coli*. In addition, the antioxidant properties of Indian lichen species *Roccella montagnei* showed significant antioxidant activity. Antioxidants play an important role in neutralizing free radicals and preventing oxidative damage associated with many diseases, including cancer, heart disease, and neurodegenerative diseases.

Further work is needed to isolate and reveal new metabolites of lichens responsible for antioxidant and antibacterial activity.

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