# *In vitro* Antioxidant Activities of Lichen Species *Dirinaria applanata* and *Parmotrema andium* Collected from Similipal Biosphere Reserve, India

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## ABSTRACT

Lichens appear to be a promising source of antioxidants due to presence of numerous metabolites that can reduce free radicals. The Lichen species were obtained from Similipal biosphere Reserve (SBR). The antioxidant activity was carried out by DPPH, $H_2O_2$, FRAP scavenging assay and further TPC and TFC was also estimated. Among the lichens tested, *Dirinaria applanata* exhibited strong antioxidant activities than *Parmotrema andium*. The methanol and acetone extracts of *D. applanata* showed DPPH radical scavenging activities ($IC_{50}$ value) as 471.16±0.85µg/ml and 519.79±1.29µg/ml whereas in *P. andium* $IC_{50}$ value was 534.77±0.75µg/ml and 600.77±0.95µg/ml respectively. Similar result was also observed in $H_2O_2$ scavenging assay and FRAP. An interesting strong relationship between total phenolic and flavonoid contents and their antioxidant activities in both the Lichen species was marked as determined with respect to gallic acid and quercetin equivalents. The results indicates that the selected lichen species possess significant antioxidant activity which may be utilized as novel sources of natural antioxidant compounds.

**KEY WORDS:** ANTIOXIDANT, DPPH, FRAP, $H_2O_2$ , LICHEN.

## INTRODUCTION

Lichen is a symbiotic organism that consists of a fungus (mycobiont) and a photosynthetic partner (photobiont) and are important constituents of many ecosystems (Obohv and Ademosun 2006; Bates et al. 2011; Hawksworth et al. 2020). Lichens are unanimously distributed in diverse climatic conditions spanning from the plains to the high mountains and from polar regions to the tropics and are susceptible to a variety of environmental stress (Felczykowska et al. 2017). As a result, they produce different secondary metabolites including some specific such as terpenes, depsides, depsidone, dibenzofuran, and xanthone which are unique to lichen species (Olivier-Jimenez et al. 2019; Stanojković 2019; Sabarwati et al. 2020). The secondary metabolites of lichen contain bioactive substances which have manifold biological activities such as antimicrobial, anticancer, anti-inflammatory and antioxidant properties (Maulidiyah et al. 2016; Mohammadi et al. 2020; Mohan et al. 2020; Nugraha et al. 2020; Studzińska-Sroka et al. 2021).

Antioxidants are compounds that can retain the quality of foods by delayed process of oxidation and protect from damage caused by free radical induced oxidative stress (Souri et al. 2008). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene toluene (BHT), tertbutylhydroquinone (TBHQ) and propyl gallate (PG) are widely accepted, but their use is being restricted nowadays due to their toxic and harmful side effects (Zhang et al. 2009; Maulidiyah et al. 2021). Thus, at present there is a growing interest towards natural antioxidants. So in the search for novel natural antioxidant sources, our target is aimed on lichens found in Similipal Biosphere Reserve (SBR) as it harbours a large variety of lichen species. Thus, the purpose of the present work is to assess the antioxidant activity of acetone and methanol extract of the lichens obtained from this region. Our investigation is the first report describing the evaluation of Similipal Biosphere Reserve lichen species as a source of natural antioxidant.

## MATERIAL AND METHODS

For the collection of lichen samples, two lichen species, *Dirinaria applanata* and *Parmotrema andium* as shown in fig.1 were collected from various localities within Similipal Biosphere Reserve (SBR). Herbarium specimen of each species was maintained in research laboratory, Department of Biotechnology, Maharaja Sriram Chandra Bhanja Deo University, Baripada, Odisha for experimental purpose.

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The lichen species were identified following available monographs and standard methods (Awasthi 1988; Awasthi 1991; Orange et al. 2001). For the preparation of lichen extract, dried lichen samples were milled into fine powder using a sterile mortar and pestle. The powdered form of material was moved through the sieve (75 microns) and 10 g of dry fine powder of sample was mixed with 100 ml of solvent (acetone and methanol) on an orbital shaker and filtered using Whatman no. 1 filter paper. Then it was vaporised in a rotary evaporator at 45°C and preserved in deep freeze for subsequent use.

For the DPPH radical scavenging activity, the antiradical activity of two test lichen extracts was evaluated by DPPH (1,1-diphenyl-2-picryl-hydrazil) assay (Kosanic et al. 2011). One ml of DPPH solution (0.1mM) was added to 3 ml of different concentration (100-1000 µg/ml) of lichen extract and the mixture was kept for 30 min at room temperature and the absorbance was recorded at 517 nm using UV-Vis. Spectrophotometer (Systronics-119). Ascorbic acid was used as positive control. The hydrogen peroxide ($H_2O_2$) scavenging activity was determined by spectrophotometric analysis according to the method of Ruch et al. (1989). $H_2O_2$ solution was prepared in $PO_4$ buffer (1 M, pH 7.4) and 0.6 ml was added to the lichen extract of various concentrations (100-1000µg/ml). The optical density was recorded at 230 nm after 10 min. Ascorbic acid was used as standard against the test extract. The DPPH/ $H_2O_2$ radical scavenging activity was calculated by the following equation:

$$\% \text{ activity} = [(\text{control Abs} - \text{sample Abs}) / \text{control Abs}] \times 100$$

$IC_{50}$ values were derived from the percentage of inhibition versus concentration plot and expressed in µg/ml.A lower $IC_{50}$ meant better radical scavenging activity.

For the ferric reducing antioxidant power assay (FRAP), the ferric reducing antioxidant power of the tested lichen species was estimated according to the method of Oyaizu (1986). Various concentration of lichen extract (100, 250, 500, 750, 1000µg/ml) was added with 2.5 ml of $PO_4$ buffer (pH 6.6, 0.2M) and potassium ferricyanide (1%) and was kept at 50°C for 25 minutes. Then it was mixed with trichloro acetic acid (10%) and centrifuged at 3000g for 20 minutes. Finally, the obtained supernatant was thoroughly mixed with 2.5 ml distilled water alongwith 0.5 ml $FeCl_3$ (0.1%) and recorded at 700nm. butylated hydroxytoluene is used as positive control for the experiment. Total phenolic content (TPC) was estimated by Folin-Ciocalteu reagent following the method of Taga et al. (1984). The lichen extract (100µl) was mixed with 2ml of Sodium carbonate (2%). After 10 min, 500 µl of Folin's reagent was supplemented and then the reaction mixture was kept under dark for 20 minutes and the absorbance was measured at 650 nm. The result was expressed as µg GAE /g dry extract. Total flavonoid content (TFC) was determined according to the method of Zhishen et al. (1999). One ml of the lichen extract was mixed with 500 µl of Sodium nitrite and Aluminum chloride (10%c 300 µl). After 10 min, Sodium hydroxide (1M, 1ml) was mixed and the volume was made up to 5ml with distilled water. Then the mixture was incubated for 30 min and the absorbance was read at 510nm. The result was expressed as quercetin equivalent i.e µg QE /g dry extract.

**Table 1. DPPH radical and $H_2O_2$ scavenging activity of methanol and acetone extract of *Dirinaria applanata* and *Parmotrema andium***

| Lichen species | Solvent | DPPH radical scavenging activity $IC_{50}$(µg/ml) | $H_2O_2$ scavenging activity $IC_{50}$ (µg/ml) |
|---|---|---|---|
| *Dirinaria applanata* | Methanol | 471.16±0.85 | 554.57±0.90 |
| | Acetone | 519.79±1.29 | 707.74±0.79 |
| *Parmotrema andium* | Methanol | 534.77±0.75 | 592.25±0.93 |
| | Acetone | 600.77±0.95 | 755.34±0.86 |
| | Gallic acid (standard) | 326.61±0.94 | 341.52±0.87 |

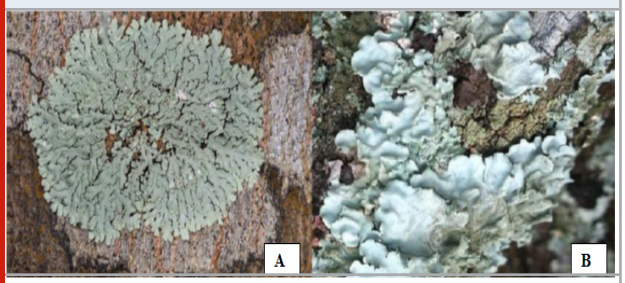
## RESULTS AND DISCUSSION

**DPPH and $H_2O_2$ scavenging assay:** The scavenging of DPPH and $H_2O_2$ radicals by the lichen extracts was determined and shown in table1.The test lichen species extracts exhibited differential scavenging ability. However, methanol extract of *D. applanata* has strong DPPH and $H_2O_2$ scavenging activity with $IC_{50}$ i.e., 471.16±0.85 µg/ml and 554.57±0.90 µg/ml respectively. The lowest $IC_{50}$ of DPPH and $H_2O_2$was recorded in acetone extract of both the species. These results are in agreement with the literature where it was reported that many lichen extracts has their ability to scavenge free radicals (Behera et al. 2009; Manojlović et al. 2012; Hawrył et al. 2020).

**Ferric reducing antioxidant power assay (FRAP):** The experimental result on assay of Ferric reducing power of two lichen species was represented in fig 2. The results infer that the higher absorbance (700nm) of lichen extracts results in higher reducing power. Accordingly, more reducing power was recorded in methanolic extract of *Dirinaria applanata* (absorbance: 0.762±0.0008) as compared to *Parmotrema andium* (absorbance:0.673±0.0008). Comparing this result it was found that *Dirinaria applanata* showed more promising antioxidant activity than *Parmotrema andium*.

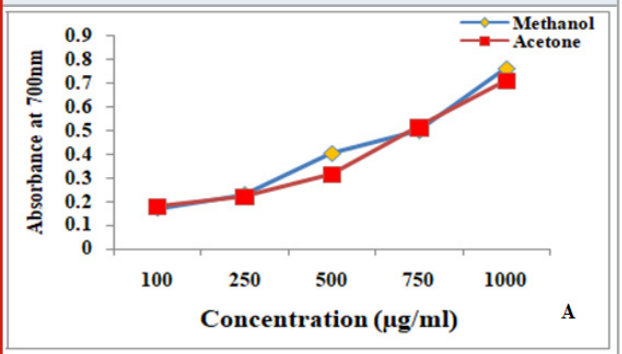
Likewise, Smitha et al. (2016) reported comparative reducing power activity with ethanol and methanol extracts of the *Ramalina pacifica* which was less when compared with *Rocella montagnei*. Similarly, the maximum reducing power was detected in methanol extract of *P. centrifuga* (Ranković and Kosanić, 2019; Aoussar et al. 2020; Hawrył et al. 2020).

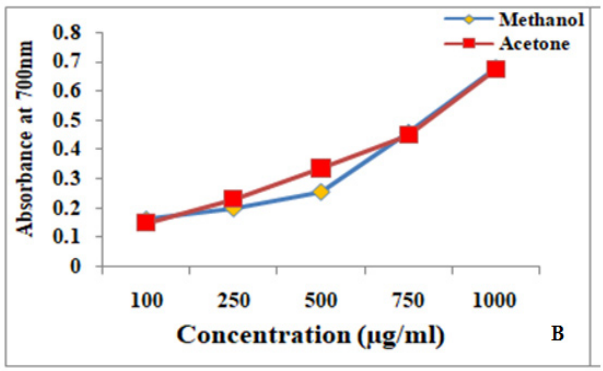
**Figure 1: (A-B) Lichens collected from Similipal Biosphere Reserve (SBR)**




A. *Dirinaria applanata* B. *Parmotrema andium*

**Figure 2: Ferric reducing antioxidant power assay of A. *Dirinaria applanata* and B. *Parmotrema andium***




**Total Phenolic and Flavonoid content (TPC and TFC):** TP and TF content of methanol and acetone extracts in both the lichen species was determined in terms of gallic acid equivalent (µg GAE/ g of extract) and quercetin equivalent (µg QE/ g of extract) respectively and the findings was summarized in table 2. Maximum phenolic content was recorded in methanolic extract of *Dirinaria applanata* (68.96±0.60 µg/ml) while acetone extracts of *Parmotrema andium* showed the lowest phenolic content (48.56±0.97µg/ml) (Manojlović et al. 2021). Similarly high flavonoid content was also found in *Dirinaria applanata*, (38.22±0.89 µg/ml) as compared to *Parmotrema andium* (27.57±1.03 µg/ml). Both Phenolic and Flavonoid compounds have antioxidant characteristics due to their redox properties, which can assist in the absorption and neutralisation of free radicals, quenching of singlet and triplet oxygen and the decomposition of peroxides. Thus, total phenolic compounds in *Dirinaria applanata* were found to be more encouraging with higher level of TPC and TFC (Manojlović et al. 2021).

It was understood and established that the antioxidant activity might be cumulative effect of different natural components and not solely of its fractions. In our result, the antioxidant activity of the *Dirinaria applanata* and *Parmotrema andium* extract might be the resultant synergistic effect of various compounds present within the extract and more interestingly encouraging antioxidant potential of *Dirinaria applanata* is due to the associated action which contributes a higher antioxidant activity in its extracts (Sargsyan et al. 2021).

**Table 2. TPC and TFC of *Dirinaria applanata* and *Parmotrema andium***

| Lichen Species | Methanol | Acetone |
|---|---|---|
| TPC(µg/ml) | | |
| *Dirinaria applanata* | 68.96±0.60 | 57.24±0.96 |
| *Parmotrema andium* | 59.82±0.85 | 48.56±0.97 |
| TFC(µg/ml) | | |
| *Dirinaria applanata* | 38.22±0.89 | 30.79±0.82 |
| *Parmotrema andium* | 32.54±0.53 | 27.57±1.03 |

## CONCLUSION

The findings of the present study demonstrate that methanol extract of *Dirinaria applanata* has impressive antioxidant activities *in vitro*. In summary, the experimental results conclude that *Dirinaria applanata* and *Parmotrema andium* may act as an agent of potential natural sources of antioxidants. Results indicated that probably the higher amount of phenolic compounds in the lichen extracts are responsible for encouraging antioxidant properties that would be of interest in food and pharmaceutical industry. Hence further work needs to be carried out to isolate and purify the active components from these species to determine their antioxidant activity.

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**Conflict of Interests:** Authors declare no conflicts of interests to disclose.

**Data Availability Statement:** The database generated and /or analysed during the current study are not publicly available due to privacy, but are available from the corresponding author on reasonable request.

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