Journal of Applied Biology & Biotechnology Vol. 10(6), pp. 34-43, Nov-Dec, 2022
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2022.100604

Antimicrobial, anticancer, and antioxidant potential of two dominant macro-lichen *Dirinaria aegialita* and *Parmotrema praesorediosum* collected from Similipal Biosphere Reserve of Odisha, India

Srimay Pradhan[1], Dalip Kumar Upreti[2], Rajesh Kumar Meher[3], Kunja Bihari Satapathy[1]*

[1]Department of Botany, School of Applied Sciences, Centurion University of Technology and Management, Odisha, India.
[2]Lichenology Laboratory, CSIR-National Botanical Research Institute, Lucknow, India.
[3]Center for Excellence in Natural Products and Therapeutics, Department of Biotechnology and Bioinformatics, Sambalpur University, Odisha, India.

ARTICLE INFO

Article history:
Received on: March 31, 2022
Accepted on: August 02, 2022
Available online: September 20, 2022

Key words:
Pharmaceutical evaluation,
Lichenized fungi,
Mayurbhanj,
Crude extract.

ABSTRACT

The present study was undertaken to evaluate the antimicrobial, antioxidant, and anticancer activity of *Dirinaria aegialita* (Afzel. ex Ach.) B.J. Moore and *Parmotrema praesorediosum* (Nyl.) Hale, the two dominant macro-lichens taxa from the Similipal Biosphere Reserve of Odisha. Both the lichens were evaluated for their efficacy against three bacterial species such as *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Bacillus subtilis,* and three fungal species such as *Aspergillus niger*, *Trichoderma harzianum*, and *Candida albicans*. The *D. aegialita* and *P. praesorediosum* showed higher inhibitory effect against *Bacillus subtilis* and *Staphylococcus aureus*, respectively. *D. aegialita also showed the higher* inhibitory activity against MCF-7 and MDA MB-231 breast cancer cell line as compared to *P. praesorediosum*. Besides, *D. aegialita* was found to have better antioxidant activity than *P. praesorediosum* in scavenging assay. Thus, the results of the above study confirmed that *D. aegialita* species is having better potential in its antibacterial, antioxidant, and anticancer activity as compared to *P. praesorediosum*.

1. INTRODUCTION

In recent years, the study on bioactivity and production of pharmaceutically important products from lichens is receiving considerable attention. Lichens are composite structure composed of algae (photobiont) and fungi (mycobiont). However, the structural composition of lichens is dominated by fungus, which ultimately control the symbiosis; hence, lichens are called as lichenized fungi. It is a great source of bioactive secondary metabolites [1]. Approximately 20000 species of lichen have been recorded so far globally, which inhabits over 10% of the terrestrial surface [2,3]. Lichens have been used as foods, medicines, cosmetics, and other ethnobotanical reasons in modern facts from prehistoric to recent days [4]. Because lichens produce secondary metabolites, they adapt to cosmopolitan distribution [5-7] and show antimicrobial, antiviral, anticancer, antioxidant, and antidiabetic activity [8-10]. The Similipal Biosphere Reserve is well known for its lichen diversity [11-14]. However, this region has not been explored enough to find out the potential bioactive compound in lichens and their pharmaceutical values [15].

*Corresponding Author:
Kunja Bihari Satapathy,
Department of Botany, School of Applied Sciences, Centurion University of Technology and Management, Odisha, India.
E-mail: kbsbotcutm @ gmail.com

Dirinaria aegialita (Afzel. ex Ach.) B. J. Moore and *Parmotrema praesorediosum* (Nyl.) Hale are two macro-lichens belongs to the Caliciaceae and Parmeliaceae family, respectively. Both the foliose species grow luxuriantly on a number of trees and rocks in Similipal Biosphere Reserve. The lichen genus *Dirinaria* has 25 species worldwide, which produce divaricatic acid and sikikaik acid and triterpenoids as their major secondary metabolites. The pharmaceutical importance of different species of *Dirinaria* is so far known for its antioxidant, antimicrobial, cytotoxic, anti-inflammatory, insecticidal, and larvicidal activity [16-22]. However, *D. aegialita* is evaluated only for its antifungal activity [23].

Parmotrema praesorediosum belongs to the genus *Parmotrema*, comprises 300 species, and considered as one of the largest genera of the family – Parmeliaceae [24]. *Parmotrema* is used as spices [25], in silk dyeing [26] and source for many bioactive compounds [27], and praesorethers E, F, and G, γ-lactonic acids, Vinapraesorediosic acid D and E [28-30], which have potential cytotoxicity activity and successfully investigated against different human cancer cell lines [31] and known for its anti-arthritic potential [32]. The presence of aliphatic acids (protopraesorediosic) makes it significant to use in synthesis of silver nanoparticles (AgNPs) to inhibit the growth of pathogenic organisms [33,34]. In view of the above, the present study was conducted with an objective to evaluate the antimicrobial, antioxidant, and anticancer activities of macro-lichen found in Similipal Biosphere Reserve of Odisha [Figure 1].

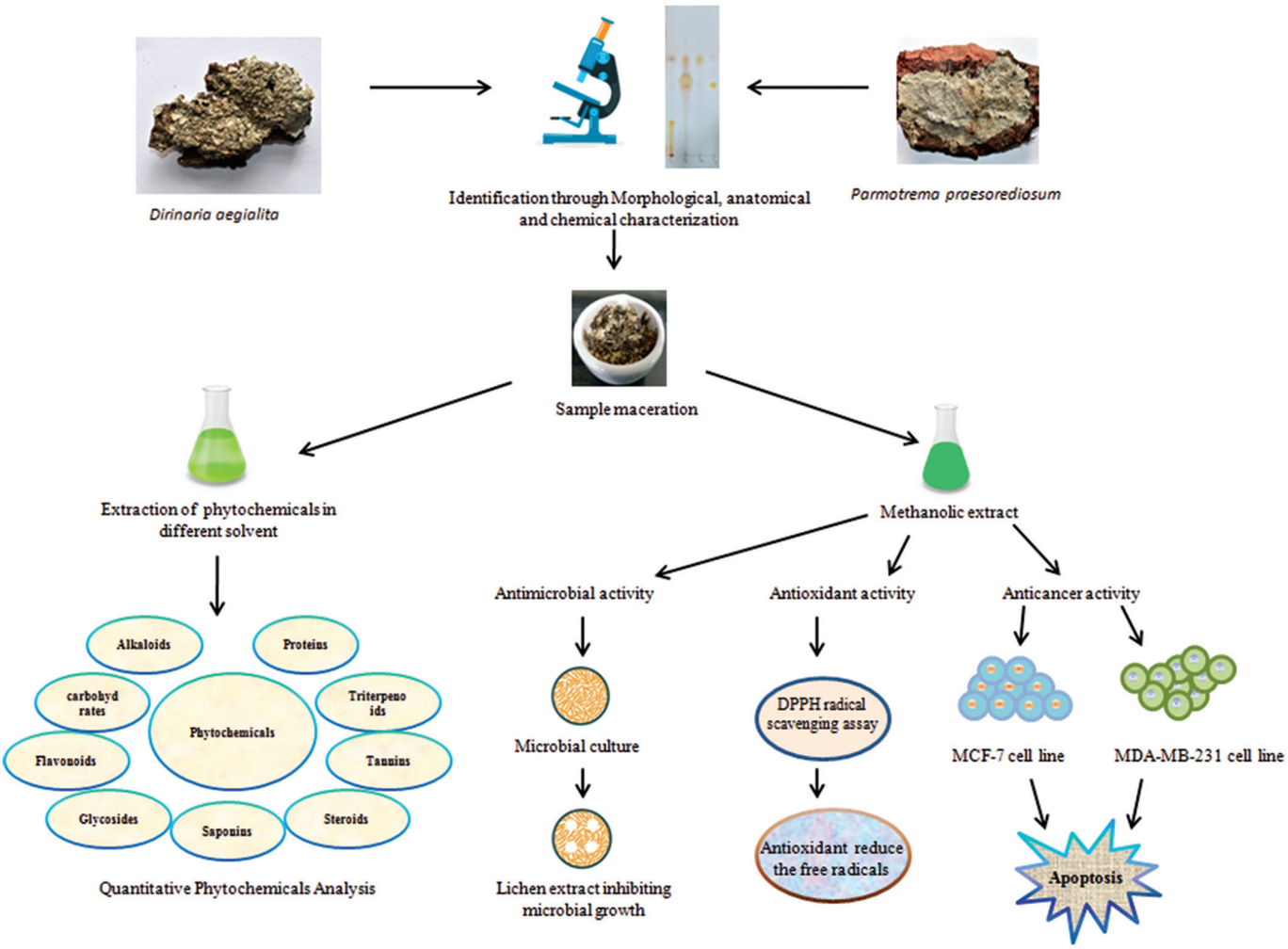

Figure 1: Schematic representation for evaluation of pharmaceutical potential of *Dirinaria aegialita* and *Parmotrema praesorediosum*.

2. MATERIALS AND METHODS

2.1. Sample Collection

Fresh lichen samples (*Parmotrema praesorediosum* and *Dirinaria aegialita*) were collected from Similipal Biosphere Reserve in Mayurbhanj district of Odisha [Figure 2]. The Similipal Biosphere Reserve of Mayurbhanj district is in North-east Odisha lying between 21°28' to 22°08' N and 86°04, to 86°37' E. It is covered with dense forested hills and valleys and dry deciduous vegetation occupying 5569 km^2 area which is almost half of the geographical area of Mayurbhanj district. On an average, the area receives 1800 mm annual rain fall with temperature ranging from 3°C to 38°C.

2.2. Lichen Identification

The collected specimens were identified according to their morphology, anatomy, and chemistry [35]. The color tests were carried out with aqueous potassium hydroxide (K), Steiner's stable paraphenylenediamine (PD), and aqueous calcium hypochlorite (C). The identified samples of *D. aegialita* and *P. praesorediosum* deposited in the lichen herbarium of CSIR-National Botanical Research Institute, Lucknow (LWG) with accession number CUTM-159 and CUTM-184, respectively.

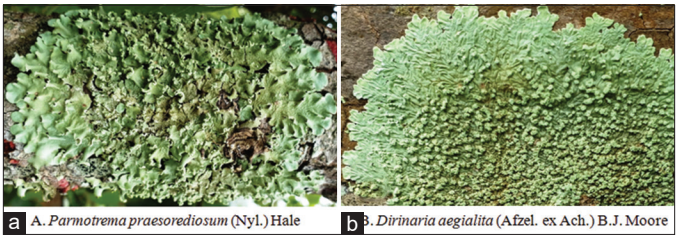

Figure 2: Lichen samples collected from the study site.

2.3. Preparation of Lichen Extract

To prepare lichen extract, the collected samples (10 mg) were added with 100ml methanol and incubated at room temperature with intermittent mixing. Then, the extract was filtered using Whatman number-1 filter paper. The solvent was allowed to evaporate from filtrate till the extract become completely dry, finally remaining residue was collected as the lichen extract. The extract obtained was stored at 4°C for further use and this extract was used to prepare five different concentrations, that is, 0.1 mg/ml, 0.2 mg/ml, and 0.3 mg/ml in methanol for the antimicrobial screening test.

Table 1: Quantitative phytochemical screening of the collected samples in different solvents.

Lichens sp.	Phytochemical constituents mg/g	Concentration of phytochemical in mg/g of lichen extract				
		Aqueous	Methanol	Hexane	Chloroform	2-Propanol
Dirinaria aegialita	Alkaloids	5.51±0.05	8.84±0.03	6.87±0.08	4.86±0.04	5.41±0.06
	Flavonoids	3.41±0.09	4.48±0.03	3.34±0.04	2.41±0.03	3.74±0.03
	Total Saponins	1.51±0.04	1.23±0.04	1.11±0.07	1.21±0.03	2.31±0.06
	Steroids	2.55±0.08	3.42±0.04	2.55±0.07	3.26±0.04	2.84±0.03
	Tannins	2.54±0.03	3.57±0.08	3.51±0.05	3.36±0.04	2.51±0.05
	Terpenoids	1.59±0.05	1.51±0.07	1.35±0.05	2.31±0.05	3.75±0.02
Parmotrema praesorediosum	Alkaloids	6.41±0.06	9.44±0.03	6.97±0.07	5.66±0.04	6.41±0.07
	Flavonoids	5.41±0.09	7.48±0.03	4.44±0.05	3.71±0.08	4.44±0.03
	Saponins	3.51±0.04	0.58±0.04	0.71±0.07	1.21±0.03	1.37±0.05
	Steroids	3.45±0.09	5.42±0.05	3.55±0.03	4.26±0.04	3.74±0.04
	Tannins	1.57±0.03	2.57±0.05	2.41±0.05	4.56±0.06	3.51±0.08
	Terpenoids	2.55±0.04	2.41±0.06	1.45±0.06	3.35±0.07	2.65±0.05

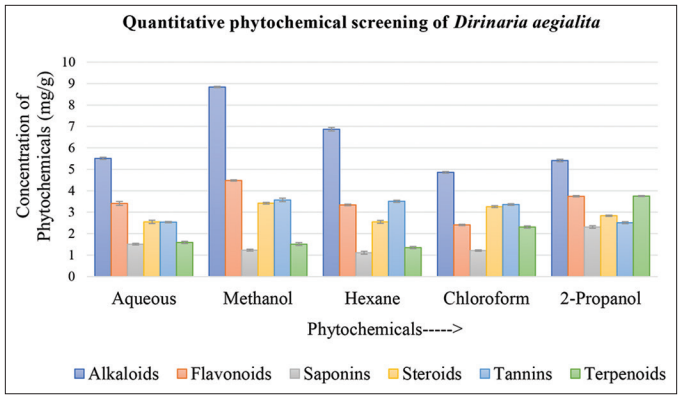

Figure 3: Quantitative screening of phytochemical in different solvent system of *Dirinaria aegialita.*

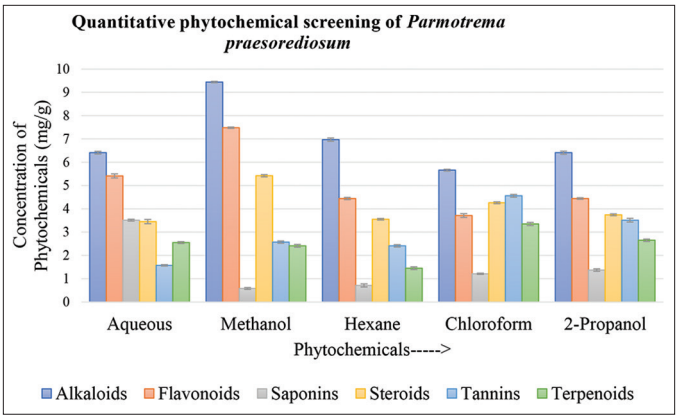

Figure 4: Quantitative screening of phytochemical in different solvent system of *Parmotrema praesorediosum*.

2.4. Qualitative and Quantitative Phytochemical Analysis

The qualitative and quantitative study of phytochemical was carried out by preparing lichen extract in water, methanol, hexane, chloroform, and 2-propanol with the help of biochemical and UV-Vis spectrophotometry method, respectively [36-40]. Major characteristics of all the solvents are provided in Table 1, Figures 3 and 4.

2.5. Microorganisms and Chemicals

Three strains of bacteria [*Staphylococcus aureus* (MTCC-96), *Pseudomonas aeruginosa* (MTCC-424), and *Bacillus subtilis* (MTCC-441)] and three strains of fungi [*Aspergillus niger* (MTCC-282), *Trichoderma harzianum* (MTCC-3178), and *Candida albicans* (MTCC-183)] were used for antimicrobial screening. The microorganisms were procured from IMTECH Chandigarh and maintained in Department of Botany, Centurion University of Technology and Management, Odisha.

2.6. Determination of Antimicrobial Potential

The antimicrobial potential was evaluated by agar well diffusion method [41]. For bacterial culture, the Muller Hinton agar media was prepared by suspending 38 g of the media in 1L water, while for fungal culture Sabouraud dextrose agar media prepared by draping 65 g of media in 1L water and autoclaved at 121°C with 15 psi for 15min. After sterilization, the media was poured into sterile glass petri-dishes followed by inoculation of 100µl of the culture broth and allowed for incubation at 37°C for 24 h.

2.7. Minimum Inhibitory Concentration (MIC)

The conventional 96 well plates with Mueller–Hinton broth (MHB) were used to determine the MIC. The different concentrations of lichen extracts, as well as MHB and pathogen as controls, were made using two-fold serial dilution. A total of 50 µg of the test bacterial inoculum with 10^5 colony forming unit/ml concentration was added [42]. After 24 and 48 h, the samples were tested for bacteria using a microplate reader (Bio-Rad, iMark-11457) at 595 nm. The MIC value was calculated as the lowest concentration of extract in the broth medium that inhibits the growth of the pathogens being examined [43].

2.8. Cell Culture and Reagents

For cell culture, all the chemical reagents and media used were procured from Invitrogen. MCF-7 and MDA-MB-231 breast cancer cell lines were obtained from the cell repository of the National Center for Cell Science, Pune, Maharashtra, India.

2.9. Antioxidant activity by 2, 2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay (DPPH)

Different aliquots of standard, that is, ascorbic acid (1mg/ml) with methanol extracts of plant ranges from (6.25 to 200 µg/ml) were taken and the total volume was adjusted to 250 µl with methanol [44]. 1 ml of DPPH at a concentration of 4mg/100ml was added to the extract and the tube was kept in dark condition at room temperature for 30 min. The absorbance was taken against the blank at 517 nm, % of free radical scavenging was calculated based on the extent of reduction in the color.

% of radical scavenging activity= Ac-As/Ac ×100, Where Ac=Absorbance of control, As= Absorbance of sample.

2.10. Anticancer Evaluation Using MCF-7 and MDA MB-231 Breast Cell Lines

The anticancer evaluation assay was performed using two human breast cancer cell lines, MCF7 and MDA MB-231. In brief, cells were allowed to grow in culture medium (MEM, DMEM) supplemented with 10% FBS, 1% penicillin/streptomycin, with maintenance of temperature at 37°C and 5% CO_2. In a 96-well plate, cancer cells were seeded at a density of 4 × 10^3 cells/well, then treated with increasing concentrations from (6.25 to 100 µg/ml) of methanolic extract of lichens, for 72 h. The cells were then stained with 0.56% of Sulforhodamine B in 1% acetic acid. To remove unbound stains by washing, 1% acetic acid was used. 10 mM Tris base with 10.5 pH was added to the 96 well plate containing fixed cells with protein bound stain and the absorbance was taken at 495 nm wavelength using a Bio Rad 96 well plate reader [45]. The IC_{50} values for the extract were calculated from the plate reader data using an online IC_{50} value calculator (AAT Bioquest, Inc., Sunnyvale, CA, USA).

2.10.1. DAPI staining for detection of apoptosis

Apoptotic cells were visualized by DAPI staining method with fluorescence microscopy. MCF-7 cells were grown on 6-well plates and were treated with the methanolic extracts of *Dirinaria aegialita* and *Parmotrema praesorediosum* at IC_{50} concentration for 72 h. After incubation, 6-well plates were fixed in 3% formaldehyde and washed with PBS, stained with DAPI having a concentration of 1mg/ml, and washed after 5 min using 1× PBS to remove unbound stain. Images were captured using a fluorescent microscope (Nikon Eclipse Ts2R-FL). Apoptotic cells were identified based on morphology of cells, for example, nuclear condensation, formation of membrane blebs and apoptotic bodies, etc. compared to untreated cells [46].

2.10.2. Ethidium bromide for detection of apoptosis

Apoptotic cells were visualized by ethidium bromide staining method with fluorescence microscopy. MCF-7 cells were grown on 6-well plates and were treated with the methanolic extracts of *D. aegialita* and *P. praesorediosum*, at IC_{50} concentration for 72h. After incubation, 6-well plates were fixed in 3% formaldehyde and washed with PBS, stained with EtBr having a concentration of 3mg/ml, and washed after 5 min using PBS to remove unbound stain. Images were captured using a fluorescent microscope (Nikon Eclipse Ts2R-FL). Apoptotic cells were identified based on stains taken by the cells. The cells stained with red represent both live and pre-apoptotic cells which are stained with EtBr displayed membrane blabbing forming apoptotic bodies as well as nuclear condensation [47].

2.11. Statistical Analysis

The results of the experiment were statistically examined. All of the experiments were done in triplicates, and the results were expressed as mean values with standard deviations (±SD).

3. RESULTS

3.1. Phytochemical's Analysis

In quantitative screening, the methanolic extract of both the species exhibited presence of alkaloid, flavonoid, steroid, and together with tannins and triterpenoids. Saponins were observed in aqueous extract of *D. aegialita* only. Steroids were found in methanolic extract of both species as well as chloroform and 2-propanol extract of *D. aegialita*. Flavonoids together with steroids were reported in methanol, chloroform, and 2-propanol extract of *D. aegialita*, while triterpenoids exhibited their presence in all extract of both the species. Hexane, chloroform, and 2-propanol extract of *P. praesorediosum* exhibit presence of alkaloids, flavonoids, tannins, and triterpenoids. Glycosides were found in methanolic and aqueous extract of both the species. Tannins were absent in chloroform and hexane extract of *D. aegialita* and *P. praesorediosum,* respectively, while present in all other solvent extracts.

The quantitative phytochemical analysis is also correlated to the qualitative data of phytochemicals [Table 1], which confirmed the presence of maximum phytochemicals in the methanolic extract [Figures 3 and 4]. In particular, the methanolic extract of both the species of lichen exhibited maximum presence of alkaloids, flavonoids, steroids, and tannins. Saponin found to be highest in 2-propanol and water in case of *D. aegialita* and *P. praesorediosum,* respectively. Similarly, terpenoids present highest in 2-propanol and chloroform in case of *D. aegialita* and *P. praesorediosum*.

3.2. Antioxidant Assay

The scavenging activity of lichen extract against DPPH radical is depicted in Figure 5, which shows that there is a significant correlation between the extract studied and the positive control (r>0.7). The antioxidant activity was increased from 32.2 to 93.1 with the increase in concentration of *D. aegialita* extract from 6.25 µg/ml to 150 µg/ml. Similarly, antioxidant activity of *P. praesorediosum* was increased from 21.8 to 86.4 with the increase in concentration from 6.25µg/ml to 150µg/ml. Among all the extract taken the methanolic, one showed highest scavenging activity against DPPH radical: 93.1 and 86.4 at 150µg/ml extract of *D. aegialita* and *P. praesorediosum,* respectively.

3.3. Antimicrobial Assay

It is revealed from the present study that *P. praesorediosum* had a pronounced activity against the microbes as compared to *D. aegialita* [Table 2 and Figure 6]. In case of *P. praesorediosum*, the

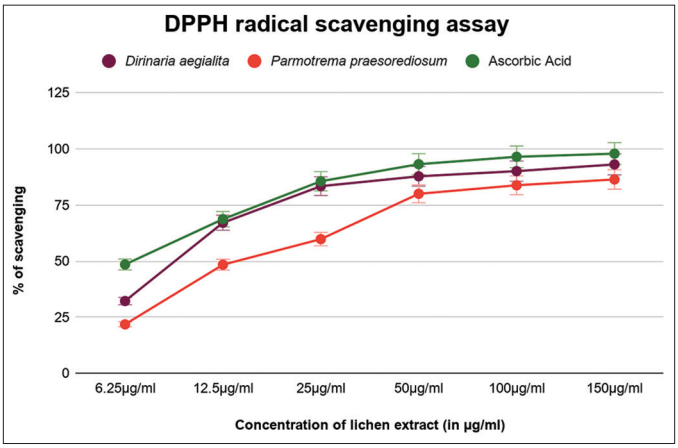

Figure 5: DPPH assay of *Dirinaria aegialita* and *Parmotrema praesorediosum*.

Table 2: The zone of inhibition (mm) obtained for the lichen extract against the bacterial and fungal isolates. DA- *Dirinaria aegialita*, PP- *Parmotrema praesorediosum*. (+) C -Positive control, (-) C -Negative control [values are expressed as mean±SD in triplicates].

Organism	Zone of Inhibition (mm)									
	0.1 mg/ml		0.2 mg/ml		0.3 mg/ml		(+) C		(-) C	
	DA	PP	DA	PP	DA	PP	DA	PP	DA	PP
Staphylococcus aureus	Nil	Nil	Nil	12±0.81	13±0.73	17±0.82	23±0.75	25±0.76	-	-
Pseudomonas aeruginosa	Nil	Nil	Nil	13±0.74	13±0.76	17±0.78	32±0.79	33±0.77	-	-
Bacillus subtilis	Nil	Nil	10±0.75	11±0.77	15±0.78	16±0.80	28±0.81	29±0.75	-	-
Aspergillus niger	Nil	Nil	Nil	Nil	12±0.71	12±0.75	18±0.78	19±0.74	-	-
Trichoderma harzianum	Nil	Nil	Nil	10±0.75	10±0.72	13±0.77	18±0.76	20±0.73	-	-
Candida albicans	Nil	Nil	Nil	Nil	13±0.74	11±0.75	20±0.78	19±0.77	-	-

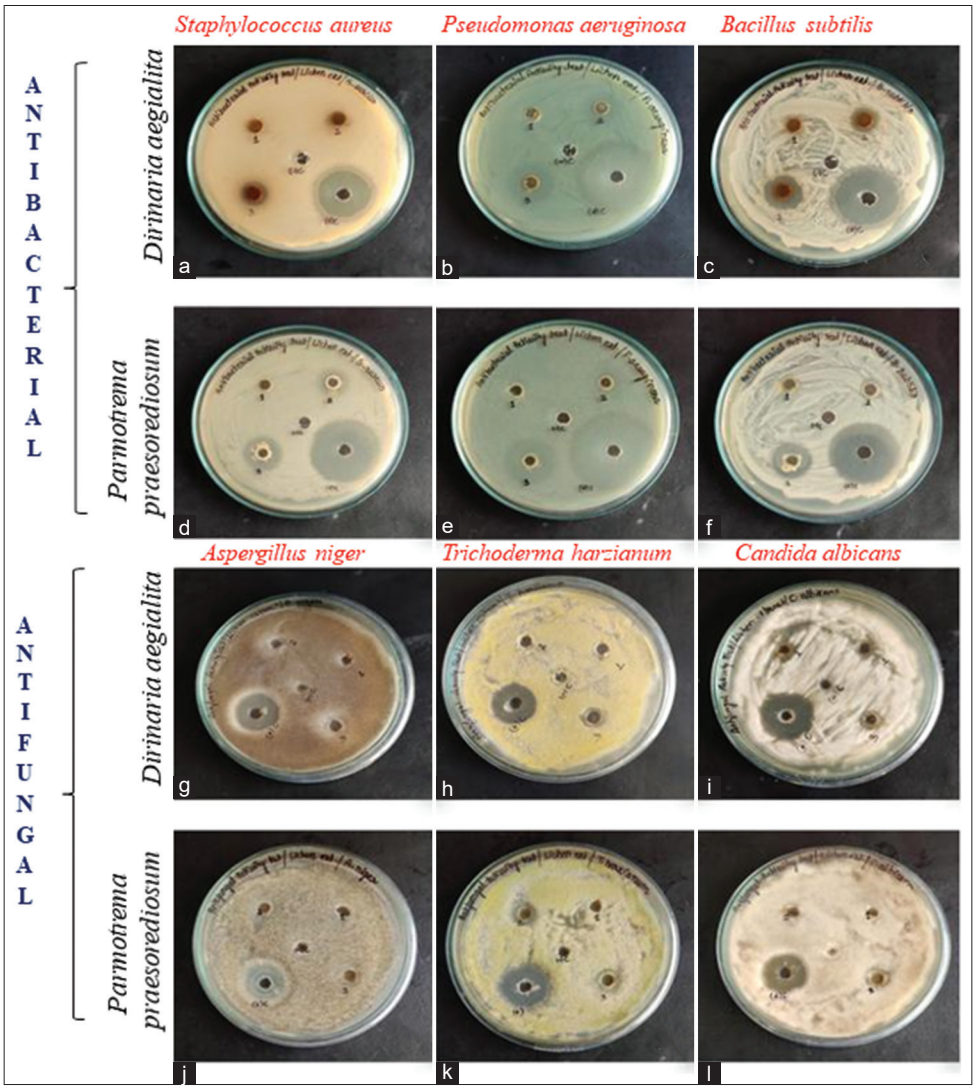

Figure 6: (a-f) Respective antimicrobial activity of *Dirinaria aegialita* and *Parmotrema praesorediosum* extract against the bacterial pathogen *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis*. (g-l) Respective antifungal activity of *Dirinaria aegialita* and *Parmotrema praesorediosum* extract against fungal isolates *Aspergillus niger, Trichoderma harzianum* and *Candida albicans*.

most susceptible bacterium is *Staphylococcus aureus* which recorded a highest inhibition of 17±0.82 mm at concentration 0.3 mg/ml, compared to *D. aegialita*, showing 15±0.78 mm inhibition against *Bacillus subtilis* at concentration 0.3 mg/ml, as well as at low concentration, which recorded 10±0.75 mm zone of inhibition at concentration of 0.2 mg/ml [Figures 7 and 8]. *P. praesorediosum* has well inhibitory zone at low concentration for all the pathogen taken except *A. niger* and *C. albicans*. However, *P. praesorediosum* showed high antifungal activity against *T. harzianum*, with 13 ± 0.77 mm zone of inhibition at concentration of 0.3 mg/ml, while *D. aegialita* showed

Table 3: MIC of methanolic extract of *D. aegialita* and *P. praesorediosum.*

Strains	MIC (µg/ml)	
	Methanol	
	Dirinaria aegialita	*Parmotrema praesorediosum*
Staphylococcus aureus	290	195
Pseudomonas aeruginosa	285	185
Bacillus subtilis	190	185
Aspergillus niger	280	275
Trichoderma harzianum	275	185
Candida albicans	245	275

Table 4: IC_{50} values of methanolic extract of Lichens-1 and 2 using two human breast adenocarcinoma cell lines, MCF-7 and MDAMB-231. Both the extracts were found to have anti proliferative activity compared to untreated one.

IC_{50} (µg/ml)	*Dirinaria aegialita*	*Parmotrema praesorediosum*
MCF-7	48.1±1.5	67.5±0.6
MDA-MB-231	86.1±1.4	125±1.2

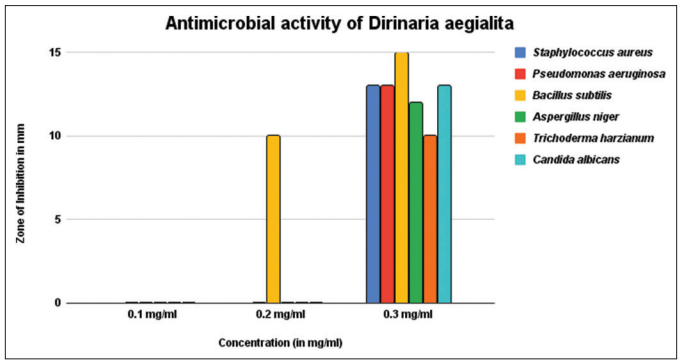

Figure 7: Activity of *Dirinaria aegialita* extract against different pathogens.

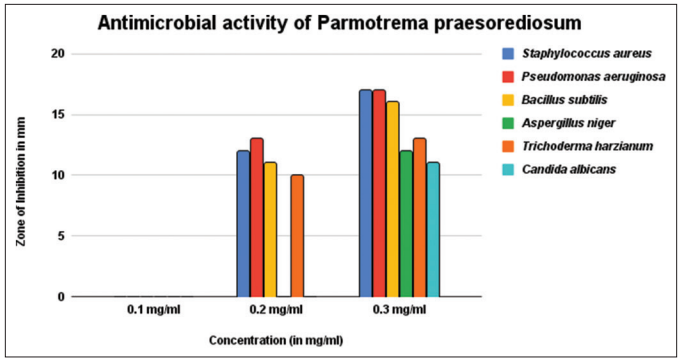

Figure 8: Activity of *Parmotrema praesorediosum* extract against different pathogens.

high antifungal activity against *C. albicans*, with 13 ± 0.74 mm of inhibition at 0.3 mg/ml. When compared with the antibacterial drug ciprofloxacin and antifungal drug luliconazole, it was found that *P. praesorediosum* had better efficacy against *S. aureus* as compared to ciprofloxacin.

3.4. Minimum Inhibitory Concentration (MIC)

Anti-microbial properties of both the lichen species against the selected pathogens were evaluated by their MIC values [Table 3]. The antimicrobial activities of the lichen extracts were identified against the test pathogens. Among the test bacteria and fungus, the MIC ranged from 185 to 290 µg/ml.

3.5. Anticancer Potential

The activity of two macro lichens against MCF-7 breast cancer cell line as evident during the present study is depicted in Figure 9a, which revealed that *D. aegialita* had higher anticancer activity against the breast cancer cell line with IC_{50} value of 48.1±1.5 µg/ml, while IC_{50} value for *P. praesorediosum* extract is around 67.5±0.6 µg/ml. *D. aegialita* showed higher anticancer activity against MDA MB-231 breast cancer cell line [Figure 9b] with IC_{50} value of 86.1±1.4 µg/ml, whereas the IC_{50} value for *P. praesorediosum* extract is around 125±1.2 µg/ml [Table 4].

Figures 10a and b panels show morphological features of MCF-7 cells stained with DAPI (4',6-Diamidino-2-phenylindole dihydrochloride), EtBr (Ethidium Bromide), from untreated cells (upper panels), and cells treated with IC_{50} concentration of methanolic extract of *P. praesorediosum and D. aegialita,* respectively, (lower panels) for 72 h using fluorescence microscopy. The apoptotic cancer cells were identified by morphology of treated cells, that is, membrane contraction, nuclear condensation, and fragmentation, etc. after 72 h of drug treatment to the cancer cell line.

4. DISCUSSION

The screening of phytochemicals for their antimicrobial, antioxidant, and anticancer properties was carried out using the extract of two different lichens from Similipal Biosphere Reserve, Odisha. The results of the investigation revealed the presence of diverse constituents with significant pharmacological and biological properties [48,49]. The phytochemical analysis confirmed the presence of alkaloids, flavonoids, saponins, steroids, tannins, and triterpenoids in the test lichen extracts. Phenolics are the most abundant and diverse group of plant metabolites, with anticancer, antibacterial, anti-inflammatory, and antioxidant properties, as well as effective in cardiovascular and neurodegenerative diseases [50-52]. The presence of flavonoids and tannins, which belongs to phenolics class of compounds, adds the bioactivity of the lichen species under studied [53,54]. The lichen extract also contains saponin, which are having importance for their anti-inflammatory, anti-microbial, anti-tumor, and anti-ulcer activities [55-57]. Steroid also has been reported for its antimicrobial activity [58]. The presence of alkaloids also adds the pharmacological activities of the plants as analgesics, anticancer, anti-inflammatory, and antimicrobial activity [59]. Maximum number of phytochemicals extracted in methanolic extract of the selected lichen, similar to the other findings [60-64].

The methanolic extract of *D. aegialita* and *P. praesorediosum* showed 93.1% and 86.4% scavenging activity, respectively, at concentration 150 µg/ml, resonating to the earlier studies [65-67]. Among both the species, *D. aegialita* showed better scavenging potential as compared to *P. praesorediosum*. The presence of flavonoids in both species is correlated by their antioxidant potential, which supports the findings of other studies [68-71].

During the present investigation, antimicrobial activity of the methanolic extract of *D. aegialita* and *P. praesorediosum* was examined

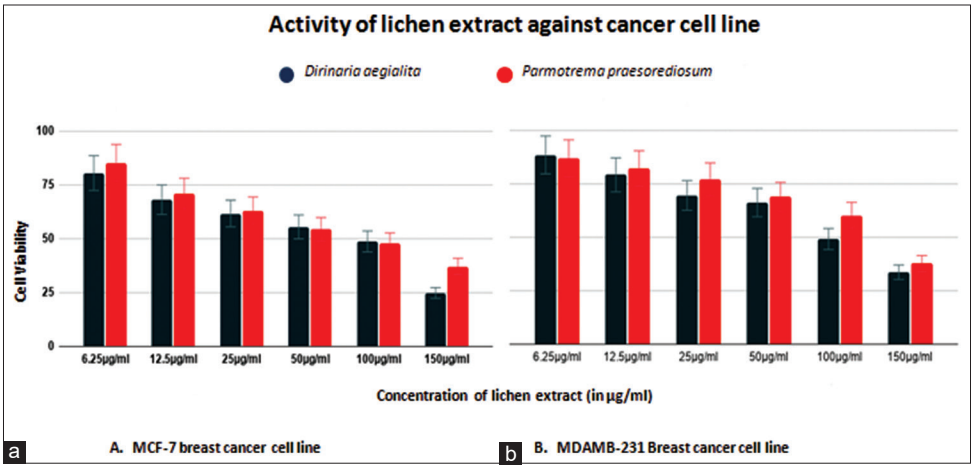

Figure 9: Activity of *Dirinaria aegialita* and *Parmotrema praesorediosum* extract *against* MCF-9 (a) and MDAMB-231 (b) breast cancer cell line.

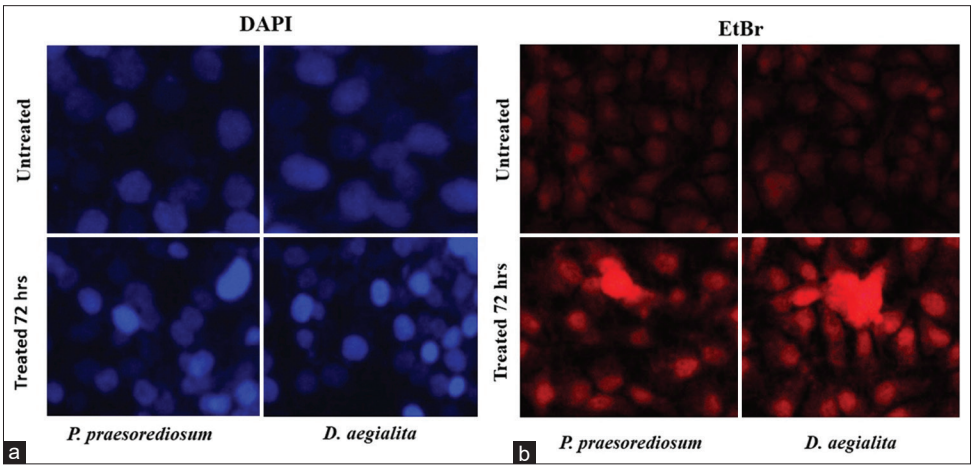

Figure 10: DAPI (a) and ethidium bromide (b) staining to morphologically detect apoptosis in MCF-7 cell line.

against six pathogens, since methanol is responsible for maximum extraction of phenolics and flavonoids compounds, thereby yielding maximum antimicrobial activity [72,73]. The findings showed that the test lichen extracts possessed very strong antibacterial activities as compared to antifungal activity. The Gram-positive bacteria were observed to be more susceptible toward the lichen extract taken, as compared to the Gram-negative strain, although they possess thick cell wall. The less sensitivity of Gram-negative bacteria can be explained by the presence of additional membrane with appendices and several pores, which makes them resistant to antibiotics [74]. The growth of microorganism is inhibited by their mechanism of enzyme action. This inhibitory mechanism may be carried out by the reaction between oxidizing compounds and the functional groups of proteins, alternatively this leads to the phenolics toxicity [75]. Antimicrobial compounds tested *in vitro* have been proven to be effective against a wide range of bacteria. This action is likely due to their capacity to form complexes with extracellular and soluble proteins, as well as with bacteria's cell walls, resulting in protein inactivation and function loss [76]. Microbial membranes may also be disrupted by more lipophilic flavonoids [77]. As a result, the potential of flavonoids as an antibacterial agent is interesting. Among the lichen species under the study, *P. praesorediosum* was found to have more antimicrobial activity than that of *D. aegialita* against all the pathogen tested. This finding was in line with another study that found that extracts of acetone, methanol, petroleum ether, and diethyl ether from three species of foliose lichen, *Dirinaria picta*, *Dirinaria papillulifera*, and *Dirinaria applanata*, had antimicrobial activity against human pathogenic bacteria and fungi using a well-diffusion method against human pathogenic bacteria and fungi [78,79]. Earlier reports also indicated that extracts of *P. praesorediosum* in methanol, acetone, hexane, and dichloromethane have inhibitory action against bacterial and fungal diseases [80].

Cancer is a public health problem in both developing and developed countries. Despite new scientific advancements, cancer claimed the lives of approximately 9.6 million people in 2018 [81]. Because of the negative side effects of chemotherapy and radiation therapy, scientists have turned to natural compounds as an alternative. Lichens have recently claimed an increasing interest in medication research because they are a symbiotic partnership of algae and fungus that produce a variety of secondary compounds. For ages, lichens have been utilized by the conventional healers [82,83]; however, there are just a few anticancer reports accessible. This is the first anticancer report of *D. aegialita* against the breast cancer cell line. The anticancer activity of *D. aegialita* is more significant than that of *P. praesorediosum*, which can be correlated to its antioxidant activity toward free radical similar to the studies on *Dirinaria consimilis* [84]. The apoptotic process was visualized through binding of ethidium bromide and DAPI to the damaged cell.

5. CONCLUSION AND FUTURE PROSPECTIVE

Among both the lichen species, *P. praesorediosum* showed higher antimicrobial activity, while *D. aegialita* showed higher antioxidant potential together with higher anticancer activity. The *Dirinaria aegialita* also shows more effectiveness than that of *Parmotrema praesorediosum*, in the preliminary investigation in terms of cell line study. The study suggests further investigation to identify the compounds and their mechanism of action toward cancer cell lines and against pathogenic strain of microorganisms for the future clinical perspective to counter cancer as well as infectious pathogenic disease. Both the species studied for their antimicrobial, antioxidant, and anticancer properties grow luxuriantly in tropical and lower temperate regions of India. As evident from the preliminary biological screening studies, both the species were observed to have good pharmaceutical potential and need further intensive investigation to identify the potent bioactive molecule for development of phytochemical for human welfare.

6. ACKNOWLEDGMENT

The authors would like to thank and acknowledge the Center for Excellence in Natural Products and Therapeutics, Department of Biotechnology and Bioinformatics, Sambalpur University and CSIR-National Botanical Research Institute, Lucknow for the necessary laboratory facilities.

7. AUTHORS' CONTRIBUTIONS

SP and RKM had conceptualized the idea, worked and prepared the data, and results along with the draft manuscript and RKM, DKU, and KBS have edited final draft manuscript. All authored have written, reviewed, and edited the manuscript.

8. FUNDING

There is no funding to report.

9. CONFLICTS OF INTEREST

All authors have read the Journal's policy on authorship agreement and disclosure of potential conflicts of interest. They have none to declare.

10. ETHICAL APPROVALS

This study does not involve experiments on animal and human subjects.

11. DATA AVAILABILITY

The authors confirmed that all the relevant data are included in the article.

12. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:
Pradhan S, Upreti DK, Meher RK, Satapathy KB. Antimicrobial, anticancer, and antioxidant potential of two dominant macro-lichen *Dirinaria aegialita* and *Parmotrema praesorediosum* collected from Similipal Biosphere Reserve of Odisha, India. J App Biol Biotech. 2022;10(6):34-43.
DOI: 10.7324/JABB.2022.100604