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Research Article

EVALUATION OF ANTIMICROBIAL, ANTIOXIDANT AND ANTICANCER ACTIVITIES OF FEW MACROLICHENS COLLECTED FROM EASTERN GHATS OF TAMIL NADU, INDIA

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ABSTRACT

The aim of the present study is to investigate the bioactivities of various solvent extracts of lichens collected from Eastern Ghats of Tamil Nadu, India. The lichen samples were identified based on color tests, chemical profiling and morphological and anatomical characteristic features. Lichens such as *P.grayanum, P.tinctorum, P.reticulatum* and *P.austrosinense* were selected and analyzed for their biological activities. Among the tested lichen samples, methanol extract of *P.tinctorum* and *P.austrosinense* showed a strong antibacterial activity with the inhibition rate of 15.13±0.76 and 13.9±0.1mm respectively. *P.reticulatum* indicated a strong antifungal activity with the inhibition zone of 10.17±0.96mm followed by *P.austrosinense* and *P.tinctorum*. Antioxidant study revealed that IC_{50} value of acetone extracts of *P. tinctorum* showed higher antioxidant activity with IC_{50} of 17.52 µg/ml than *P.austrosinense* exhibited IC_{50} value of 26.39 µg/ml. These activities were confirmed through *in vitro* cytotoxic studies in which *P. austrosinense* registered the maximum control over HeLa cell lines with the IC_{50} value of 39.06µg/ml.

Key words: Lichens, antimicrobial, antioxidant, lichen compounds, macrolichens, anticancer activities.

INTRODUCTION

Long-term use of synthetic drugs causes numerous side effects and sometimes resistances to the drugs. Lichens have economic benefits to human beings which has antibiotic properties that are valuable commercially for biomedical applications. Lichens and their metabolites yield significant bioactive substances for the treatment of various human diseases caused by different pathogenic microorganisms. There are about 2040 species of lichens present in India[1] in which around 10 lichen species are being used for food, more than 15 lichens used for biomedical applications and few are employed for environmental monitoring and natural dye extraction purposes. Lichens can be efficiently used for monitoring the level of pollution in the atmosphere and analysis of lichen samples can be used to estimate the extent and pollutant emissions around an industry or a particular locality[2]. Lichens provide warning signal before severe damages occur on ecosystem and health.

It has been observed that the majority of lichen populations are unexplored for commercial exploitation[3]. Lichens can produce a wide array of both intracellular and extracellular compounds. These lichen compounds are called secondary metabolites which are extracellular in nature often called lichen acids. These are unique to lichens. Since lichens are composed of mycobionts and phycobionts, the former are playing pivotal role for the production of secondary metabolites[4]. The quantities of these secondary metabolites may vary up to 30% of the dry weight of the lichen thalli. So far number of lichen compounds have been extracted for various investigations. Lichen secondary metabolites comprise of amino acid derivatives, aromatic compounds, dibenzofurans, depsides and depsidones etc. which exhibited manifold biological activities such as antibiotic, anti-inflammatory, analgesic, antipyretic and cytotoxic activities[5,6]. The aim of the present study is to evaluate the antimicrobial, antioxidant and anticancer activities of the acetone and methanol extracts of few lichens collected from Eastern Ghats of Tamil Nadu, India.

MATERIALS AND METHODS

Collection and identification of lichen samples

Lichen samples were collected from Yercaud and Kolli hills belonging to Eastern Ghats of Tamil Nadu for the present study. The collected lichen samples were dried and preserved for further study[7]. Moreover, lichen samples were identified by spot tests and chemical profiling along with morphological and anatomical characters. The spot test was carried out by direct application of the reagents such as aqueous solution (5%) of potassium hydroxide (K), 2% calcium hypochlorite (C) and solution of KC (application of K, quickly followed by C).

Preparation of lichen extracts and phytochemical analysis

The lichen extracts were prepared by cold maceration method using acetone, methanol, toluene, chloroform, benzene and water. Approximately 5 gm of lichen material was soaked in 25 ml of selected solvent and kept at room temperature on a rotary shaker for 24 hours. This solution was filtered by Whatman No. 1 filter paper. The filtrate was used for the phytochemical screening. The presence of phytochemical compounds of various solvent extracts were tested for the presence of various phytochemical

constituents such as tannins, glycosides, alkaloids, saponins, flavonoids, terpenoids, phenols, carbohydrates and proteins by standard methods.

Antimicrobial activity of lichen extracts

The extracted compounds were then analyzed for the antimicrobial activity. The test microorganisms such as *E.coli, B.subtilis, S.aureus, A.flavus* and *A.niger* were maintained on Muller-Hinton agar and potato dextrose agar (PDA); respectively for bacterial and fungal cultures. Antimicrobial ability of the lichen extracts were carried out by well-diffusion method[8]. The plates were inoculated with 50 µl (1 mg/ml) of lichen extracts and 30mg of tetracycline were added to the respectively labelled wells. The plates are allowed to stand for 30 minutes and were incubated at 37°C for 24 hours and the zone of inhibition was recorded[9]. For fungal isolates, the spore suspension of test fungi was swab inoculated aseptically on the sterile PDA plates. Nystatin was used as standard antifungal agent. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Experiment was carried in triplicates and average values were recorded.

Minimum inhibitory concentration (MIC) was determined by Micro well dilution method[10], all the lichen species including *P.grayanum, P.tinctorum, P.reticulatum* and *P.austrosinense* against *E.coli, B.subtilis, S.aureus, A.flavus* and *A.niger.* The test samples were dissolved in 10% DMSO and the selected bacterial and fungal cultures were prepared using nutrient broth and PD broth and incubated for 24h and 72h respectively. A series of dilutions with concentrations ranging from 10 to 50 µg/ml of was used against selected bacterial and fungal cultures. The 96 well plates were prepared by adding 100 µl of bacterial and fungal culture with various concentrations of extracts (acetone and methanol) and kept for incubation for 12 h. The absorbance was measured at 600 nm.

Antioxidant activity
DPPH assay

Free radical scavenging effect of lichen samples were determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH) method [11]. 50µL of various concentrations of the extracts in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. After that the absorbance was measured at 517 nm using spectrophotometrically. The percentage of inhibition was calculated by

$$\% \text{ Inhibition} = (A_{control} - A_{sample} / A_{control}) * 100$$

The inhibition concentration (IC_{50}) was calculated to compare the radical scavenging activity.

Reducing power[12]

Reducing power of lichen extracts were determined. One milliliter of extract (1 mg/mL) was mixed with 2.5 mL of phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 1%). The mixture was incubated for 20 min at 50^0 C. Then, trichloroacetic acid (10%, 2.5 mL) was added and the mixture centrifuged. Finally the upper layer was removed and mixed with distilled water (2.5 mL) and FeCl3 (0.5 mL; 0.1%). The absorbance of the solution was measured at 700 nm using spectrophotometer. Ascorbic acid was used as positive control.

Cytotoxicity assay

HeLa (cervix adenocarcinoma) cell lines were obtained from National Centre for Cell and Science, Pune, India for the present study. The cell lines were sub-cultured and maintained at CO_2 Incubator at 37ºC. Then it was subjected to treat with various concentrations of crude extracts at various concentrations to analyze the cytotoxicity using MTT assay[13]. The inhibitory concentration (IC_{50}) was calculated for the various solvent extracts against the cancer cells. Cell count and viability was done using hemocytometer and trypan blue assay[12]. Cell death and viability was calculated by the following formulae:

$$\text{Cell death} = [(C - T) / C] * 100$$

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary phytochemical screening revealed that methanolic and acetone extracts showed the presence of most of the phytochemical constituents present in lichen thallus including alkaloids, glycosides, flavonoids, carbohydrates, proteins and saponins (Table 1). The results were coincided with the report of Rashmi et al. (2014)[14]. Methanolic extract of *P.tinctorum* and *P. austrosinense* and acetone extract of *P.tinctorum* were confirmed the presence of phenols.

Antimicrobial activity

Antimicrobial activity of lichen extracts against bacterial and fungal strains were presented in the Table 2. Methanolic and acetone extracts of lichen species revealed significant antibacterial effect against the tested organisms. *P.grayanam* and *P.tinctorum* showed significant antibacterial activity against *P.aureus*[15], it was evident from the zone of inhibition values of 10.83±1.26 mm and 10.23±0.75mm respectively. The antibacterial activity of *P.tinctorum* is due to the presence of atronorin and lecanoric acid. *P.austrosinense* was found to be active against *B.subtilis* with the inhibition zone of 13.9±0.1mm; *P.tinctorum* showed positive response against *B.subtilis* and *A.flavus* with the inhibition of 15.13±0.76 mm and 6.5±0.87 mm; respectively. *P.reticulatum* showed significant activity against *A.flavus*. *P.tinctorum* was found to be effective against *E.coli*[16]. No activity was recorded for *P.austrosinense* and *P.tinctorum* against *A.niger*. Hence, it is evident that *Parmatrema* lichens are promising microbial agents. The MIC values of tested lichen species were showed in the Table 3 which indicated almost all the selected lichen species shown antimicrobial activity against the tested organisms.

Antioxidant activity

Antioxidant activities determined by DPPH radical scavenging power and ferric ion reducing power were presented in the Table 4. Antioxidant activity of lichens are evidenced by the presence of phenols[17]. A decrease in absorbance of the reaction mixture indicated a higher free radical scavenging activity. From the value of inhibitory concentration at 50% inhibition, methanol extract of *P.tinctorum* and *P.austrosinense* showed greater radical scavenging activity with IC_{50} value of 17.52 µg/ml and 26.39 µg/ml respectively. The increasing absorbance showed the increasing reducing power. The results of reducing power assay indicated that acetone extract of *P.reticulatum* and methanolic extract of *P.austrosinense* exhibited greater reducing power. *P.austrosinense* can be used as natural antioxidant sources[18]. Methanol and ethanol extracts of *P. reticulatum* have shown DPPH radical scavenging activity[19]. The presence of phenols confirmed the antioxidant property of lichens and it is important

to confirm the phenolic contents in order to justify their contribution to antioxidant activity[20]. The antioxidant property of lichens were already confirmed by several studies[21].

Cytotoxicity assay

The cytotoxicity effect of the methanolic lichen extracts of *P.austrosinense* and *P.tinctorum* was shown in the Table 5. These two extracts were selected based on their result of free radical scavenging activity. Better cytotoxicity activity was exhibited by *P.austrosinense* over HeLa cell lines with the IC_{50} value of 39.06 µg/ml. Lichen compounds are responsible for anticancer activities in which the growth of cells are inhibited significantly[22,23]. Bogo et al (2010)[24] reported that *P.tinctorum* had good anticancer activity against HEp-2 larynx carcinoma, MCF7, 786-0 and B16-F10 cell lines. It has been reported that lichen compounds are having inherent properties towards growth inhibition of tumor cells[25].

Table 1: Phytochemical analysis of lichen species

Phytochemical tests	*Parmotrema grayanum*						*Parmotrema reticulatum*						*Parmotrema austrosinense*						*Parmotrema tinctorum*					
	T	M	W	B	C	A	T	M	W	B	C	A	T	M	W	B	C	A	T	M	W	B	C	A
Tannins	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+
Alkaloids	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+
Terpenoids	-	+	-	-	-	+	-	+	-	-	-	-	-	+	-	+	+	+	-	+	-	-	+	+
Glycosides	-	+	+	+	-	+	-	-	+	-	+	+	+	-	-	-	+	+	-	-	+	-	+	+
Carbohydrates	+	+	-	-	+	-	+	+	-	+	+	-	-	+	-	-	+	+	+	+	-	-	+	-
Flavonoids	-	+	-	+	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-	+	-	-	+	-
Proteins	-	-	-	-	-	-	+	+	-	+	+	+	-	+	-	-	-	-	-	+	-	+	+	+
Saponins	-	-	-	+	+	-	-	-	-	+	+	-	+	-	-	+	+	-	-	-	-	+	+	-

Table 2: Zone of inhibition by lichen species extracts through agar well diffusion method against various microorganisms

Test organisms	Zone of inhibition (mm)			
	Parmotrema grayanum	*Parmotrema tinctorum*	*Parmotrema reticulatum*	*Parmotrema austrosinense*
E.coli	9.67±1.53	11.83±1.04	8.53±0.5	9.83±0.15
B.subtilis	2.50±0.50	15.13±0.76	2.97±0.25	13.90±0.10
S.aureus	10.83±1.26	10.23±0.75	2.73±0.70	7.87±0.23
A.flavus	6.33±1.53	6.50±0.87	10.17±0.96	8.77±0.21
A.niger	3.67±1.53	ND	8.23±1.08	ND

ND - Not detected

Table: 3 Minimum inhibitory concentration (MIC) of lichen extracts against various microorganisms

Test organism	Acetone extract (µg/ml)				Methanol extract (µg/ml)			
	P. grayanum	*P. reticulatum*	*P. austrosinense*	*P. tinctorum*	*P. grayanum*	*P. reticulatum*	*P. austrosinense*	*P. tinctorum*
E.coli	10	10	40	10	20	20	20	10
B.subtils	10	10	30	10	10	10	10	10
P.aureus	10	30	10	10	30	20	40	10
A.flavus	40	10	40	30	40	20	10	20
A.niger	10	10	10	20	10	10	10	20

Table: 4 Antioxidant activities of lichen species

Lichen species	DPPH radical scavenging activity IC_{50} (µg/ml)		Reducing power Absorbance at 700 nm	
	Acetone	Methanol	Acetone	Methanol
Parmotrema grayanum	74.48	1451	0.617	0.401
Parmotrema reticulatum	34.45	1101.7	1.686	0.313
Parmotrema austrosinense	26.39	1178.7	1.393	1.117
Parmotrema tinctorum	17.52	1059.7	1.359	0.215

Table: 5 Anticancer activity of methanolic extracts of lichen species

Lichen Samples	*Parmotrema austrosinense*		*Parmotrema tinctorum*	
Concentration	25 µg/ml	50 µg/ml	25 µg/ml	50 µg/ml
Cell death	72.54	32.47	78.92	36
Cell viability	27.45	67.52	21.07	64
IC_{50} (µg/ml)	39.06		41.88	

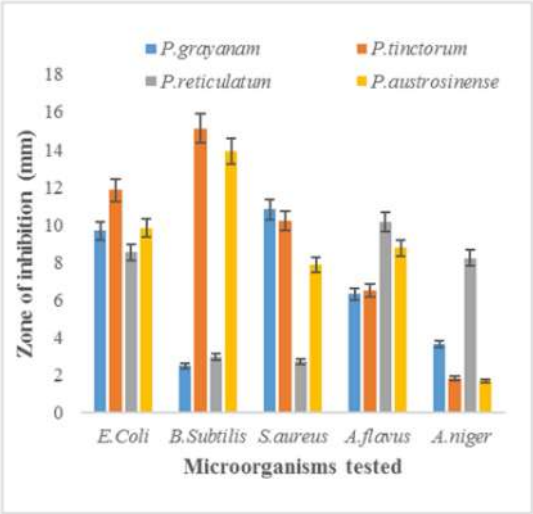

Figure 1: Zone of inhibition for lichen extracts against tested organisms

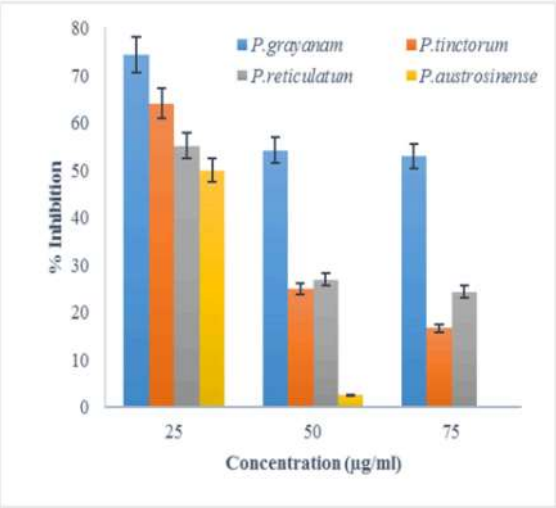

Figure 2: Percentage inhibition of various lichen extracts

CONCLUSION

Even though synthetic drugs have been widely used to treat various diseases, continuous and prolonged usage of these drugs cause undesired side effects which in turn leads to the need for discovering formulations of natural origin. Hence lichens become the potential source to get promising drugs molecules to prevent various life threatening diseases. The present study revealed that salazinic acid and chloroatronorin, lecanoric acid and atronorin are the compounds responsible for the biological activities of lichen species. Further studies on isolation of bioactive lichen compounds without depleting the natural resource becomes the promising sector of lichen research.

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REFERENCES

1. Awasthi DD, Bishen Singh, Mahendra Pal Singh A. Compendium of the Macrolichens from India, Nepal and Sri Lanka. The Bryologist 2007; 1-580.
2. Negi HR. Lichens A Valuable Bioresource for Environmental monitoring and sustainable development, Resonance2003; 1:51-58.
3. Ayyappadasan G, Madhu Preetha T, Ponmurugan P, Jeya Prakash S, Nayaka S. Antioxidant and antibacterial activity of Parmeliod lichens from Shevaroy hills of Eastern Ghats, India. International Journal of Pharm Tech Research 2015; 8 (9): 13-23.
4. Ponmurugan P, Ayyappadasan G, Verma RS Nayaka S. Survey, distribution pattern and elemental composition of lichens in Yercaud hills of Eastern Ghats in southern India. Journal of Environmental Biology 2016; 37: 407-412.
5. Kosanic M, Rankovic B, Stanojkovic T. Antioxidant, antimicrobial and anticancer activity of Umbilicaria species. Journal of Food Science 2012; 71:T20-T25.
6. Manojlovic N, Vasiljevic P, Gritsanapan W, Supabphol R, Manojlovic I. Phytochemical and antioxidant studies of Laurera benguelensis growing in Thailand. Biological Research 2010; 43: 169-176.
7. Nayaka S. Methods and techniques in collection, preservation and identification of lichens. In plant Taxonomy and Biosystematics–Classical and Modern Methods (Eds T.S. Rana, K.N. Nair and D.K. Upreti). New India Publishing Agency, New Delhi 2014; 101-128.
8. Kaur S, Mondal P. Study of Total phenolic and flavonoid content, antioxidant activity and antimicrobial properties of medicinal plants. Journal of Microbiology & Experimentation 2014; 1(1): 00005
9. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 2015; 45: 493 -496.
10. Sahin F, Karaman I, Gulluce M, Ogutcu H, Sengul M,, Adiguzel a, Azturk S, Kotan R. Evaluation of antimicrobial activities *Satureja hortensis L.* Journal of Ethnopharmacology 2002; 87: 61-65.
11. Dorman HJ, Bachmayer O, Kosar M, Hiltunen R, Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. Journal of Agricultural and Food Chemistry 2004; 52:762–770.
12. Oyaizu, M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 1986; 44: 307-315.
13. Mosmann T. Rapid colorimetric assay for cellular growth and survival; application to proliferation and cytotoxicity assays Journal of Immunological methods 1983; 65:55-63.
14. Rashmi S, Rajkumar HG. Preliminary phytochemical screening of different solvent extracts of lichens from Kodagu district, Karnataka. Journal of Pharmacognosy and Phytochemistry 2014; 3 (4): 209-212.
15. Ramya K, Thirunalasundari T. Pharmaceutically relevant metabolites from Lichens of Kodaikanal, India. International Journal of Current Microbiology and Applied Sciences 2011; 3(11):779-788.
16. Ayyappadasan Ganesan, Madhu Preetha Thangapandian, Ponmurugan Ponnusamy1, Jeya Prakash Sundararaj, Sanjeeva Nayaka. Antioxidant and antibacterial activity of Parmeliod lichens from Shevaroy hills of Eastern Ghats, India. International Journal of Pharm Tech Research 2015; 8(9): 13-23.

17. Behra BC, Verma N, Sonone A, Makhjia U. Evaluation of antioxidant potential of the cultured mycobionts of a lichen *Usnea ghattensis*. Phytotherapy Research 2005; 19: 58-64.
18. Subhash Gaikwad, Neeraj Verma, Sharma BO, Behra BC. Growth promoting effects of some lichen metabolites on probiotic bacteria. Journal of Food Science and Technology 2012; 51(10):2624-2631.
19. Sharma BC, Kalikoty S. Screening of antioxidant activity of lichens Parmotrema reticulatum and Usnea sp. from Darjeeling hills, India. IOSR Journal of Pharmacy 2012; 2(6): 54-60.
20. Choi Y, Jeong H, Lee J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chemistry 2007; 103: 130-138.
21. Ayyappadasan Ganesan, Deepak Kumar Purusothaman, Uthira Muralidharan, Rubavathy Subbaiyan. Metabolite profiling and *in vitro* assessment of antimicrobial and antioxidant activities of lichen Ramalina inflate. International Research Journal of Pharmacy 2016; 7(12):132-138
22. Bucar F, Schneider I, Ogmundsdottir H, Ingolfsdottir K. Anti-proliferative lichen compounds with inhibitory activity on 12(S)-HETE production in human platelets. Phytomedicine 2004; 11(7-8): 602-606.
23. Burlando B, Ranzato E, Volante A, Appendino G, Pollastro F, Verotta L. Anti-proliferative effects on tumor cells and promotion of keratinocyte wound healing by different lichen compounds. Planta Medica 2009; 75:607–613.
24. Bogo D, Matos MFC, Honda NK, Pontes EC, Oguma PM, Santos ECS, De Carvalho JE, Nomizo A. *in vitro* antitumor activity of orsellinates. Journal of Biosciences.2015; 65: 43–48.
25. Jayaprakasha GK, Rao LJ. Phenolic constituents from the lichen Parmotrema stuppeum (Nyl.) Hale and their antioxidant activity. A Journal of Biosciences 2000; 55: 1018-1022.

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