Research Article

METABOLITE PROFILING AND *IN VITRO* ASSESSMENT OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF LICHEN *RAMALINA INFLATA*

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ABSTRACT

Lichen, a symbiotic form of fungi and algae has been known for its metabolites, but, much of the metabolites remain unexplored. It's excellent biomedical potential with active substances are being applied for medicinal purposes for the past few years. By considering the active compounds, the present study aims at reporting the anti-microbial and anti-oxidant activities of less explored lichen, *Ramalina inflata*. Lichen sample was collected from Kolli hills of Eastern Ghats in the range of above 1000MSL. Further, it was subjected to extraction (Soxhlet) using five solvents and purification of bioactive metabolites. The result indicated the compounds that were present in the different extracts were found to be salazinic acid, divaricic acid and some triterpenes. Antimicrobial activity of methanolic extract showed a high zone of inhibition against *Klebsiella pneumoniae* (13.01±0.12 mm). Petroleum ether showed the best inhibition against *Staphylococcus aureus* with 10.17±0.07 mm and against *Candida krusei* with an inhibition of 8.47±0.08 mm. Antioxidant assays of Phenolics, DPPH, Ferric reducing power assay, CUPRAC, and Phosphomolybdenum assay were done for various crude extracts. The result observed that benzene extract showed a high content of total phenolics, which was 30.790±0.903 µg/ml whereas, its percentage of inhibition in DPPH assay was 12.987%. The petroleum extract of *R. inflata* showed a moderate activity in ferric ion reducing power assay and molybdenum reducing assay and a very low cupric ion reducing capability. Methanol extract showed a high cupric ion reducing power with an optical density of 0.829±0.036.

Keywords: *Ramalina*, Salazinic acid, DPPH, CUPRAC, metabolite profiling.

INTRODUCTION

The world is in need of alternative safe medications and therapeutics due to the increasing rate and incidence of diseases. Since the primordial age, plants have been used widely in therapeutics which are still continuing to serve their purpose as medicines. Due to the advent of industrial bioprocess technologies, large quantity of commendable medicines was obtained from microorganisms too. The first microorganisms to be used as medicines was fungi after which, it has been investigated widely to have several medicinal uses such as anti-microbial, anti-oxidant, anti-inflammatory, anti-diabetic, etc.,. One such type of fungi is lichen which is a symbiotic relationship or restricted parasitism¹ between a fungus and one or more algae. The algal partner, which is the source of nutrition for the fungus may be either green or blue-green. Lichens are less explored area for valuable metabolites. One such lichen is *Ramalina inflata* (Ramalinaceae), a fruticose lichen and this study will reveal its pharmaceutical uses. As lichen metabolites are very unique, a separate chromatographic system must be adopted in order to purify the individual compounds. The present study reports the extraction, purification and analysis of unique bioactive metabolites from lichen symbionts for various biomedical applications. Most of the valuable compounds of lichens are the secondary metabolites of fungal partner which are mostly insoluble in water and can be extracted with organic solvents². Majority of the compounds are from the acetyl-polymalonyl pathway in lichen whereas other pathways such as shikimic acid and mevalonic acid pathways also contribute certain compounds^{3, 4}. In order to prevent the colonization of other microorganisms, lichens produce certain secondary

metabolites, for example, usnic acid. The metabolites of lichen have antibiotic properties⁵ and the novel metabolites are important in the current scenario as the development of multidrug resistant pathogenic bacteria poses as a serious problem in the pharmaceutical industry due to the recurrent use of conventional antibiotics⁶. Hence the antibiotic activity of the lichen *Ramalina inflata* has been investigated in the present study.

Free radicals are highly reactive atoms which are formed as a result of imbalance of homeostasis of anti-oxidants⁷. The free radicals serve various purposes of the body such as cell signalling, energy production, phagocytosis, and generation of biologically important compounds⁸, but, at high concentrations, the unstable radicals have the ability to damage a large number of biomolecules of the body by the process of chain oxidation reaction. This is called as oxidative stress and this leads to different chronic diseases such as cancer, diabetes, neurodegenerative diseases, and arthritis. Antioxidants prevent the free radicals from oxidising the biomolecules, by providing with the electrons that are needed to stabilise and detoxify the radicals. The current world is searching for high potent natural drugs rather than synthetic molecules to minimize the co-effects. Plants are excellent source of antioxidants and lichens are also investigated to have good antioxidant properties. We have reported the antioxidant activities of the Petroleum ether, Benzene, 2-propanol, Ethyl acetate and Methanol extracts of the lichen *Ramalina inflata*, a valuable lichen species collected from Kolli and Shevroy hills of Tamilnadu, by performing a number of radical scavenging assays.

MATERIALS AND METHODS

Chemicals and reagents

Folin & ciocalteu's phenol reagent (L. R.), Pyrocatechol dimethyl ether, 2, 2-Diphenyl-1-picrylhydrazyl, Trichloroacetic acid (A. R.), Potassium ferricyanide (A. R.), Neocuproine (A. R.), Ammonium molybdatetetrahydrate (A. R.), 3, 5-Dinitrosalicylic acid (A. R.) were purchased from Ponmani & Co., Coimbatore. All other chemicals used in this study were of analytical grade.

Sample Collection and Identification

Lichen samples were collected from various areas of Kolli and Shevroy hills which are parts of the Western Ghats in Southern India, at an altitude of 800 MSL – 1250 MSL. The collected samples were subjected to different procedures⁹ such as Morphological observations, K, C, KC and PD biochemical tests, Chromatographic separation tests for their identification. Once the lichens were identified, the samples were dried under shade conditions for about 48-72 hours and were packed in standard measure 25*35 cm Herbarium sheets. The Herbarium sheets must be acid free and must remain in a neutral pH¹⁰. The samples were maintained at the Lichenology Herbarium of K. S. Rangasamy College of Technology, Tiruchengode (accession number KSRBT08).

Compound extraction

The lichen Herbarium with the voucher specimens of *R. inflata* were subjected to compound extraction¹⁰. A solid-liquid extraction setup was followed and the Soxhlet apparatus served for the purpose¹¹. The extraction was carried out at 50° C and the organic solvents like Petroleum ether, Benzene, 2-Propanol, Ethyl acetate, Methanol were used since the secondary metabolites of lichen are poorly soluble in water. 1.5 g of lichen thallus was taken with 150 ml each of the above-mentioned solvents. Once the extraction was over, the compounds were dried completely for the evaporation of solvents. The extracted compounds were weighed and properly stored under inert conditions.

Preparation of crude extract and determination of lichen metabolites

The working stock was prepared by dissolving 10mg of the extracted compound with 10ml of corresponding solvent for the complete dissolution of the extract and was maintained at 4°C in a refrigerator for further analysis. The lichen metabolites were observed using the standard optimized Thin Layer Chromatography (TLC).

Thin Layer Chromatography (TLC)¹²

To perform TLC, commercially available silica-coated plates made of aluminium sheets (Silica Gel 60F₂₅₄, Merck) were employed. The loaded plates were exposed to optimized solvent systems (Solvent System A & C) consisting of Toluene, Dioxane and Acetic acid in the ratio of 180:45:5 and Hexane, Diethyl ether and Formic acid in the ratio of 130:80:20 respectively with a drop of formic acid in each. Once the solvent has reached the maximum distance of 13 cm, the TLC plate was taken out and the R_f values were calculated.

$R_f = (\text{Distance travelled by the compound} / \text{distance travelled by the solvent front})$

The plates were observed under UV Light to detect the UV absorbing compounds and the dried plates were sprayed with 10% sulfuric acid and heated in an oven at 110°C, till the development of spots. On comparison with the R_f values and colours of standard lichen compounds, the separated compounds were identified.

Evaluation of Antimicrobial properties¹³

Antimicrobial property was evaluated by the agar well diffusion method. For the assessment of antibacterial property, Mueller Hinton Agar plates were prepared for five extracts against four pathogenic organisms- *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*. Similarly, Potato Dextrose Agar (PDA) plates were prepared for *Candida tropicalis*, *Candida krusei*, *Aspergillus flavus*, *Aspergillus fumigates*. Chloramphenicol, a commercial antibiotic was used as the positive control for bacteria and Amphotericin was used as a control for fungi. The diameter of the inhibition zone (measured in millimetre) produced by each extract was measured and compared against the control antibiotic to determine the antimicrobial capability of the extracts.

Estimation of Antioxidant potentials

The ability of the lichen extract to convert an unstable free radical into a stable one is termed as its antioxidant potential. Various assays have been devised to assess the antioxidant activity of extracts. The five basic assays to determine the antioxidant capacities of the five lichen extracts obtained were performed.

Total Soluble Phenolics¹⁴

The amount of total phenolics in a sample will help to assess its antioxidant activity. Total soluble phenolics in the different soxhlet extracts were determined by the Folin-Ciocalteu (FC) method described by Slinkard & Singleton, using Pyrocatechol as a standard. The absorbance was measured at 760 nm.

$$\text{Absorbance} = 0.001 \times \text{Total phenols in extract} \\ (\mu\text{g pyrocatechol}) + 0.0033$$

The total phenolic content (TPC) of each of the extracts were calculated from the above equation which was obtained from the standard graph of Pyrocatechol and the values were tabulated.

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay¹⁵

The underlying principle of this method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donor (antioxidant) which results in the formation of the non-radical form of DPPH which is DPPH-H¹⁶. Khalaf *et al.*, method of DPPH assay was followed. Ascorbic acid was used as a standard in the increasing concentration range of 20-100 µg/ml. The optical density was measured at 517 nm using Hitachi-U2900 Spectrophotometer. The solution mixture without sample/standard served as the control. The Percentage inhibition was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = [(A_c - A_t)/A_c] \times 100$$

where, A_c - absorbance of the control,
A_t - absorbance in the presence of sample/standard

A graph between concentration and DPPH scavenging effect of the five lichen extracts was plotted.

Reducing power assay¹⁷

Those compounds with reducing power must be electron donors and hence reduce the oxidized intermediates, so that the compounds can act as significant antioxidants¹⁸. The Reducing power assay was done for the lichen extracts as per a previously described protocol. The absorbance was measured at 700 nm. Ascorbic acid in the concentration range of 20-100 µg/ml was used as standard and the mixture without sample/standard served as the control.

$$\text{Increase in reducing power (\%)} = (A_t - A_b / A_b) * 100$$

where, A_t = Absorbance of test, A_b = Absorbance of blank

A graph was plotted between concentration and absorbance of the test samples at 700 nm.

Cupric ion reducing antioxidant capacity (CUPRAC) assay¹⁹

The chromogenic oxidising agent used in this method, bis (neocuproine) copper (II) chloride reacts with polyphenols where the protons released are buffered with the relatively concentrated ammonium acetate buffer solution. In this reaction, the aromatic hydroxyl groups of the polyphenols are oxidized to the corresponding quinones and the neocuproine reagent is reduced to a highly coloured chelate²⁰. A control was prepared

without the presence of cupric chloride. The absorbance was taken at 450 nm. Ascorbic acid was used as the standard. A graph between the concentration of the extracts and their corresponding absorbance was plotted.

Phosphomolybdenum assay²¹

The basic principle of this method is based on the reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V) by the sample at acidic pH which results in a green coloured complex²². The procedure followed was as described by Berk *et al.*, The final absorbance was taken at 695 nm. Ascorbic acid (10 mg/ml in Dimethyl sulfoxide (DMSO)) was used as standard and the mixtures without the sample was taken as the control. The total antioxidant activities of the samples were expressed as ascorbic acid equivalents. A graph between the concentration of the extracts and their absorbance at 695 nm was plotted.

Statistical analysis

All the experiments and assay procedures were done in triplicates. Graph Pad Prism version 5.01 and MTR test were done for the conduction of statistical analyses. All the graphs were plotted using the mean values with the error bars shown for each point in the graph.

Table 1: Details of the compounds present in the five lichen extracts which were separated using TLC

Spot number	Extract	Inference after comparison with standard lichen compounds
1.	Petroleum ether (6 th spot)	Yellow Spot – Salazinic acid
2.	Benzene (7 th spot)	Upper two spots – Triterpenes Below triterpenes – Divaricatic acid
3.	2-Propanol (9 th spot)	Upper two spots – Triterpenes
4.	Ethyl acetate (5 th spot)	No detectable compounds
5.	Methanol (8 th spot)	Yellow Spot – Salazinic acid

Table 2: Measured zones of inhibition of four pathogenic bacteria by the lichen extracts

Lichen extract	Concentration (µg)	Diameter of zone of inhibition (Mean±Standard deviation) (mm)			
		<i>Klebsiella pneumonia</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
Petroleum ether	20	-ND-	7.32±0.06 ^{cl}	0.8±0.10 ^a	-ND-
	30	-ND-	9.40±0.13 ^{hi}	10.17±0.07 ⁱ	-ND-
Benzene	20	0.99±0.08 ^b	6.31±0.09 ^{cd}	5.98±0.18 ^{bcd}	0.7±0.11 ^a
	30	12.29±0.16 ^{fg}	11.36±0.07 ^j	7.97±0.05 ^c	10.0±0.14 ^{fg}
2-Propanol	20	1.02±0.14 ^{bc}	0.60±0.15 ^a	8.22±0.13 ^{ef}	6.27±0.08 ^{bcd}
	30	11.79±0.17 ^c	9.08±0.05 ^h	8.36±0.06 ^e	7.96±0.17 ^c
Ethyl acetate	20	7.26±0.08 ^d	6.20±0.11 ^c	-ND-	-ND-
	30	0.12±0.07 ^a	8.09±0.13 ^g	5.88±0.11 ^b	6.16±0.09 ^{bc}
Methanol	20	13.01±0.12 ^h	7.31±0.07 ^e	5.90±0.15 ^{bc}	6.06±0.14 ^b
	30	12.17±0.05 ^f	1.38±0.18 ^b	8.99±0.06 ^h	9.88±0.09 ^f
Chloramphenicol	30	19.04±0.05 ⁱ	14.23±0.21 ^k	28.14±0.07 ^j	25.0±0.07 ^h
SE±		0.45	0.73	0.34	1.06

-ND- Not determined

Table 3: Antifungal activity of various extracts of lichen *Ramalina inflata*

Fungal Strains	Diameter of zone of inhibition (Mean±Standard deviation) (mm)					
	Petroleum ether	Benzene	Ethyl acetate	2-Propanol	Methanol	Amphotericin
<i>Candida tropicalis</i>	7.29±0.13 ^c	5.12±0.21 ^{bcd}	6.13±0.10 ^a	8.19±0.11 ^b	7.12±0.07 ^b	13.57±0.04 ^{abc}
<i>Candida krusei</i>	8.47±0.08 ^d	4.58±0.12 ^b	7.12±0.02 ^c	9.12±0.03 ^d	6.18±0.10 ^a	12.87±0.08 ^a
<i>Aspergillus flavus</i>	4.12±0.17 ^a	3.18±0.08 ^a	6.34±0.12 ^b	6.25±0.14 ^a	7.67±0.08 ^c	13.16±0.11 ^{ab}
<i>Aspergillus fumigates</i>	5.12±0.12 ^b	4.87±0.01 ^{bc}	7.17±0.04 ^{cd}	8.76±0.20 ^e	8.12±0.11 ^d	15.89±0.10 ^d
SE±	0.38	0.94	0.19	0.29	0.67	0.48

Table 4: Concentration of total phenolics present in the five lichen extracts

Extracts	Total soluble phenolic content (Mean±Standard deviation) (µg/ml)
Petroleum ether	16.147±1.394 ^d
Benzene	30.790±0.903 ^c
2-Propanol	1.943±0.896 ^{ab}
Ethyl acetate	1.087±0.467 ^a
Methanol	9.650±1.041 ^c
SE±	2.12

Figure 1. TLC plate with the spots of separated compounds of the five crude extracts of *R. inflata* Figure 2. Percentage of inhibition of DPPH radical by *R. inflata* in various concentrations Figure 3. Ferric Reducing anion power of the crude extracts of *R. inflata*

Figure 4. Graph showing the cupric ion reducing capacity of the five lichen extracts

Figure 5. The ability of the five extracts to reduce molybdenum was assayed and demonstrated

RESULTS & DISCUSSION

Visualization of lichen metabolites using a chromatographic technique-TLC

The spots that were developed in the TLC Plate in Figure 1, after acidic heat incubation gives a clear realisation about the chemical composition of the five solvent extracts of the lichen under study. The spots obtained were compared with the standard spots of lichen compounds in terms of R_f value and the colour on the TLC plate after separation. Table 1 gives the complete results of the spots obtained.

Earlier studies have shown that *Ramalina* species have excellent inhibition of amylase activity, particularly those with salazinic acid²³. Two of the extracts, petroleum ether and methanol extracts in the present study has salazinic acid which showed a good anti-oxidant capacity. Salazinic acid, in its pure form also proved to be a successful and potent anti-diabetic agent in a previous study²⁴. So, this metabolite must have contributed for the activity of petroleum ether and methanol extracts. Benzene extract which also showed good antioxidant activity has triterpenes and divaricatic acid. But, 2-Propanol extract, which

also has triterpenes, did not show significant anti-oxidant activity. So, it can be concluded that, divaricatic acid of Benzene extract is responsible for its significant anti-oxidant properties.

Antimicrobial properties of the lichen extracts

The antimicrobial properties of the five lichen extracts which were assessed against four pathogenic bacteria in terms of the diameter of the zones of inhibition are as listed in Table 2 and against the four fungal pathogens are listed in Table 3. Petroleum ether showed good inhibition against *S. typhi*, *S. aureus* and *Candida species* and did not show any inhibition activity against *K. pneumoniae* and *E. coli*. Benzene, methanol and 2-Propanol showed a significant inhibition against all the four bacterial pathogens. Benzene showed a moderate inhibition against the fungal pathogens, whereas 2-Propanol showed good inhibition against *C. krusei*. Methanol's antifungal activity was good against *A. fumigates*. Ethyl acetate showed a considerable inhibition against *K. pneumoniae*, *S. typhi*, *C. krusei* and *A. fumigates*.

In a previous study²⁵, various lichens, including *Ramalina pollinaria* and *Ramalina polymorpha* methanolic extracts were tested against a large number of pathogens, particularly that against *E. coli* was found to be 10 mm for both the lichens. This is similar to the results obtained in the present study, where the zone of inhibition of the methanolic extract was 9.88 ± 0.09 mm. In another study²⁶, which tested *R. farinacea* against a number of clinical pathogens, the activity of ethanolic extract against *Candida albicans* was found to be 24 mm and against *Aspergillus niger* was found to be 22 mm. This is much greater than the observed values in the present study.

Antioxidant potentials of the lichen extracts

The total phenolic content in the extracts is a direct relevance of their antioxidant potential. Lichens are known for their high phenolic content which is also a reason why they have varied biological applications²⁷. This is due to the redox properties of their hydroxyl groups which make them as strong antioxidants²⁸. The results of the phenolic estimation of the extracts are as tabulated in Table 4. Benzene extract had the maximum total soluble phenolics while petroleum ether and methanol had significant amount of the same. The other two extracts had very less amount of total soluble phenolics. From a previous study¹⁹ of *Ramalina nervulosa*, the phenolic content of petroleum ether extract was very high when expressed in gallic acid equivalents. It was about 158.23 ± 1.77 mg GAE/g dw. This was comparatively less in our study which showed only 16.147 ± 1.394 µg/ml.

The percentage of inhibition of the DPPH radical by the lichen extracts are as plotted in the Figure 2. From the graph, the Petroleum ether, Benzene and methanol extracts showed a high activity when compared with the other two extracts. The range of the activities were from 1.08 % to 13.71 % for all the five extracts at the highest concentration used in the study. There is a consistent increase in the activity of the benzene extract when the concentration is increased. So, it can be concluded that if the concentration of benzene is increased, it can inhibit DPPH radical to a higher extent. 1mg/ml of methanol extract showed a percentage inhibition of 12.55 % in DPPH assay but in a study²⁹ of *Ramalina roesleri*, the methanolic extract showed a high inhibition % of 66.5 % for 250 µg/ml.

The reducing power assay results of all the five extracts of *R. inflata* showed varied activities at different concentrations (Figure 3). Petroleum ether and methanol extracts showed a constant rise when the concentration was increased up to 80 µg/ml at which the highest activity was shown and their activity started to reduce. The benzene extract showed the consistent rise in reducing power when the concentration was increased and showed a maximum at 100 µg/ml. 2-Propanol gave a high activity at a very low concentration than other extracts, that is, at 40 µg/ml. Ethyl acetate had a comparatively low activity similar results also observed with the activity. Our results of the petroleum ether extract are low when compared with a previous study⁶, in which the petroleum ether extracts of *P. tinctorum* showed an absorbance of 1.46. But when the methanolic extract is considered the absorbance value of *P. tinctorum* was 0.125, which was less than our value.

The property of reducing cupric ion to cuprous ion was significantly high only on the methanolic extract of the lichen *R. inflata*. All the other four extracts had very low property of reducing cupric ions. The capacities of the lichen extracts to reduce cupric ion are as shown in Figure 4. The benzene extract of the lichen showed a high ability of reducing molybdenum at 100 µg/ml. All the other extracts also showed a good reducing

ability but were less when compared to the benzene extract. This analysis can be understood from Figure 5.

CONCLUSION

In the present study, the anti-microbial and anti-oxidant activities of the lichen, *Ramalina inflata* were evaluated. The extracts showed a good anti-microbial activity, inhibiting both bacterial and fungal pathogens. Antioxidant activity is a direct measure of the pharmacological activity. In order to produce a novel natural drug, we investigated a lichen for its anti-microbial and anti-oxidant activity. Lichens are already known for their excellent biomedical applications most of which are uninvestigated. *Ramalina* is known for its excellent anti-diabetic activity³⁰, but the species *Ramalina inflata* has not been explored for its anti-oxidant activities. So, this study poses a significant contribution to the lichenology field. From the different radical scavenging assays performed, Benzene, Petroleum ether and Methanol showed a high anti-oxidant capacity. Due to their high anti-oxidant capacity, this lichen can be further studied for their other biomedical applications. If the concentration of the metabolites responsible for anti-oxidant activity is increased by further purification steps or if some modifications are done to the existing metabolites of lichens, it is possible to transform the metabolites of lichens to appealing active pharmaceutical ingredients (APIs). The potent extracts of *R. inflata*, can be assessed further for their anti-diabetic, anti-cancerous activities. From this study of lichens, we can come to a conclusion that lichens can contribute greatly to natural drug research because of their abundant and diversified metabolites.

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REFERENCES

- Ahmadjian V. The Lichen Symbiosis. New York: John Wiley. 1993
- Elix JA, Stocker-Wargotter. Biochemistry and secondary metabolites: In: Nash TH, editor. Lichen Biology. 2nd ed. 2008; pp 104-133.
- Culbertson CF, Elix JA. Lichen substances: In: P.M. Dey and J.B. Harborne, editors. Methods in Plant Biochemistry, Vol. 1: Plant Phenolics, 1989; pp. 509-535.
- Huneck S. New results on the chemistry of lichen substances: In: W. Herz, H. Falk, G.W. Kirby and R.E. Moore, editors. Progress in the Chemistry of Organic Products, 2001; pp. 1-276.
- Francolini I, Norris P, Piozzi A, Donelli G, Stoodley P. Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. Antimicrobial Agents and Chemotherapy 2004; 48:4360-4365
- Ganesan A, Thangapandian M, Ponnusamy P, Sundararaj JP, Nayaka S. Antioxidant and antibacterial activity of parmelioid lichens from Shevaroy hills of Eastern Ghats, India. International Journal of PharmTech Research 2015; 8(9), pp 13-23.
- Nikita Rijhwani, Rajkumar Bharty. In vitro antioxidant activity and total phenolic content of leaf extracts of *Dalbergia sissoo* (Roxb.). Int. Res. J. Pharm. 2016;7(8):16-21 <http://dx.doi.org/10.7897/2230-8407.07891>

8. Kosanic, Marijana & Rankovic, Branislav & Vukojevic, Jelena. Antioxidant properties of some lichen species. Journal of Food Science and Technology 2011; 48(5):584–590
9. Awasthi DD. A hand book of Lichens. Dehradun, India: Bishen Singh Mahendra Pal Singh. 2000.
10. Byers, Sheila C, Metsger, Deborah A. Managing the Modern Herbarium: An Interdisciplinary Approach. Society for the Preservation 1999.
11. Soxhlet F. "Die gewichtsanalytische Bestimmung des Milchfettes". Dingler's Polytechnisches Journal (in German) 1879; 232: 461–465
12. Culberson CF, Kristinsson HD. A standardized method for the identification of lichen products. Journal of Chromatography A 1972; 45: 85-93.
13. 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.
14. Slinkard K, Singleton VL. Total phenol analysis: Automation and comparison with manual methods. American Journal of Enology and Viticulture 1977; 28: 49-55.
15. Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z, Farah H. Antioxidant activity of some common plants. Turkish Journal of Biology 2008; v.32, p.51-55.
16. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181: 1199-1150.
17. Prakruthi Appaiah, Sunil L., Gopala Krishna A. G. and Suresh Kumar G. Phytochemicals and antioxidant activity of testa extracts of commercial wet and dry coconuts and cakes. Int. Res. J. Pharm. 2016;7(9):9-13 <http://dx.doi.org/10.7897/2230-8407.079106>
18. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry 1995; 43: 27-32.
19. Sundararaj JP, Kuppuraj S, Ganesan A, Ponnusamy P, Nayaka S. *In vitro* assessment of antioxidant and antimicrobial activities of different solvent extracts from lichen *Ramalina Nervulosa*. International Journal of Pharmacy and Pharmaceutical Sciences 2015; 7 (8): 200-204.
20. Alam MN, Bristi NJ, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal 2013; 21, 143–152.
21. Berk S, Tepe B, Arslan S, Sarikurkcü C. Screening of the antioxidant, antimicrobial and DNA damage protection potentials of the aqueous extract of *Asplenium ceterach* DC. African Journal of Biotechnology 2011; 10:8902–8908.
22. Ashwini, Prabhu, Krishnamoorthy M. Antioxidant activity of Ethanolic extract of *Cassia tora* L. Int. J. Res. Ayurveda Pharm. 2011; 2(1), 250-252.
23. Vinayaka KS, Karthik S, Nandini KC, Kekuda P, T.R., Amylase Inhibitory Activity of Some Macrolichens of Western Ghats, Karnataka, India. Indian Journal of Novel Drug Delivery 2013; 5(4):225-228.
24. Verma N, Behera BC, Sharma BO. Glucosidase inhibitory and Radical scavenging properties of lichen metabolites Salazinic acid, Sekikaic acid and Usnic acid. Hacettepe Journal of Biology and Chemistry 2012; 40 (1), 7-21.
25. Gulluce M, Aslan A, Sokmen M, Sahin F, Adiguzel A, Agar G, Sokmen A. Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollinaria*, *Ramalina polymorpha* and *Umbilicaria nylanderiana*. Phytomedicine 2006; 13:515–521.
26. Esimone CO, Adikwu MU. Antimicrobial activity and cytotoxicity of *Ramalina farinacea*. Fitoterapia 1999; 70:428-431.
27. Ganesan A, Mahesh A, Sundararaj JP, Mani K, Ponnusamy P. Antihyperglycemic and anti-oxidant activity of various fraction of *Parmotrema hababianum* in streptozotocin induced diabetic rats. Bangladesh Journal of Pharmacology 2016; 11: 935-39.
28. Materska M, Perucka I. Antioxidant Activity of the Main Phenolic Compounds Isolated from Hot Pepper Fruit (*Capsicum annuum* L.). Journal of Agricultural and Food Chemistry 2005; 53, 1750–1756.
29. Sisodia R, Geol M, Verma S, Rani A, Dureja P. Antibacterial and antioxidant activity of lichen species *Ramalina roesleri*. Natural Product Research 2013.
30. Shivanna R, Parizadeh H, Garampalli RH. Screening of lichen extracts for *in vitro* antidiabetic activity using alpha amylase inhibitory assay. International Journal of Biological & Pharmaceutical Research 2015; 6(5): 364-367.

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