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EVALUATION OF ANTIOXIDANT ACTIVITY AMONG THE EPIPHYTIC LICHENS OF KODAIKANAL

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ABSTRACT

Many chronic and degenerative diseases are caused because of the oxidative stress induced due to reactive oxygen species. Phenolic compounds are important part of phytochemicals in natural resources that have high antioxidant ability to scavenge the free radicals and prevent the oxidative stress. There is an increasing attention towards identifying novel and natural antioxidants for treating stress related diseases like cancer, cardiovascular and neurodegenerative diseases. Lichens are one among the natural resources that have exorbitant number of polyphenolic compounds, which place them as good antioxidants. In the present study, the acetone, methanol and ethanol extracts of the lichens; *Ramalina conduplicans, Ramalina subpusilla, Parmotrema reticulatum, Parmotrema cristeferum* and *Usnea cineraria* has been screened for their antioxidant potential by standard methods (DPPH radical scavenging, superoxide anion radical scavenging and determination of total phenolic compounds). Effective antioxidant activity was observed among all the lichens tested. The methanolic extract of the lichen, *Ramalina conduplicans* showed the highest quantity of total phenolic content as well as DPPH radical scavenging activity. *Usnea cineraria* showed the best superoxide dismutase radical scavenging activity in the acetone extract.

KEYWORDS: Lichens, DPPH, Super oxide dismutase, Total phenols, Antioxidant.

INTRODUCTION

As a part of normal cellular function, free radicals and oxidants are ubiquitously and endogenously produced in all cells by normal physiological processes. These radicals can also form due to exogenous sources, like pollution, radiation, tobacco, drugs, smoke, heavy

metals etc.[1] At higher concentrations, free radicals can be hazardous that cannot be destroyed and thereby leads to oxidative stress in the body. This causes serious diseases like cancer, aging, arthritis, neurodegenerative and cardiovascular disorders. An effective way of eliminating the free radicals is, to use antioxidants. Antioxidants can decrease the oxidative damage caused in normal cells either directly by reacting with the free radicals or indirectly by generating enzymes to inhibit the activity of free radicals. Enzymes like superoxide dismutase (SOD) and catalase as well as certain specific compounds like tocopherol, ascorbic acid and glutathione owe a key role towards protecting human cells from the damage caused by free radicals.[2]

Natural antioxidants are present in fruits, vegetables, spices, grains and herbs in the form of phenolics (phenol and polyphenols), flavonoids, carotenoids, steroids and thiol compounds.[3] Significant amount of phytochemicals from natural resources have been exploited as potential antioxidants. However, the quest for discovery of newer natural bioactive principles as natural antioxidants continues. Apart from plants and microbes, many number of lichen species have been explored to have a considerable amount of antioxidants.[4]

Lichens are self-supporting obligate and composite organisms that comprises a fungal (mycobiont) and an algal partner (photobiont). Having known the multifold biological activities of lichens,[5, 6, 7, 8] these species have been explored worldwide for various interesting economic uses like preparing dyes, as food, in folk medicine etc. Lichens produce a large number of phenolic compounds, such as depsides, depsidones and dibenzofurans. These phenolic compounds have strong antioxidant properties because they act as hydrogen donors and singlet oxygen quenchers and, therefore, have redox capacity.[9]

In the present study, the total phenolics present in lichens as well as the DPPH radical scavenging and superoxide dismutase radical scavenging activity of the chosen epiphytic lichens are reported.

MATERIALS AND METHODS

Lichen samples

The lichen samples of *Ramalina conduplicans* Vain. *Ramalina subpusilla* (Nyl.) Krog & Swinsc., *Parmotrema reticulatum* (Taylor) Choisy, *Parmotrema cristiferum* (Taylor) Hale and *Usnea cineraria* Mot., were collected from *Prunus persica* tree in Kodaikanal area,

Tamil Nadu, India. The samples were identified and have been preserved in the LWG herbarium, NBRI, Lucknow.

Preparation of Lichen extracts

Lichen specimens were air dried at room temperature. The dried lichen materials were finely ground into powder using mechanical blender. 5 grams of each of the powdered lichen material were extracted in acetone, methanol and ethanol (50 ml each) by shaking for 48 hours in orbital shaking incubator. The extracts were then filtered using Whatmann No: 1 filter paper and the solvent was evaporated. The residue obtained was used for antioxidant studies.

Antioxidant activity

DPPH radical scavenging activity

The free radical scavenging activity of the extracts were measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH). The antioxidant potential of the sample was determined by the assay as described in Kitts *et al*.,[10] Briefly, 1mL of DPPH working solution was mixed with 100µl of the lichen sample at various concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml). The mixture was incubated in dark for 30 minutes at room temperature. The absorbance of the DPPH radicals present in the mixture was measured at 517 nm. Ascorbic acid was used as standard.

The free radical scavenging property of DPPH was calculated using the following equation:

DPPH scavenging effect (%) = $[(A_o-A_1)/A_o] \times 100$

Where, A_0 is the absorbance of the negative control (consisting of all the reaction agents except the extract) and A_1 is the absorbance of reaction mixture or standards.

Superoxide dismutase activity

The superoxide anion radical scavenging activity of lichen extracts was detected according to the method of Nishimiki et al.[11] 0.1 ml of the extracts were mixed with 1 ml of nitroblue tetrazolium (NBT) solution (156 µM in 0.1 M phosphate buffer, pH 7.4) and 1 ml NADH solution (468 µM in 0.1 M phosphate buffer, pH 7.4). The reaction was started by adding 100 µL of phenazine methosulphate (PMS) solution (60 µM in 0.1 M phosphate buffer, pH 7.4). The mixture was incubated at room temperature for 5 min, and the absorbance was measured at 560 nm against the blank sample (0.1M phosphate buffer). Ascorbic acid was used as

standard. The percentage inhibition of the superoxide anion generated was calculated using the formula:

Superoxide anion scavenging activity (%) = $[(A_o-A_1)/A_o] \times 100$

Where A_0 is the absorbance of the negative control (consisting of all the reaction agents except the extract) and A_1 is the absorbance of reaction mixture or standards.

Determination of total phenolic compounds

The total phenolic content of the lichen extracts was determined spectrophotometrically by Folin-Ciocalteu method.[12] 0.5 mL of the lichen extract at various concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml) and 2.5 mL of 1:10 Folin-Ciocalteau reagent were mixed in a test tube. 2 mL of sodium carbonate (75 g/L) was added and incubated for 15 mins at 45°C. The absorbance was measured at 765 nm. The total phenolic concentration was calculated from catechol calibration curve. The phenolic content of the extracts were expressed as catechol equivalents (CE)/g of the respective extracts.

All the values of the antioxidant activity obtained were expressed as Mean ± S.D of three parallel measurements in MS EXCEL.

RESULTS

DPPH free radical scavenging activity

Free radical scavenging action is considered as one among the various antioxidant tests. Fig.1 to Fig.5 represents the dose dependent DPPH radical scavenging activity of the lichen extracts used in this study. Concentration dependent radical scavenging activity is clearly observed for all the extracts. All the three extracts of the lichens tested showed good scavenging activity on DPPH radicals. However, the methanolic extract of *R.conduplicans* showed the best activity ranging from 32.35% to 81.47% of the lichen extract when compared to others. It is observed that all the lichens had higher DPPH activity in the methanolic extracts only. This is followed by better activity in the ethanolic extracts of all the lichens while acetone extract had the lowest activity.

Superoxide anion scavenging activity

Results of superoxide anion scavenging activities of tested extracts are as shown in Fig.6 to Fig.10. All extracts revealed marked superoxide dismutase scavenging activity. It was interesting to note that only in *R.conduplicans*, the ethanolic extract had higher superoxide anion scavenging activity ranging from 9.61% to 42.17%, while all other lichens showed

good activity in the acetone extracts. Also, very high activity has been exhibited by all the extracts than the control in this study. *U.cineraria* reports the highest superoxide anion scavenging activity even at the least concentration of 50µg/ml with 80.12% when compared to ascorbic acid standard which has inhibition percentage of 11.21% only.

Determination of total phenolic compounds

The total phenolic content was calculated based on catechol equivalent in the lichen sample. Considerable amount of total phenols were observed in all the extracts of the lichens used for this study (Table.1, Fig.11). A high amount of phenolic compounds was seen in *R.conduplicans* among all the extracts with 32.06 ± 16.86 mg/g of catechol equivalent. Methanolic extract of all the species portrayed better yield among the extracts while acetone extract of all the lichens revealed the least amount of phenolic content.

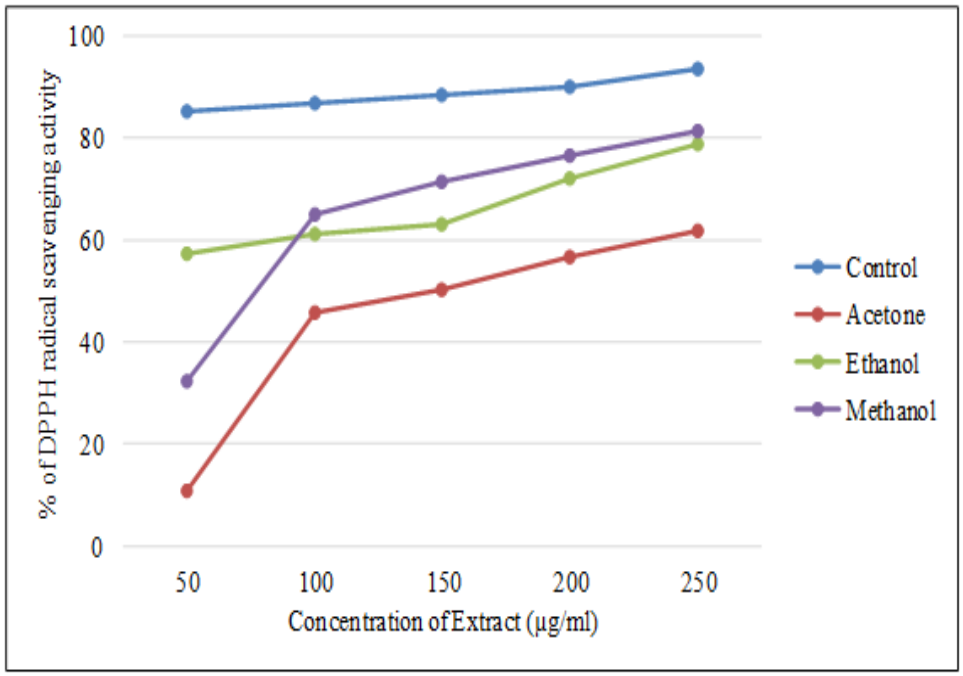

Fig. 1: DPPH radical scavenging activity of different solvent extracts of *Ramalina conduplicans.*

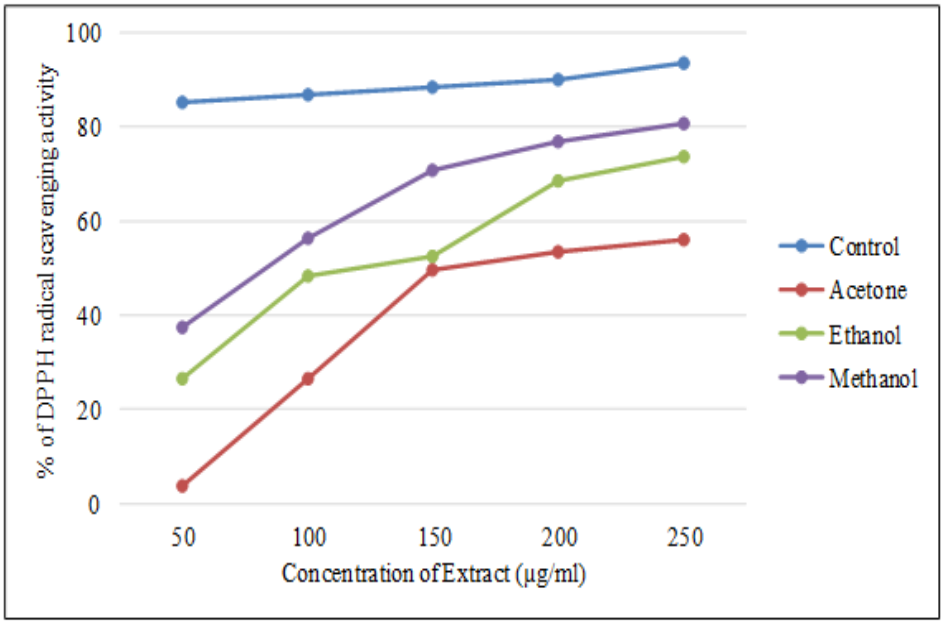

Fig. 2: DPPH radical scavenging activity of different solvent extracts of *Ramalina subpusilla.*

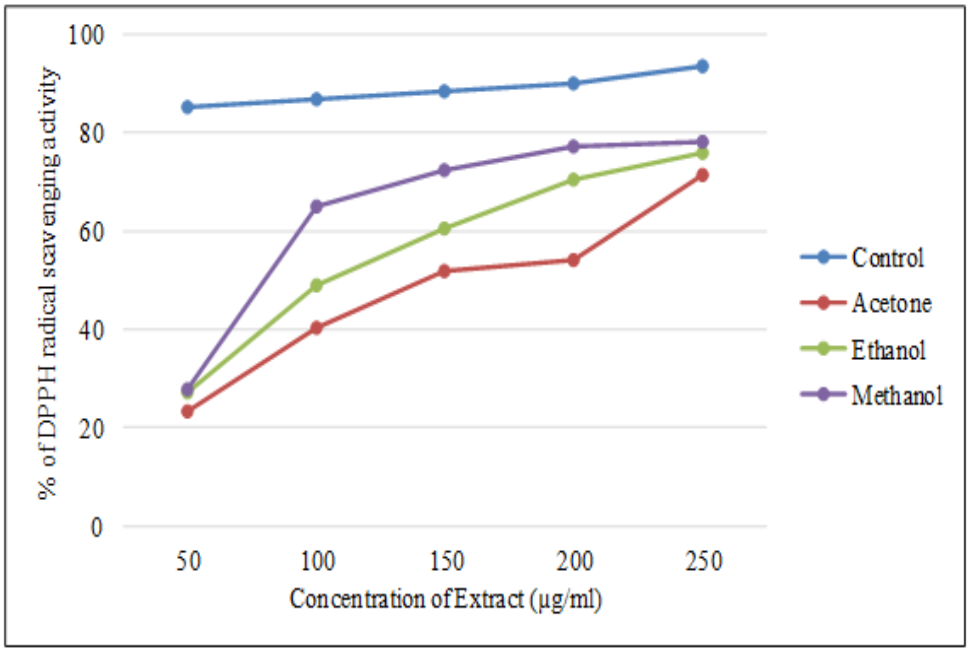

Fig. 3: DPPH radical scavenging activity of different solvent extracts of *Parmotrema reticulatum.*

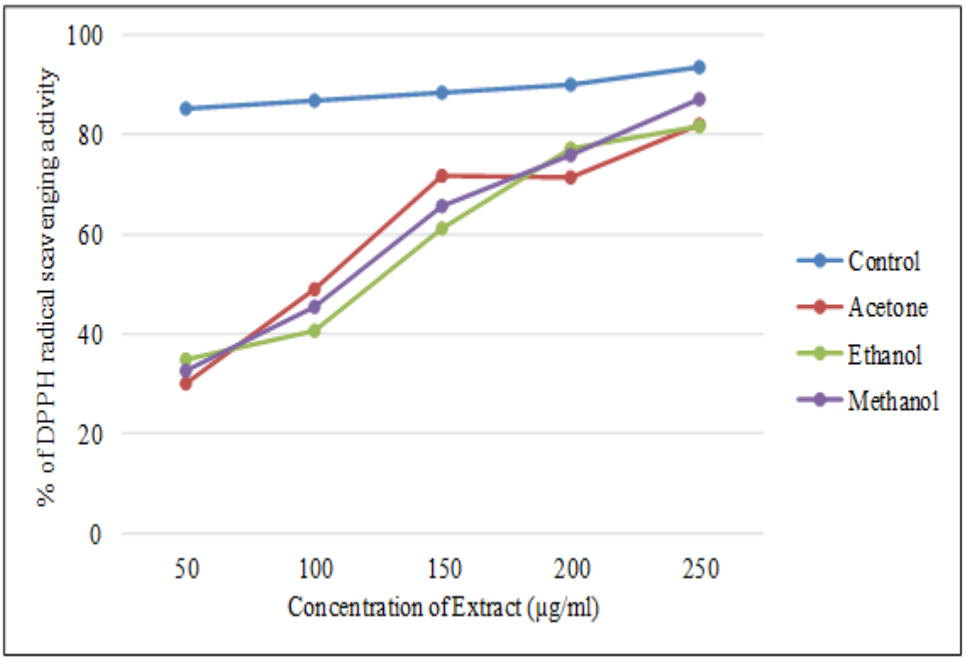

Fig. 4: DPPH radical scavenging activity of solvent extracts of *Parmotrema cristeferum*.

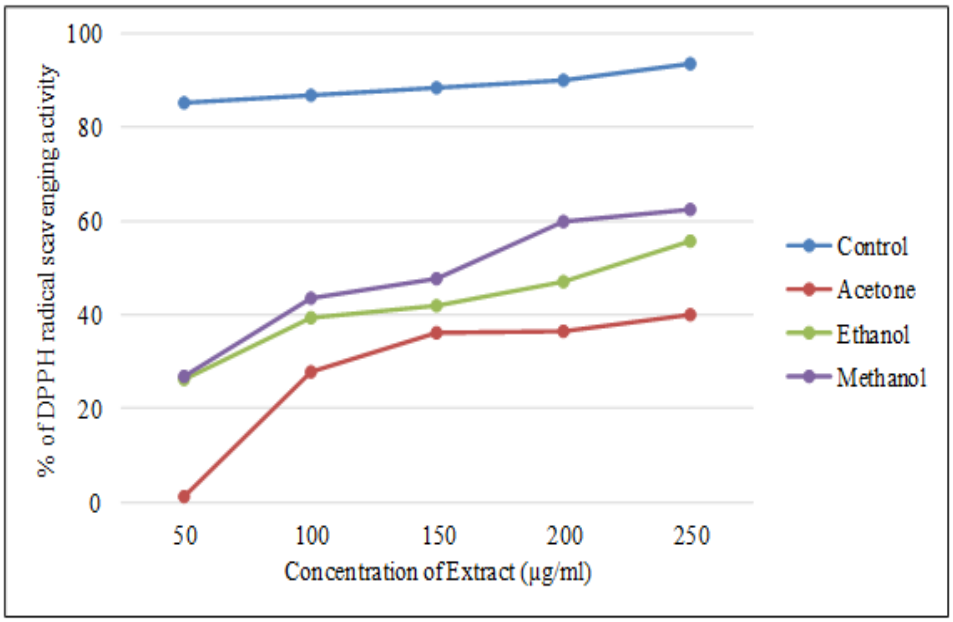

Fig. 5: DPPH radical scavenging activity of different solvent extracts of *Usnea cineraria*.

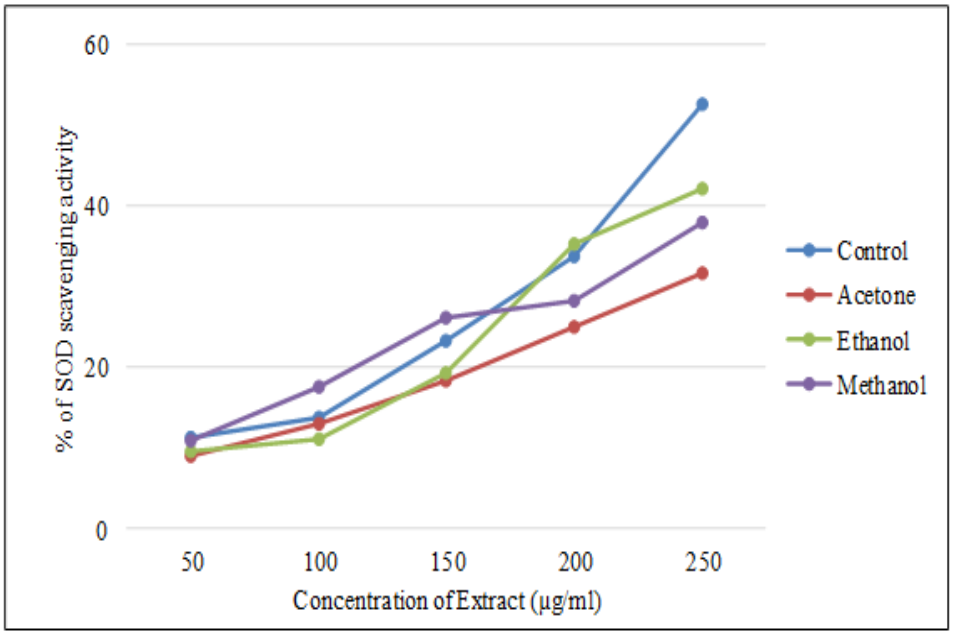

Fig. 6: Super oxide dismutase radical scavenging activity of different solvent extracts of *Ramalina conduplicans.*

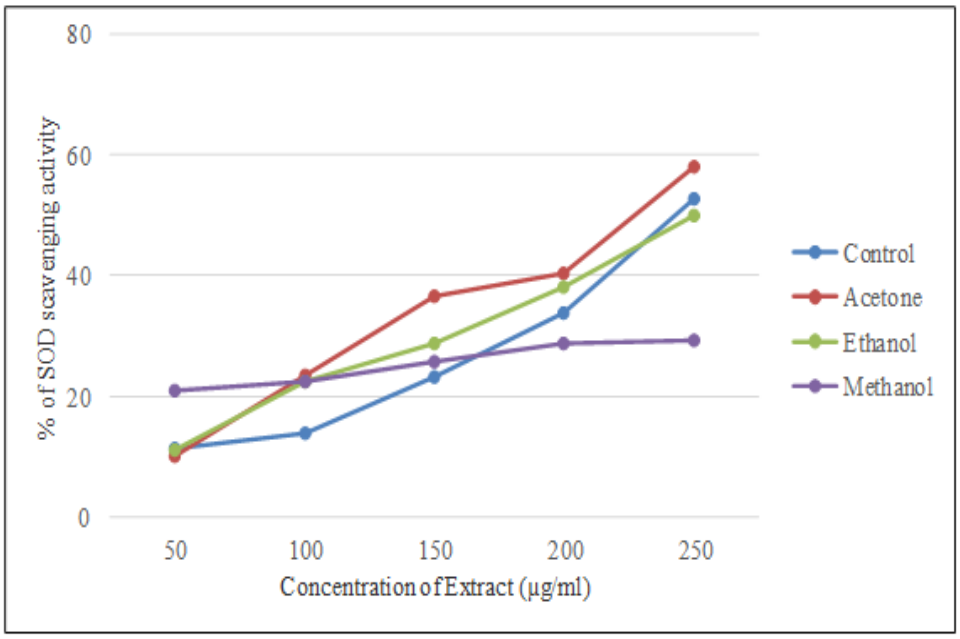

Fig. 7: Super oxide dismutase radical scavenging activity of different solvent extracts of *Ramalina subpusilla.*

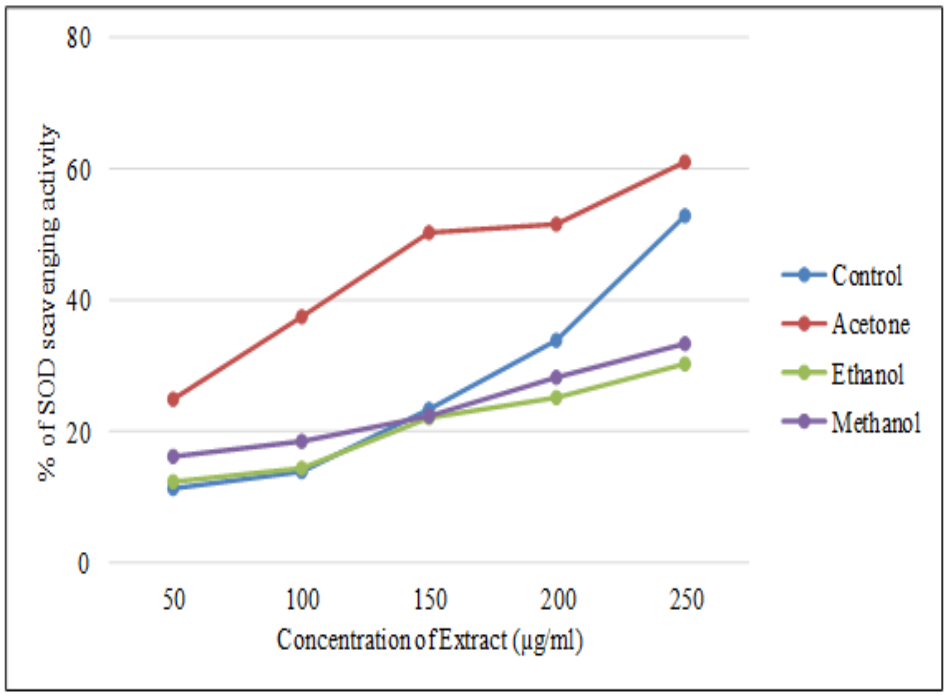

Fig. 8: Super oxide dismutase radical scavenging activity of different solvent extracts of *Parmotrema reticulatum.*

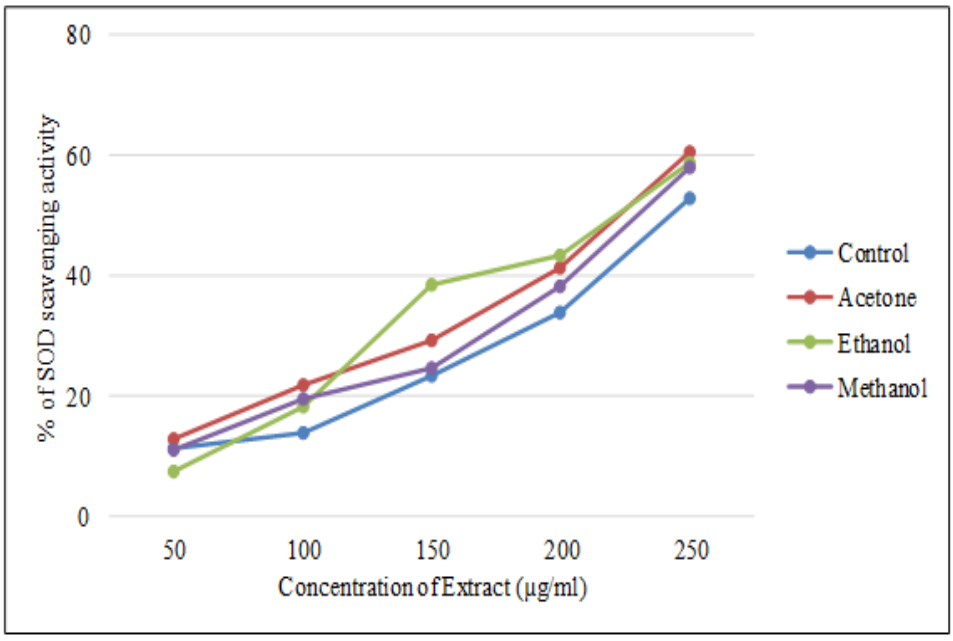

Fig. 9: Super oxide dismutase radical scavenging activity of different solvent extracts of *Parmotrema cristeferum.*

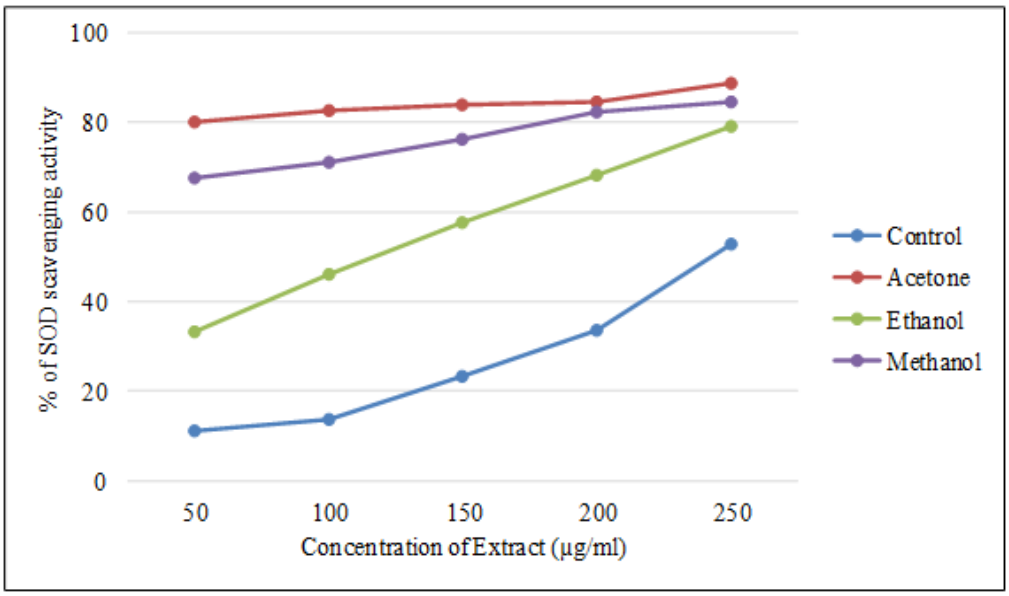

Fig. 10: Super oxide dismutase radical scavenging activity of different solvent extracts of *Usnea cineraria.*

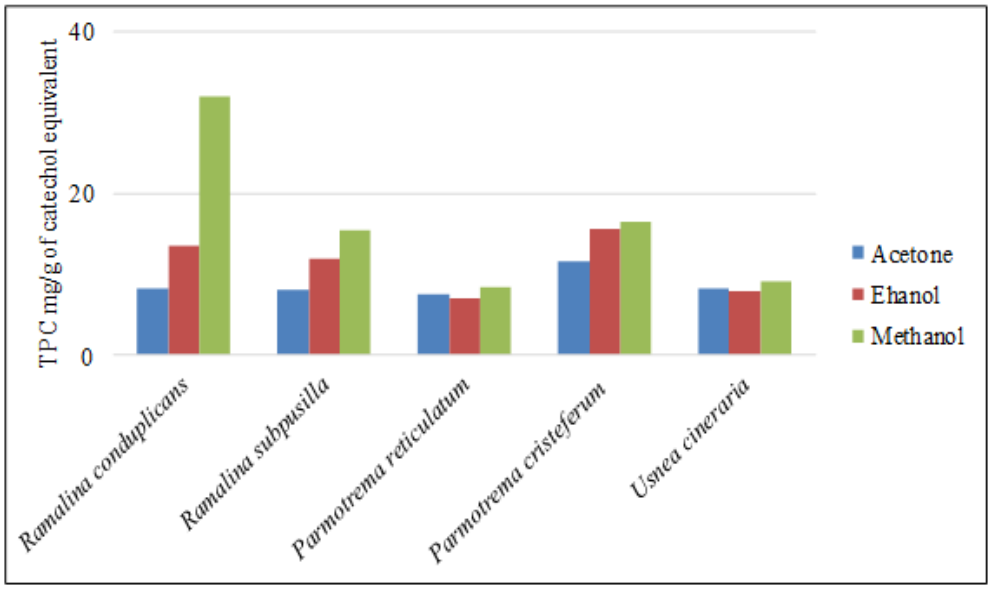

Fig. 11: Total Phenolic content of the lichen extracts.

Table 1: Total Phenolic Content of the Lichen Extracts.

Sample	TPC (mg/g) of CE		
	Acetone	Ethanol	Methanol
Ramalina conduplicans	08.27±4.46	13.58 ± 1.35	32.06± 16.86
Ramalina subpusilla	08.08±2.11	11.96±3.51	15.53±14.45
Parmotrema reticulatum	07.50±3.61	06.99±1.22	08.38±3.36
Parmotrema cristiferum	11.66±3.04	15.58±3.22	16.58±2.32
Usnea cineraria	08.24±4.04	07.86± 3.41	09.05±3.39

DISCUSSION

The antioxidant activity of lichen extracts might be due to the presence of various groups of secondary metabolites in them. Abundant literatures support this property of lichens and its metabolites.[13, 14, 15]

While considering both total phenolic content in the lichen extracts and DPPH radical scavenging activity, extraction by using methanol has provided significantly better results than other solvents. This is supportive with the information from many reports on the antioxidant activity of lichens that has shown better activity in the methanolic extracts.[9, 8, 16, 17] The depside compounds present in *R.conduplicans* like sekikaic acid and homosekikaic acid is the major reason for the lichen to exert high DPPH radical scavenging activity.[9, 18]

All extracts showed a relatively good superoxide anion scavenging activity. The superoxide anion scavenging activity of the different lichens ranged from 12.74 to 88.68%. Interestingly, this analysis reports the superoxide dismutase activity of lichens on a higher side when compared to the standard, ascorbic acid. Maximum scavenging activity was found in the acetone extracts of the lichens. This result is concomitant with the report on several lichen species having higher activity than ascorbic acid in the elimination of superoxide radicals.[19] Maximum superoxide anion scavenging activity has also been observed in the acetone extracts by Marijana et al., in 2011.[8] Among the lichens tested, *U.cineraria* had strong activity in all the three extracts. This may be because of higher usnic acid content in the lichen.[20]

In this study, methanolic extract of all the lichens have higher total phenol content followed by ethanol while it is found to be lower in the acetone extracts. Methanol has been described as a very efficient solvent for extraction of lower molecular weight polyphenols.[21] As lichens are rich in polyphenolic compounds and the polarity of methanol has more soluble properties, thereby, the methanolic extracts display better results of the scavenging activity in almost all the lichens in this study. Similar reports of previous studies support these results.[8, 20] The phenolic compounds in lichens are majorly monocyclic phenols, depsides, depsidones and dibenzofurans that are derived through acetyl-polymalonyl pathway.[22, 4] Also, such compounds have a higher red-ox potentials that helps in absorbing and neutralizing the free radicals.[23]

CONCLUSION

The present study confirms that all three lichen extracts represent a significant source of phenolic compounds, which makes the lichen species as potent natural antitoxidants. Further *in-vitro* and *in-vivo* investigation on the biologically significant lichen compounds, can help in the discovery of important leads for the development of conventional drugs towards treatment of oxidative stress related diseases.

REFERENCES

1. Alugoju Phaniendra, Dinesh Babu Jestadi, and Latha Periyasamy. Indian J Clin Biochem. 2015 Jan; 30(1): 11–26. Antioxidative Properties: A Review. Oxidative Medicine and Cellular Longevity Volume 2017, Article ID 2079697.
2. Niki E, Shimaski H, Mino M. Antioxidantism-free radical and biological defense. Gakkai Syuppn Center, Tokyo. 1994; 3–16.
3. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med. 2006; 41: 1727–46.
4. Vinitha M. Thadhani and Veranja Karunaratne. Potential of Lichen Compounds as Antidiabetic Agents with Antioxidative Properties: A Review. Oxidative Medicine and Cellular Longevity Volume 2017.
5. Perry NB, Benn MH, Brennan NJ, Burgess EJ, Ellis G, Galloway DJ, Lorimer SD and Tangney S. 1999 – Antimicrobial, antiviral and cytotoxic activity of New Zeland lichens. Lichenologist, 31: 627–636.
6. Huneck S. 1999 – The significance of lichens and their metabolites. Naturwissenschaften, 86: 559– 576.
7. Conti, M. E., & Cecchetti, G. (2001). Biological monitoring: lichens as bioindicators of air pollution assessment--a review. *Environmental Pollution*, *114*(3): 471–92.
8. Marijana Kosanić & Branislav Ranković & Jelena Vukojević. Antioxidant properties of some lichen species. J Food Sci Technol, (September–October 2011); 48(5): 584–590
9. H. Luo, X. Wei, Y. Yamamoto et al., "Antioxidant activities of edible lichen *Ramalina conduplicans* and its free radicalscavenging constituents," *Mycoscience*, 2010; 51(5): 391–395.
10. Kitts, D.D., Y.V. Yuan, A.N. Wijewickreme and C. Hu, 2000. Antioxidant properties of a North American Gingseng extract. Mol. Cell. Biochem, 203: 1-10.

11. Nishimiki M, Rao NA, Yagi K (1972). The occurrence of super-oxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun, 46: 849–853.
12. Singleton, V.L.; Orthofer, R.; Lamuela, R.R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth. Enzymology, 1999; 299: 152–178.
13. Behera BC, Verma N, Sonone A, Makhija U (2005) Antioxidant and antibacterial activities of lichen *Usnea ghattensis* in vitro. Biotechnol Lett, 27(14): 991-995.
14. Poornima G, Kekuda PTR, Vinayaka KS. Antioxidant efficacy of *Olea dioica* Roxb (Oleaceae). Biomedicine. 2012; 32(4): 506-510.
15. N. Verma, B. C. Behera and B. Om Sharma, "Glucosidase inhibitory and radical scavenging properties of lichen metabolites salazinic acid, sekikaic acid and usnic acid," *Hacettepe Journal of Biology & Chemistry*, 2012; 40(1): 7–21.
16. N. Manojlovic,´ B. Rankovic,´ M. Kosanic,´ P. Vasiljevic,´ and T. Stanojkovic,´ "Chemical composition of three *Parmelia* lichens and antioxidant, antimicrobial and cytotoxic activities of some their major metabolites," *Phytomedicine*, 2012; 19(13): 1166 – 1172.
17. Ghate NB, Chaudhuri D, Sarkar R, Sajem AL, Panja S, Rout J, et al. (2013) An Antioxidant Extract of Tropical Lichen, Parmotrema reticulatum, Induces Cell Cycle Arrest and Apoptosis in Breast Carcinoma Cell Line MCF-7. PLoS ONE, 8(12): e82293. https://doi.org/10.1371/journal.pone.0082293
18. V. M. Thadhani, M. I. Choudhary, S. Ali, I. Omar, H. Siddique, and V. Karunaratne, "Antioxidant activity of some lichen metabolites," *Natural Product Research*, 2011; 25(19): 1827– 1837.
19. Antônio Sérgio Nascimento Moreira, Raimundo Braz-Filho, Vicente Mussi-Dias and Ivo José Curcino Vieira. Chemistry and Biological Activity of *Ramalina* Lichenized Fungi. *Molecules*, 2015; *20*(5): 8952-8987.
20. Pavithra G.M, Vinayaka K.S, Rakesh K.N, Syed Junaid, Dileep N, Prashith Kekuda T.R, Saba Siddiqua, Abhishiktha S. Naik. Antimicrobial and antioxidant activities of a macrolichen Usnea pictoides G.Awasthi (Parmeliaceae) Journal of Applied Pharmaceutical Science, August, 2013; 3(08): 154-160.
21. J. Dai, R.J. MumperPlant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 2010; 15: 7313-7352.

22. Thadhani VM, Choudhary MI, Ali S, et al. (2011). Antioxidant activity of some lichen metabolites. Nat Prod Res., 25: 1827–37.
23. Saha M R, Hasan SMR, Akter R, Hossain MM, Alam MS, Alam MA, Mazumder MEH 2008. *In vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* Linn. *Bangl J Vet Med, 6*: 197-202.