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Antioxidant and antibacterial activity of parmeliod lichens from Shevaroy hills of Eastern Ghats, India

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Abstract: In recent decades, the occurrence of new diseases associated with pathogens in humans has become very common and has spurred efforts to exert control over various diseases. Lichen species such as *Parmotrema austrosinense, P. hababianum*, and *P. tinctorum* were collected from the Eastern Ghats of India, covering the Shevaroy hills in Tamil Nadu. The extracts were obtained by using various solvents; they were then subjected to antibacterial and various antioxidants assays, such as 2,2-diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power, and hydrogen peroxide. Benzene extract of *P. tinctorum*, showed an inhibition zone of 13.77 mm against *Salmonella typhimurium*, whereas in case of *P. hababianum* and *P. austrosinense* inhibition zones were 8.2 and 12.23 mm, respectively. Total antioxidant activity of acetone extract (IC_{50} 18.41 µg/mL) of *P. tinctorum* was higher than that of other species. Methanol and benzene extracts of *P. hababianum* and *P.austrosinense* showed significant effects on free radical scavenging activity.
Keywords: Lichen, Antibacterial, Antioxidant activity, DPPH assay, FRAP assay, IC_{50}..

1. Introduction

Antioxidant substances have the potential to capture free radicals formed as a result of stress; free radicals can be damaging to healthy cells and can trigger various diseases in the human body. Thus, they are very important in protecting against diseases, such as arthritis, diabetes mellitus, and carcinogenesis and cardiovascular diseases, developed in response to oxidative stress [1]. Antioxidant compounds can be derived from many sources, using both natural and synthetic methods. Synthetic antioxidant compounds exert adverse effects on human and animal cells [2]. Natural antioxidant compounds are highly useful in medicinal chemistry and exert no negative effect on naturally occurring cells [3, 4]. They can be derived from bioresources, and plant-derived drugs are very useful in both pharmaceutical and medicinal chemistry. Hence, search for drugs derived from different natural resources is gaining importance [5].

This study discusses a search for new molecules from unique organisms called lichens. Lichens are composite organisms consisting of fungi and other species either algae or cynobacteria. Lichens produce about 1000 metabolites through their acetyl-polymalonyl, shikimic acid, and mevalonic acid pathways [6]. Lichen metabolites spur diverse biological activities, such as antimicrobial, antitumor, antimutagenic, antiherbivore, and allergenic [7]. The biological activity of an extract can be determined by the presence of phenolic compounds such as flavonoids, phenolic acids, and total phenols [8, 9]. Lichens are also known for having

higher phenolic content, which has various applications. The development of multidrug resistance in pathogenic bacteria is a serious problem in current clinical chemistry, as it occurs because of the excessive use of existing antibacterial drugs [10]. Bacteria resistant to multiple antibiotics may become endemic in health-care settings, making treatments for human diseases very difficult. To overcome this issue, this study aimed to investigate the antibacterial activity of various extracts of lichen species against human pathogens [11]. It also discusses unexplored drug components and their viability against multidrug-resistant pathogens.

This study will enable researchers to understand various pharmaceutically active compounds in various lichen sample extracts [12]. It discusses the correlation of phenolic content with the antioxidant potential of various extracts obtained from parmeliod lichens collected from the Shevaroy hills of Tamil Nadu, India. Parmeliod lichens are large, lobed thalli that grow well both in trees and on the surfaces of rocks. Lichens such as *P. austrosinense*, *P. hababianum* and *P. tinctorum* were selected for this study.

2. Materials and Methods

2.1 Plant source

Lichen samples were collected from the Shevaroy hills region of the Eastern Ghats of Tamil Nadu. The samples were identified by following standard procedures with respect to studying their morphology, anatomy, and chemistry. Thin-layer chromatography (TLC) was conducted following the procedure established by Walker and James [13], using solvent system A to identify secondary metabolites. Voucher specimens were deposited in the lichen herbarium of the K.S. Rangasamy College of Technology, Tiruchengode, for reference purposes.

2.2 Preparation of Lichen Extracts

Approximately 8-g samples of each species were powdered and used for extraction with different solvent systems with increasing polarity (petroleum ether, ethyl acetate, acetone, ethanol, and water) using Soxhlet extractor [14]. Using a vacuum desiccator, we subjected the extracts to evaporation in order to form a dry powder. Powdered extracts were stored at –80°C in a deep freezer for further study.

2.3 Antibacterial Activity of Lichen Extracts

Antibacterial activity of lichen extracts against clinical pathogens was determined with Muller–Hinton agar plates using well-diffusion method [15]. Bacteria such as *Proteus vulgaris* (NCIM 2857), *Bacillus cereus* (NCIM 2150), *Staphylococcus aureus* (NCIM 5021), *Klebsiella pneumoniae* (NCIM 2957), *Pseudomonas aeruginosa* (NCIM 2608), *Escherichia coli* (NCIM 2563), and *S. typhimurium* (NCIM 2501) were selected for this study. These were acquired from the National Collection of Industrial Microorganisms (Pune, India).Experiments was carried out in triplicate and zones of inhibition were recorded by comparing those of tetracycline and polymyxin antibiotics.

2.4 Analysis of Lichen Compounds

2.4.1 Bioautography using thin-layer chromatography of various lichen extracts

Lichen compounds in extracts were subjected to TLC to identify their active components, following the procedure established by Walker and James [13]. Extract powder was dissolved into a 1 mg/mL concentration of corresponding solvents and spotted onto the TLC plate using a capillary tube. The TLC plate, which contained a toluene/1,4-dioxane/acetic acid (180:45:5) mix, was kept in the TLC chamber for 1 h. The plate was then air-dried and sprayed with 10% sulfuric acid solution. Charring was carried out both to visualize the colors and to separate the patterns of the compounds present in the extracts.

2.4.2 High-performance liquid chromatography analysis

Lichen extracts were subjected to high-performance liquid chromatography (HPLC) (Agilent 1220, Boeblingen, Germany, C18, methanol/water/acetic acid, 80:20:1) analysis for identifying various compounds present in the parmeliod lichens, following the procedure used by Yoshimura et al.[16]. The 20-µL sample volume was injected into the column, and peak area and retention factors were calculated for individual lichen

compounds.

2.5 *Antioxidant Activity of Lichen Extracts*

2.5.1 2, 2-Diphenyl-1-picrylhydrazyl free radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of different concentrations of lichen extracts (20, 50, 100, 200, 400, 800, and 1000 µg/mL) in corresponding solvents (w/v) with ascorbic acid as the standard was tested [17]. DPPH solution (2mL, 0.002%) was mixed with 2 mL lichen extracts. The mixtures were incubated at room temperature in the dark for 30 min and their optical density was measured at 517 nm using a UV–Vis spectrophotometer (U-2900, Hitachi, Japan).

2.5.2 Ferric reducing power assay

A ferric reducing antioxidant power (FRAP) assay was carried out on 20, 50, 100, 200, 400, 800, and 1000µg/mL samples of lichen extracts, with ascorbic acid as the standard. Phosphate buffer (2.5mL; 200 mM, pH 6.6) and 1% potassium ferricyanide solution (2.5 mL) were added to each concentration. The amounts of iron (II)–ferricyanide complexes formed were determined by measuring the formation of Perl's Prussian blue at 700 nm using a UV–Vis spectrophotometer. The higher absorbance of the reaction mixture indicated a steep reduction in power [18].

2.5.3 Hydrogen peroxide–reducing activity assay

Hydrogen peroxide (H_2O_2) activity was assayed by measuring 200, 400, 600, 800, and 1000µg/mL concentrations of lichen extracts. These extracts were added to 0.6 mL H_2O_2 (40 mM). The reaction mixtures were then incubated at room temperature for 10 min, and the mixture was read at 230 nm against the blank solution with phosphate buffer (pH 7.4) using a UV–Vis spectrophotometer. The percentage of inhibition was calculated and then compared with that of ascorbic acid[19].

2.6 Determination of Total Phenolic Content of Lichen Extracts

The total phenolic content of the lichen extracts was determined by Folin–Ciocalteu method, which was described by Slinkard and Slingleton[20]. Lichen extracts were diluted at a concentration of 1 mg/mL with respective solvents, from which 0.1 mL extracts was taken and added to 1 mL Folin-Ciocalteu reagent (1:1) and 3 mL Na_2CO_3 (2%). Color intensity was measured at 760 nm using a UV–Vis spectrophotometer, and Gallic acid was used as a standard.

2.7 Statistical Analysis

Analysis of variance was carried out to identify the significance level of various treatments ($p<0.05$) using Graph Pad 5.0 Software. Means and standard error were compared for significance level using Dungan's multiple range test. Principal component analysis (PCA) was used to evaluate the important components responsible for the each experiment. PCA and correlation matrix were generated using PAST 2.09.

3. Results and discussion

3.1 Antibacterial assay

The antibacterial activity of various extracts of parmeliod lichens is shown in Table 1. The benzene extracts of all the tested lichen species were highly significant in inhibiting the growth of most of the bacteria. Methanol extract of *P. tinctorum* showed the highest potential to control the growth of *S.typhimurium* with the inhibition zone of 15.7 mm, which is better than the standard antibiotic tested. Acetone and methanol extracts were found to be more potent against *P.vulgaris*, with the inhibition zones of 15.33 and 13.17 mm, respectively. Similar effects were also observed in the extracts of *P.hababianum.* It revealed that both benzene and methanol extracts significantly inhibit all tested pathogenic bacteria. Extracts of *P.hababianum*

Table 1. *In vitro* growth of inhibition of bacterial pathogens using lichen extracts

Lichens	Solvents	Diameters of zone of inhibition against various pathogens (mm) (20µg/ml)						
		S. typhimurium	***K. pneumoniae***	***S. aureus***	***B. cereus***	***P. vulgaris***	***P. aeruginosa***	***E.coli***
P. tinctorum	P.Ether	ND	ND	ND	ND	ND	ND	10.4^{cd}
	Benzene	13.77^{e}	10.17^{c}	11.37^{c}	9.1^{ab}	9.7^{b}	13.5^{cde}	ND
	Acetone	8.06	ND	13.8^{e}	ND	15.33^{f}	15.87^{f}	ND
	Methanol	15.7	8.2^{a}	ND	ND	13.17^{d}	ND	ND
	Water	ND	ND	ND	ND	ND	ND	ND
P. hababianum	P.Ether	ND	ND	ND	ND	ND	ND	ND
	Benzene	8.2^{a}	10.27^{cd}	15.17^{ef}	10.77^{b}	15.37^{fg}	13.27^{cd}	12.5^{e}
	Acetone	ND	ND	ND	ND	ND	ND	ND
	Methanol	10.6^{c}	10.5	ND	ND	ND	12.4^{c}	8.23^{a}
	Water	ND	ND	ND	ND	ND	ND	ND
P.austrosinense	P.Ether	ND	ND	ND	ND	ND	ND	ND
	Benzene	12.23^{d}	10.33^{cde}	9.67^{b}	10.23^{abcd}	9.93^{bc}	10.43^{ab}	15.27^{f}
	Acetone	ND	ND	ND	ND	ND	ND	ND
	Methanol	ND	ND	12.17^{d}	9.87^{abc}	ND	ND	8.23^{a}
	Water	ND	ND	ND	ND	ND	ND	ND
Standard 1		14.1^{ef}	14.2^{f}	18.1^{g}	18.17	13.37^{de}	19.23^{g}	10.23^{c}
Standard 2		9.17^{ab}	9.4^{b}	6.17^{a}	9.0^{a}	7.17^{a}	10.23^{a}	9.067^{ab}
*SEM ±		1.029	0.696	1.46	1.422	1.181	1.193	0.965

Standard 1- (Tetracyclin (30 mcg) Standard 2- (Polymyxin B (25 mcg), ND- Not detected

*SEM calculated as per the triplicate values of experiment (n=3) and*P.austrosinense* were observed to have good activity against *E.coli*, where as that of *P.tinctorum*was ineffective against *E.coli.* The result further revealed that *P. austrosinense*has better inhibition ability against *E.coli* (with 15.27 mm zone of inhibition) followed by *S. typhimurium*(12.23 mm). Petroleum ether extracts showed no activity against any of the bacteria tested. The overall result indicated that benzene and methanol extracts are superior in terms of inhibiting the growth of bacteria when compared with standard polymyxin B, whereas growth inhibition was on a par with tetracycline. In case of *E.coli*, extracts of parmeliod lichens were found to be superior to the tetracycline antibiotics. Our results are in line with those of Rankovic [21] showing highest inhibition ability of Gram-positive bacteria than Gram-negative bacteria in various lichen extracts. Antibacterial activity of lichen extracts was more potent against various human pathogens. It leads to discover the broad-spectrum antibiotics over various diseases. The results of the present study showed acetone and methanol extracts of *P. tinctorum*to have the highest antibacterial activity. The similar results were also obtained by Priti [22] against antifungal activity.

3.2 Total polyphenol content

Phenolic content was found to be more in benzene extracts (154.2 mg gallic acid equivalent (GAE)/g of lichen extracts) whereas least content was observed in the petroleum ether extract for*P. tinctorum*(Fig. 3C). All the extracts showed sufficient amount of phenolic contents, which may be crucial in various activities such as antioxidant and antibacterial activities. The antioxidant activity of extracts of parmeliod lichens showed that higher total polyphenol content (TPC) has the highest potential of capturing free radicals. *P. austrosinense* showed the lowest amount of phenolic contents, which shows the moderate potential of capturing both hydrogen and hydroxyl ions. It may be the poor specificity of various substances in the extracts to Folin reagents that exerts the most effect on the antioxidant activity [23]. Moreover, the present assay depends on the number of phenolic groups present in the particular extracts, which may be the reason for high phenolic content showing the low level of antioxidant activity [24]. *P. hababianum* showed the positive correlation with respect to antioxidant activity. The overall result exhibits that the various extracts of lichens with highest content of polyphenolic substances have the highest power of antioxidant activity.

3.3 Analysis of Chemical Compounds in Various Lichen Extracts using Chromatography

3.3.1 Thin-layer chromatography

The result showed the separation of components based on the refractive values (Figure 1A). Spot 1 shows the three components with various colors, which corresponds to *Parmeliella wallichiana*, whereas *P. tinctorum* and *P. austrosinense*are seen in spots 2 and 4, respectively, which indicates that same pattern of compounds might be present in the species. *P.hababianum* showed atranorin in R_f clause 7. Species were fractioned based on the increasing polarity of various solvents. Methanol extract of *P. hababianum* is seen at spot1 on the TLC plate (Fig. 1B), which shows that there are about five compounds observed with different colors. The study by Divakar and Upreti [25] also supports the presence of atranorin and protolichesterinic acid in *P.hababianum*. Benzene and methanol extracts of *P.austrosinense* were observed to have both atranorin and lecanoric acid. On the other hand, spot 4 shows unidentified compounds along with atranorin. Methanol extract of *P.tinctorum*did not show any compounds visible in different solvent systems. Benzene extract of *P.tinctorum* showed atranorin in R_f clause 7 with yellow color. It also agrees with the report of Vagn Alstrup [26] indicating that *P.tinctorum* has atranorin and lecanoric acid. The lichen extracts were illustrated with different solvent system for compound development (Fig. 1C & 1D) which revealed that R_f value has been changed for individual compound.

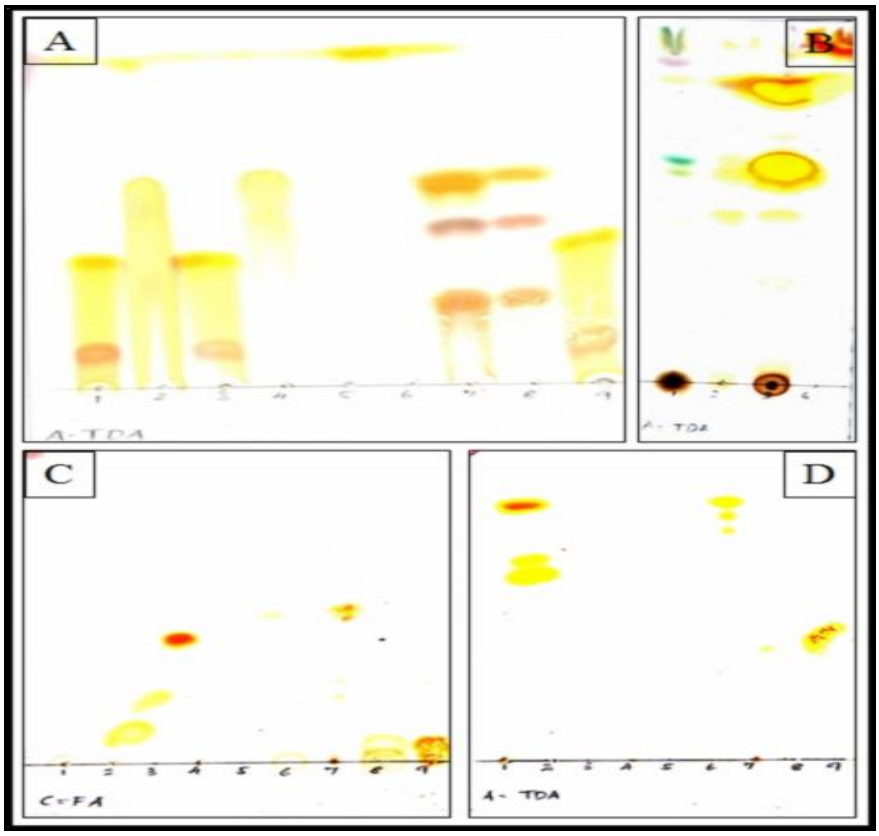

Figure 1. Chemical profile of various lichen extracts in TLC plate A) Parmeliod lichen species B) Crude extract of *P.hababianum and P.austrosinense*C)&D) Crude fraction of various extracts of lichen species

3.3.2 High-performance liquid chromatography analysis of lichen thalli

In *P. austrosinense* the absorbance peak indicated the presence of various compounds, such as atranorin (Rt 20.719), lecanoric acid (Rt 6.662), salazinic acid (Rt 3.299), and chloroatranorin (Rt 27.482), which is on par with the results of Hiromi et al[27]. The same compounds were found to be present in two other species, namely *P. tinctorum* and *P. hababianum*, which is in line with the results of Laily et al. [28]. *P. wallichiana* is used as standard for the analysis of all three species. In addition, some of the unidentified compounds were found in all the species at different retention time, which may influence the antioxidant activity (Fig. 2).

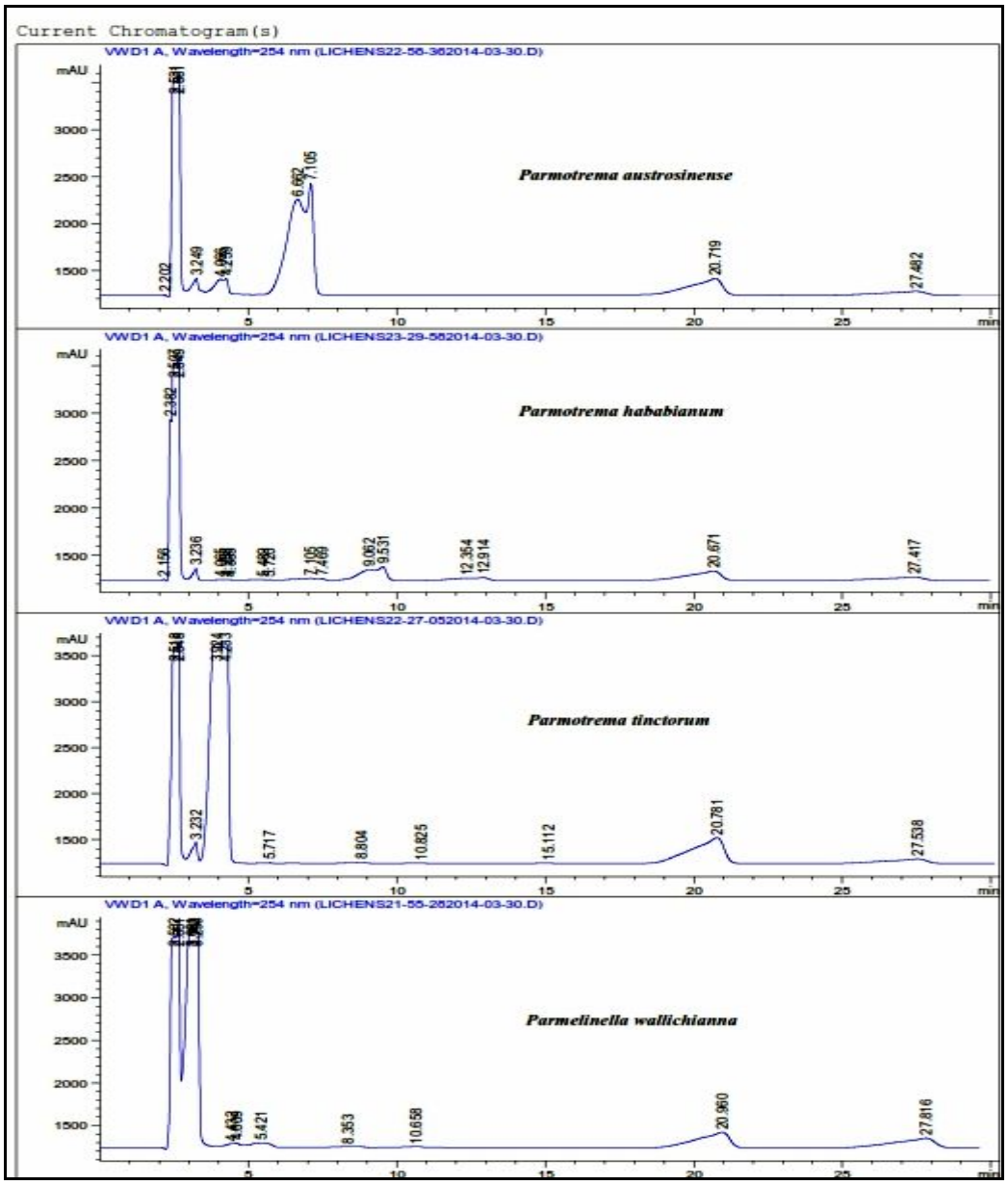

Figure 2. HPLC chromatogram for Lichen species with respect to the chemical compound

3.4 *Evaluation of Antioxidant Activity of Various Lichen Species*

3.4.1 2, 2-Diphenyl-1-picrylhydrazyl free radical scavenging activity

Stable DPPH was used to assess the free radical capturing power of hydrogen ions. It can donate hydrogen molecule to nearby atoms that can form hydrogen free radicals [29]. Free radical capturing power was measured in terms of reduction in the DPPH molecule by monitoring the absorbance of 517 nm. Decrease in the absorbance indicated that the free radicals were captured by the active molecules present in the extracts. Percentage of inhibition for each lichen extract was calculated and compared with that of commercial antioxidant molecules. All the values were calculated in the ascorbic acid equivalent antioxidant capacity ($AEAC_{DPPH}$) of per gram of lichen extracts. *P. tinctorum* showed very good antioxidant activity followed by *P. hababianum* and *P. austrosinense*(Fig. 3B). With respect to IC_{50}, acetone extracts of *P. tinctorum* were found to be superior (18.41µg/mL) followed by methanol (60.3 µg/mL) and petroleum ether (74.5 µg/mL) extracts. This is in line with an earlier report by Praveen [30] obtained for *P. pseudotinctorum* of IC_{50}< 100 µg/mL.There is no difference in the antioxidant activity of methanol extracts of *P.tinctorum* and *P.hababianum*. But these extracts showed better activity than the other solvents tested. On the other hand, acetone, petroleum ether, and water extracts of both *P.habababianum* and *P. austrosinense* did not show any activity against hydrogen ion scavenging

assay. The result revealed that methanol and benzene extracts have a number of antioxidant compounds in the crude extracts of lichen species.

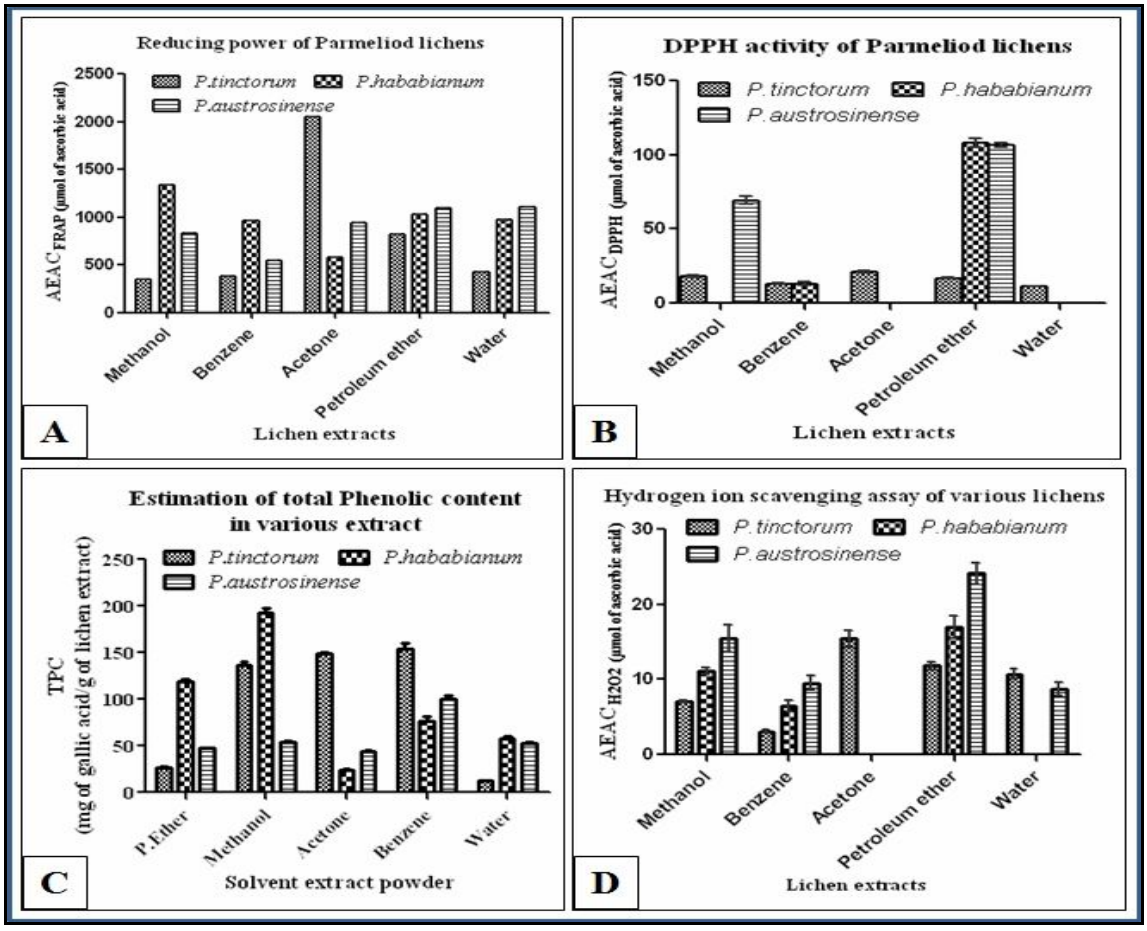

Figure 3. AEAC of various methods of antioxidant activity

3.4.2 Ferric reducing ability of lichen extracts

Ferric reducing power assay was used to study the reducing power of various chemical components present in the lichen extracts. It was also used to evaluate the capacity of extracts to transfer single hydrogen atom from the ferric ions [23]. Ferric ions from potassium hexaferricyanide (Fe^{3+}) were reduced to ferrocyanide (Fe^{2+}). Then it was reacted with ferric chloride to form a colored complex. Intensity of absorbance was measured at 700 nm. The highest absorbance of extracts revealed the highest reducing power. In view of the observation, acetone extracts of *P.tinctorum*showed the highest reducing power as 3.77 at 1000 µg of concentration followed by petroleum ether extracts (1.46), whereas the least activity was measured for methanol extracts (0.125). In case of *P.hababianum*, methanol extracts showed highest reducing power (0.687) followed by water extracts (0.45). In contrast,water extracts of *P.austrosinense* showed better activity (0.48). Similar effects were seen for both methanol and petroleum ether extracts of *P. austrosinense* I observed as 0.46. The reduction potential was calculated in terms of ascorbic acid equivalents per gram of lichen extracts, which shows the direct comparison of potential with the standard antioxidant molecule (Fig. 3A).. The result indicated that selected lichen species showed moderate levels of reducing the ferric ions, which can act as prooxidants for the food system [31]. Moderate level of reducing ability was more beneficial to the human system.

3.4.3 Hydrogen peroxide scavenging assays

H_2O_2 can form highly reactive hydroxyl ions that can be captured by the lichen extracts. Thus, the scavenging of H_2O_2 was much useful to protect the cells from damage [32]. Among the various solvents tested, methanol and benzene extracts of *P. hababianum* showed the highest potential of scavenging whereas other extracts did not show any activity (Fig. 3D). Benzene extract of *P.tinctorum* showed highest scavenging power as 26.5 followed by acetone extracts (35.32). Similar effects were observed in both methanol and benzene

extracts of *P.austrosinense* for scavenging power as 80.99 and 89.85, respectively. According to Wettasinghe and Shahidi [33], H_2O_2 scavenging is highly contributed by the phenolic substances present in their various extracts. It is also supported by our research attributing phenolic substance in *P. austrosinense* in the scavenging of H_2O_2.

3.5 *Statistical analysis*

3.5.1 Correlation

3.5.1.1 Correlation of $AEAC_{DPPH}$ and $AEAC_{H2O2}$ with total polyphenol content

Phenolic substance has been reported to be responsible for the antioxidant activity of various plant extracts. Present correlation involves the scavenging power of both hydrogen and H_2O_2 with phenolic substances. Antioxidant activity can be expressed in terms of ascorbic acid equivalence (µmol of ascorbic acid/g of lichen extracts) with corresponding lichen species. $AEAC_{DPPH}$ was calculated for different lichen species. The highest content of AEAC was obtained from petroleum ether extracts of *P. hababianum* and *P.austrosinense*. It required almost 105.5 µmol of ascorbic acid equivalence. Benzene extracts *P. austrosinense* showed the

Table 2. Correlation coefficient of antioxidant activity of various assays

Lichen species		$AEAC_{FRAP}$	Phenol	$AEAC_{DPPH}$	$AEAC_{H2O2}$
Parmotrema tinctorum	$AEAC_{FRAP}$		0.5390457	0.8362572	0.7930273
	Phenol	0.5390457		0.3464293	0.176656
	$AEAC_{DPPH}$	0.8362572	0.3464293		0.5936297
	$AEAC_{H2O2}$	0.7930273	0.176656	0.5936297	
P.hababianum	$AEAC_{FRAP}$		0.5390457	0.8362572	0.7930273
	Phenol	0.5390457		0.3464293	0.176656
	$AEAC_{DPPH}$	0.8362572	0.3464293		0.5936297
	$AEAC_{H2O2}$	0.7930273	0.176656	0.5936297	
P.austrosinense	$AEAC_{FRAP}$		0.9555258	0.4220838	0.5992189
	Phenol	0.9555258		0.5510398	0.725337
	$AEAC_{DPPH}$	0.4220838	0.5510398		0.9451377
	$AEAC_{H2O2}$	0.5992189	0.725337	0.9451377	

least content as 7.10 µmol of AEAC. Benzene and methanol extracts of *P. tinctorum* showed the least content of $AEAC_{H_2O_2}$, whereas benzene and water extracts showed less ascorbic acid equivalence of antioxidant activity. In *P.tinctorum*, $AEAC_{DPPH}$ showed a moderate correlation of 0.59, whereas $AEAC_{H_2O_2}$ with TPC showed very less correlation of about 0.18. On the other hand, $AEAC_{DPPH}$ with TPC was found to be 0.35, which is superior than the H_2O_2 scavenging power. In case of *P.hababianum*, moderate correlation was obtained for $AEAC_{DPPH}$ with TPC (0.55). $AEAC_{H_2O_2}$ Showed the highest influence toward the total phenolic content (0.73). Both species revealed that phenolic compounds are moderately responsible for the antioxidant activity (Table 2). *P. hababianum*was found to be influenced significantly by phenolic substances rather than *P. tinctorum*. On the other hand, *P. austrosinense*had the negative correlation of phenolic compounds with antioxidant activity. But the correlation between $AEAC_{DPPH}$ and $AEAC_{H_2O_2}$ was much higher in the species tested. The results indicated that free radical scavenging of all the extracts were highly correlated with various scavenging assays. Phenolic substance was moderately contributing the antioxidant activity in DPPH assay whereas the contribution was very low in H_2O_2 scavenging activity of lichen species. It may be due to the presence of non-phenolic substance that may feature the antioxidant activity [34].

3.5.1.2 Correlation $AEAC_{FRAP}$ with TPC

Reducing the power of ferric ion was correlated with the TPC of corresponding extracts. AEAC of FRAP assay was measured in the different extracts. *P.tinctorum* showed moderate correlation with the phenolic content (0.54), whereas *P.hababianum* showed highly significant phenolic content (0.96). It revealed that

phenolic compounds were responsible for reducing the power of various extracts. On the other hand, *P.austrosinense* showed negative correlation with phenolic compounds, which indicated that the non-phenolic substances may be involved in the antioxidant activity. The present result was supported by Pulido et al. [35] for the strong contribution of phenolic content in the lichen extracts toward the reducing ability of ferric ions.

3.5.2 Principal Components Analysis

The result revealed that acetone extracts of *P. tinctorum* were highly loaded on the PC1 axis, which showed a strong effect on the DPPH, FRAP, H_2O_2 assays, as well as total phenolic compounds. Benzene extracts of *P.tinctorum* were strongly loaded onto the TPC (Fig. 4A). Water and methanol extracts loaded less on the PC2 axis, whereas methanol extracts of *P. hababianum* were highly loaded on the PC1 axis, which shows a strong influence on the phenolic content as well as reducing power. In contrast, benzene extracts were less loaded on the PC2 level, which strongly affects the hydrogen scavenging power assay. FRAP as well as H_2O_2were found to be similarly loaded, which revealed that both are very closely related to antioxidant activity (Figure 4B). On the other hand, antioxidant activity was slow loaded with the TPC of *P. austrosinense*. Methanol extracts were found to be highly loaded onto FRAP, H_2O_2, and DPPH assays of antioxidant property (Figure 4C). DPPH, H_2O_2, and FRAP assay were equally loaded onto the PC1 axis and also moderately related to the antioxidant activity [23].

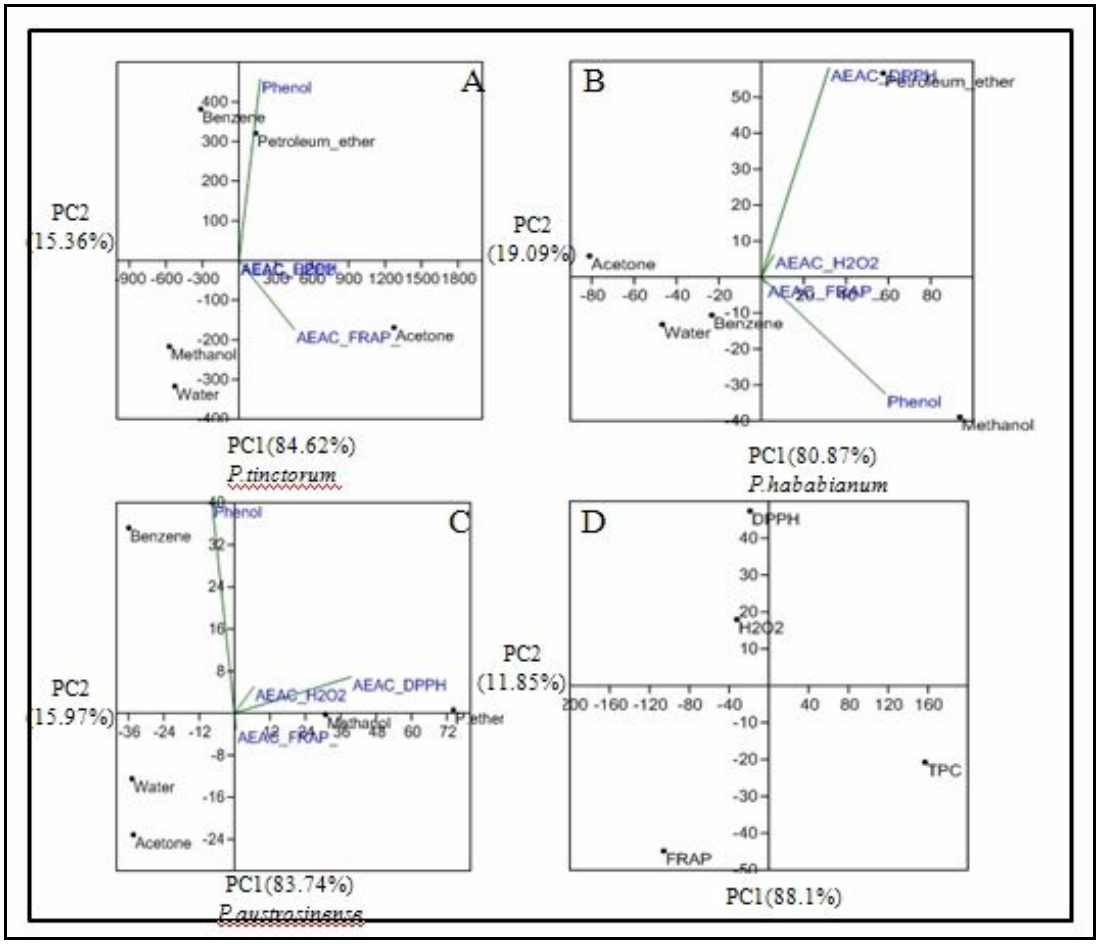

Figure 4. Analysis of principal components in antioxidant activity against various lichen species A) PCA analysis of *P.tinctorum* B) *P.hababianum* C) *P.austrosinense* D) Antioxidant activity

4. Conclusion

On the basis of the result obtained, *in vitro* assessing of different solvent extracts of parmeliod lichens shows better antioxidant and antimicrobial activity. In HPLC analysis, atranorin, lecanoric acid, salazinic acid and chloroatranorin were the major compounds present in the four different species of parmeliod lichens. In

addition, finding of these activities could lead to the formulation of pharmaceutically important products for various ailments.

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