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Research Article

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Evaluation of anti-oxidant properties of *Foliose lichens*

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ABSTRACT

This is the first attempt reported on the antioxidant activities of Heterodermia boryi, Sticta weigelii and Dermatocarpon vellereum.The levels of super oxide dismutase were evaluated in Heterodermia boryi, Sticta weigelii and Dermatocarpon vellereum. Among which SOD levels were found to be higher in Heterodermia boryi (4460 U/g). Catalase was found to be higher in Dermatocarpon vellereum (113.01 U/g). Peroxidase activity was higher in Dermatocarpon vellereum (829.12 U/g). GST level was higher in Sticta weigelii (454.87 U/g). Polyphenol oxidase was estimated in the form of catechol and laccase. COX and LAC levels were higher in Dermatocarpon vellereum (9.973 and 9.33 U/g) and total polyphenol oxidase was found to be higher Dermatocarpon vellereum (19.23 U/g) Ascorbic acid level was higher in Sticta weigelii (24.29 mg/g) Tocopherol were reflected higher in Sticta weigelii (1474 µg/g). Reduced glutathione was found to be higher in Heterodermia boryi (11.63 nano moles/g). ABTS radicals were highly scavenged by Sticta weigelii (36.91%) followed by Dermatocarpon vellereum (35.02%) and Heterodermia boryi (31.81%).DPPH radicals were highly scavenged by Heterodermia boryi (90.66%). The inhibitory effect on lipid peroxidation by the lichens was estimated and was found that Dermatocarpon vellereum (92.26%). Comparatively Heterodermia boryi and Sticta weigelii possessed lesser LPO (94.17% and 85.51% respectively). Inhibition of oxidant induced DNA damage in herring sperm was evaluated, Sticta weigelii greatly inhibited (92.24%).

Key words: *Heterodermia boryi*, *Sticta weigelii*, Lipid peroxidation, Herring sperm DNA, Laccase, Polyphenol oxidase.

INTRODUCTION

Lichens and lichen products have been used in traditional medicine worldwide, including the Indian system of medicine, these lichen species are said to effectively cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders and many disorders of blood and heart [1, 2, 3].

Since manifold activities of lichens substances have now been recognized, because of the difficulties in collection of substantial amounts of lichen biomass, their therapeutic potential has not been fully explored yet and thus remains pharmaceutically unexploited [4]. In their natural habitats, lichens can survive in extreme environments, some of which are rather dry habitats such as hot deserts, arctic tundras, heaths and tree canopies. They are able to exist a great part of the time in a dry state with a very low level of metabolic activity [5, 6] but can rapidly resume normal physiological activities upon rehydration. Metabolic activities, mainly respiration and photosynthesis, frequently result in production of ROS. These are enhanced during stresses like nutrition limitation, exposure to xenobiotics, or desiccation and/or rehydration.

Potentially toxic oxygen species occur in all aerobic organisms. They are ubiquitously generated in many biological oxidations as products of enzymatic reactions and of cellular redox reactions. The toxicity has been attributed to their interaction with other cellular components, particularly lipids and pigments. Unfavorable environmental

conditions can enhance the production of such toxic oxygen radicals. Free radicals (hydroxyl radical, superoxide anion and hydrogen peroxide and reactive nitrogen species such as nitric oxide) play an important role in many chemical processes in cells, but they are also associated with unwanted side effects, causing cell damage. They attack proteins, nucleic acids as well as unsaturated fatty acids in cell membranes [7].

Antioxidants, both synthetic and natural, are substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [8]. However, in current scenario, it is suspected that synthetic antioxidants like BHA, TBHQ, BHT and PG have toxic and carcinogenic effects [9]. Because of that, natural antioxidants are needed to be developed for their capacity to protect organisms without any negative effects from damage induced by oxidative stress [10, 11]. Natural antioxidants should be required from various sources such as plants, macromycetes and lichens. In the search for new antioxidant sources, the present research is focused on lichens.

Lichens have been found to contain a variety of secondary metabolites called 'Lichen substances' with strong anti - oxidant activity. These substances have high ability to scavenge toxic free radicals due to their phenolic groups. The highly reactive potential of ROS responsible for some human diseases e.g. cancer and cardiovascular diseases cause oxidative damage to proteins, DNA and lipids [12]in both humans and microorganisms. Plants have to counteract these negative effects by diverse effective enzymatic and non-enzymatic mechanisms [13]. Several enzymes like superoxide dismutase, catalase, peroxidase are able to scavenge ROS [14].

EXPERIMENTAL SECTION

ESTIMATION OF SUPEROXIDE DISMUTASE

The activity of superoxide dismutase was assayed spectrophotometrically [15]. Superoxide dismutase uses the photochemical reduction of riboflavin as oxygen generating system and catalyzes the inhibition of NBT reduction, the extent of which can be assayed spectrophotometrically at 600nm.Each of the lichen species (500mg) were ground separately with 3.0ml of potassium phosphate buffer. The homogenates were centrifuged at 2000 rpm for 10 minutes. The supernatants were used for the assay. The reaction mixture contained, in a final volume (3.0ml) having 50mM potassium phosphate buffer (pH 7.8), 45µM methionine, 5.3mM riboflavin, 84µM NBT and 20µM potassium cyanide. The reaction mixtures were placed in aluminium foiled tubes maintained at 25°C and equipped with 15W fluorescent lamps for 10 minutes. The reduced NBT was measured spectrophotometrically at 600nm. The maximum reduction was observed in the absence of enzyme. One unit of enzyme activity was defined as the amount of enzyme giving 50% inhibition of the reduction of NBT. The values were calculated as U/gram lichen.

ESTIMATION OF CATALASE

Catalase activity of the lichen samples were determined [16]. Calorimetric estimation was done at 230-250nm. On hydrogen peroxide decomposition by catalase, the absorption decreases with time. The enzyme activity can be estimated by this decrease in absorption. A 20% homogenate of each lichen extract were prepared in phosphate buffer and the homogenate was employed for the assay. The samples were read against a control without homogenate but with hydrogen peroxide and phosphate buffer. To the experimental reaction setup, 3ml of hydrogen peroxide buffer was added, followed by immediate addition of 40µl lichen extract and mixed thoroughly. The time interval required for a decrease in absorbance by 0.05 U was recorded at 240nm. The enzyme solution containing hydrogen peroxide free phosphate buffer served as control. One unit of enzyme was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 U.

ESTIMATION OF PEROXIDASE

The activity of peroxidase in lichen samples was estimated according to the method described [17]. Peroxidase catalyses the chemical conversion of hydrogen peroxide in to water and oxygen in the presence of hydrogen donor pyrogallol. Oxidation of pyrogallol to a colored product called purpurogalli can be measured spectrophotometrically at 430nm with the specified time interval. The intensity of the product is proportional to the activity of the enzyme. Lichen samples were prepared as 20% homogenate in 0.1M phosphate bufferand used for the assay. 3.0 ml of pyrogallol solution and 0.1 ml of enzyme extract was pipette into cuvette. 0.5ml of 1% hydrogen peroxide was rapidly added and mixed. Change in absorbance was recorded every 30 seconds up to 3 minutes. One unit of peroxidase is defined as the change in absorbance per minute at 430nm.

ESTIMATION OF GLUTATHIONE-S-TRANSFERASE (GST)

The Glutathione-S-transferase activity was estimated by the method previously described [18]. Each of the lichen samples (0.5g) was homogenized with 5.0ml of phosphate buffer. Homogenates were centrifuged at 5, 000rpm for 10 minutes and the supernatant was used for assay. Enzyme activity was determined by monitoring the change in absorbance at 430nm in a spectrophotometer. The assay mixture contained 0.1ml of GSH, 0.1ml CDNB. Reaction

mixture was made up to 2.9 ml with phosphate buffer and initiated by the addition of 0.1ml of lichen extract. The values were recorded against blank for minimum 180 seconds. Control was prepared without sample to monitor non-specific binding of substrates. One unit of GST activity was defined as the nano moles of CDNB conjugated per minute.

ESTIMATION OF POLYPHENOL OXIDASE

The activity of polyphenol oxidase, expressed in the form of catechol oxidase and laccase, can be assayed simultaneously as described previously [19].Each lichen sample was homogenized (5.0g) in 20ml medium containing Tris HCl, Sorbitol and Sodium chloride. Homogenates were centrifuged at 2,000rpm for 10 minutes and the clear supernatant was taken for assay. The assay mixture constituted 2.5ml of 0.1M phosphate buffer and 0.3ml of catechol and was measured at 495nm. Lichen extract (0.2ml) was added to same tube and the change in absorbance was recorded every 30seconds up to 5minutes. One unit of enzyme (catechol oxidase or laccase) can be defined as the amount of enzyme transforming 1micromole of dinitrophenol to 1micromole quinine per minute.

The activity of PPO was calculated as follows:

Enzyme unit = K x (ΔA/min)
K value (for catechol oxidase) = 0.272
K value (for laccase) = 0.242

ESTIMATION OF ASCORBIC ACID

The amount of ascorbic acid present in lichens was estimated according to the method described [20]. The lichens samples (1g each) were homogenized in 4% TCA and the volume was made up to 10ml with 10% TCA. Standard ascorbic acid (1mg/ml) was treated with same way. Samples were then centrifuged at 2000rpm for 10 minutes and the supernatants were treated with a pinch of activated charcoal and incubated for 10 minutes. Samples were spun to remove traces of charcoal. Each supernatant was made up to 2.0ml with 4% TCA and standards were prepared with concentrations having 20-100µg of ascorbic acid. DNPH was added to all tubes, followed by 10% thiourea solution. The reaction setups were kept at 37°C for 3 hours. The reaction mixtures were treated with 2.5ml of 85% sulphuric acid under cold condition. Samples were read at 540 nm after incubating at room temperature for 30 minutes. Ascorbic acid levels were expressed as mg/gram sample.

ESTIMATION OF TOCOPHEROL

The levels of tocopherol in lichen samples were determined by spectrophotometric method [21]. Lichen samples (2.5g each) were homogenized and made up to 50ml with 0.1N sulphuric acid. The contents were allowed to stand for overnight in dark and were shaken rapidly, filtered and filtrates were used for the assay. All the tubes (sample extract, standard and blank) were added with 1.5ml of ethanol and xylene and centrifuged at 4, 000rpm for 10minutes. After centrifugation, the xylene layer was removed from all tubes and was transferred to new set of tubes. To 1.0ml of xylene layer, 1.0ml of 2, 2′ -dipyridyl was added and mixed well. This reaction mixture was measured at 460nm. All the tubes were then treated with 0.33ml of ferric chloride and incubated for 15 minutes. OD was read against blank at 520nm. The amount of tocopherol was calculated as follows:

$$\text{Tocopherol } (\mu g) = \frac{A_{520} - A_{450}}{Std_{A520}} (0.29 \times 15)$$

ESTIMATION OF REDUCED GLUTATHIONE

Reduced glutathione levels were estimated as previously [22] described. A 20% homogenate was prepared by homogenizing 0.5g of lichen samples in 2.5ml of 5% TCA. Homogenates were immediately acidified by adding 125µl of 25% TCA to prevent aerial oxidation of glutathione. The precipitated protein was centrifuged at 1, 000 rpm for 10minutes.The homogenate was cooled on ice and 0.1ml of the supernatant was taken for estimation. The supernatants were made up to 1.0ml with. 2.0ml of freshly prepared DTNB solution was added to all tubes. The intensity of yellow color formed was read at 412nm in a spectrophotometer after 10minutes.A standard GSH was prepared using concentration ranging from 2-10 nano moles of GSH were set to linear regression mode. The values are expressed as nano moles of GSH/gram lichen tissue.

DPPH SCAVENGING EFFECT

The ability of lichen extracts to scavenge the stable free radical DPPH was determined by the method described [23]. The lichen extracts and 0.48ml of methanol was added to 0.5ml of methanolic DPPH. The reaction setup was allowed to stand for 30minutes. Methanol alone served as blank and methanolic DPPH without lichen extracts, served as positive control. After 30minutes of incubation, the discoloration of the purple color was measured at 518nm. The radical scavenging activity was calculated as

$$\text{Scavenging activity (\%)} = 100 - \frac{A_{518}\text{ (std)} - A_{518}\text{ (blank)}}{\text{A518 (blank)}} \times 100$$

ABTS SCAVENGING EFFECT

The ability of lichens to scavenge the free radical ABTS was studied by the method described [24]. All the three lichen extracts (0.5ml each) were dissolved in 0.3ml of ABTS solution, were made up to 1ml with ethanol and measured at 745nm. The percentage inhibition of lichen extracts was calculated as,

$$\text{Inhibition (\%)} = \frac{\text{Control - test}}{\text{Control}} \times 100$$

EFFECT ON LIPID PEROXIDATION INHIBITION

Oxidizing agents (ferrous ions and ascorbate or hydrogen peroxide) impose a stress on membrane lipids which can be quantified as the extent of TBARS formed. The extent of inhibition of lipid peroxidation by lichen extracts on goat RBC membrane was evaluated as described [25, 26].Goat blood (50ml) was collected from slaughterhouse and was immediately de-fibrinated using acid-washed stones. The de-fibrinated blood was diluted with saline and transported to laboratory on ice. The RBCs were collected by centrifuging at 3, 000rpm for 10 minutes and washed thrice with isotonic KCl. The cells were then treated with hypotonic KCl and allowed to lyse completely at 37°C for one hour. The lysate was centrifuged at 5, 000 rpm for 10 minutes at 4°C. The pellet was washed several times with hypotonic KCl until most of the hemoglobin was washed off and a pale pink pellet was obtained. The pellet was suspended in 1.5ml of TBS. 50µl aliquots of pellet were used for the assay. The control tubes were prepared for each sample containing respective lichen extract (50µl corresponding to 20mg), membrane aliquot (RBC membrane - 50µl) and TBS (100µl) to make a final volume of 500µl. Ferrous sulphate (100µl) was added all the tubes except control at a final concentration of 10µmoles. Blank was prepared without lichen extract, membrane aliquots but with ferrous sulphate and TBS. An assay medium corresponding to 100% oxidation was prepared by adding all the other constituents except lichen extracts. Experimental medium corresponding to auto oxidation contained only membrane preparations. All the tubes were incubated at 37°C for 60minutes. After incubation, all the tubes were incubated with H_2O_2 and subjected to TBARS quantification. Lipid peroxidation in all the tubes was arrested by adding 0.5ml of 70% ethanol. All the tubes were added with 1ml of 1% TBA and incubated in boiling water bath for 20 minutes. After cooling to room temperature, 500µl of acetone was added and the TBARS measured at 535nm. The rate of lipid peroxidation inhibition is calculated as follows

$$\text{Inhibition (\%)} = \frac{\text{Control - test}}{\text{Control}} \times 100$$

EFFECT ON INHIBITION OF OXIDANT INDUCED DNA DAMAGE

DNA damage was assessed *in vitro* in commercially available preparations of DNA. The bio molecular protective effect of the lichen extracts on the damaged DNA was determined by method as previously described [27].The assay reaction mixture contained 0.5ml (containing 0.05ml of herring sperm DNA, 0.167ml of H_2O_2, 0.05ml of $MgCl_2$ and 0.05ml of $FeCl_3$) and sample (20µl containing 10mg of lichen extract diluted in tris buffer). The reaction was terminated by the addition of 0.05ml of 0.1M EDTA after incubating at 37°C for 1hour.The color was developed by adding 0.5ml of thiobarbutyric acid and 0.5ml of HCl. DNA damage was measured by the increase in absorbance at 532nm. The amount of TBARS formed in the group treated with H_2O_2 alone was fixed as 100% and the extent of damage caused in other groups were calculated that are related to this value.

STATISTICS

The data were subjected to one way analysis of variance (ANNOVA) to determine the significance of individual differences. Significant means were compared by the Duncan's multiple range tests. All the statistical analyses were carried out using SPSS software package (SPSS Version 16.0, Chicago, USA).

RESULTS

This is the first attempt reported on the antioxidant activities of *Heterodermia boryi*, *Sticta weigelii* and *Dermatocarpon vellereum*. The levels of super oxide dismutase were evaluated in *Heterodermia boryi*, *Sticta weigelii* and *Dermatocarpon vellereum*. Among which SOD levels were found to be higher in *Heterodermia boryi* (4460U/g; $P< 0.001$). Next *Sticta weigelii* constituted 4394.6U / gram; $P< 0.001$. Least amounts (compared with *Heterodermia* and *Sticta*) of SOD were observed in *Dermatocarpon vellereum* (3638 U/g; $P< 0.001$). The amount of

catalase was found to be higher in *Dermatocarpon vellereum* (113.01 U/g; *P*< 0.001) and it was 69.44 U/g (*P*< 0.001) in *Sticta weigelii*. Least amounts were found in *Heterodermia boryi* (42.11U/g; *P*< 0.001). The activity of peroxidase in all the three lichens was evaluated and was found to be higher in *Dermatocarpon vellereum* (829.12U/g; *P*< 0.001). The levels were comparatively lesser in *Heterodermia boryi* (139.15U/g; *P*< 0.001) and *Sticta weigelii* (137.40U/g; *P*< 0.001) (Table 1).

GST levels were evaluated in lichens and were found to be comparatively higher in *Sticta weigelii* (454.87 U/g; *P*< 0.001). The GST levels were moderate (333.69U/g; *P*< 0.001) in *Dermatocarpon vellereum* and low in *Heterodermia boryi* (169.61 U/g; *P*< 0.001). The polyphenol oxidase was estimated in the form of catechol and laccase. COX and LAC levels were higher in *Dermatocarpon vellereum*(9.973 and 9.33U/g respectively; *P*> 0.05). Their levels were low in *Sticta weigelii* (5.313; *P*< 0.01 and 4.36U/g; *P*< 0.001 respectively) and *Heterodermia boryi* (3.046 and 3.086U/g respectively; *P*< 0.001) (Table 1).

Total polyphenol oxidase was found to be least in *Heterodermia boryi* (3.97 U/g; *P*< 0.01) followed by *Dermatocarpon vellereum* (19.23 U/g; *P*< 0.001 - significant increase than standard) and *Sticta weigelii* (7.17 U/g; *P*> 0.05) (Table 1).

Total ascorbic acid level was expressed in mg/g of lichen. The level was higher in *Sticta weigelii* (24.29 mg/g; *P*< 0.001) and comparatively less in *Dermatocarpon vellereum* (12.7mg/g; *P*< 0.001) and *Heterodermia boryi* (10.04mg/g; *P*> 0.05) (Table 1).

Table 1. Levels of enzymatic anti-oxidants of lichens

*Values (enzyme units/gram lichen) are expressed as mean ±SEM (n=6). *** - P<0.001, **- P<0.01, NS - non significant (P>0.05)*

Enzymatic anti-oxidant	Standard	*Sticta weigelii*	*Dermatocarpon vellereum*	*Heterodermia boryi*
	Enzyme units/gram lichen			
Superoxide dismutase	1000±0.00	4645.1±93.66***	3418.8±77.15***	4631±68.67***
Catalase	100±0.00	69.44±1.42***	113.01±1.49***	42.11±2.01***
Peroxidase	200±0.00	137.4±1.62***	829.12±6.72***	139.15±0.82***
Glutathione-S-transferase	200±0.00	454.87±17.77***	333.69±38.23**	169.61±27.79^{NS}
Catechol oxidase	10±0.00	5.31±1.05**	9.973±0.93^{NS}	3.04±0.59***
Laccase	10±0.00	4.36±0.86***	9.33±0.91^{NS}	3.08±0.68***
Poly phenol oxidase	10±0.00	7.17±0.52^{NS}	19.23±1.89***	3.97±0.03**

The levels of tocopherol were reflected as µg/g. It was to be higher in *Sticta weigelii* (1474µg/g; *P*< 0.001) and comparatively less in *Dermatocarpon vellereum* (1111.98µg/g; *P*< 0.01) and *Heterodermia boryi* (273µg/g; *P*< 0.001)(Table 2).GSH level was estimated in all the lichens. The levels of GSH were expressed in nano moles/g lichen tissue. It was found to be higher in *Heterodermia boryi* (11.63 nano moles/g; *P*> 0.05), and less in *Dermatocarpon vellereum* (6.28nano moles/g; *P*> 0.05) and *Sticta weigelii* (4.23nano moles/g; *P*< 0.05) (Table 2).

ABTS radicals were highly scavenged by *Sticta weigelii* (36.91%; *P*< 0.001), followed by *Dermatocarpon vellereum* (35.02%; *P*< 0.001) and *Heterodermia boryi* (31.81%; *P* < 0.001).DPPH radicals were highly scavenged by *Heterodermia boryi* (90.66%; *P*< 0.001), followed by *Sticta weigelii* (82.23%; *P*< 0.001) and *Dermatocarpon vellereum* (78.41%; *P* < 0.001) (Table 2).The inhibitory effect on lipid peroxidation by the lichens was estimated and was found that *Dermatocarpon vellereum* (92.26%; *P* < 0.05) possessed maximum percentage of LPI. Comparatively *Heterodermia boryi* and *Sticta weigelii* possessed lesser LPI (94.17%; *P*< 0.01and 85.51%; *P*< 0.001). Oxidant (H_2O_2) induced DNA damage in herring sperm was evaluated for the lichen extracts. Extracts of *Sticta weigelii* greatly inhibited (92.24%; *P*< 0.001) the damage inflicted by H_2O_2 on herring sperm DNA compared to other two lichen extracts [*Heterodermia boryi* (67.17%; *P*< 0.001) and *Dermatocarpon vellereum* (38.66%; *P*< 0.001)] (Table 2).

Table 2. Levels of non - enzymatic anti-oxidants of lichens

*Values (enzyme units/gram lichen) are expressed as mean ±SEM (n=6). *** - P < 0.001, **- P < 0.01, * - P< 0.05, NS - non significant (P>0.05)*

Non-enzymatic anti-oxidant	Standard	*Sticta weigelii*	*Dermatocarpon vellereum*	*Heterodermia boryi*
Ascorbic acid (mg/gram lichen)	10±0.00	24.29±0.33***	12.7±0.09***	10.04±0.14^{NS}
Tocopherol (mg/gram lichen)	1000±0.00	1415.83±30.26***	1111.98±8.02**	285.68±15.49***
Reduced glutathione (nano moles/ gram lichen)	10±0.00	4.23±0.50*	6.28±2.03^{NS}	11.63±1.07^{NS}
ABTS scavenging activity (%)	100±0.00	36.91±0.43***	35.02 ±3.10***	31.81±1.22***
DPPH scavenging activity (%)	100±0.00	82.23±1.37***	78.41±0.97***	90.66±1.23***
Inhibition of lipid peroxidation (%)	100±0.00	85.51±3.27***	95.26±2.19*	94.17±2.64**
Inhibition of herring sperm DNA damage (%)	100±0.00	92.24±3.40***	38.66±5.82***	67.17±6.04***

SOD provides therapeutic benefits in protecting tissue injuries like ischemia, inflammation, hyperoxia, arthritis etc. In our findings, *Dermatocarpon vellereum* shown lesser activity which may be attributed by the result of inactivation of enzymes by interacting with ROS produced during dehydration. ROS such as hydroxyl radical and hydrogen peroxide were shown to cause inactivation and fragmentation of SOD enzymes [28]. The increased activity of SOD in such lichens also contributed due to the growth of lichens in higher altitudes [29]. This higher activity might be included as a response to multiple stress conditions, particularly to ozone stress, which may occur at these regions. A decrease in cellular activities of antioxidant enzymes in the hydrated thalli and increased activities after desiccation may reflect general changes in total cellular proteins. Selective decrease in total cell proteins during desiccation occurs as a part of the preparation for survival in dried state. Such effects were reported for plants and seeds undergoing dehydration were dependent on an increase in proteolytic activities [30, 31].

In our study, *Dermatocarpon vellereum* have shown least SOD activity. The decreased SOD activity might be countered by prolonged desiccation and rehydration by dew during night or by rainfall. Decreased catalase activity in *Heterodermia boryi* might be due to the conversion catalase into its inactive derivatives by interaction with H_2O_2[32]. *Dermatocarpon vellereum* was found to grow in highly moisture areas like waterfalls rocks. They are highly lithotrophic and mainly depend on rocky silica. Such inhabiting lichen thallus frequently exposed to high moisture content. The rhizinomorphs are highly tender and delicate to flooding and moisture accumulation. In order to secure themselves from destruction by moisture, they possess a set of enzymes that could degrade oxidized water (formed during oxidative stress) in to water. Since this is a vital part of lichens having impact on environment, they possess catalase.

Certain lichen species requires copper and manganese for nutrient accumulation. The fungal partners in lichens possess copper and manganese dependent SOD. Few other algae requires Fe-SOD, were protein products of different genes underwent certain modifications. Alternatively this multiplicity may be the result of post translational modifications which alters the induction activity of elements on the formation of hydrogen peroxide. Excess hydrogen peroxide in turn switches on catalase genes to express on appropriate levels. Peroxidases have been difficult to study through classical genetics and Biochemistry. There are large number of peroxidase isoenzymes and even more peroxidase genes. Peroxidases are a convenient physiological marker because of the simplicity of their activity assay, and this feature has allowed peroxidase to be a useful marker in plant development, physiology and infection [33, 34]. Although peroxidase is a highly catalytic enzyme, it has very little specificity, and there exists a multitude of isoenzyme forms [35]. This makes it difficult to understand the actual plant functions of peroxidase and its role in plant growth and adaptation to its environment.

The levels of COX were found to be very low in *Heterodermia boryi* (2U/g). The presence of phenolic compounds inhibits catechol [36] and hence low COX activity was reported in this study. Plants use different pathways and produce a diverse array of secondary metabolites involved in resistance against microbial pathogens. Although there are defense compounds that are specific for selected plant families, some chemical compounds, such as phenyl propanoid derivatives, are often involved in these protective processes [37].

Polyphenol oxidase activities were found in lichenized ascomycetes belonging to different taxonomic groups. Most of the epigeic and epilithic lichens of *Peltigerales* and *Lecanorales* were found to possess laccase activity. Water soluble polyphenol oxidases were present only in peltigerous lichens: activity that could be washed out from intact thalli comprised 10% of that released from disrupted thalli. Laccases catalyze the oxidation of a variety of phenolic compounds, diamines and aromatic amines, with concomitant reduction of molecular oxygen to water [38]. Recently, laccases have been discovered in representatives of the order *Peltigerales*. The activity of the peltigerous lichens and the release of soluble polyphenol oxidases into the medium increased when thalli were rehydrated quickly. Polyphenol oxidases discovered so far may play an important role in phenolic metabolism of lichens and be involved in biochemical reaction of humus synthesis during primary soil formation, which may be previously an unknown geochemical function of symbiotic microorganisms [39].

The non - enzymatic antioxidants, for the cells of many life forms, the major water-soluble low molecular weight antioxidants are glutathione and ascorbic acid [40] which were hydrophilic and their major function is cellular protection from oxidative damage in liquid phases, particularly in cytoplasm. *L. pulmonaria* and *P. polydactyla* produced more α-tocopherol during rehydration than did *P. furfuracea*, but their concentrations after 120 min of rehydration decreased with increasing desiccation time. Re-establishment of normal levels of reduced glutathione during rehydration was also negatively correlated with length of desiccation in *L. pulmonaria* and *P. polydactyla*, but not in *P. furfuracea* [41] indicating that the latter is more tolerant to desiccation. It may therefore be tempting to conclude that *P. polydactyla* contains more antioxidants in order to prevent desiccation induced oxidative damage. Some species show a powerful oxidative burst during rehydration following desiccation. This was best developed in members of *Peltigerales* [42, 43].

Glutathione occurred in a reduced form (GSH) in *L. polytropa* apothecia, suggesting that no serious oxidative stress occurred in the copper rich lichen. In contrast to higher plants, GSH may be the main non enzymatic antioxidant in lichens [44]. Glutathione, the principal low molecular thiol present in living organisms, plays a critical role in detoxification and cellular defense against oxidative damage. The GSH level in the copper loaded apothecia of *L. polytropa* was two to four folds lower than those recorded in foliose lichens *Pseudevernia furfuracea*, *Lobaria pulmonaria*, *Peltigera polydactylon* and *Xanthoria parietina* [41, 45] which were not exposed to metals. Interestingly, phytochelatins2 and 3, were also detected in copper rich *L. polytropa* apothecia. These cysteine-rich peptides were previously induced in lichen photobionts in response to metal toxicity [46].

DPPH is one of the few, stable, N - centred, commercially available organic free radical and has UV-visible absorption maxima at 515-518 nm in methanol. On accepting hydrogen from a corresponding donor, the solution of DPPH loses the characteristic deep purple color and becomes yellow colored diphenyl picryl hydrazyl. DPPH radical scavenging activity is one of the widely used assays to determine antioxidant activity of many compounds including plant extracts [47, 48]. The radical nature of DPPH was lost due to the donation of hydrogen by Lichen extract leading to the formation of the stable DPPH-H molecule [49]. The radical scavenging activity of methanol extract was stronger when compared to other solvent extracts. The extracts possessing high phenolic content displayed stronger scavenging of DPPH radicals. The results obtained are similar to previous studies where extracts containing high phenolic contents exhibited stronger scavenging activities [50, 51, 52].

Although the scavenging abilities of lichen extracts were lesser than that of ascorbic acid, it was evident that the extracts showed hydrogen donating ability and could serve as free radical scavengers, acting possibly as primary antioxidants [53]. Previous studies on antioxidant potential of *Parmotrema pseudotinctorum* [54] and *Everniastrum cirrhatum* [55] showed similar result where the extract was found to exhibit lower scavenging potential when compared to reference standard.

In the present study too, the extracts of *Dermatocarpon vellereum* inhibited lipid peroxidation. The lipid peroxidation was proved by electron donating ability and tyrosinase inhibitory potential of certain extracts of *A. awasthii*. Several plant extracts also show inhibition of lipid peroxidation as like *Phyllanthus niruri*. Aqueous extracts of leaf, fruit and seed of *Moringa oleifera* have shown significant inhibition of hydroxyl radical-dependent damage of pUC18 plasmid [56]. The aqueous extract of edible sea weed *Gracilaria tenuistipitata* shown a reduction in H_2O_2 induced plasmid breaks [57]. The methanolic extract of*Nelumbo nucifera* inhibited H_2O_2 induced damage on fatty acid peroxidation and plasmid damage in Swiss albino mice [58].

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