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Mineral Composition, Total Phenol Content and Antioxidant Activity of a Macrolichen *Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae)

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Abstract: In the present study, we investigated for the first time mineral composition, total phenol content and antioxidant activity of a foliose macrolichen *Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae) from Bhadra wildlife sanctuary, Karnataka, India. Mineral content of the lichen was estimated by Atomic absorption spectrophotometer after acid digestion. The secondary metabolites were detected by thin layer chromatography (TLC) and phytochemical assays. The lichen material was extracted with methanol in soxhlet apparatus. Total phenol content was estimated by folin ciocalteu method. Antioxidant activity was determined by DPPH, Ferric reducing and metal chelating assays. Among the principal elements, calcium was found in high concentration followed by magnesium, potassium and phosphorus. Among trace elements, iron was detected in high amount followed by zinc, manganese and copper. The DPPH radical scavenging activity was found to be dose dependent with an IC_{50} of 6.73 µg/mL. In ferric reducing assay, the absorbance increased with the concentration of extract suggesting reducing power. The extract exhibited good metal chelating activity with an IC_{50} value of 29.28 µg/mL. Total phenol content was 101.2 mg tannic acid equivalents per gram of extract. Phytochemical analysis revealed the presence of alkaloids, saponins, tannins and terpenoids. TLC revealed atranorin, salazinic acid and protolichesterinic acid. The lichen can be consumed as a source of minerals required for the body as appreciable amount of minerals has been detected. The marked antioxidant activity may be attributed to the presence of phenol content in the extract. Further studies on isolation of metabolites and their bioactivities are under investigation.

Keywords: *Everniastrum cirrhatum* (Fr.) Hale, Bhadra wildlife sanctuary, Mineral composition, Total phenol content, Antioxidant activity

Introduction

Lichens comprise a unique group that consists of two unrelated organism, a fungus and an alga, growing together in a symbiosis. Lichens with blue green symbionts, contribute significantly for forest nitrogen fixation. Lichens are distributed universally and are occurring in varied climatic conditions ranging from the poles to the tropics. They may look like crust, spreading rapidly over the surface (crustose) or leafy and loosely attached to the surface (foliose) and branched and shrubby, hanging from tree twigs or branches, with a single attachment (fruticose). Besides many other uses, lichens are also used as pollution monitors[1,2]. *Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae) is a foliose lichen in which phycobiont is a chlorolichen alga. It usually grows on the barks of trees in temperate regions[3,4]. It grows luxuriantly in tropical himalayas, central India and higher altitudes of southern India. It is characterized by linear, laciniate lobes which are grey in color. Thallus is tapering apically, 2-6 mm wide and 10 cm long. Thallus is loosely attached to the substratum and pendulous in nature. Apothecia are laminal, margins are inflexed, cilia are black in color, and spores are large[5]. In ayurveda, it is mentioned as astringent, resolvent, laxative, carminative and aphrodisiac. It is also useful in bleeding piles, leprosy and excessive salivation. It is used as spices in Madhya Pradesh. Whole boiled material used as vegetable in Nepal and north Sikkim[6,7]. *E. cirrhatum* is traditionally used as antiseptic, used to heal wound and bronchitis in Sikkim[8]. *E. cirrhatum* has been used as material for sacrificial fire by Gaddi tribe of Kangra valley. It is used as a spice and flavoring agent for meat and vegetables by Bhaiga, Bhil, Bhilala, Gond, Korka, Muria of Madhya Pradesh. It is used as vegetables in Lepchas and Nepalese of Sakyong valley, North Sikkim. In Uttaranchal, Uttar Pradesh and Sikkim, it is commercially sold as spice[9]. The mycobiont (lichen forming fungus) of *E. cirrhatum* was shown to cause mycelial growth inhibition of hot pepper anthracnose pathogen, *Colletortichum acutatum*[10]. Ethanol extract of *E. cirrhatum* showed antimycobacteral properties against *Mycobacterium tuberculosis* H37Rv and H37Ra strains with minimum inhibitory concentration of 500 µg/mL[4]. It was reported that the whole thallus of *E. cirrhatum* yielded a red brown dye[11]. Although it is a used as traditional medicine in Indian subcontinent, literature surveys reveals that many of the bioactivities of this lichen has not yet been documented. Its ethnobotanical claim prompted us to undertake this investigation. In this study, we have investigated mineral composition, total phenol content and antioxidant activity of methanol extract of *E. cirrhatum* collected from Bhadra wildlife sanctuary, Karnataka, India.

Experimental

The lichen *E. cirrhatum*, growing on barks of trees, was collected from the Bhadra wildlife sanctuary, Karnataka, India, during August 2010. The lichen specimen was identified by morphological, anatomical, chemical tests[12]. The voucher specimen of the lichen was deposited in the University herbaria, Department of PG Studies and Research in Botany, Shankaraghatta-577451, Karnataka, India for future reference.

Detection of secondary metabolites by thin layer chromatography (TLC)

The lichen was shade dried, powdered, extracted with acetone, spotted on the silica plate and developed with solvent A (180 mL toluene: 60 mL 1-4, dioxine: 8 mL acetic acid) to detect secondary metabolites using standard protocols[13,14].

Preparation of extract using methanol

The lichen was shade dried at room temperature under shade. After air-drying, the lichen material was ground to fine powder and extracted by soxhlet apparatus using methanol as solvent. The extract was filtered using Whatman filter paper No. 1 and concentrated at 40 ^{o}C under reduced pressure. The condensed methanol extract was stored at 4 ^{o}C until use[15].

Phytochemical analysis of methanol extract

The extract obtained after solvent evaporation was subjected to standard tests for detection of alkaloids (Dragendorff's reagent and Mayer's reagent), tannins (ferric chloride test), saponins (frothing test and hemolysis test), glycosides (Salkawski test and Keller-Kiliani test), sterols (Burchard test), flavonoids (Shinoda test) and terpenoids (Salkowski test)[16,17].

Elemental composition of E. cirrhatum

For elemental analysis, a known amount of powdered lichen material (1.0 g) was digested using a mixture of concentrated nitric acid and perchloric acid (10 mL) in a beaker. The powdered material was left in acid mixture for 24 h and was digested on the hot plate until complete digestion. After digestion, 10 mL of 10% nitric acid was added to the beaker and the beaker was left for two hours for residue to settle down. The supernatant liquid was filtered though Whatman No. 1. The filtrate was subjected to estimation of elements using Atomic absorption spectrophotometer[18].

Antioxidant activity of methanol extract of *E. cirrhatum*

DPPH assay

The radical scavenging ability of methanol extract of *E. cirrhatum* and the ascorbic acid (standard) was tested on the basis of the radical scavenging effect on the DPPH free radical. Different concentrations of extract and standard namely 2.5, 5.0, 10.0, 25.0, 50.0, 100, 200 and 400 µg/mL were prepared in methanol. In clean and labeled test tubes, 2 mL of DPPH solution (0.002% in methanol) was mixed with 2 mL of different concentrations of extract and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using UV-Vis Spectrophotometer. The absorbance of the DPPH control was also noted. The scavenging activity of the extract was calculated using the formula: Scavenging activity (%) = A – B / A X 100, where A is absorbance of DPPH and B is absorbance of DPPH and extract/standard combination. The IC_{50} value for the extract was calculated by Origin 6.0 software. IC_{50} denotes the concentration of extract required to scavenge 50% of DPPH free radicals[19].

Ferric reducing assay

Different concentrations of extract and standard (Tannic acid) namely 2.5, 5.0, 10.0, 25.0, 50.0, 100, 200 and 400 µg/mL in 1 mL of methanol were mixed in separate tubes with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The tubes were placed in water bath for 20 minutes at 50 ^{o}C, cooled rapidly and mixed with 2.5 mL of 10% trichloroacetic acid and 0.5 mL of 0.1% Ferric chloride. The amount of iron(II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussian blue at 700 nm after 10 minutes. The increase in absorbance of the reaction mixtures indicates increased reducing power[20].

Metal chelating activity

The chelating of ferrous ions by different concentrations of methanol extract of *E. cirrhatum* and EDTA (standard) was estimated by the method of Dinis *et al*[21]. Briefly, the extract and standard (2.5-100 µg/mL) were added to a solution of 2 mM FeCl2 (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for ten minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm in a spectrophotometer. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given by the formula:

Percent inhibition = $A_0 - A_1 / A_0$ X 100, where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the extract and standard. The control contains $FeCl_2$ and ferrozine[22]. The IC_{50} value of the extract was calculated by origin 6.0 software.

Total phenol content of extract

Total phenol content of methanol extract of *E. cirrhatum* was determined by Folin-Ciocalteu method. A dilute concentration of extract (0.5 mL) was mixed with 0.5 mL of 1:1 diluted Folin-Ciocalteu reagent and 4 mL of sodium carbonate (1 M). The mixtures were allowed to stand for 15 minutes and the total phenol content was determined colorimetrically at 765 nm. A standard curve was prepared by using an increasing concentration of tannic acid in methanol. Total phenol value was expressed in terms of tannic acid equivalent[23].

Statistical analysis

All data were expressed as mean ±SD of the number of experiments (n = 3). Past software version 1.92 was used.

Results and Discussion

Preliminary phytochemical analysis of methanol extract of *E. cirrhatum* was determined by chemical tests. Phytoconstituents namely alkaloids, saponins, tannins and terpenoids were detected in the extract (Table 1). TLC in solvent A showed the presence of Atranorin ($C_{19}H_{18}O_8$, mol.wt. 374.33), Salazinic acid ($C_{18}H_{12}O_{10}$, mol.wt. 388.28) and Protolichesterinic acid ($C_{19}H_{32}O_4$, mol.wt. 324.45) in the lichen material. The structures of atranorin, salazinic acid and protolichesterinic acid is shown in Figure 1.

Table 1. Phytochemicals detected in methanol extract of *E. cirrhatum*

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's test	+
	Mayer's test	+
Saponins	Frothing test	+
	Hemolysis tst	+
Flavonoids	Shinoda test	-
Glycosides	Salkowski test	-
	Keller-Kiliani test	-
Tannins	Ferric chloride test	+
Sterols	Burchard test	-
Terpenoids	Salkowski test	+

'+' Present; '-' Absent

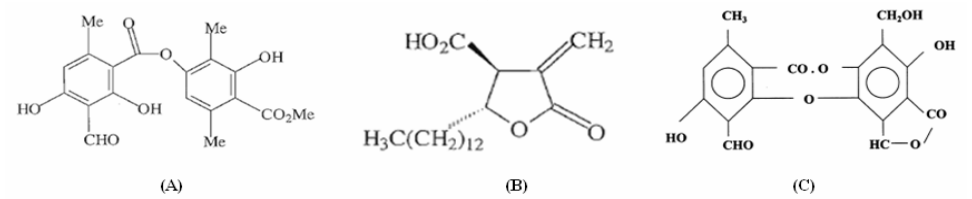

Figure 1. Structure of (A) Atranorin (B) Protolichesterinic acid and (C) Salanizic acid[24,25]

The mineral composition of the lichen material was determined using atomic absorption spectrophotometer and the result is shown in Table 2. Among the principal elements, calcium was present in high concentration (5190 ppm) followed by magnesium (1505 ppm),

potassium (1540 ppm) and phosphorus (24 ppm). Among trace elements, iron was detected in high amount (893.2 ppm) followed by zinc (65.9 ppm), manganese (52.9 ppm) and copper (5.5 ppm).

Table 2. Mineral composition of *E. cirrhatum*

Element	Quantity, ppm
Phosphorus (P)	24.67±0.57
Potassium (K)	1542±1.52
Magnesium (Mg)	1506±1.15
Calcium (Ca)	5191±1.00
Iron (Fe)	893.7±1.10
Zinc (Zn)	66.3±0.60
Manganese (Mn)	53.13±0.32
Copper (Cu)	5.83±0.35

The significance of the mineral elements in humans, animals and plants nutrition can not be overemphasized. The mineral elements are separate entities from the other essential nutrients like proteins, fats, carbohydrates and vitamins. Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. These include calcification of bone, blood coagulation, neuromuscular activity, acid base equilibrium, enzyme activity, osmotic regulation *etc.*[26,27].

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS[28,29]. The synthetic antioxidants like BHA, BHT, gallic acid esters *etc.,* have been suspected to cause or prompt negative health effects. Strong restrictions have been placed on their application[30,31]. In recent years much attention has been devoted to natural antioxidant and their association with health benefits[32]. The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging[33]. Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, metal chelating, ROS quenching assays are commonly used for evaluation of antioxidant activities of extracts[34,35].

Antioxidant activity of different concentrations of methanol extract of *E. cirrhatum* and ascorbic acid in terms of free radical scavenging ability was evaluated using DPPH free radical assay (Figure 2). The extract exhibited marked antioxidant activity by scavenging $DPPH^*$ (free radical) and converting into DPPHH and the activity was found to be dose dependent. The scavenging activity of ascorbic acid was greater than that of methanol extract. The IC_{50} value for the extract was found to be 6.73 µg/mL. The effect of antioxidants on DPPH free radical scavenging is due to hydrogen donating ability. DPPH is relatively stable nitrogen centred free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule[36]. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses color stoichometrically depending on the number of electrons taken up[37]. The DPPH assay is a widely used method to evaluate antioxidant activities in a relatively short time compared to other methods[38]. The reduction capability on the DPPH radical is determined by the decrease in its absorbance at 517 nm

induced by antioxidants. The maximum absorption of a stable DPPH radical in methanol is at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. This is visualized as a discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidant activity[34,35]. In this study, a significant decrease in the concentration of DPPH radical due to the scavenging ability of the extract was observed. Though the DPPH radical scavenging abilities of the extract was less than that of standard, the study showed that the extract has proton-donating ability and could serve as free radical inhibitors or scavenger, acting possibly as primary antioxidant.

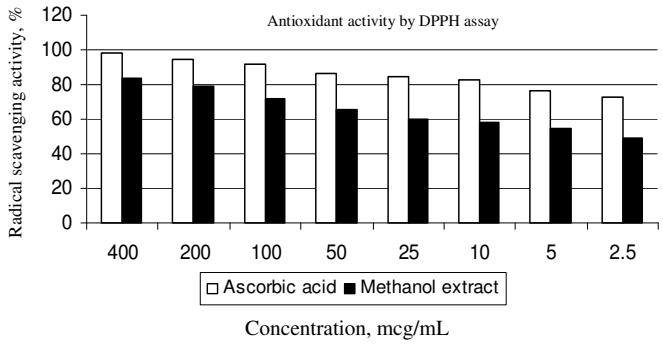

Figure 2. DPPH radical scavenging activity of methanol extract of *E. cirrhatum*

To examine the reducing power of extract, the reduction of Fe^{3+} to Fe^{2+} was investigated in the presence of extract. The result of reducing power of different concentrations of methanol extract and tannic acid is presented in Figure 3. In this study, the absorbance was found to increase with the concentration of extract and standard which is suggestive of reducing power. The reducing ability of a compound generally depends on the presence of reductones, which exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom[39]. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue[35] at 700 nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[40]. The reducing power, as indicated by the absorbance at 700 nm, of extract arose with an increasing concentration of extract.

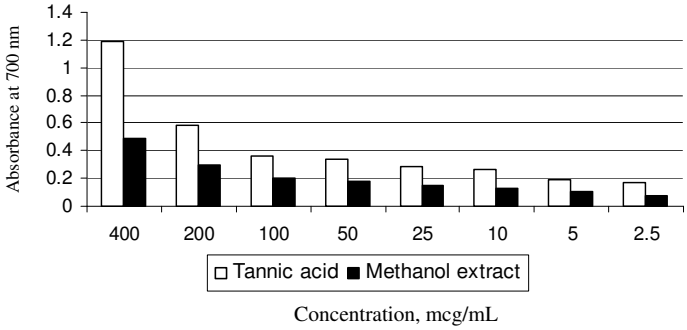

Figure 3. Ferric reducing activity of methanol extract of *E. cirrhatum*

The chelating of ferrous ions by methanol extract of *E. cirrhatum* was estimated and the result is shown in Figure 4. The formation of the Fe^{2+}-ferrozine complex was not completed in

the presence of methanol extract, indicating that the extract chelates the iron. The absorbance of Fe^{2+}-ferrozine complex was linearly decreased dose-dependently (from 2.5 to 100 µg). IC_{50} value for the extract was 29.286 µg/mL. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted and eventually that the red color of the complex fades. Measurement of colour reduction therefore allows estimation of the chelating activity of the co-existing chelator[41]. In this assay the extract and standard interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation[35,42]. Metal chelating capacity is important since it reduced the concentration of the catalysing transition metal in lipid peroxidation[34]. It was reported that chelating agents form bonds with a metal and are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion[39]. The data obtained reveal that the extract demonstrate a marked capacity for iron binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity.

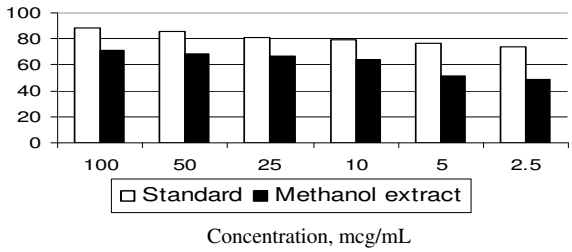

Figure 4. Metal chelating activity of methanol extract of *E. cirrhatum*

Total phenol content was expressed in terms of milligram tannic acid equivalents per gram of dry extract. The phenol content was found to be 101.20±1.68 mg/g dry weight of extract. Phenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds[43,44]. Phenols are very important constituents because of their scavenging ability due to their hydroxyl groups[45]. According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species[46-48]. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[49]. In fact, many medicinal plants contain large amounts of antioxidants such as polyphenols. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases[50,51]. In this study, appreciable phenol content (100.20 mg/g dry weight of extract) was detected in the methanol extract which accounts for the antioxidant activity of the extract. It is suggested that polyphenolic compounds may have inhibitory effects on mutagenesis and carcinogenesis in humans[52]. In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation[53].

Conclusion

An appreciable amount of most of the minerals has been detected in this study and thus the lichen can be consumed as a source of minerals required for the body. Antioxidant assays showed that extract has reducing power, metal chelating activity and scavenging activities.

The major contribution to the observed antioxidant activity could be due to phenol content. Further studies on isolation of metabolites and their bioactivities are under investigation.

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References

1. Kumar B, *J American Sci.,* 2009, **5(4),** 107-112.
2. Slack N G, *Bib Lichenol.,* 1988, **30,** 23-53.
3. Nayaka S, Ranjan S, Saxena P, Pathre U V, Upreti D K and Singh R, *Curr Sci*., 2009, **97(4)**, 538-545.
4. Gupta V K, Darokar M P, Saikia D, Pal A, Fatima A and Khanuja S P S, *Pharm Biol.,* 2007, **45(3),** 200–204.
5. Divakar P K and Upreti D K, Parmelioid lichens in India; Bishen singh Mahendra Pal Singh Pub, Dehra Dun: India, 2005, 97-98.
6. Lal B, Upreti D K and Kalakoti B S, *J Eco Tax Bot.,* 1985, **7,** 203-204.
7. Chandra S and Singh A, *J Res Ind Med.,* 1971, **6,** 209-225.
8. Saklani A and Upreti D K, *J Ethnopharmacol.,* 1992, **37,** 229-233.
9. Upreti D K, Divakar P K and Nayaka S, *Econ Bot*., 2005, **59(3),** 269-273.
10. Wei X, Jeon H, Han K S Koh Y J and Hur J S, *Plant Pathol J.,* 2008, **24(2),** 202-206.
11. Gaur R D, *Nat Prod Rad.*, 2008, **7(2),** 154-165.
12. Awasthi D D, A Compendium of the Macrolichens from India, Nepal and Sri Lanka. Bishen Singh Mahendra Pal Singh Pub, Dehra Dun: India, 2000, 1-580.
13. Culberson C F, *J Chromatogr.,* 1972, **72,** 113–125.
14. Walker F J and James P W, *Bull Brit Lich Soc.,* 1980, **46**, 13-29.
15. Manjunatha B K, Patil H S R, Vidya S M, Kekuda T R P, Mukunda S and Divakar R, *Indian Drugs.,* 2006, **43,** 150-152.
16. Mallikarjuna P B, Rajanna L N, Seetharam Y N and Sharanabasappa G K, *E-J Chem.,* 2007, **4(4),** 510-518.
17. George N J, Obot J B, Ikot A N, Akpan A E and Obi-Egbedi N O, *E-J Chem.,* 2010, **7(3),** 1071-1079.
18. Sarangi R K, Kathiresan K and Subramanian A N, *Ind J Mar Sci.,* 2002, **31(3),** 251-253.
19. Ravikumar Y S, Mahadevan K M, Kumaraswamy M N, Vaidya V P, Manjunatha H, Kumar V and Satyanarayana N D, *Environ Toxicol Pharmacol.,* 2008, **26(2),** 142-146.
20. Oyaizu M, *Jpn J Nutr.,* 1986, **44,** 307-315.
21. Dinis T C P, Madeira V M C and Almeida L M, *Arch Biochem Biophy.,* 1994, **315,** 161-169.
22. Gulcin I, Buyukokuroglu M E and Kufrevioglu O I, *J Pineal Res.,* 2003, **34(4),** 278-281.
23. Sudharshan S J, Valleesha N C, Chinmaya A, Kekuda T R P, Murthuza S and Rajeshwara A N, *Asian J Exp Sci.,* 2010, **24(1),** 11-15.
24. Karunaratne V, Bombuwela K, Kathirgamanathar S and Thadhani V M, *J Natn Sci Foundation Sri Lanka.,* 2005, **33(3),** 169-186.
25. Behera B C and Makhija U, *Curr Sci.,* 2001, **80(11),** 1424-1427.
26. Satyanarayana U, Biochemistry. Books and Allied (P) Ltd, Kolkata: India, 2005, 449-465.
27. Soetan K O, Olaiya C O and Oyewole O E, *African J Food Sci.,* 2010, **4(5),** 200-222.
28. Kumpulainen J T and Salonen J T, Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease, The Royal Society of Chemistry, UK, 1999, 178-187.

29. Cook N and Samman S, *J Nutr Biochem.,* 1996, **7(2),** 66-76.
30. Barlow S M, Toxicological Aspects of Antioxidants Used as Food Additives. In Food Antioxidants, Hudson BJF (Ed.,) Elsevier: London, 1990, 253-307.
31. Branen A L, *J American Oil Chemists Soc.,* 1975, **5,** 59-63.
32. Ali S S, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A. and Bora U, *Food Res Int.*, 2008, **41(1),** 1-15.
33. Diplock A T, *Free Rad Res.,* 1997, **27,** 511–532.
34. Duh P D, Tu Y Y and Yen G C, *Lebensmittel-Wissenchaft und Technologie.,* 1999, **32,** 269–277.
35. Chang L W, Yen W J, Huang S C and Duh P D, *Food Chem.,* 2002, **78,** 347–354.
36. Jayaprakasha GK, Singh R P and Sakariah K K, *J Agric Food Chem.,* 2001, **55,** 1018-1022.
37. Blois M S, *Nature,* 1958, **29,** 1199-1200.
38. Soares J R, Dins T C P, Cunha A P, Ameida L M, *Free Rad Res.,* 1997, **26,** 469–478.
39. Gordon M H, The Mechanism of the Antioxidant Action *In Vitro*. In: Hudson, B J F, (Ed.), Food Antioxidants. Elsevier: London, 1990, 1-18.
40. Meir S, Kanner J, Akiri B and Hadas S P, *J Agric Food Chem.,* 1995, **43,** 1813-1817.
41. Yamaguchi F, Ariga T, Yoshimira Y and Nakazawa H, *J Agric Food Chem.,* 2000, **48,** 180-185.
42. Halliwell B, *American J Med.*, 1991, **91,** 14-22.
43. Okudu T, Yoshida T and Hatano T, Food phytochemicals for cancer prevention II. In Ho C T, Osawa T, Huang M T and Rosen R T (Eds.,) Chemistry and antioxidative effects of phenolic compounds from licorice, tea and Compositae and Labiateae herbs. American Chemical Society, Washington DC: 1994, 132-143.
44. Tepe B, Sokmen M, Akpulat H.A and Sokmen A, *Food Chem.,* 2006, **95,** 200-204.
45. Hatano T, Edamatsu R, Mori A, Fujita Y and Yasuhara E, *Chem Pharm Bull.,* 1989, **37,** 2016–2021.
46. Oktay M, Gulcin I and Kufrevioglu O I, *Lebensmittel-Wissenchaft und Technologie,* 2003, 36, 263-271.
47. Velioglu Y S, Mazza G, Gao L and Oomah B D, *J Agric Food Chem.,* 1998, **46(10),** 4113– 4117.
48. Vinson J A, Yong H, Xuchui S and Zubik L, *J Agric Food Chem.,* 1998, **46,** 3630–3634.
49. Zheng W and Wang S Y, *J Agric Food Chem.,* 2001, **49,** 5165–5170.
50. Anderson K J, Teuber S S, Gobeille A, Cremin P, Waterhouse A L and Steinberg F M, *J Nutr.,* 2001, **131,** 2837-2842.
51. Djeridane A, Yousfi M, Nadjemi B, Boutassouma D, Stocker P and Vidal N, *Food Chem.,* 2006, **97,** 654-660.
52. Tanaka M, Kuei C W, Nagashima Y and Taguchi T, *Nippon Suisan Gakkaishi.,* 1998, **54,** 1409-1414.
53. Yen G C, Duh P D and Tsai C L, *J Agric Food Chem.,* 1993, **41,** 67-70.
54. Boustie J and Grube M, *Plant Genetic Resources,* 2005, **3**, 273-287.
55. Lal B and Upreti D K, *Lichenologist,* 1995, **27**, 77-79.
56. Negi H R and Kareem A, *Amrut.,* 1996**, 1(4),** 3-6.