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BIOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT POTENTIAL OF *CYATHODIUM TUBEROSUM* KASH COLLECTED FROM DIFFERENT ENVIRONMENTAL CONDITIONS

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ABSTRACT

Environment plays important role in determining the biochemical properties of plants. It also affects metabolite production which leads to change in morpho-physiological properties of plants. *Cyathodium tuberosum* Kash (Bryophyte) was evaluated for its antioxidant potential, total phenolics, chlorophyll, anthocyanin, carotenoid content, lipid peroxidation and amino acids content exposed to three different environmental conditions. The total chlorophyll, anthocyanin and carotenoid content were observed to be highest in the methanol solvent system from location 3 to that of ethanol and acetone. The radical scavenging potential and total phenolic content of methanol extracts collected from the location 3 showed high percent radical scavenging activity (60.06±3.8 %) and total phenolic content (5.37±0.01 mg/ml) than ethanol and acetone extracts. While ethanolic extract from location 1 showed maximum percent protection (76.88±4.5%) evaluated by TBARS assay. The polyphenol content (gallic acid, cathechol, vanillin) varied from location to location along with amino acid content (arginine, histidine, thyrosine, alanine) which were identified by HPLC analysis. The present study has revealed that methanol is the best solvent system for the extraction of phenolic compounds compare to ethanol and acetone. The findings of

this study are in agreement with the other work on bryophytes that *Cyathodium tuberosm* can be used as bioindicator for environmental changes.

KEYWORDS: *Cyathodium tuberosum*, antioxidant activity, DPPH activity, RP–HPLC, TBARS, Total phenolic content, Bryophytes, amino acids.

1. INTRODUCTION

The bryophytes are taxonomically placed between the algae and the pteridophytes with more than 20,000 species all around the world including hornworts (Anthocerotophyta), liverworts (Marchantiophyta) and mosses (Bryophyta). However bryophytes can be used in more restricted sense to refer the mosses alone (Family: Bryophyta). Bryophytes are an essential part of this planet's biodiversity and are used as reliable indicators of air pollution.[1-2] They are exploited as bryometer instrument for measuring phytotoxic air pollution. Lichens and bryophytes either independently or together are valuable organisms in developing an index of atmospheric purity (IAP) which is based on the number, frequency-coverage and resistance factor of species. This index provides a fair picture of the long-range effects of pollution in a given area.[3] Bryophytes are very sensitive to pollution and show visible symptoms of injury even in the presence of minute quantities of pollutants. These plants trap and prevent recycling of pollutants in the ecosystem for different periods of time. Analysis of such plants gives a fair idea about the degree of pollution.[4]

Bryophytes have been investigated extensively for active constituents and pharmacological activity earlier. The active constituents include phenolics,[5] bibenzyls,[6] mono, di, tri and sesquiterpenoids,[7] flavonoids,[8] dihydrostelbenes[9] etc. Cytotoxic, anticancer and antitumour activity,[10-12] antifungal,[13-14] antibacterial property,[15-16] anti-inflammatory response[16] in mosses have also been reported. Bryophytes are traditionally used in Chinese, European, North American and Indian medicine as medicinal plants to cure cuts, burns, external wounds, bacteriosis, pulmonary tuberculosis, fractures, convulsions, scalds, uropathy, pneumonia and neurasthenia.[17,1] It is estimated that 80% of the bryophytes contain a bitter principle while few species show surprisingly pungent taste and others can induce allergenic contact dermatitis.[18]

Bryophytes possess strong antioxidative enzymatic machinery which helps them to cope up with extreme climatic conditions and stresses. Bryophytic species such as *Frontinalis antipyretica* is used as potential biomarker in freshwater biomonitoring system.[18-19] They

serve as important bioaccumulators, because of their high absorbing and ion exchange capacities.[20] *Thuidium cymbifolium* and *Cyathodium tuberosum* are used as biomarkers in pollution monitoring of heavy metals like lead and nickel. Desiccation tolerance in *Tortula ruralis* and certain species of bryophytes growing in extreme environmental conditions like Antarctic region have developed a mechanism of tolerance to ultraviolet rays.[21-23]

Though bryophytes posses many medicinal properties and are used as bioindicator in order to determine air pollution, but very little work has been reported on the effects of various air pollutants on the antioxidant potential and amino acid content in bryophytes. Hence in the present study an attempt has been made to evaluate how environmental pollution is affecting the antioxidant potential using *Cyathodium tuberosum* as a bioindicator.

2. MATERIALS AND METHODS

2.1 Plant material

Cyathodium tuberosum Kash was collected from three different locations (Table 1) from Maharashtra (India) during rainy season. Fresh samples were used for the determination of anthocyanin and carotenoid content, whereas air dried samples in a well ventilated room was used for biochemical assays.

Table 1 Environmental condition and locations for samples collection

Locations	Height	Temperature	Geographic Location	Climatic condition	Environmental condition
Location 1	624 m	20-28°C	18°44´00.00”N, 73°43´00.00"E	Heavy rainfall, windy	Pollution free, 98-100% humidity
Location 2	28 m	28-30°C	19°4’48”N, 73°0’36” E	Moderate-Heavy rainfall, steady wind	Moderate pollution, 70-80% humidity
Location 3	10m	28-32°C	19°4’48”N, 73°0’36”E	Moderate rainfall, steady wind	Heavy pollution, 70-80% humidity

2.2 Extraction of Sample

The dried plant material was crushed to fine powder using liquid nitrogen. Five gram of crushed sample was suspended in 50 ml of three different solvents system viz. methanol, ethanol and acetone respectively for overnight extraction. Extracts were filtered using Whatman No.1 paper and the filtrates were concentrated to 10ml using rotary evaporator at 40°C. Extracts were resuspended in respective extracting solvents to make the stock solution of 100mg/ml.[24]

2.3 Determination of chlorophyll content

Chlorophyll content was estimated as per Arnon.[25] Fresh thallus tissue (0.1 g) was grinded and homogenized in 10 ml DMSO. The extract was further kept in a water bath at 65ºC for 4 h. The extract was centrifuged at 5000Xg for 5 min at room temperature. Supernatant was collected and absorbance was measured at 665 and 645 nm respectively. Chlorophyll content was expressed in mg chlorophyll/g of leaf tissue. Chlorophyll a, b and total chlorophyll were measured using equations of Arnon.[25]

2.4 Determination of anthocyanin and carotenoid content

Anthocyanin and carotenoid content were determined as per methodology described by Sims et al. and Lichtenthaler et al.[26, 27]

Anthocyanin (µmol/ml) = $0.08173 \times A_{537} - 0.00697 \times A_{647} - 0.00228 \times A_{663}$

Carotenoid (µg/ml) = $\{1000 \times A_{470} - 3.27$ [Chl a]-104 [Chl b]$\}/227$

2.5 Determination of Total Phenolic Content

Folin-Ciocalteu reagent was used for the determination of total phenolic content (TPC) as per Ghatak et al.[28] 0.2 ml of the plant extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 2ml of 20% aqueous sodium carbonate. The mixtures were incubated for 15 min at room temperature. The absorbance was taken at 650 nm. Total phenolic values were expressed as mg/ml using tannic acid as a standard.

2.6 Radical scavenging activity assay

The free radical scavenging activity was estimated using DPPH as described by Chaturvedi et al.[29] A solution of 0.3mM DPPH in methanol was prepared and 2 ml of this solution was mixed with 1.9 ml of distilled water and 100µl of plant sample dissolved in methanol, ethanol and acetone respectively. The reaction mixture was incubated in dark at room temperature for 30min. The absorbance of the mixture was measured using spectrophotometrically at 517nm. The ability to scavenge DPPH radical was calculated using following formulae:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.7 Isolation of Mitochondrial fraction from Rat Liver

Wistar rats (weighing 240 ± 20 g and 3 months old) were used due to their close resemblance to human system for preparation of mitochondria. Rat liver was excised and homogenized in

0.25M sucrose solution containing 1mM EDTA. The homogenate was centrifuged at 3000 × g for 10 minutes, to remove cell debris and the nuclear fractions. The supernatant was centrifuged at 10,000 × g for 10 min to sediment the mitochondria.[28]

2.8 Protein estimation of mitochondrial extract by Bradford Reagent

Bradford reagent was prepared according to Stoscheck[30] by dissolving 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol; add 100 ml 85% (w/v) phosphoric acid. Dilute the prepared reagent to 1 liter when the dye has completely dissolved, and filter it through Whatman No.1 paper just before use. The Bradford reagent should be a light brown in color. Filtration may have to be repeated to get rid of the blue components. Protein content was estimated at 595 nm and mitochondrial pellets were suspended in potassium phosphate buffer at the concentration of 10 mg protein/ml.

2.9 Exposure of rat liver mitochondria to oxidative stress

Oxidative damage was induced by ascorbate-Fe^{2+}-system as described by Devasagayam.[31] The pink color of thiobarbituric acid reactive substances (TBARS) formed was estimated spectrophotometrically at 532nm as malondialdehyde equivalents after accounting for appropriate blanks. Malondialdehyde standard was prepared by the acid hydrolysis of tetraetoxypropane.

2.10 Amino acid profiling

Sample was dissolved in 800 µl of 6N HCl and then 100 µl of 0.1 N HCl. Thereafter 100 µl of L-Norleucine std. (1000 ppm) and 10 µl of phenol were added. The solution was hydrolysed for 24 hr at 110°C and then neutralizes the hydrolysed sample with 50% NaOH. The mixture was then diluted up to 10ml with diluents. Thereafter 100 µl of diluents, standard and sample was mixed in stopper test tubes. 900 µl of Borate Buffer, 1 ml of FMOC solution were added and 4.0 mL of n-pentane was added, kept in shaker for 10 min and centrifuged at 1000 rpm for 3 min to separate the layer. n-pentane layer was discarded. The previous step was repeated twice. Thereafter injection of the aqueous layer was done for the analysis.[32]

Amino acid profiling was carried out using Ultimate 3000 RSLC HPLC System (DIONEX) using Acclaim PA2, C18, 3µ and dimensions L_1 Type 250 x 4.6 mm, 5µ column. Amino acid content for each sample was identified by comparing their relative retention time with the standards of mixture. Standard amino acids were obtained from Sigma (USA).

Calculations

$$\text{Amino Acids (mg/ml)} = \frac{\text{Area ratio of AA in sample X Wt. of std. (mg) X 0.25 X 5}}{\text{Mean area ratio of respective AA in std. X 10 X 25 X Sample taken (mL)}}$$

2.11 Estimation of phenolic compounds using RP-HPLC

Analysis of individual phenolic compound present in the different solvent extracts were performed on a Waters HPLC (Model 2487), using a hypersil C18 reversed phase column 15cm with 5µ particle size. A constant rate of 0.75ml/min was used with two mobile phases: (A) 25% methanol in 1% Acetic acid and solvent (B) 75% methanol in 1% Acetic acid. The elution gradient was linear starting with (A) and ending with (B) over 60 min, using an UV detector set at wavelength 280 nm. Phenolic compound from each sample were identified by comparing their relative retention time with the standards of mixture chromatogram. Standard phenolic compounds were obtained from Sigma (USA). The concentration of an individual compound was calculated on the basis of peak area measurements and then converted to ppm. All the chemicals and solvents used were HPLC spectral grade.[28]

2.12 Statistical analysis

All the observations were taken in triplicate and the data was analyzed by student's T-test to determine the significance.

3. RESULT AND DISCUSSION

3.1 Chlorophyll content

The chlorophyll content of the *Cyathodium tuberosum* Kash was estimated and was observed that the solvents played an important role in the extraction of pigments (Table 2). Methanol extract from location 3 showed higher concentration of chlorophyll a, b (0.3247±0.04, 0.1553±0.03 mg/g leaf tissue) and total chlorophyll content (0.49552±0.17 mg/g leaf tissue) followed by location 2 and location 1. Significant variation in chlorophyll content of three locations was observed, which may be due to varied level of air pollution and the atmospheric humidity. The results obtained were similar to previous studies reported.[33]

3.2 Anthocyanin and Carotenoid content

Methanol extract from location 3 showed high content of anthocyanin (6.862±0.94 µmol/ml) and carotenoid (23.2±2.22 µg/ml) followed by location 2 (anthocyanin 4.99±0.28 µmol/ml and carotenoid 28±0.55 µg/ml) and location 1 (anthocyanin 5.8±0.25 µmol/ml and carotenoid

22.8±2.7 µg/ml) (Table 2). Anthocyanin may act as antioxidants and the relation between anthocyanin and oxidative stress has the ability to reduce excitation pressure and oxidative damage. Furthermore carotenoids are essential component of the photosynthetic apparatus in plants, algae, and cyanobacteria, in which they are protected against photo oxidative damage and contribute to light harvesting for photosynthesis.[33]

Table 2 Anthocyannin, Carotenoid, Chlorophyll a, Chlorophyll b and Total Chlorophyll content in the different extracts

Location	Solvent system	Anthocyannin (µmol/ml)	Carotenoid (µg/ml)	Chlorophyll a (mg/gm leaf tissue)	Chlorophyll b (mg /gm leaf tissue)	Total Chlorophyll (mg/gm leaf tissue)
L1	**Methanol**	5.80±0.25	22.8±2.7	0.16±0.04	0.05±0.002	0.21±0.04
	Ethanol	2.62±0.11	12.4±1.4	0.14±0.08	0.03±0.001	0.15±0.06
	Acetone	4.62±0.31	18.4±3.6	0.13±0.03	0.04±0.003	0.03±0.004
L2	**Methanol**	4.99±0.28	28±0.55	0.15±0.01	0.06±0.007	0.21±0.03
	Ethanol	2.793±1.3	11.8±1.63	0.137±0.02	0.028±0.004	0.1456±0.06
	Acetone	3.667±0.88	17.6±1.44	0.126±0.09	0.067±0.008	0.024±0.001
L3	**Methanol**	6.862±0.94	23.2±2.22	0.3247±0.04	0.1553±0.03	0.49552±0.17
	Ethanol	3.228±1.43	13.6±1.51	0.282±0.06	0.024±0.005	0.345±0.003
	Acetone	5.288±0.97	19.3±2.34	0.124±0.02	0.035±0.01	0.134±0.045

Values represent mean ± standard deviation (SD)

3.3 Total phenolic content

The methanol extracts of plant showed highest phenolic content to that of extract prepared in ethanol and acetone (Table 3). Thus methanol was observed as a best solvent system for the extraction of phenolic compounds from thallus tissue due to its ability to inhibit the action of oxidizing polyphenols.[28] The methanol extract of the sample collected from location 3 showed high amount of total phenol content (5.37±0.01 mg/ml) to that in ethanol (2.49±0.56 mg/ml) and in acetone (3.63±0.09 mg/ml). Similarly methanolic extract from location 1 (4.31±1.3 mg/ml) and location 2 (4.28±0.66 mg/ml) showed phenolic content (Table 3). Phenolic compounds posses an aromatic ring to which carboxylic group is attached, they are commonly found in the plants either in the free form or combined into esters or glycosides. Many of these phenolic compounds are good source of natural antioxidants. The presence of these phenolic compounds and secondary metabolites confers the pharmacological potentials of the bryophytic species.[34] It was also observed that the use of Folin-Ciocalteu reagent is based on color measurement which is non specific to particular phenolic compounds, perhaps it can also react with ascorbic acid and various other phenolic compounds in response to this

assay.[35] However, the measurement of color change in 30 min may be due to the existence of phenolic and other non phenolic compounds present in the samples.

3.4 Radical scavenging activity

Plants are the potential source of natural antioxidants and produce antioxidative compounds in order to survive and counteract with reactive oxygen species (ROS).[35] The methanol extracts of *Cyathodium tuberosum* Kash from location 3 showed high percent scavenging activity (60.06 ±3.8 %) compared to location 1 (55±5.7 %) and location 2 (32.76±3.5 %). Similar study was performed by Chobot et al.[36] in which they showed that ethanol extracts have highest radical scavenging properties. However methanol extracts showed significant difference in antioxidant capacity to that of the other solvent systems used in the study (Table 3). Methanol was found to be a good solvent system for the extraction of total phenolic compound. The phenolic and polyphenolic compounds are natural antioxidants which enhance the free radical scavenging activity.[36-40] While Ghatak et al.[24, 28, 37] showed that there is a correlation between antioxidant capacity and phenolic content; however, Kahkonen et al.[41] stated that it is not necessary to correlate antioxidant activity with the high amounts of phenolics, but in the present study it was observed that there is a correlation between the antioxidant capacity and phenolic content of extracts of these brayophyte species studied with statistical significance. Bryophytes possess strong antioxidative enzymatic machinery which helps them to cope up with extreme climates and stresses. A number of antioxidative enzymes were found to be activated due to stress response. Some bryophytes were found to sequester and accumulate toxic metals. These bryophytes are used as biomonitoring agents.[18] Bryophytes from the extreme environment can be exploited as a commercial source of antioxidants.[42] The data indicates its potentiality to be used as a possible source of antioxidant for medicinal and cosmetic purpose as well as a bioindicator of pollution.

Table 3 Total phenolic content and DPPH radical scavenging activity of the extracts from different locations

Locations		**L1**			**L2**			**L3**	
Solvent System	**Methanol**	**Ethanol**	**Acetone**	**Methanol**	**Ethanol**	**Acetone**	**Methanol**	**Ethanol**	**Acetone**
Total phenolic Content (mg/ml)	4.31±1.3	1.1±0.8	3.15±0.54	4.28±0.66	2.15±0.52	3.4±0.76	5.37±0.01	2.49 ±0.56	3.63±0.09
DPPH radical scavenging activity (%)	55 ± 5.7	14.67 ±2.6	23.20 ± 2.4	32.76 ± 3.5	23.89 ±1.7	30.41 ± 5.4	60.06 ± 3.8	28.20 ± 3.44	34.67 ± 5.7

Values represent mean ± standard deviation (SD)

3.5 Lipid peroxidation assay

Reactive oxygen species (ROS) causes lipid peroxidation by the formation of toxic compounds which leads for deterioration of food quality. Antioxidative compounds increases the shelf life of food due to their redox potential activity. These compounds retard the oxidative degradation and improve the quality and nutritive value of food. [28, 29, 38] The data in the Table 4 represents inhibitory effect of various extracts of plant tissue on lipid peroxidation in rat liver mitochondria. Among all the solvent systems, ethanol extract of *Cyathodium tuberosum* Kash from location 1 was found to be most effective and gave 76.88±4.5 % protection followed by acetone extract from location 1 (66.17±3.2 %) and ethanol extract from location 2 (64.69±4.8%). The concentration of peroxide decreases with the increase in the antioxidant activity, while the absorbance values are much smaller with higher antioxidant activities of the samples. Ethanol extract has demonstrated a significant antioxidant activity on lipid peroxidation compared with methanol and acetone extracts. Ethanol extracts showed higher inhibitory effect because of the presences of phenolic and flavonoid compounds in the bryophytic species.[29]

Table 4 Effect of various extracts on lipid peroxidation in rat liver mitochondria. Lipid peroxidation was measured as formation of thiobarbituric acid reactive substances (TBARS), peroxidation (damage) was induced by ascorbate Fe^{2+} system.

Locations				**L1**			**L2**			**L3**	
Extracts	**Control**	**Damage**	**Methanol**	**Ethanol**	**Acetone**	**Methanol**	**Ethanol**	**Acetone**	**Methanol**	**Ethanol**	**Acetone**
TBARS (nmoles/ mg protein)	1.99 ±0.5	24.36 ±2.4	16.42 ± 0.10	5.63 ± 0.18	8.24 ± 0.10	9.75 ± 0.27	8.6 ± 0.10	9.09 ± 0.15	18.36 ± 0.18	8.83 ± 0.11	12.86 ± 0.18
Percent (%) protection	----------	----------	32.59 ± 2.10	76.88 ± 4.5	66.17 ± 3.2	59.97 ± 2.7	64.69 ± 4.8	62.68 ± 5.6	24.63 ± 1.5	63.75 ± 4.2	47.2 ±3.7

Values represent mean ± standard deviation (SD)

3.6 Amino acid profiling: Individual amino acids such as arginine, serine, aspartic acid, glutamic acid, alanine, tyrosine and histidine were identified in methanol extract of *Cyathodium tuberosum* Kash (Table 5). It was observed that sample collected from location 2 showed maximum concentration of serine (0.22±0.01 mg/ml) followed by tyrosine (0.22±0.08 mg/ml) and alanine (0.18±0.05 mg/ml). Similarly location no 1 and 3 showed maximum concentration of alanine (0.18±0.08 mg/ml).The amino acids identified were non-

essential amino acids which only become essential in certain stress conditions, out of which only histidine belongs to the group of essential amino acid. Plants that can take up amino acids directly from the soil solution may have a competitive advantage in ecosystems where inorganic nitrogen sources are scarce. Therefore it is hypothesized that diverse mosses in cold and stressed ecosystems share this ability.[43]

Table 5 Amino acid profiling of the sample by Ultimate 3000 RSLC System for HPLC analysis

Locations	Solvent Systems	Arginine (mg/ml)	Serine (mg/ml)	Aspartic acid (mg/ml)	Glutamic acid (mg/ml)	Alanine (mg/ml)	Tyrosine (mg/ml)	Histidine (mg/ml)
L1	**Methanol**	0.11±0.03	0.02±0.005	0.05±0.01	0.01±0.006	0.18±0.08	0.06±0.004	0.04±0.001
L2	**Methanol**	0.11±0.04	0.22±0.01	0.04±0.002	0.17±0.07	0.18±0.05	0.22±0.08	0.17±0.07
L3	**Methanol**	0.11±0.02	0.04±0.01	0.03±0.004	0.03±0.008	0.18±0.08	0.07±0.03	0.05±0.005

Values represent mean ± standard deviation (SD)

3.7 HPLC analysis for phenolic composition: Individual phenolic compounds like gallic acid, cathechol, ferulic acid, caffic acid, p-coumaric acid and vanillin in *Cyathodium tuberosum* Kash were analyzed and quantified using RP- HPLC (Fig. 1) and it was observed that, all the solvent system used showed presence of Gallic acid (Table 6). While vanillin, caffic acid, p-coumaric acid and ferulic acid were detected in trace amount in different solvent extracts from plants. The methanol extracts from location 1 showed the presence of cathechol in higher concentration (35.12±0.45 ppm) to that of other locations and solvent systems. Thus methanol can be used as best solvent system for polyphenol quantification.

Table 6 Polyphenolic concentration in the extracts by RP-HPLC Analysis

Locations	Solvent Systems	Gallic acid (ppm)	Cathechol (ppm)	Vanillin (ppm)	Caeffic Acid (ppm)	Ferullic acid (ppm)	p-coumeric acid (ppm)
L1	**Methanol**	2.02±0.5	35.12±0.45	7.12±0.27	---	2.14±0.34	---
	Ethanol	6.05±0.23	---	24.1±0.17	5.07±0.2	---	3.01±0.77
	Acetone	4.01±0.84	2.14±0.77	14.1±0.9	3.02±0.8	---	---
L2	**Methanol**	2.12±0.33	15.06±0.4	2.14±0.5	---	4.12±0.22	1.44±0.1
	Ethanol	2.33±0.2	---	18.1±1.37	8.06±0.54	6.02±0.78	---
	Acetone	2.22±0.54	18.05±1.76	---	2.22±0.43	---	---
L3	**Methanol**	2.07±0.6	20.24±1.2	4.11±0.46	6.047±0.7	---	6.01±0.53
	Ethanol	6.01±0.71	1.73±0.16	22.12±2.7	---	1.23±0.63	---
	Acetone	3.06±0.42	14.14±1.8	7.12±0.58	---	---	---

***ppm - parts per million**

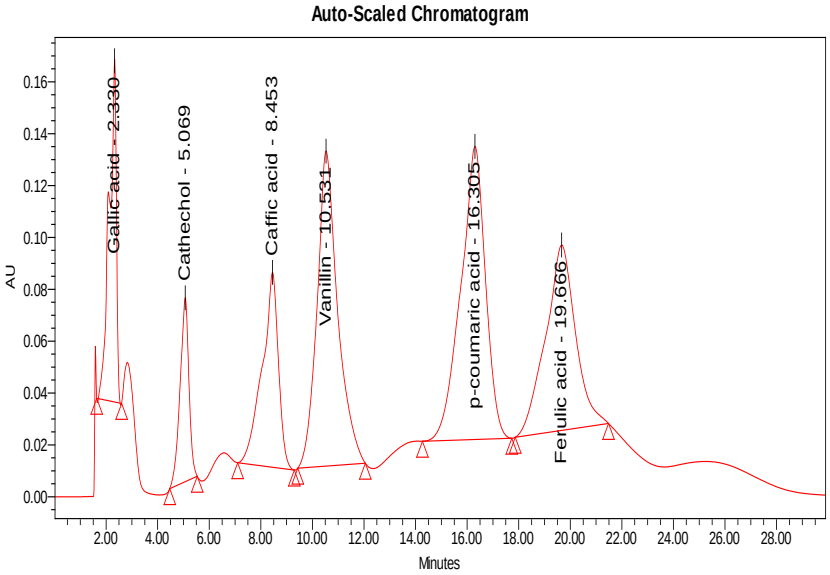

Fig.1 HPLC fingerprinting: HPLC chromatogram of standard, separated on C18 column, hypersil, USA (Revere phase column 15cm; particle size 5µm) using gradient elution – Acetic acid and methanol at a total flow rate of 0.75ml/min. The chromatograms at 280nm were analyzed and compared

4. CONCLUSION

Bryophytes are excellent indicators for a wide range of environmental pollution. They have been mainly used as accumulation indicators especially for heavy metals, radionucleides and for toxic organic compounds due to their presence in the areas where wide range of species are unable to grow or colonized. Bryophytes are unique and having tremendous therapeutic potential. It holds good antioxidant capacity and has high phenolic content however; significant difference was also observed in the solvent system used along with the difference in the antioxidant capacity in the plant sample collected from different locations. The maximum antioxidant capacity was observed in the plant material collected from location 3 where it was observed to be moderate rainfall, 70-80 % humidity, steady wind and heavy pollution area as compared to location 2 and location 1. It has also been observed that bryophytes response sensitively during environmental changes, they show decline vitality for example color changes following damages in the chloroplast structure or less vigorous growth by individual or populations. Due to accumulation of the toxic substances there is large effect in their nutrient cycle and nutrient uptake mechanism. Hence antioxidant activity can be used as stress indicator and *Cyathodium tuberosum* requires further studies for pollution indicator as these small and evolutionary plant group has worldwide distribution.

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