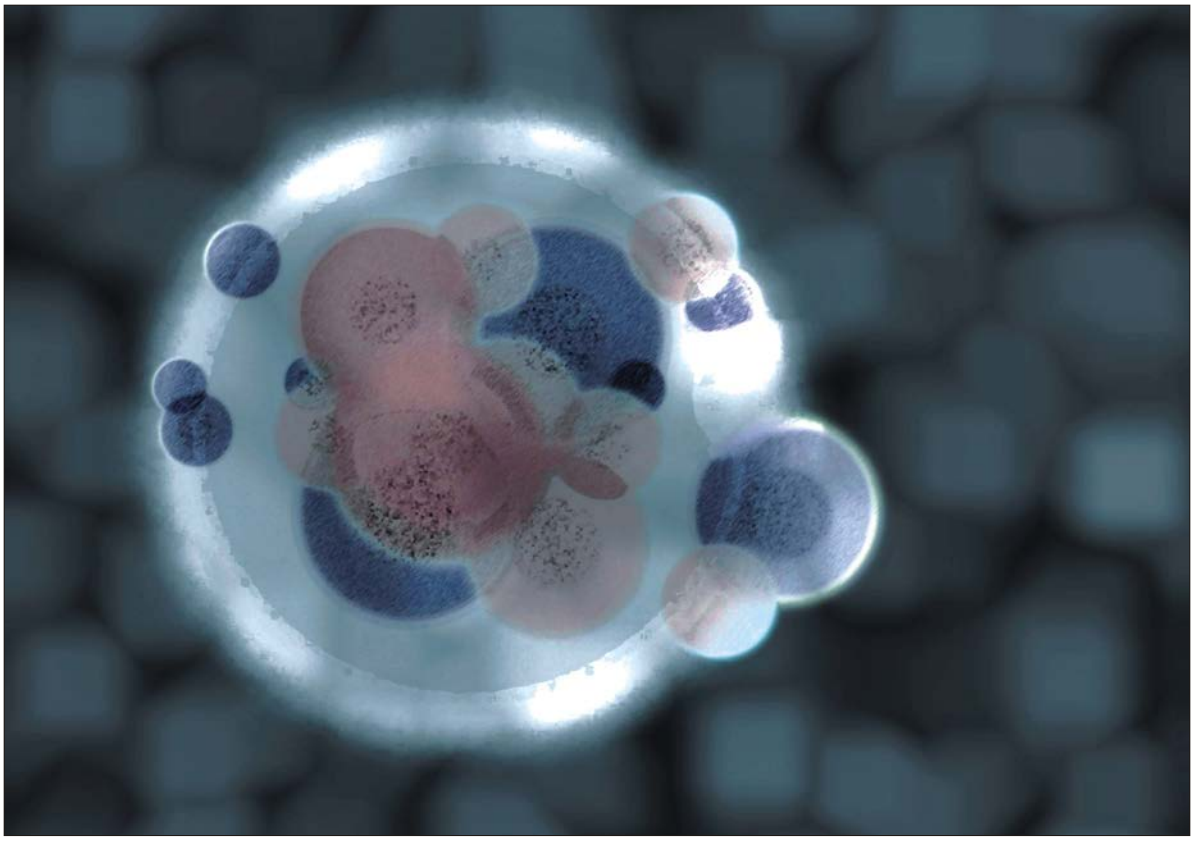

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Altitude and Tissue Type Influence Antioxidant Potential of *Pellia endiviifolia* from Darjeeling Himalaya

[1]Abhijit Dey, [2]Arijit De, [2]Pinky Ghosh and [2]Souryadeep Mukherjee
[1]Department of Botany, Presidency University, West Bengal, India
[2]Department of Zoology and Molecular Biology and Genetics, Presidency University, West Bengal, India

Abstract: Herbal remedy is considered as one of the popular forms of alternative and complementary medicines. Plants are considered to possess a number of chemical constituents with diverse pharmacological efficacies. Bryophytes, a small group of plants, are known to contain unique secondary metabolites having pharmacological and potential therapeutic value. The primary focus of the study is to depict the role of altitude and tissue types on antioxidant capacity of the liverwort *Pellia endiviifolia* (Dicks.) Dumort. (Pelliaceae). In the present investigation, an attempt has been made to explore the antioxidative potential of vegetative and reproductive tissues of *P. endiviifolia* collected from five different altitudes of Darjeeling Himalaya, West Bengal, India. Total phenolics and flavonoids contents of the liverwort samples were also determined. Methanol extract of the thalloid liverwort was investigated for antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, total phenolic and flavonoid estimation. Maximum radical scavenging activity was found to be 89.336%±4.3. Maximum total phenolics content in 1 mg of the extract was 58±0.175 μg of Gallic Acid Equivalent (GAE) per mg dry weight. Maximum flavonoids content in 1mg of the extract was 80.3±331 μg of Quercetin Equivalent (QE) per mg dry weight. The results indicate, for the first time, the antioxidative potential and possible use of the liverwort as a natural antioxidant. It also shows a variation of antioxidant capacity of the liverwort depending on their tissue type and their altitude of occurrence.

Key words: *Pellia endiviifolia*, liverwort, antioxidant activity, methanol extract, altitude, vegetative, reproductive, DPPH, phenolics, falvonoids

INTRODUCTION

Reactive Oxygen Species (ROS) generates oxidative stress in the body as a result of endogenous or exogenous factors (Klaunig and Kamendulis, 2004) which is associated with different ailments (Pejin *et al*., 2012). Beneficial effects of antioxidants against various types of diseases such as diabetes (Kaneto *et al*., 1999), leprosy (Vijayaraghavan *et al*., 2005), Alzheimer's disease (Mancuso *et al*., 2007), cancer (Nishino *et al*., 2004), AIDS (Shabert *et al*., 1999) etc. have been recorded. Dietary antioxidants have been used against tumor (Black and Chan, 1975) and cancer (Khan *et al*., 2008). Herbs, spices, fruits and vegetables have always served as a natural source of antioxidants (Nakatani, 2000; Yanishlieva *et al*., 2006; Peschel *et al*., 2006; Podsedek, 2007).

Plants are reported to possess medicinal properties (Dey and De, 2012a,b) which have been tested by pharmacological investigations (Dey *et al*., 2011; Mukherjee *et al*., 2012a). Bryophytes are known to produce a number of secondary metabolites with diverse biological functionality to resist various types of biotic and abiotic stresses (Xie and Lou, 2009). Bryophytes are reported as a natural storehouse of bioactive molecules having immense pharmacological potential. They have been reported for antibacterial (Kamory *et al*., 1995), antifungal (Dey and De, 2011), antioxidative (Dey and De, 2012c), nitric oxide inhibitory (Harinantenaina *et al*., 2006) and cytotoxic (Scher *et al*., 2002) properties. Antioxidative activities of bryophytes have been reported in *Bryum moravicum* Podp. (Bryaceae), *Brachythecium rutabulum* (Hedw.) Schimp. (Brachytheciaceae), *Calliergonella cuspidata* (Hedw.) Loeske (Hypnaceae) and *Hypnum mammillatum* Funck (Hypnaceae) (Pejin and Bogdanovic-Pristov, 2012; Pejin *et al*., 2012), *Marchantia polymorpha* L. (Marchantiaceae) (Gokbulut *et al*., 2012) and many others (Chobot *et al*., 2008).

Pellia endiviifolia (Dicks.) Dumort. (Pelliaceae) is a terrestrial liverwort which grows on moist rocks either in pure population or in mixed population with other bryophytes (Singh and Singh, 2009). The liverwort is

Corresponding Author: Souryadeep Mukherje, Department of Zoology and Molecular Biology and Genetics, Presidency University, 86/1, College Street, Kolkata-700073, West Bengal, India Tel: +919831165969

reported to possess bis (bibenzyl) compounds (Hashimoto *et al*., 1991) which are known to exhibit prolific bioactivities. Cell suspension culture of the species has also yielded diverse types of metabolites (Ono *et al*., 1992) and the species is yet to be reported for its antioxidative activity. Other species of the genus *Pellia* such as *Pellia epiphylla* (L.) Corda (Pelliaceae) (Cullmann *et al*., 1993, 1997; Cullmann and Becker, 1998) and *Pellia fabbroniana* Raddi (Pelliaceae) (Matsuo *et al*., 1971) have been reported for phyto-constituents. The present investigation first time focuses on the antioxidative potential of the liverwort *P. endiviifolia*.

MATERIALS AND METHODS

Plant material: The vegetative and reproductive thalli of *P. endiviifolia* were collected by A. Dey and S. Mukherjee from five different altitudes of Darjeeling district of the eastern Himalayas (Tiger Hill, Lava, Lolegaon, Rishyap and Kalimpong) during the years 2010-2011 (Table 1). The voucher specimen (PE 01) was identified from the key to the specimen (Singh and Singh, 2009) and deposited at the Department of Zoology and Molecular Biology, Presidency University, Kolkata, India.

Extraction: Before extraction, the plant material was first washed with detergent Teepol® followed by Bavistin to remove microbial contamination. Final rinsing was done in autoclaved distilled water. The plant material was air dried and powdered (40 mesh size) using an electrical mixer grinder. Extraction was done by soaking 1 g of dried powder in 80% methanol for 96 h. Filtration was done using Whatman's No.1 filter paper. The extract was concentrated using a rotary evaporator to give 0.02 g of residue (yield 0.02%). The extract was diluted in 80% methanol to make a 10 mg mL^{-1} solution and stored at +4°C.

Antioxidant activity and estimation of total phenolics and flavonoids

Total phenolics estimation: Folin-Ciocalteu method described by Liu *et al*. (2008) with some modifications was followed to estimate the total phenolics content. Optical density was measured at 765 nm. The obtained result was expressed as µg GAE per mg dry weight.

Table 1: Places of collection of the plant materials with respective altitude and latitude

Place of collection	Altitude (ft)	Latitude
Tiger Hill, West Bengal, India	8202	27°0'00"N 88°28'33"E
Lava, West Bengal, India	7251	27°0'86"N 88°74'88"E
Lolegaon, West Bengal, India	5400	27°03'76"N 88°24'65"E
Rishyap, West Bengal, India	8400	27°6'30"N 88°38'59"E
Kalimpong, West Bengal, India	4150	27°06'00"N 88°47'00"E

Total flavonoids estimation: Total flavonoid was estimated following Zhishen *et al*. (1999) with some modification. Optical density of the solution was measured at 510 nm. Total flavonoid content was expressed as µg of QE per mg dry weight.

DPPH radical scavenging activity: A 0.5 mL of extract (1mg mL^{-1}) was added to 3 mL of 0.1 mM solution of DPPH following the method given by Ohnishi *et al*. (1994) with slight modifications. The concentrations of the extract were 0.0625 to 1 g mL^{-1}. The mixture was incubated at room temperature for 20 min. Optical density was measured at 517 nm.

Statistical analyses: All calculations were performed in triplicate with their mean values and standard error (Pagano and Gauvreau, 2000). Values are given as Mean±S.D. Student's t-test was used to determine statistical significance. Values with $p<0.05$ were taken as significant. Tables were prepared using Microsoft Office Excel (2007).

RESULTS

Methanol extract of plant body of *P. endiviifolia* showed high DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity in a dose-dependent manner. Maximum radical scavenging activity was 89.336±2.77 (Mean±SE, n = 3) from the Tiger Hill population (Table 2). The IC_{50} value was found to be 0.1±0.07 mg mL^{-1}.

Table 2: DPPH assay for *P. endiviifolia* vegetative and reproductive thalli

Populations at different altitudes	Concentration (mg mL^{-1})	Activity (%)	
		Vegetative thallus	Reproductive thallus
A1	0.0625	38.930±1.120	36.100±1.030
	0.125	51.145±1.480	49.870±1.670
	0.25	65.252±2.020	64.652±1.987
	0.5	79.412±2.410	75.569±2.021
	1.0	89.336±2.770	88.392±2.227
A2	0.0625	39.730±1.020	33.400±0.980
	0.125	47.202±0.180	44.860±1.320
	0.25	66.762±1.990	61.760±1.760
	0.5	77.613±2.370	74.570±1.913
	1.0	87.761±2.890	85.720±2.333
A3	0.0625	33.040±1.890	29.441±1.970
	0.125	49.330±1.870	42.651±0.980
	0.25	64.562±0.980	59.340±1.980
	0.5	75.620±1.980	71.670±1.751
	1.0	82.650±2.650	79.712±2.450
A4	0.0625	30.671±1.650	30.341±0.760
	0.125	47.321±1.801	46.791±0.490
	0.25	66.451±0.610	59.695±0.470
	0.5	71.593±1.820	69.762±0.993
	1.0	87.546±2.081	84.450±1.980
A5	0.0625	34.342±1.451	31.320±1.090
	0.125	49.093±1.650	44.540±1.590
	0.25	61.738±1.120	57.103±1.190
	0.5	74.220±0.731	63.550±1.872
	1.0	88.986±1.450	87.706±1.120

Table 3: Phenolics content of vegetative and reproductive thalli of *P. endivifolia*

	Phenolics content (Expressed as μg of GAE mg^{-1} of extract)	
Altitude	Vegetative thallus	Reproductive thallus
A1	58.3±175	57.4±169
A2	57.1±121	56.5±280
A3	54.2±260	52.3±340
A4	55.3±370	53.8±143
A5	54.3±116	52.9±365

Table 4: Flavonoids content of vegetative and reproductive thalli of *P. endivifolia*

	Flavonoid content (Expressed as μg of QE/mg of extract)	
Altitude	Vegetative thallus	Reproductive thallus
A1	80.3±331	77.5±213
A2	76.1±442	75.2±316
A3	79.2±260	78.1±252
A4	75.4±273	73.8±121
A5	78.5±141	76.9±217

Maximum total phenolic content in 1mg of the extract was 58.3±175 μg of gallic acid equivalent from the Tiger Hill population. The flavonoid content in 1mg of the extract was 80.3±331 μg of quercetin equivalent (Table 3, 4) form the same population. Interestingly, slight differences were found regarding antioxidative capacity, total phenolic and flavonoid content of the hepatic collected from various altitudes and the vegetative thallus has shown higher activity and content than the reproductive plant body.

DISCUSSION

The results indicate considerable amount of phenolic and flavonoid content in the tested extract, which may be interpreted as the reason for the significant radical scavenging activity of the bryophyte. Results also depict altitudinal and tissue specific variation of bioactivity which may be attributed to differential exposure of different bryophyte tissues to UV radiation and temperature at various altitudes. Altitude seems to play a significant role in the secondary metabolite profile of certain plants (Spitaler *et al*., 2006; Ganzera *et al*., 2008). In another report, temperature was depicted as the key factor in such variations (Albert *et al*., 2009). In higher altitudes, dual role of enhanced UV-B radiation and decreased temperature seems to influence the antioxidant phenolics content in certain higher plants (Spitaler *et al*., 2008). In *Ginkgo biloba*, altitude was found to control the plant's neutraceutical formulations by modulating secondary metabolite profiling (Kaur *et al*., 2012). In another eastern Himalayan hepatic, *Dumortiera hirsuta*, tissue type (vegetative/reproductive) and altitude are reported to influence the plants bioactivity against a number of human pathogenic bacteria (Mukherjee *et al*., 2012b). Antioxidant and antidiabetic potential of a *Vigna* sp. was also found to vary depending on altitude (Yao *et al*., 2012).

Plants are reported to possess antioxidative activities (Muchuweti *et al*., 2006; Kumbhare *et al*., 2012) and bryophytes are no exception. These tiny plants are also reported from the ethnic uses for their diverse healing properties (Harris, 2008). The therapeutic ability of the bryophytes may be ascribed to their diverse pharmacological properties including antioxidative potentials. The data indicate potential antioxidative properties of the liverwort of *P. endiviifolia* from Darjeeling Himalaya which can be exploited as a natural source of antioxidants with possible therapeutic applications. Antioxidants have been tested against aging (Pejin and Bogdanovic-Pristov, 2012) and age related disorders (Snodderly, 1995), ethanol intoxication (Xie and Lou, 2009) and in cosmetics (Darvin *et al*., 2006). The growing demand of antioxidants in medical science and cosmetic industry can be complemented with the newer sources of antioxidants from natural sources.

CONCLUSION

Bryophytes are subjected to face a number of stresses starting from its pioneer colonization on barren rock surface to their continuous exposure to UV, cold temperature, predation and microbial attack. A strong antioxidative response is present in bryophytes which may be exploited for medicinal and cosmetic use. Further analyses may reveal the presence of bioactive compounds with pharmacological activity which can be implicated to their potential as antioxidants.

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