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Screening of Antimicrobial and Antioxidative Potential of Selected Eastern Himalayan Mosses

Subhra Talai Mukhopadhyay[1], Souvik Mitra[2], Anashuya Biswas[2] Nilansu Das[3] and Mousumi Poddar-Sarkar[2*]

[1]Department of Botany, Barasat Govt. College, Kolkata 700124, India.
[2]Department of Botany, Centre of Advanced Study, University of Calcutta, Kolkata 700019, India.
[3]Department of Microbiology, Surendranath College, Kolkata 700009, India.

Authors' contributions

The entire work was done jointly in a collaborative manner with active participation of all the authors. Author STM designed the experiments for biochemical portion of the work while author ND designed experiments for microbiology portion. Field work and identification of the samples were done by the author SM. Author AB performed all the experiments and prepared the draft manuscript. The corresponding author MPS initially highlighted the problem and did the literary review. All authors read and approved the final manuscript.

Short Communication

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ABSTRACT

The present study was aimed to determine the antibacterial activity of some mosses [*Octoblepharum albidum, Hyophila involuta, H. perannulata, Campylopus introflexus, Syrrhopodon subconfertus, Erythrodontium julaceum* and *Sematophyllum subhumile*] collected from different altitudes of Eastern Himalaya on Gm+ and Gm- bacteria. The antioxidative potential of these genera against 2, 2-Diphenyle-1-pycril-hydrazyl hydrate (DPPH) was also measured to assess their pharmacological importance. Antimicrobial assay was carried out by considering the zone of Inhibition (ZOI) through agar well diffusion method after extraction with two solvent systems (aquous and hydro-ethanol). *Bacillus subtilis* (B), *Staphylococcus aureus* (S), *Escherichia coli* (E) and *Klebsiella pneumoniae* (K) were used for experimentation. The percent inhibition of methanolic DPPH by plant extracts was measured spectrophotometrically. The free radical

**Corresponding author: Email: mpsarkar1@rediffmail.com;*

scavenging activities were examined and expressed in comparison with Vitamin C. Among seven genera studied, *S. subconfertus* showed antimicrobial activity both on Gm+ and Gm- bacteria although their percentage of DPPH reduction was quite less in all the tested concentrations. In contrast, *E. julaceum* exhibited dose-dependent-antimicrobial activity on Gm+ and *E. coli* bacteria and also had appreciable antioxidant property. Therefore, it can be concluded that the antimicrobial potential is not indicative of the antioxidative potential of the respective genera. However, the presence of an important species-specific active compound or ensemble of many active compounds or their relative concentrations might be responsible for their efficacy against bacteria. Thus, survey on Himalayan bryoflora was the primary effort on the way to understand their therapeutic application and for formulation of nutraceuticals.

Keywords: Bryophytes; mosses; Eastern Himalaya; antimicrobial; Gm+; Gm–; antioxidant; DPPH.

1. INTRODUCTION

Bryophytes being the oldest known land plant in the Universe constitute one of the largest spore-producing, nonvascular groups of plant assemblages [1,2]. The phylogenetic placement between algae and pteridophytes, underlines the taxonomic and evolutionary importance of the bryophytes, particularly mosses, as an unique division in the plant kingdom [3]. Among three major divisions, Marchantiophyta (liverworts), Anthocerotophyta (hornworts) and Bryophyta (mosses) the last one is the largest division, constituting over 22,000 species [2]. Due to their boreal habitat, poikilohydric nature and abundance in the Eastern Himalayan forest ecosystems, they play a significant role in community structure and ecology of the region. They dominate some plant communities in terms of their productivity and biomass cover, particularly at high altitudes [4]. Eastern Himalayan Biodiversity zone is a rich bioresource having many genera of acrocarpous and pleurocarpous mosses.

Mosses normally grow in humid condition, and their relative resistance to microbial and fungal attack indicates their ability to produce some active inducible antimicrobials [5-7]. Though their antimicrobial potentiality and clinical prospects have not been explored exhaustively till now, the study of literature indicates that in bryophytes flavonoids, biflavonoids, and isoflavonoids constitute the active components providing resistance against microorganisms [8,9]. On the other hand, the presence of flavonoids, tannins and phenolic compounds of plant systems play a major role as free radical scavengers, thereby acting as natural antioxidants [10,11]. Some Indian Bryophytes are also known to produce different biologically active compounds [12] and their antimicrobial activity was also observed [13,14].

Antioxidants for counteracting oxidative damage have been reported in angiosperms, gymnosperms and pteridophytes [15,16]. Antioxidants constitute an endogenous defensive mechanism against Reactive Oxygen Species (ROS) and hence find extensive usage in cosmetic and pharmaceutical industries [17]. Himalayan bryophytes as natural resources of antioxidant have not been explored extensively to date. The antioxidant activity of three European mosses *Brachythecium rutabulum* (Hedw.) Schimp., *Calliergonella cuspidata* (Hedw.) Loeske and *Hypnum mammillatum* (Brid.) Loeske were measured from aqueous extract with the help of ABTS (2,2'-azino-bis(3--ethylbenzthiazoline-6-sulphonic acid) [18].

The Antarctic polar moss *Sanionia uncinata* has been reported as a natural antioxidant for medicinal and cosmetic purposes [19].

The present study explored the antimicrobial potential of some high altitude mosses and investigated their antioxidative potential to assess their possibility for therapeutic uses and for their clinical and cosmetic prospects.

2. MATERIALS AND METHODS

All the samples viz. *Octoblepharum albidum* Hedw. (1N) [Octoblepharaceae], *Hyophila involuta* (Hook.) A. Jaeger (3A) [Pottiaceae], *Hyophila perannulata* Ren. et Card. (3K) [Pottiaceae], *Campylopus introflexus* (Hedw.) Brid. (4E) [Dicranaceae], *Syrrhopodon subconfertus* Broth. (4I) [Calymperaceae], *Erythrodontium julaceum* (Hook. ex Schwägr.) Paris (4L) [Entodontaceae], and *Sematophyllum subhumile* (Müll. Hal.) M. Fleisch. (5N) [Sematophyllaceae] were collected from different altitudes [ca. 1320m – ca. 2680m] of Darjeeling, Mungpoo, Kalimpong, Lava Hill-range of Eastern Himalaya during different seasons throughout the year and were identified following the key of the monograph of H.C. Gangulee, 1969-1980 [20]. Voucher specimens were submitted at the Calcutta University Herbarium (CUH). Pure cultures of bacteria were maintained in the Dept of Microbiology, Surendranath College, University of Calcutta.

Sample biomass [3-5 g, depending on availability of the samples; both gametophytic and sporophytic plant parts used for experimentation] were thoroughly washed with double distilled water to make it dirt free and dried on blotting paper for future work. Samples were crushed under liquid nitrogen, weighed and extracted with 10ml of two solvent systems [W= Aqueous extract and WA = Ethanol and Water (1:1)] and then centrifuged twice at 10,000 rpm for 5 min. Different extracts were then lyophilized by Freeze Dryer, FDU-2100 (Eyela World, Japan) and the volume of each extract was reduced to 1 ml. The final concentration was made according to the value mentioned in Table 1 by addition of required amount of solvent for application within bore-holes. Extracts were preserved in air-tight vials under N_2 gas to prevent oxidation of compounds and kept under refrigeration for future use.

Sterilized Petri plates containing solid Luria Bertani media were inoculated with two Gm+ [*Bacillus subtilis* (B); *Staphylococcus aureus* (S)] and two Gm- bacteria [*Escherichia coli* (E); *Klebsiella pneumoniae* (K)]. Plant extracts were poured by making bore-holes on Petri plates. Control sets with the extractable solutions were prepared simultaneously following the same procedure for comparison. All the experimental plates were incubated at 37ºC for 48 hrs. Experiments were repeated twice. Zones of Inhibition (ZOI) were recorded following standard procedure. The inhibitory zone of 15mm along the diameter outside bore hole was taken into consideration as antimicrobial. The relative percentages of 2-15 mm ZOI was indicated in Table 1.

For measurement of antioxidant activity of plant extract, samples were extracted with methanolic water (9:1 v/v) at a concentration of 5.0mg/ml initially and serially diluted according to the required concentrations of 0.5, 1.0, 2.0 and 5.0 mg/ml. One ml of each concentration was taken in a vial and 3 ml of 0.16 mM methanolic DPPH (2,2-Diphenyl-2-pycryl-hydrazyl hydrate) was added. The mixture was incubated at room temperature in the dark for 15 min. The degree of decolourisation for scavenging free radicals by DPPH was determined by measuring the absorbance at 517nm in a spectrophotometer (Systronics, Visiscan-167, India). Similar protocol was followed for Vitamin C for comparison. In both

cases, methanolic DPPH was used as the blank solution. The radical scavenging activity was calculated using the following formula [21,22]:

$$\text{Percentage of inhibition} = [\{Abs_{blank} - Abs_{sample}\} / Abs_{blank}] \times 100$$

The experiment was done in triplicates and the results were expressed as mean ± standard error of means.

3. RESULTS AND DISCUSSION

It is evident from Table 1 that the moss *S. subconfertus* (4I) was inhibitory on normal growth of both Gm+ bacteria *B. subtilis* and *S. aureus* and Gm- bacteria like *E. coli and K. pneumoniae* depending on the solvent system used for extraction. Aqueous extract of *E. julaceum (4L)* showed inhibition on growth of *B. subtilis, S. aureus* and *E. coli* when applied in lower concentration than 4I. But no conclusive result could be drawn against *K. pneumoniae* for both aqueous and hydro-ethanol extracts. Therefore, the antimicrobial potentiality is not only dependent on concentration but also on the nature of active compounds present in these two genera as well as nature of applied test micro-organisms. Two species of *Hyophila*, *H. involuta* (3A) *and H. perannulata* (3K) did not show any zone of inhibition for both types of extractable solutions against all four test micro-organisms. The hydro-ethanol extract of *O. albidum* (1N) showed 20% area of inhibition in *K. pneumonae,* at a conc. of 0.33 g/ml. Videl et al. [23] reported that the MIC value of this same genus *O. albidum* (1N) collected from Brazil against *E. coli* and *K. pneumonae* was 512 µg/ml. *O. albidum* was also known to be used externally for reducing body ache during fever [24]. Both types of extracts of *C. introflexus* (4E) and *S. subhumile* (5N) did not show any inhibition of growth for both Gm+ and Gm- bacteria. These two above polar solvent systems were used for extraction of active compounds considering their compatibility for usage in our physiological system.

Table 1. Antimicrobial activity of mosses' extracts on four bacterial strains

Sample No.	Solvent of extraction (W/WA)	Conc. of plant extract (g/ml)	Zone of inhibition of bacteria			
			Gm+		Gm-	
			B	S	E	K
1N	W	0.33	×	-	×	-
	WA	0.33	×	×	×	20%
3A	W	0.25	×	×	×	×
	WA	1.00	×	-	×	-
3K	W	0.33	×	-	×	-
	WA	0.84	×	×	×	×
4E	W	1.14	×	×	×	×
	WA	1.04	×	×	×	×
4I	W	1.83	√	>50%	√	×
	WA	2.70	×	×	√	√
4L	W	0.73	√	>25%	√	×
	WA	0.63	×	×	×	×
5N	W	1.00	×	×	×	×
	WA	1.00	×	×	×	×

[W]aqueous extract; [WA]extract in water: ethanol (1:1 v/v)

[√]Positive inhibition (100% by15mm or above); [×]No inhibition; --Data not available.

The antioxidant assay by using DPPH provided information on the free radical scavenging activity of these seven genera. It was evident from Fig. 1 that *C. introflexus* (4E) and *E. julaceum* (4L) had appreciable antioxidant potential which was comparable to Vitamin C for almost all the concentrations. *E.julaceum* (4L) also showed antimicrobial activity on *B.subtilis, S. aureus* and *E. coli* at a concentration of 730 mg/ml and had highest degree of reduction of DPPH even at lowest concentration (0.5 mg/ml). These results indicated its highest therapeutic value among all the genera. *S. subconfertus* (4I) revealed antimicrobial activity on both Gm+ and Gm- bacteria, its DPPH inhibition data was quiet less for 0.5 mg/ml and 1.0 mg/ml of plant extract. The free radical scavenging activity for both the species of *Hyophila* did not show much variation in lower concentrations. The antioxidant activity of *O. albidum* (1N) showed more or less similar antioxidant activity at all the concentrations. The percent inhibition of DPPH of *S. subhumile* (5N) was considerably with higher values at 1.0, 2.0 and 5.0 mg/ml but at a concentration of 1.0 g/ml of extract it did not show any antimicrobial activity.

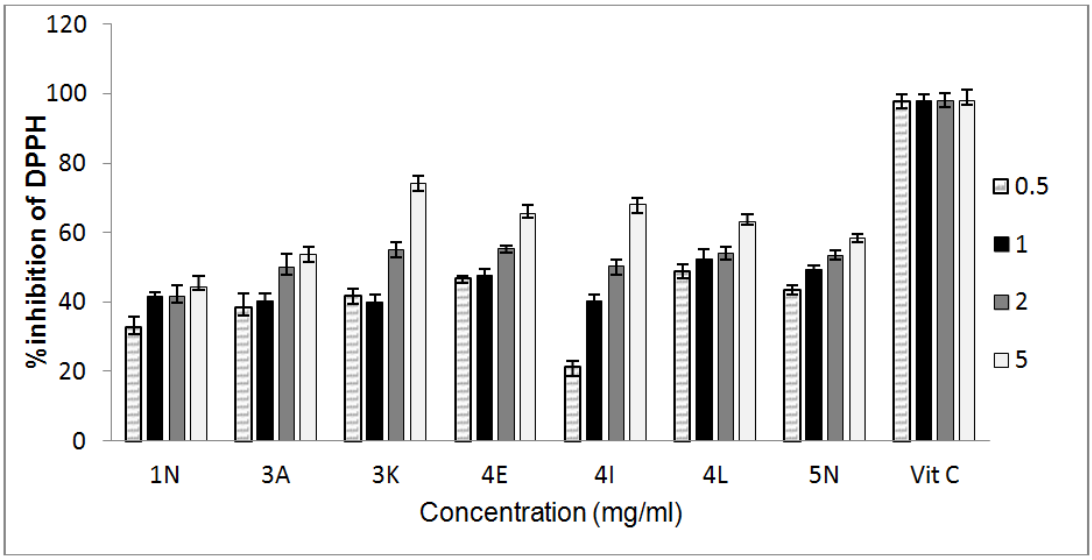

Fig. 1. Variations in antioxidant activity of different concs. of moss extract on percentage inhibition of DPPH [1N- *Octoblepharum albidum*; 3A- *Hyophila involuta*; 3K- *Hyophila perannulata*; 4E- *Campylopus introflexus*; 4I- *Syrrhopodon subconfertus*; 4L- *Erythrodontium julaceum*; 5N- *Sematophyllum subhumile*]

Mean ± S.E.M = Mean values ± Standard error of means of three replicates.

4. CONCLUSION

This preliminary work has revealed that the antimicrobial and antioxidative potentiality of some mosses depends on the presence of specific chemical groups and not only on their relative concentrations [as revealed by the antibacterial activity of *Syrrhopodon subconfertus, Erythrodontium julaceum* in Table 1]. Considering the above results *E. julaceum* could be considered as a potential source for antioxidants and can be pharmaceutically explored for its antimicrobial activity in future. The nature of antimicrobial affectivity depends on strain-specificity of test organisms. The milieu of compounds responsible for antimicrobial activity may or may not take part in preventing oxidative

damage [for e.g. *Sematophyllum subhumile*]. These experiments may pave the path for taking future quantitative approach on bioactive component(s) and screening of more genera of Bryoflora from Eastern Himalayan Biodiversity, National pride of India, considering their therapeutic and clinical utility. The need to explore more natural resources of virgin forest flora from Himalayan terrain in order to satiate the demands of overburden population of this country is the impetus for the study of this relatively unattended plant community.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

The manuscript was prepared only for academic interest and with full consent of all the authors. Authors have declared that no competing interests exist.

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