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Phytochemical screening and total phenolic content in the extract of bryophyte *Plagiochasma appendiculatum* and *Dicranum scoparium*

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Abstract

Since ancient times, people explored the nature, chiefly plants in search of new drugs. This has resulted in the use of great number of medicinal plants with curative properties to treat a variety of diseases and hold medicinal significant bioactive compounds. Along with higher plants lower plants also posses this quality and bryophytes are just one of them as they are relatively free from microbial attacks which point toward their antimicrobial ability. Unfortunately, very little is known about the chemistry of bryophytes and information concerning research results is very scattered. Hence, preliminary phytochemical screening of different extracts (petroleum ether, acetone, methanol, chloroform, ethanol) of *Plagiochasma appendiculatum* and *Dicranum scoparium* was carried out along with total phenolic (GAE/ gm) and total flavonoid (mg QE/ gm) content of different extract of two tropical bryophytes, i.e. *P. appendiculatum* and *D. scoparium* using standard procedures, to identify the phytochemical constituents. The extracts were screened for the presence and quantities of total flavonoids and total phenols with a view to assess their therapeutic values in ethnomedicine. The results of the phytochemical screening revealed the presence of total flavonoids and total phenols in varying quantities in the two bryophytes. These results suggest that the undertaken bryophytes can be veritable and potential source of useful drugs in treatment of ailments.

Keywords: Bryophytes, flavonoid, endomedicine, phenolics, phytochemicals.

Introduction

Plants has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from plants, many based on their use in traditional medicine (Cragg and Newman, 2013). The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties (Hoareau and DaSilva, 1999). In fact, plants produce a varied range of bioactive molecules, making them a rich source of dissimilar types of medicines (Singh and Srivastava, 2013; Srivastava and Singh, 2007). A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda (Murugan and Saranraj, 2011). Phytochemicals are naturally occurring in plants, leaves and other vegetative parts and roots. These phytochemicals have a role in defense mechanism of plants and in protection of plants from various diseases. Chemical constituents and ethno-bryology of bryophytes are not elaborated very well (Sabovljevic and Sabovljevic 2008). Bryophytes are extremely rich in phenols (flavonoids and bibenzyl derivatives), terpenoids, glycosides, fatty acids and also some aromatic compounds. It also considered as a remarkable reservoir of new, natural products or secondary compounds, many of which have shown interesting biological activities and provide a great potential for biotechnological and biopharmaceutical applications (Krishnaiah *et al.,* 2007). Phytochemicals are primary compound which included chlorophyll, proteins and common sugars and secondary compounds which have

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terpenoids, alkaloids and phenolic compounds .Bryophytes are rich storehouse of biologically active component such as terpenoids, flavonoid, glycoside, tannin and other aromatic compound (Xie and Lou, 2009). In past few years more than 400 novel chemical compounds were isolated from bryophytes and they were structurally elucidated. Some important biologically active compounds isolated from bryophytes includes biflavonoids, terpenes and terpenoids and flavonoids as well as aromatic compound (Asakawa, 2011). In spite of the fact that many plant secondary metabolites found in bryophytes are biologically active substances and these biologically active compounds are likely involved in pathogen defense and protection against microbial infection (Basile *et al*., 1999; Merkuria *et al*., 2005) Infact, higher plants and bryophytes have similar evolutionary history but search for novel therapeutic compounds within biodiversity of bryophyte remained neglected due to small size and lack of awareness among people (Asakawa, 2007).

Material and Methods

Collection and identification of Plant materials:

Fresh thallus of bryophytes plant *Plagiochasma appendiculatum* and *Dicranum scoparium* were collected from various places like Almora, Ranikhet, Jageshwar and Nanital of Kumaon hills, India in the rainy season of year 2017. The taxonomic identification of collected sample was done on the basis of available literature (Chopra, 1975; Smith, 1978; Gangulee, 1969; Dhabdhe, 1998). A voucher specimen of plant material was preserved in the Department of Environmental Science, ITM University, Gwalior.

Sample cleaning and Extraction: Collected samples were carefully inspected to remove contaminants like soil and other plant materials. Plant samples were then washed with tap water, air dried at room temperature to remove the water content and then grinded to fine powder. Organic solvents such as petroleum ether, chloroform, methanol, ethanol and acetone were employed for the extraction of different bioactive principles. Extraction was done in different solvent by soxhlet apparatus for 24 hours. The extracts were filtered, concentrated and then stored at 4º C for further investigation.

Phytochemical Screening: All the extracts in different solvent were subjected to various chemical tests in order to detect the presence of different phytochemicals such as glycosides, terpens, carbohydrate, alkaloids, saponins, flavonoids, phenolic, steroids, and lipid. Chemical tests were carried out using ethanol, methanol, chloroform, petroleum ether and acetone extracts of *P. appendiculatum* and *D. scoparium* to identify various constituents using the standard procedures as described by Evans (2006), Ramaan (2006) and Harborne (1973).

Total Phenolic Content: The total phenolic content of extract was estimated by the method of Singleton and Rossi, (1965) and Singleton *et al.,* (1999) with few modifications using Folin-Ciocalteu reagent. Gallic acid is used as standard to estimate total phenols present (Figure 1). 1 ml 95% ethanol, 5 ml distilled water and 0.5 ml 50% Folin Ciocalteu reagent was added with 1 ml extract. After 5 minutes, 1 ml of 5 % sodium carbonate was added and the mixture was incubated for 1 hours. Absorbance was measured at 760 nm. Standard curve was prepared with different concentrations of gallic acid. Experimentations were carried out in triplicate. The total phenol values are expressed in terms of gallic acid equivalent (mg GAE/g of extract), which is a common reference compound.

Results and discussion

Preliminary phytochemical screening:

The preliminary phytochemical screening of various extracts viz., petroleum ether, chloroform, methanol, ethanol and acetone were performed for the presence of various phytoconstituents namely alkaloid, flavonoid, carbohydrate, saponins, glycoside, steroids, terpenes, phenolic and lipid.

The results depicts that, methanolic and ethanolic extracts of *P. appendiculatum* showed the presence of alkaloids, flavonoids, carbohydrates, phenols, terpenes, steroids, glycosides and lipids, whereas saponins were tested negative (Table 1). However, chloroform extracts of *P.appediculatum* showed the presence of flavonoid, carbohydrates, phenols, terpenes and lipids, whereas, alkaloid, flavonoid, saponins, steroids and glycosides were found

Table 1: Preliminary phytochemical screening of different extracts of *Plagiochasma appendiculatum*

Phytochemicals	Bryophytes extracts				
	Petroleum ether	Methanol	Chloroform	Ethanol	Acetone
Alkaloid					
Dragendroff's test	-	+	-	+	-
Mayer's test		+	-	+	-
Hager test	-	+	-	+	-
Flavonoid					
NaOH Test	+	+	-	+	-
Shinoda Test	-	+	-	+	-
H_2SO_4 test	+	++	-	-	-
Carbohydrate					
Fehling solution test	+	+	+	+	+
Saponins					
Foam Test	-	-	-	-	-
Glycosides					
Borntragor's test	-	-	-	+	-
Steroids					
LibermannBruchard test	-	+	-	+	-
Terpenes					
Salkowski's test	+	++	+	++	+
Phenolic					
$FeCl_3$ Test	+	++	+	+	-
Lead acetate test	-	++	+	+	-
Lipid					
AACC Approved method	+	+	+	+	+

negative (Table 1). In addition, petroleum ether extract of the same plant showed the presence of flavonoids, carbohydrates, phenols, terpenes and lipids, while, alkaloids, saponins, glycosides and steroids were not present. In acetone extract of undertaken bryophyte, only carbohydrates, terpenes, and lipids were present. (Table 1). On the other hand methanol and ethanol extract of *D. scoparium* showed the presence of alkaloids, flavonoids, carbohydrates, phenols, terpenes and lipids, whereas, saponins, steroid and glycosides were absent. Furthermore, in chloroform extract carbohydrate, terpenes, phenolic and lipid was present, while, alkaloids, flavonoids, saponins, glycosides, steroids were tested negative. In petroleum ether and acetone extract same result was observed as in *P. appendiculatum* (Table 2). The results showed, particularly, methanol and ethanol extracts of undertaken bryophytes were good sources of different classes of compounds. This indicates that, these solvents are effective to isolate active biological compounds due to their high polarity (Khanam *et al.,* 2015). Flavonoids were detected in methanol, ethanol and petroleum ether extracts and were found absent in chloroform and acetone extract in studied bryophytes. The analyzed flavonoids are well known for their health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties and also reduced risk of cardiovascular and cancer diseases (Sofawara, 1993; Aiyelaagbe and Osamudiamen, 2009; Yang *et al.,* 2001; Alothman *et al.,* 2009; Bhat *et al.,* 2011). The study indicates that, the extract of *P.*

Table 2: Preliminary phytochemical screening of different extracts of *Dicranum scoparium*

Phytochemicals	Bryophytes extracts				
	Petroleum ether	**Methanol**	**Chloroform**	**Ethanol**	**Acetone**
Alkaloid					
Dragendroff's test	-	+	-	+	-
Mayer's test	-	+	-	+	-
Hager test	-	+	-	+	-
Flavonoid					
NaOH Test	-	-	-	-	-
Shinoda Test	-	+	-	+	-
H_2SO_4 test	+	+	-	+	-
Carbohydrate					
Fehling solution test	+	+	+	+	+
Saponins					
Foam Test	-	-	-	-	-
Glycosides					
Borntragor's test	-	-	-	-	-
Steroids					
Libermann Bruchard test	-	-	-	-	-
Terpenes					
Salkowski's test	+	+	+	+	+
Phenolic					
$FeCl_3$ Test	+	++	+	+	-
Lead acetate test	-	+	-	+	-
Lipid					
AACC Approved method	+	+	+	+	+

appendiculatum and *D. scoparium* are rich in carbohydrates, flavonoids, glycosides, alkaloids, phenolics and lipid and shows high medicinal potential and physiological activities.

Total Phenolic content:

Total phenolic content showed dose depended increase with increasing concentrations. The minimum values were observed in petroleum ether i.e. 30.17 mg GAE/ gm in *P. appendiculatum* and 22.64 mg GAE/ gm in *D. scoparium*. In addition, maximum values were recorded in methanol extract i.e. 62.94 and 60.43 mg GAE/ gm in *P. appendiculatum and D. scoparium* respectively (Table 3 and 4). The results obtained are in agreement with the results documented by Goli *et al.*, 2005; Atmani *et al.*, 2009. The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used in the extraction process. Furthermore, solvent polarity will play a key role in increasing phenolic solubility (Naczk and Shahidi, 2006). As the results indicated, it is apparent that the recovery of phenolic compounds was dependent on the solvents used and their polarity. This may be attributed to the fact that wide range of phenols can dissolve in aqueous methanol mixtures. On the other hand, methanol is a good solvent system for the extraction of polar antioxidants (Luximon-Ramma *et al.*, 2003). There is a strong link between phenolic content and free radical scavenging activity (Oki *et al.*, 2002; Mohammad *et al.*, 2010). Phenolics possess a broad spectrum of biochemical activities such as antioxidant, antimutagenic, anti-carcinogenic and even the ability to modify the gene expression (Marinova *et al.*, 2005). Polyphenols are the largest group of

Table 3: Total phenolic content in mg GAE/ gm of different extract of Plagiochasma appendiculatum

Nature of Extract	Concentration µg/ml				
	20	40	60	80	100
Methanol	28.14	35.84	48.45	55.67	62.94
Ethanol	15.48	22.18	35.27	42.15	55.14
Acetone	14.23	18.43	35.70	38.54	45.51
Chloroform	9.62	13.88	14.28	21.08	31.54
Petroleum ether	8.32	10.15	18.61	28.12	30.17

Table 4: Total phenolic content in mg GAE/ gm of different extract of *Dicranum scoparium*

Nature of Extract	Concentration µg/ml				
	20	40	60	80	100
Methanol	25.45	32.46	45.42	52.64	60.43
Ethanol	13.48	18.18	25.27	32.15	45.14
Acetone	12.25	15.45	25.70	30.55	40.56
Chloroform	10.33	12.55	15.66	18.16	25.75
Petroleum ether	7.93	10.66	12.08	16.88	22.64

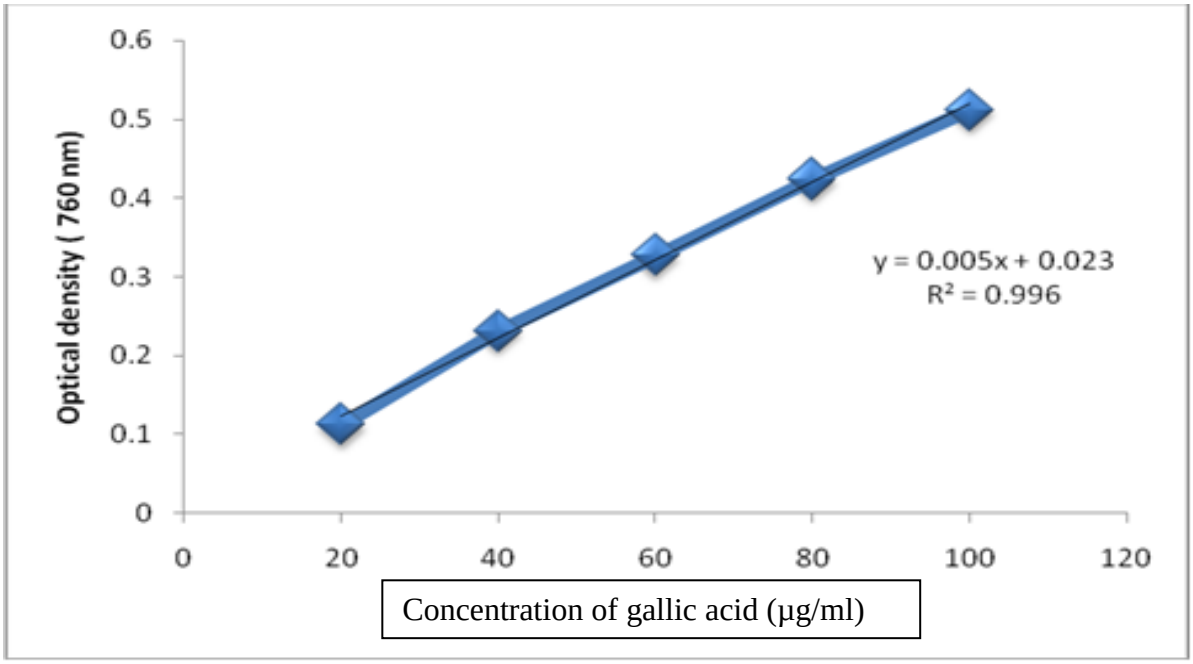

Figure 1: Standard curve of gallic acid for estimation of total phenolics

phytochemicals that account for most of the therapeutic activity in plants or plant products (Sulaiman *et al.*, 2013). Methonol extract of undertaken bryophytes showed the higher total phenolic content at all concentration in compression to all other extract studied. The higher values of total phenolic content in suggests to be possessing good antioxidant potential. Phenolic compounds present in plants are considered to be responsible for the significant free radical scavenging activity. This free radical scavenging activity is considered to be due to their redox properties, which allow them to act as antioxidants. Plant secondary metabolites are considered natural antioxidants and have gained importance in recent years because of the increasing awareness of herbal remedies as potential sources of phenolic oxidants. It is well documented that phenolic compounds has

great contribution in improving the quality and nutritional value in terms of modifying colour, taste, aroma and flavor besides providing health beneficial effects (Aliyu *et al.*, 2010).

Conclusion

The study led to the conclusion that these small creatures (bryophytes) are the rich storehouse of the secondary metabolites *viz*. alkaloids, flavonoids, carbohydrate, terpenoids, tannins and phenolic. The phytochemical analysis of the bryophytes are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. The ant diuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the plants are due to the presence of the above mentioned secondary metabolites. The experiments performed shows that bryophytes are good sources of nutrients, mineral elements and phytochemicals and therefore, their use as nutritional supplements and as medicine is highly promising.

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