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Phytochemical Analysis and Characterization of the Bioactive Compounds of *Mnium cuspidatum* by GC-MS and FTIR

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Abstract: **In the present study, primary and secondary metabolites were estimated quantitatively from *Mnium cuspidatum* – a moss commonly found in Himalayan region. *M. cuspidatum* is used as traditional medicine to cure burns and inflammation. GC-MS and FTIR analyses revealed the presence of bioactive compounds with medicinal properties in the studied moss indicating its potential to be used as an herbal alternative for various diseases.**

Keywords: **Bryophytes, bioactive compounds, *Mnium cuspidatum*, GC-MS (Gas Chromatography-Mass Spectrometry) and FTIR (Fourier Transform Infrared Spectroscopy).**

1. INTRODUCTION

Plant products have been used to cure various ailments since ancient times. The discovery of herbal drugs depends on phytochemical and bioactive constituents of plants (Foye *et al*., 2008). Due to increased cases of resistance of microbes to synthetic drugs, the plant based medicines are gaining popularity because of their low toxicity. Chinese system of medicines has given detailed information about 24 lichens, 74 sea-algae, 22 mosses, 5 liverworts, 112 fungi and 329 ferns with their morphological features and pharmacological activities (Ding, 1982). Bryophytes are non-vascular cryptogams with potential medicinal properties. *Plagiochasma appendiculatum* has been used to cure skin diseases by the people of Gaddi tribes, Himachal Pradesh, India (Kumar *et al*., 2000). Volatile compounds present in bryophytes also got attention due to their bioactive properties (Saritas *et al*., 2001). The extract prepared from powdered form of mosses *Calymperes afzelii*, *Bryum coronatum*, *Thuidium gratum* and *Barbula lambarenensis* have been used against the stem borers of maize (Ande *et al*., 2010; Abay *et al*., 2013).

Since older times, bryophytes have been used as medicines to cure various diseases like fever, inflammation, burns and skin diseases (Frahm, 2004; Singh *et al*., 2006). Environmental factors play an important role in the growth and development of these plants which directly affect the chemical constituents of plants (Kokate *et al*., 2004). Quantitative analysis of primary and secondary metabolites helps in extraction, purification and identification of bioactive compounds which are helpful for various pharmaceutical purposes. Bryophytes possess a large number of secondary metabolites which have antimicrobial, cytotoxic, anti-inflammatory properties. These medicinal properties of bryophytes could be due to the presence of secondary metabolites with antioxidant potential (Yayintas *et al*., 2017).

Potential of antioxidants derived from plants to prevent coronary heart diseases, inflammation and cancer has increased the interest of scientists and researchers towards the medicinal plants (Löliger, 1991).

Phenolic compounds in plants play a major role in medicinal properties including antioxidant potential (Kähkönen *et al*., 1999; Valenzuela *et al*., 2003). There is direct relation between antioxidant activity and phenolic content due to redox properties of phenolic compounds (Rasineni *et al*., 2008).

Species of *Bryum*, *Mnium* and *Philonotis* have been used traditionally to reduce burn pains (Flowers, 1957). *Mnium cuspidatum* Hedw. has been used traditionally to cure hemostasis and nose bleeding (Pant, 1998; Asakawa,2007). *Mnium* species have been used to reduce soreness and inflammation caused by burns or bruises (Azuelo *et al*., 2011).

Due to smaller size of bryophytes, only few efforts have been made to explore phytochemical composition of bryophytes in comparison to higher plants. Although bryophytes have been used as medicinal plants since vedic period, yet chemical characterization of bryophytes with the help of GC-MS and FTIR is still restricted. The purpose of this study was to estimate primary metabolites content, determination of total flavonoid and phenolic content and phytochemical characterization of *M. cuspidatum* by GC-MS and FTIR.

2. MATERIAL AND METHODOLOGY

Plant material: The plant sample of moss *M. cuspidatum* was collected from Shimla in October, 2017. *M. cuspidatum* is a moss belonging to family Mniaceae, commonly found in west Himalayan region, brown to green in colour, up to 3 cm in height and leaves are bordered with two rows of marginal teeth. Upper leaves are crowded and form a comal tuft while lower leaves are smaller in size. The specimen has been deposited in the herbarium of Panjab University, Chandigarh under reference no. PAN 6312.

Quantitative determination of primary metabolites: The following primary metabolites which play a major role in life processes like growth and development of the plants have been analysed presently:

Water soluble carbohydrates

Water soluble carbohydrates were determined by Anthrone method of Yemm and Willis (1954) using Glucose as standard.

Water soluble proteins

Water soluble proteins were estimated by Lowry *et al*. (1951) using Bovine Serum Albumin as standard.

Total free amino acids

Total free amino acids were determined by Lee and Takahashi (1966) using Glycine as standard.

Chlorophyll and carotenoid content:

Chlorophylls were estimated using the method of Arnon (1949). Chlorophylls were extracted in 80% chilled acetone. From 250 mg of fresh plant material was homogenized in cold pestle with mortar in dark. A pinch of $MgCO_3$ was added to neutralize the acids released during extraction. The extract was filtered through Whatman No.1 filter paper using Buchner's funnel under suction. Final volume of the filtrate was made to 25 ml with 80% acetone. The filtrate was transferred into a conical flask wrapped with black paper to prevent photo-oxidation of the pigments. Absorbance was read at 663nm and 645 nm on a double beam spectrophotometer using 80% acetone as a blank.

Carotenoids

Carotenoids were extracted in 80% chilled acetone from the weighed amount of material as per the procedure described above for chlorophylls earlier. Carotenoids were estimated following the method described by Kirk and Allen (1965). The absorbance was recorded at 480 nm on a double beam spectrophotometer.

Quantitative determination of secondary metabolites: Secondary metabolites in plants have medicinal properties and play an important role in defence mechanisms like disease resistance.

Extraction: The moss sample was washed and cleaned under running water to remove any soil and adherents. The sample was shade dried and then ground to powder form. The plant material was extracted in ethanol. The extract was filtered and stored at ±4°C for further use.

Total phenolic content: Total phenolic content was determined by Folin-Ciocalteu method using Gallic acid as standard (Nampoothiri *et al*., 2012; Dar *et al*., 2014).

Total flavonoid content: Total flavonoid content was determined by Aluminium Chloride colorimetric method (Pourmorad *et al*., 2006; Bag *et al*., 2015) using quercetin as standard.

GC-MS and FTIR analysis

The GC-MS analysis of extract was performed using Thermo Trace 1300GC coupled with Thermo TSQ 800 Triple Quadrupole MS equipped with TG 5MS (30m X 0.25mm, 0.25µm) column. Injector temperature was set at 250° C, whereas mass transfer line temperature was 280° C. The injection volume was 1.0µl. The name, molecular weight and structure of the components of the methanol extract of *M. cuspidatum* were ascertained.

Statistical analysis: The samples were taken as triplicates and the data were expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

In the present study, primary and secondary metabolites of *M. cuspidatum* were analysed quantitatively and the characterization of bioactive compounds has been done using GC-MS and FTIR. The results are presented in Figs. 1, 2 and 3.

The quantitative analysis of primary metabolites in *M. cuspidatum* has revealed the presence of carbohydrates, proteins and amino acids. Primary metabolites are directly responsible for the growth and development of the plants. Chlorophyll (the green pigment) is one of the most important primary compounds as it is the only substance that helps in photosynthesis by capturing sunlight (Geetha and Geetha, 2014). The chlorophyll *a* content in *M. cuspidatum* was found to be higher (8.80 mg/g) than chlorophyll *b* (4.88 mg/g) as shown in Fig. 1. Carotenoids are the organic pigments that aid in photosynthesis and protect the plants from heat stress. Carotenoid content in the studied moss is 0.22 mg/g. Sabovljević *et al*. (2010) observed concentration of chlorophyll *a* and chlorophyll *b* 13.40 nmol/gm and 3.56 nmol/g respectively in *Bryum argenteum*. Kanchna *et al*. (2015) studied seasonal variation in photosynthetic pigments of three species of Marchantiaceae and observed that chlorophyll *a* content was higher than chlorophyll *b* in all the seasons and total chlorophyll content was found higher during the end of the growing season.

Carbohydrates are the product of photosynthesis and are required to provide energy used during defence mechanisms and act as signalling molecules for defence gene’s regulation (Ehness *et al*., 1997; Roitsch *et al*., 2003; Bolton, 2009). Carbohydrate content was found to be 15.58 mg/g. The soluble carbohydrates helps during water stress as soluble carbohydrates get accumulated during desiccation in plants (Hoekstra *et al*., 2001; Oliver *et al*., 2005). The high carbohydrates content in the plant indicates that it can be used as a good source of energy and can be used as alternative source for artificial sweetener (Freeze, 1998). Kapila *et al.* (2014) reported higher carbohydrate content during end of the growing season as the bryophytes tend to store carbohydrates towards the end of favourable growth period.

Total protein content in the studied taxon was 12.77 mg/g. Higher protein content in the moss sample is the indicator of increased food value so that protein based bioactive product could be extracted in future (Thomsen *et al*., 1991). Total amino acids content was 21.84 mg/g. Amino acids are one of the stress reducing agents in plants especially during oxidative stress and heavy metal stress (Zhao, 2010; Maeda and Dadareva, 2012). Recently, Thakur and Kapila (2016) studied carbohydrate, total proteins and total amino acid content in *Marchantia papillata* subspecies *grossibalba*. Carbohydrate content was found to be 11.24 mg/g, total protein and total amino acid content was found to be 13.58 and 8.33 mg/g respectively in vegetative thalli of *M. papillata*. Kanchna *et al*. (2018) studied seasonal variation in biochemical changes in three species of *Plagiochasma* and observed that total carbohydrate content was maximum during the end of the growing season; total protein content was highest during winter season and total free amino acids was maximum in the rainy season.

Secondary metabolites mainly phenolics and flavonoids play an important role in disease resistance, plant growth and development. Total phenolic content (66.92 mg/g) in the studied plant material is found to be higher than total flavonoid content (28.18 mg/g) as shown in Fig.1. Similar results were reported by Bhadauriya *et al.* (2017) in *Plagiochasma appendiculatum* and *Dicranum scoparium* where total phenolic content was observed as 62.94 mg/g and 60.43 mg/g respectively. Higher phenolic content attributes to the disease resistance, anticancerous, anti-inflammatory and good antioxidant properties of the plant. Wang *et al.* (2017) reported total flavonoid content in epiphytic and aquatic bryophytes ranging between 1.8 to 22.3 mg/g and concluded that epiphytic bryophytes had more total flavonoid content than aquatic bryophytes.

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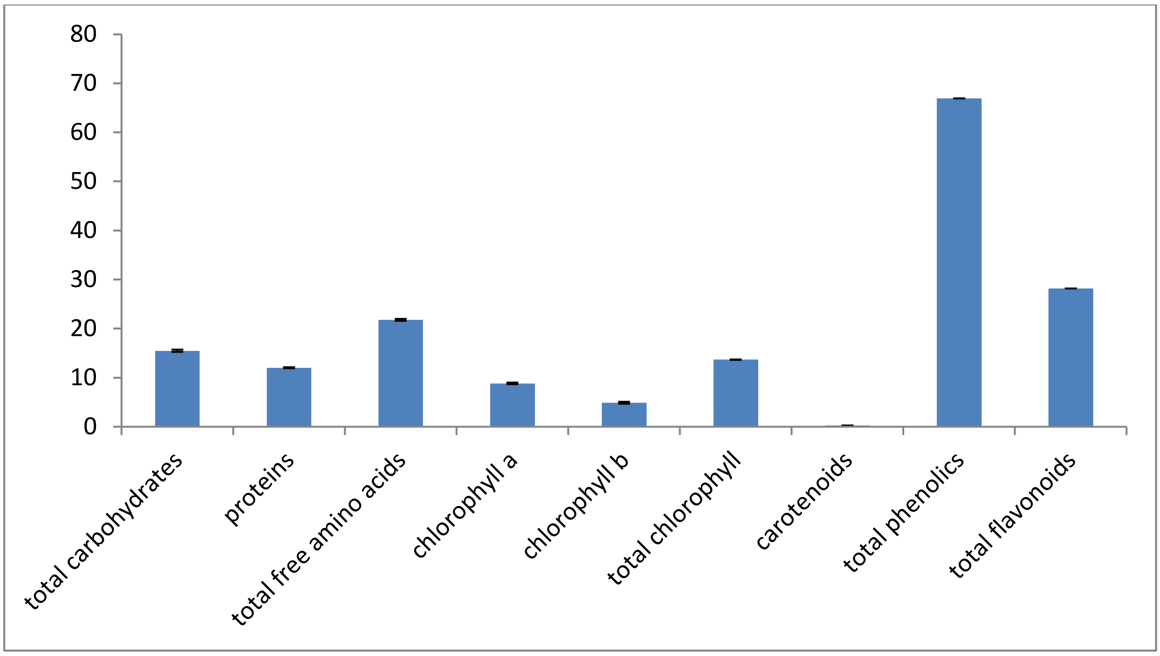

Fig 1: Quantitative analysis of primary and secondary metabolites in *M.cuspidatum*

FTIR Analysis: FTIR spectrum of *M. cuspidatum* is given in Fig. 2. The common IR absorption frequency values of *M. cuspidatum* suggested that N-H and O-H stretching of amides corresponds to the presence of alkaloids, phenolics, saponins and tannins. C=O stretching of amides, esters, carboxylic acids, aldehydes and ketones corresponds to the presence of carbohydrates, flavonoids, phenolics and tannins. N-H bending of amides and NO_2 group absorption corresponds to the presence of alkaloids. C-H bending corresponds to the presence of steroids and terpenoids, whereas C-O stretching in alcohols, esters and ethers corresponds to the presence of carbohydrates, flavonoids, glycosides, gums and mucilages and reducing sugars.

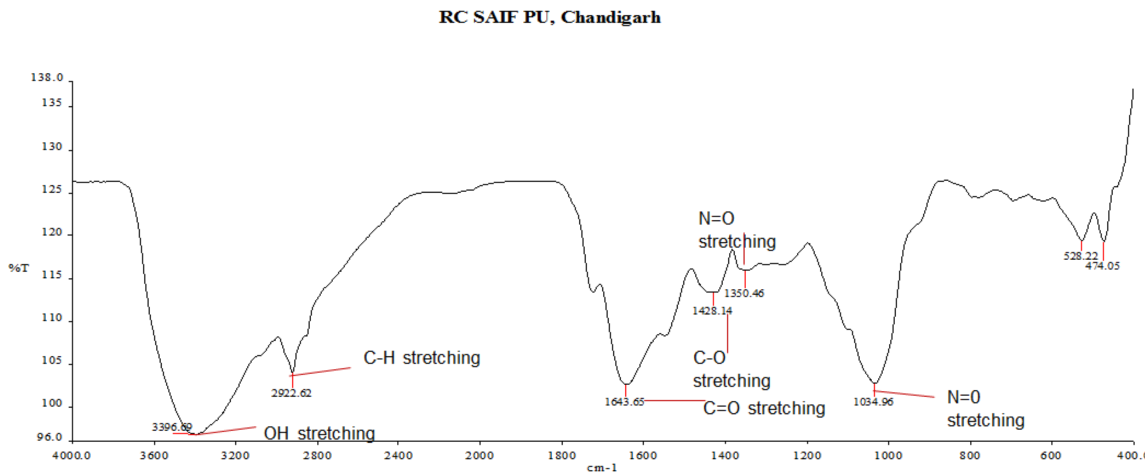

Fig. 2: FTIR spectra analysis of *Mnium cuspidatum*

Table 1: FTIR peak values of methanol extract from *Mnium cuspidatum*.

Absorption spectrum, Frequency (cm^{-1})	Components
3396.69	O-H stretching for hydroxyl, N-H stretching for amenes, N-H stretching for amides
2922.62	C-H stretching for alkyls,; carboxylic acids; O-H stretch
1643.65	C=O stretching for carbonyls; C=C stretching for aromatics

1428.14	C-O stretching for alcohols; C-O stretching for carboxylic acids
1350.46	N=O stretching for nitro compounds; alkanes; C-H stretching
1034.96	N=O stretching for nitro compounds

FTIR analysis revealed the presence of several bioactive compounds like alkaloids, phenolics, saponins, tannins, glycosides and flavonoids as shown in Table 1 which have various biological activities. Steroids are one of the stress releasing agents, help in lowering cholesterol levels, aid in improving immune system and also have cytotoxic effect (Grandhi, 1994, Panda and Kar, 1997; Sharma *et al*., 2011). Terpenoids are aromatic compounds which are the major constituents of herbal medicines have antiviral, anti-inflammatory, anti-cancerous properties (Mahato and Sen, 1997). Flavonoids are phytoestrogens, hydroxylated polyphenolic compounds and possessing antioxidant properties (Middleton, 1998). Alkaloids which are nitrogenous compounds have antimicrobial and anti-cancerous properties along with sedative effect (Rahaman, 2010). Phenols are one of the major secondary metabolites present in plants and have antiseptic, anti-inflammatory, antioxidant and cytotoxic effect (Rahaman, 2010). Saponins possess wound healing, anti-inflammatory capacity, have antiviral, antimicrobial activities and can also be used as detergents and pesticides. Tannins are polyphenolic compounds which provide disease resistance against microbes (Sharma *et al*., 2011; Okwu and Josiah, 2006). Glycosides provide relief during dry cough and are diuretic (Sharma *et al*., 2011).

GC-MS ANALYSIS: GC-MS analysis revealed the presence of fifteen components in methanol extract of *M. cuspidatum* (Fig. 3). The identified compounds of *M. cuspidatum* are presented in the Table 2 with retention time, percentage peak area and molecular formula.

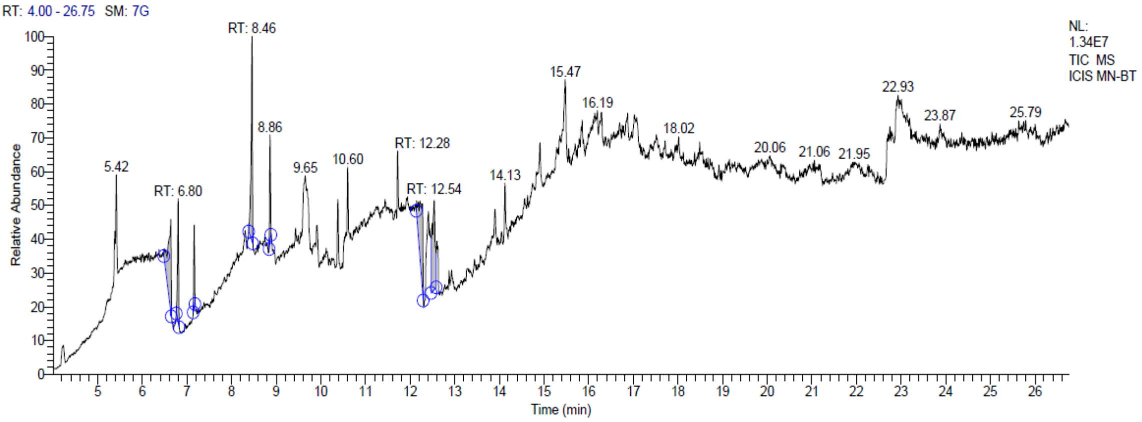

Figure 3: GC-MS chromatogram of methanolic extract of *M. cuspidatum*

Table 2: Phytochemical identification of methanolic extrat of *Mnium cuspidatum* by GCMS

S.No.	% Peak area	RT(min.)	Compounds detected	CAS No.	Mol. Formula
1	8.27	6.80	Silane, dimethylisobutoxybutoxy	NA	$C_{10}H_{24}O_2Si$
2	8.27	6.80	Dibutoxy(dimethyl)silane	NA	$C_{10}H_{24}O_2Si$
3	8.27	6.80	Dimethyl[bis(2methylpropoxy)] silane	NA	$C_{10}H_{24}O_2Si$
4	25.64	12.56	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13 tetradecamethyl	19095-23-9	$C_{14}H_{44}O_6Si_7$
5	25.64	12.56	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15, 15 hexadecamethyl	19095-24-0	$C_{16}H_{50}O_7Si_8$
6	25.64	12.56	Bis(trimethylsilyl)2[hydroxytris(ethoxy)phenyl] 2[hydroxybis(ethoxy)phenyl]propane	NA	$C_{27}H_{44}O_5Si_2$
7	13.73	15.31	7,15Dihydroxydehydroabietic acid, methyl ester,di(trimethylsilyl)ether	NA	$C_{27}H_{46}O_4Si_2$

8	13.73	15.31	Corticosterone, bis(trimethylsilyl) ether	NA	$C_{27}H_{46}O_4Si_2$
9	13.73	15.31	3Hydroxybromoazepam,bis(trimethylsilyl)deriv	NA	$C_{20}H_{26}BrN_3O_2Si_2$
10	25.02	15.46	7,15Dihydroxydehydroabietic acid, methyl ester,di(trimethylsilyl)ether	NA	$C_{27}H_{46}O_4Si_2$
11	25.02	15.46	Bis(pentamethylcyclotrisiloxy)tetramethyldisilo xane	17909-18-1	$C_{14}H_{42}O_9Si_8$
12	9.65	16.18	1,2Di(2,3,7,8,12,13,17,18Octaethyl7,8dihydro21 H,23Hporphinyl10) ethane	75066-13-6	$C_{74}H_{98}N_8$
13	9.65	16.18	2Hydroxy4(methylsulfonyl)isophthalic acid tritms	NA	$C_{18}H_{32}O_7SSi_3$
14	9.65	16.18	Butanedioic acid, 2,3bis[(tertbutyldimethylsilyl) oxy], bis(tertbutyldimethylsilyl) ester	NA	$C_{28}H_{62}O_6Si_4$
15	7.02	16.86	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11dodecamethyl	995-82-4	$C_{12}H_{38}O_5Si_6$

GC-MS analysis revealed the presence of compounds which have biological activity (Phytochemical and Ethnobotanical Databases of Duke). (Dimethyl[bis(2methylpropoxy)] silane has antifungal properties (Wang *et al.*, 2012); Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl and Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11 dodecamethyl have antimicrobial properties.

4. CONCLUSIONS

Plant based products have gained popularity owing to their less toxicity. Bryophytes with medicinal properties are one of the major sources of traditional medicinal system. The phytochemical analysis showed that *M. cuspidatum* contains phytochemicals such as reducing sugars, glycosides, phenolic compounds, flavonoids, phenolic compounds, saponins, tannins and alkaloids. The results of the present study are very promising and can be used after scientific validation for the economical formulation of herbal drugs.

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