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PRELIMINARY PHYTOCHEMICAL ANALYSIS AND CHARACTERIZATION OF *BRYUM CORONATUM* SCHWAGER

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ABSTRACT

The present investigation was focused on the preliminary phytochemical and Fourier Transform Infrared Spectral analysis of *Bryum coronatum* Schwager. The organic solvent extracts (ethanol) from the aerial part of *Bryum coronatum* were tested for the availability of alkaloids, phenols, flavonoids, saponins, steroids, tannins and terpenoids and glycosides. Flavonoids and steroids detected from moss species have commercial interest in pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. The FT-IR spectrum showed the presence of alcohols, nitrite group, carbonyl groups, phenolic esters, ethers, aromatic compounds, alkyl halides and alkene. In GC-MS analysis twenty different compounds were detected. The results confirm the fact that this moss posses' important bioactive constituents useful for our health so further scientific investigation is needed.

KEYWORDS: *Bryum coronatum*, FT-IR, GC-MS analysis, Bioactive constituents.

INTRODUCTION

Mosses belong to class Bryophyta, which earlier also includes hornworts and liverworts but now hornworts and liverworts are separate divisions. Mosses are an advanced class of bryophytes which includes about 17,000 species of 900 genera and 89 families of 4 orders under 3 subclasses distributed in the world (Richardson, 1981). Many microbiologists and botanists have documented the presence of biologically active compounds and antibiotic substances in bryophytes such as glycosides, phenols, terpenoids, and fatty acids (Banerjee and Sen, 1979, Glime and Saxena 1991, Zhu *et al*., 2006 and Sabovljevic *et al.,* 2009). They contain several potential compounds including polysaccharides, amino acids, sugars, alcohols and phenolic compounds (Pant and Tiwari 1990). The constituents that have been isolated from *Hypnum cupressiforme* are, biflavonoids, hypnogenols and dihydroflavonols. Some of these flavonoids were revealed to have marked antibacterial effects (Dulger *et al.,* 2005). Phenolic, Terpenoids and volatile constituents have also been scrutinized in some bryophytes. Many of the terpenoids were isolated and described mainly from liverworts (Saritas, 2001).

Asakawa (1981, 1984) stated that the presence of lipophilic aromatic compounds and terpenoids in liverworts as potent source of antibiotics. By using Gas Chromatography and Mass Spectroscopy (GC-MS) techniques compounds like monoterpenoids, sesquiterpenoids, diterpenoids, bicarbocyclic diterpenoids, triterpenoids, phenolic compounds, sterols, flavonoids, and fatty acids can be found out (Banerjee, 2001). Wankhede and Manik, (2005) trace the possible chemical compounds from crude methanolic extract of *Plagiochasma appendiculatum* by Gas Chromatography and Mass Spectroscopic analysis. They revealed the presence of compounds like Caryophyllene, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, Phytol, Hexacosane and Heneicosane.

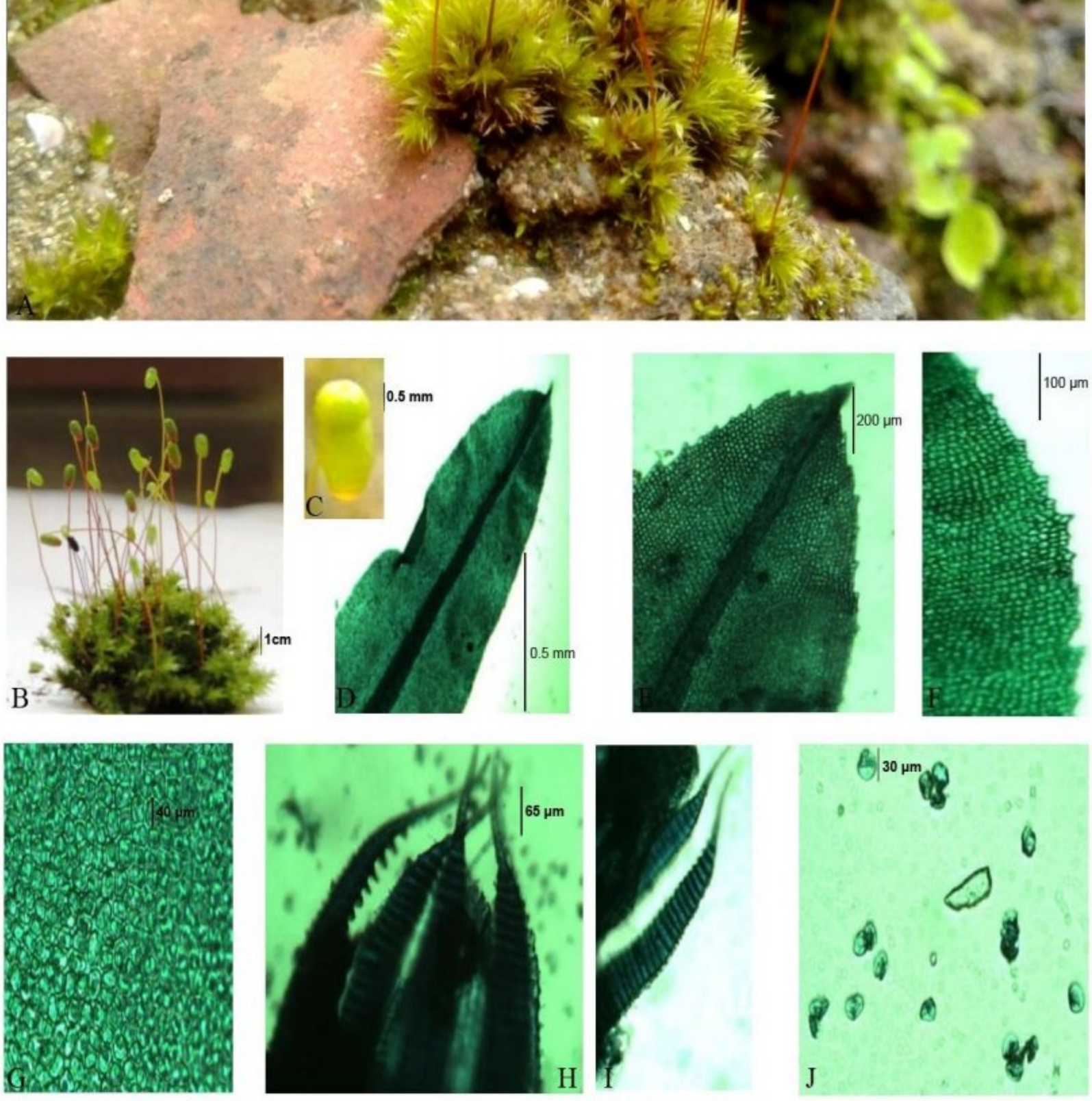
MATERIAL AND METHODS

1. Collection of plant material: The different moss species *Bryum coronatum* collected from various localities such as Purandar, Lonawala, Sinhagad, Kaas Plateau and Koynanagar during from moist shady places in rainy season. Before evaluation, material has cleaned along with water and used for analysis.

2. Qualitative test for phytochemical analysis: The collected moss were properly washed and dried. After drying it was used for extract preparation. The plant material weighted was grinded in mortar and pestle with equal amount methanol till the formation of fine paste and left for overnight, then it was filtered. This filtrate was used as (100%) crude extract. The freshly prepared extract was used for standard phytochemical analysis to

check the presence or absence of phytochemicals such as glycosides, terpenoids, alkaloids, tannins, saponins, phenols, flavonoids and steroids by standard procedures (Horborne, 1998).

Bryum coronatum Schwaegr.



A- Plant habit, B-Entire plant with capsule, C- Single capsule, D-Entire leaf, E- Leaf apex F- Leaf margin, G - Median leaf cell, H, I - Peristome teeth and J - Spores.

Photo Plate No. 1.

Table 1: Qualitative screening of phytochemical constituents (Horborne, 1998).

Sr. No.	Experiments/ Test	Observation and Inference
1.	Test for alkaloids 1 ml of test solution shaken with 2N HCl. Aqueous layer formed, decanted and to which one or two drops of Mayer's reagent added.	White turbidity or precipitate develops. Presence of alkaloids
2.	Test for steroids 1 ml of test solution + 3 – 4 drops of chloroform and few drop of acetic acid, acetic anhydride and 2 drops of Con. H_2SO_4 and heated gently.	Blue or green colour develops. Presence of steroids.
3.	Test for tannins 1 ml of test solution + H_2O + lead acetate	White precipitate develops Presence of tannins
4.	Test for saponins 1 ml of test solution + 1 ml of distilled water and mixed well	Foamy lather develops Presence of saponins
5.	Test for flavonoids In a test tube few drops of 1% NH_3 solution is added to the 1 ml test solution	Appearance of yellow colour. Presence of flavonoids
6.	Test for terpenoids 5 ml of plant sample is mixed with 2 ml of $CHCl_3$ in a test tube.3 ml of con H_2SO_4 is carefully added to the mixture to form a layer	An interface with a reddish brown coloration is formed. Presence of terpenoids
7.	Test for glycosides 5 ml of the plant sample is mixed with 2 ml of glacial acetic acid containing 1 drop of $FeCl_3$. The above mixture is carefully added to the 1 ml con H_2SO_4 so that the con H_2SO_4 is underneath the mixture.	A brown ring will appear Presence of glycosides
8.	Test for phenolic compounds Alcoholic solution of test solution (1 ml) + one drop of ferric chloride	Intense colour develops Presence of phenolic groups.

3. Fourier transform infrared spectroscopy (FTIR)

A known weight of sample (mg) was taken in mortar and pestle and ground with 2.5 mg of dry potassium bromide. The powder obtained was filled in 2 mm interval diameter micro cup and loaded on FTIR set at 26 ^{0}C ± 1 ^{0}C. The samples were scanned using infrared in range of 4000-500 cm^{-1} using FTIR. The spectrum obtained was compared with reference chart to identify functional groups present in sample.

4. Chromatographic analysis of moss secondary metabolites

A. Preparation of extract for chromatography.

1. The processed 1gm plant material was weighted and grinded in mortar and pestle with 10 ml HPLC grade methanol till the formation of fine paste and left for overnight.
2. Then it was filtered by using Whatman filter paper no. 40.
3. This filtrate was centrifuged.
4. From supernant 0.1 ml is taken and 1ml methanol is added. Then it was used for GC-MS.

B. Gas Chromatography- Mass Spectroscopy (GC-MS).

Chromatography generally used in biochemistry for separate, identify, quantify and analysis of active compounds. Qualitative and quantitative analysis of phytochemicals can be done using Gas Chromatography-Mass Spectroscopy (GC-MS). GC-MS can be applied to solid, liquid and gaseous samples. In this method gas phase is flowing and the liquid phase is stationary. First the samples are converted into gaseous state then analysis is carried out on the basis of mass to charge ratio. The methanolic extract of *Bryum coronatum,* was subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds.

C. Method used for GC-MS.

GC-MS analysis of methanolic extract of *Bryum coronatum* was carried out using Shimadzu Make QP-2010 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature was set with initial temperature at 50 °C and held for 5- 8 min and the final temperature of the oven was 280 °C. A 2-µL sample was injected with split mode. Plunger speed (Injection) and syringe insertion speed was high but Injection mode was normal. Detector gain mode was relative to the tuning result and detector gain was 1.07 kV + 0.00 kV. Pressure was 117.6 kPa. Total flow was 25.0 mL/min. Column Flow was 2.00 mL/min. Mass spectra was recorded over 35 - 650 amu range with electron impact ionization energy 70 eV

The chemical components from the methanolic extract of moss was identified by comparing the retention times of chromatographic peaks using Quadra pole detector with

NIST Library to relative retention guides. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the extract were find out.

RESULTS AND DISCUSSION

1. Preliminary screening of secondary metabolites: The Preliminary phytochemical analysis of extracts of *Bryum coronatum* revealed that presence of flavonoids, phenols and steroid but alkaloids, tannins, terpenoids, saponins and glycosides are completely absent. The phytochemical analysis of the medicinally important mosses 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.

2. Identification of functional groups using FTIR

FT-IR spectra of extracts showed the normal stretching frequency for alcohols at 3635.1 cm^{-1} and characteristic stretching bands of C=N in 2361.94 cm^{-1} region indicating the nitrite group. FT- IR spectra exhibits the intensive band of C = C group at 1643.42 cm^{-1} and characteristic bending bands of C = O in 1419.67 cm^{-1} region indicating the carbonyl groups of phenolic esters. The spectra showed the C-O stretching bands of ethers and alcohols at 1013.64 cm^{-1} and bending at 873 cm^{-1} for aromatic compounds. The intensities of absorption bands in 522.73 – 658.72 cm^{-1} region exhibits stretching frequency for alkyl halides (R-x). FT- IR spectra of extracts showed the normal stretching frequency for alkene = C-H bending at 444.61 cm^{-1}. Thus the ethanolic extract of *Bryum coronatum* showed considerably higher concentration of phenolic compounds (1643.42 cm^{-1}).

Table 2: Phytochemical analysis of secondary metabolites.

Sr. No	Name of secondary metabolites.	Test Present or Absent
1	Alkaloids	-
2	Flavonoids	+
3	Phenols	+
4	Tannins	
5	Steroids	+
6	Terpenoids	-
7	Saponins	-
8	Glycosides	-

Present: +, Absent: -

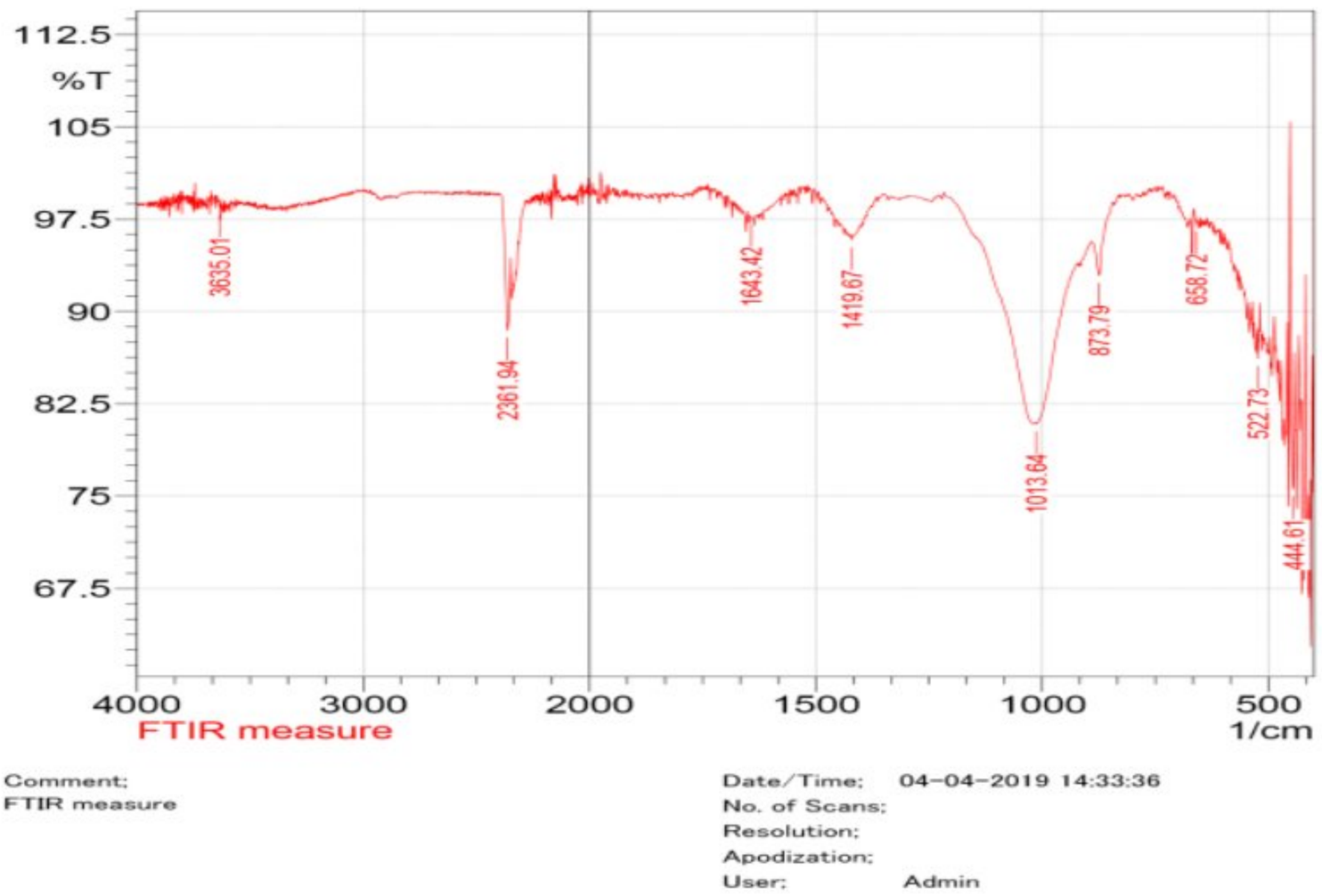


Fig: 1. FT-IR absorption spectra of *Bryum coronatum* Schwager methanolic extract.

3. Gas Chromatography and mass spectroscopy

To trace the possible chemical compounds, Gas Chromatography and Mass Spectroscopic analysis of crude methanolic extract was done. The compound obtained from GC-MS were identified by compare with mass spectral analysis of the *Bryum coronatum* (Table. 3) and also revealed the presence of compounds like Butanal, 2-ethyl-3-methyl-, Undecane, 3-Allyl-6-

methoxyphenol, Methyleugenol, 2,4-Di-tert-butylphenol, Diethyl Phthalate, Tetradecanoic acid, Decane, 1-iodo-Hexadecanoic acid, methyl ester, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dienn- 9-Octadecenoic acid (Z)-, methyl ester, Oleic Acid, Octadecanoic acid, Hexadecanoic acid, Dibutylphthalate, 1Heptacosanol, 11,14- Eicosadienoic acid, methyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymet, 4,4'- (p-Phenylene) diisopropylidene) diphenol, Octadecanoic acid, 2,3-dihydroxypropyl ester and 1,3-Docosenamide, (Z). All these compounds show biological activity. Detected compounds Undecane, shows antioxidant activitiy, 3-Akalyl-6-methoxyphenol shows antimicrobial and antioxidant, Methyleugenol act as hepatocarcinogenic, 2,4-Di-tert-butylphenol is effective on carcinoma cell line, Diethyl Phthalate acts as chemotactic factor, Hexadecanoic acid, methyl ester shows antifungal and antibacterial activities, 9-Octadecenoic acid (Z)-, methyl ester shows antioxidant activity, Oleic Acid shows apoptotic activity(death of tumour cells) and cytotoxic, Octadecanoic acid and Hexadecanoic acid act as anti-inflammatory, anti-cancer and shows antioxidant activity, Dibutyl phthalate used against endocrine disruption and neurotoxicity, (www Pub chem ncbi com).

The chemical constituents from the methanolic extract of *Bryum coronatum* was identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

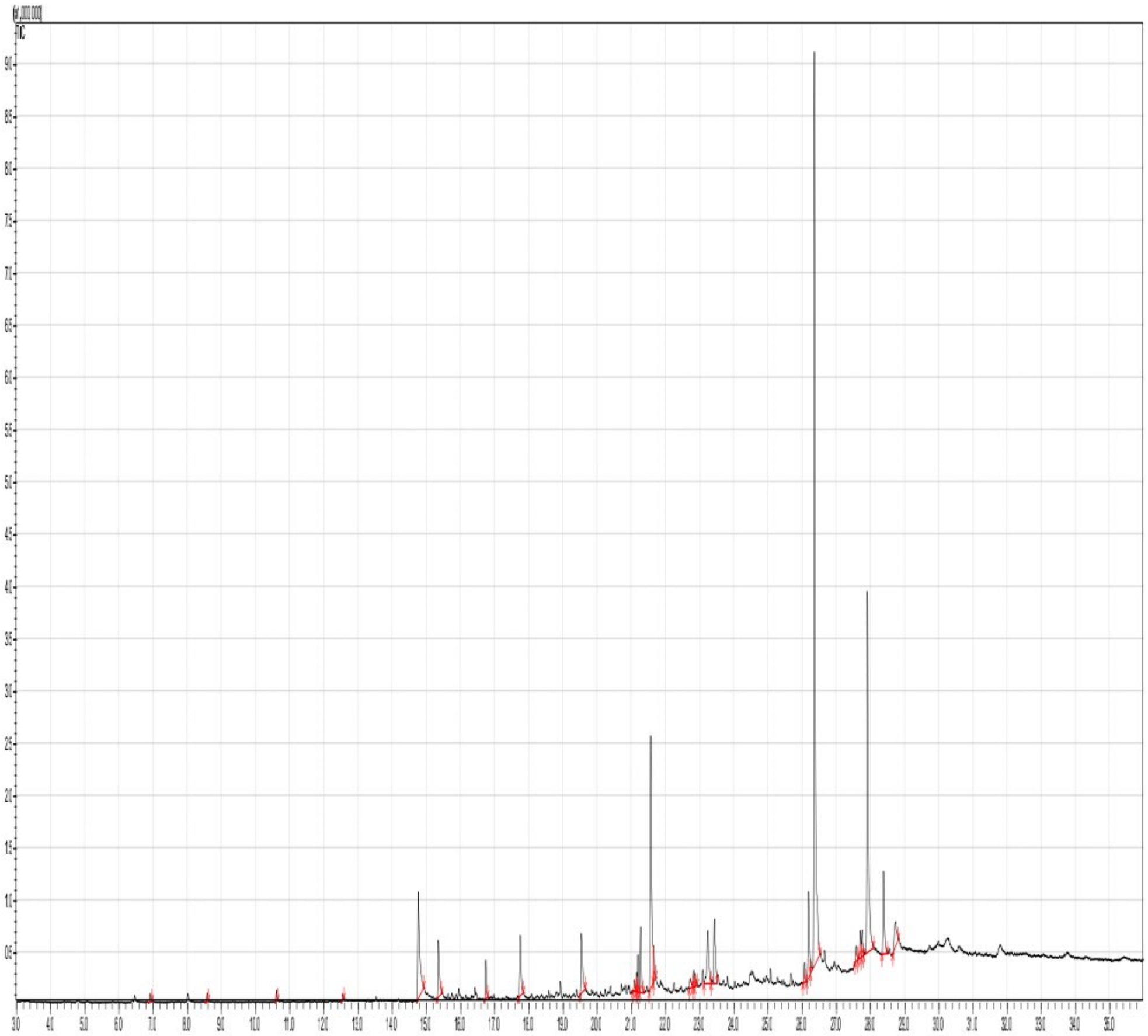


Fig. 2: GC-MS Chromatogram of *Bryum coronatum* Schwager.

Table: 3 Compounds analyzed from mass spectrum of *Bryum coronatum* Schwager.

Sr. No.	Retention time	Area of the peak%	Compound analyzed	Molecular formula	Molecular weight g/mol	Activity reported
1	6.912	0.28	Butanal, 2-ethyl-3-methyl-	$C_7H_{14}O$	114.19	against asthma
2	8.578	0.25	Undecane	$C_{11}H_{24}$	156.31	antioxidant
3	14.760	5.97	3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	164.2	antimicrobial and antioxidant
4	15.347	2.29	Methyleugenol	$C_{11}H_{14}O_2$	178.23	act as hepatocarcinogenic
5	16.732	1.13	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	effective on carcinoma cell line
6	17.746	2.80	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24	acts as chemotactic factor
7	19.534	2.81	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Used in cosmetics
8	21.155	0.56	Decane, 1-iodo-	$C_{10}H_{21}I$	268.18	-
9	21.192	1.06	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	362.6	antifungal and antibacterial activities
10	21.267	2.03	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-dien	$C_{17}H_{24}O_3$	276.4	-
11	21.564	9.89	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	antifungal and antibacterial activities
12	21.645	1.04	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	euro toxicity
13	22.710	0.46	1-Heptacosanol	$C_{27}H_{56}O$	396.7	Nematocidal activity
14	22.820	0.60	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322.5	-
15	22.870	0.39	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.5	antioxidant activity
16	23.233	3.20	Oleic Acid	$C_{18}H_{34}O_2$	282.5	apoptotic activity and cytotoxic
17	23.429	3.16	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	anti-inflammatory, anti-cancer and shows antioxidant activity
18	26.354	33.64	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet)	$C_{19}H_{38}O_4$	330.5	anti-inflammatory, anti-cancer and shows antioxidant activity
19	27.750	1.05	4,4'- (p-Phenylene) diisopropylidene) diphenol	$C_{24}H_{26}O_2$	346.5	analgesic
20	27.901	15.60	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358.6	anti-inflammatory, anti-cancer and shows antioxidant activity
21	28.382	3.30	13-Docosenamide, (Z)	$C_{22}H_{43}NO$	337.6	Induces physiological sleep

(Source. Pubchem.ncbi.nlm.nih.gov)

SUMMARY AND CONCLUSIONS

Preliminary screening of methanolic extract of *Bryum coronatum* for some metabolites ensures the presence of flavonoids steroids and phenols. In GC-MS analysis twenty different compounds were detected and in FT-IR alcohols, nitrile group phenolic esters, aromatic compounds, alkyl halides and alkene were detected. Isolation and characterization of secondary metabolites of such compounds may lead to the introduction of new active compounds for possible application in pharmacy after further pharmacological tests to control various human, animal and plant diseases.

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