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Semibarbula orientalis (Web.) Wijk. and Marg: A Potential Source of Bioactive and High Value Phytochemicals

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Authors' contributions

This work was carried out in collaboration between both the authors. Author VD performed the experimental work, wrote the protocol and first draft of the manuscript. Author GSD designed the study, managed the literature and supervised the entire work. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Present study aims to evaluate the presence of bioactive compounds of *Semibarbula orientalis* (Pottiales: Pottiaceae), a bryophyte whole plant methanolic extract by Gas Chromatography-Mass Spectrometry (GC-MS) which are important medicinally as well flavouring and colouring agents.
Study design: Qualitative preliminary phytochemical and GC-MS analysis.
Place and duration of study: The study was carried out at Department of Botany, Center of Advanced Study, Jai Narain Vyas University, Jodhpur-Rajasthan (India) from January 2017 to December 2020.
Methodology: The Preliminary phytochemical screening of *S.orientalis* was carried out qualitatively following the standard methods of Harbourne, Trease and Evans. GC-MS analysis was performed by GC-MS-QP 2010 Shimadzu, Japan equipped with thermal desorption system TD 20.
Results: Preliminary phytochemical analysis revealed the presence of carbohydrates, proteins, phenols, sterols, flavonoids and terpenoids. GC-MS analysis of methanolic extract of whole plant

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revealed the presence of 49 bioactive phytoconstituents which include mainly n-Hexadecanoic acid, Cis-vaccenic acid, Azulene, Hexadecanoic acid metyl ester, 1,3-Propanediol, 2-(hydroxyl methyl)-2-nitro, 9-12 Octadecadienol chloride (z,z), Octadecanoic acid etc.
Conclusion: Preliminary phytochemical and GC-MS profiling of methanolic extract *Semibarbula orientalis* whole plant showed the presence of high value bioactive compounds with important medicinal properties and other uses in food industries as flavouring and colouring agents.

Keywords: Bryophytes; Semibarbula orientalis; GC-MS; phytochemicals; methanolic extract.

1. INTRODUCTION

The use of plants and plant products for the treatment of human diseases as old as our civilization. Man's interest and observations towards the mother's nature led to discloses of various curative properties of plants and these properties were acquired by the plants during evaluation, as its own adaptive strategies for survival, especially for protecting against different types of abiotic and biotic challenges faced by the plants**.** In addition to this, changing climatic conditions of the earth also played an important role in designing plant's adaptive abilities and man has utilized these adaptive abilities of the plants for ensuring his own survival. Needless to discuss, before the introduction of modern medicine, the diseases were treated or managed only by herbal remedies. Plants from algae to angiosperms were found to be rich sources of phytoconstituents or therapeutic agents therefore, contributed to the drug industries till date. Even today, many medicinally important components are derived from plant sources. There is huge potential to further harness these resources by exploring the vast diversity present in the plants [1].

Increasing awareness and health consciousness among the people, their attitude towards medicine and diet has undergone a dramatic transformation especially after worldwide COVID-19 pandemic. Now man is focusing on plant-based diet and health care supplements because natural supplements are comparatively healthier and free from other side effects of harmful chemicals.

Plants contain different types of phytochemicals also known as secondary metabolites [2] and these chemicals play an important role in pharmaceutical industry to develop new drugs and preparation of therapeutic agents [3].

The screening of plant extract is a new approach to find out therapeutically active components in plant species [2]. Phytochemicals such as flavonoids, tannins, saponins alkaloids and terpenoids have several biological properties which include antioxidant, anti-inflammatory, anti-diarrhea, anti-cancer and anti-ulcer activities [4].

Bryophytes are the second-largest taxonomic group in the plant kingdom [5]. These plants are resistant to pathogenic attacks which suggest that they are rich in antimicrobial phytochemicals but these have been neglected for a long time due to their small size and identification problems [6] and thus the phytochemistry and bioactivity of these plants have not been much studied from the perspective of their application potential. Nowadays, interest in the chemical composition of bryophytes is growing, as the numbers of biologically active compounds have been identified and isolated from these plants. Liverworts reported containing aromatic compounds like bibenzyls, benzoates, cinnamates, and naphthalene's as well as lipophilic mono-, di-, and sesquiterpenoids whereas bioflavonoids, terpenes, terpenoids, and flavonoids reported in mosses [6]. These bioactive compounds isolated from liverworts and mosses have been recorded to possess antifungal [7], antibacterial, antiviral [8], antioxidative [9], and anti-inflammatory [10], potential. Thus the problem of emergence of multiple drug resistance in pathogenic strains can be solved using novel biomolecules isolated from this group of plants. Various extracts of bryophytes have been screened for their antibacterial activity [11, 12].

The Great Indian Thar Desert is the ninth-largest subtropical desert, which comprises of a range of arid to sub humid climatic conditions. Reports on the exploration of bryophytes from this particular region are very limited [13]. *Semibarbula orientalis* [synonym= *Barbula indica* (Hook.)] belongs to a Pottiaceae family is a commonly growing moss in the Indian Thar Desert. Traditionally it has been used in the treatment of intermittent fever and during menstrual pain [14]. But the plant is negligibly investigated for its bioactive phytochemicals. Thus in the present

study, the plant was investigated for the identification of bioactive compounds by preliminary phytochemical screening and GC-MS analysis which are important for pharmaceutical as well as food industries.

2. MATERIALS AND METHODS

2.1 Sample Collection and Extract Preparation

The plant material of moss *Semibarbula orientalis*, were collected during the year from 2017 to 2020, particularly in the rainy season from different localities of Jodhpur and Bikaner. The collected materials were brought to the laboratory in polythene bags. Fresh plant materials were then extensively washed under running tap water followed by distilled water to remove attached debris and then shade dried on filter paper for 15 days. This air–dried plant material was then kept in paper packets and stored for further use in the laboratory Department of Botany Jai Narain Vyas University, Jodhpur (Rajasthan), India. Bryophytes are free from any kind of pathogenic attack till date due to the presence of self defending proteins or secondary metabolites hence experimental material was taken from this undamaged/unspoiled stored material of four years.

20 gm of air-dried plant material was grinded in double-distilled water in the ratio of 1:10 w/v with the help of an electric grinder till the formation of fine paste and remained soaked in solvent for 24 hrs in a beaker. Then the extract was filtered with sterile muslin cloth followed by Whatmann filter paper 1. The filtrate was then centrifuged at 2500 rpm for 15 minutes. The supernatant thus obtained was used as 100 per cent crude aqueous extract.

For the organic solvent extraction (ethanolic and methanolic), 20 gm air-dried material was grinded with respective alcoholic solvent (1:10 w/v) and was kept in a beaker for 48 hrs with occasional stirring. The extract was then filtered and centrifuged at 2500 rpm for 15 minutes. The obtained supernatant was evaporated until dry on water bath to obtain crude extract. The extract was preserved in brown bottles at 4°c till further use.

2.2 Preliminary Phytochemical Screening

Preliminary phytochemical screening of aqueous, ethanolic and methanolic crude extracts of *Semibarbula orientalis* was carried out to evaluate the presence or absence of primary and secondary metabolites such as carbohydrates, proteins, alkaloid, sterol, phenol, glycosides, terpenoids and flavonoids etc. following the standard methods of Harbourne [15], Trease and Evans [16].

2.3 GC-MS Analysis

(a). Extract preparation: 10 g dried powder of plant material was soaked in 100 ml of HPLC grade methanol (1:10 w/v) in a beaker for 48 hrs with occasional stirring. The extract was filtered with sterile muslin cloth followed by Whatmann filter paper no.1 Further this filtrate was centrifuged at 2500 rpm for 15 minutes to get clear solution. The extract thus obtained was evaporated to dryness on water bath to obtain crude extract. The extract was preserved in brown bottle till further use [17]. For GC-MS analysis crude extract was redissolved in methanol to make stock solution, from this stock solution, 1µl was used in GC-MS analysis. Chromatographic separation was carried out at USIC, AIRF, JNU, New Delhi with GC-MS-QP 2010 Shimadzu, Japan equipped with thermal desorption system TD 20.

(b). Analytical parameter: Helium (99.99%) was used as carrier gas at a constant flow rate of- Total Flow: 16.3 ml/min and Column Flow: 1.21 ml/min. The temperature was programmed at - Column Oven Temp. : 50.0°C, Injection Temp. : 260.00 °C; Ion Source Temp.: 220.00 °C and Interface Temp.: 270.00 °C respectively. Injection was performed in the split less mode. Mass spectra were obtained by electron ionization (EI) at 70 ev. Total running time of GC-MS was 50 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass.

(c). Identification of compounds: Identification was based on the interpretation of the Retention time (RT) and GC-MS spectrum using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of unknown compounds was compared with the spectrum of known compound stored in the NIST library. The name, molecular formula, molecular weight, and structure of the components of the test materials were ascertained.

3. RESULTS AND DISCUSSION

Biologically active compounds from natural sources have played a major role in the development of new drugs. Bryophytes are extremely rich in secondary metabolites many of which have shown important biological activity. The results of a preliminary phytochemical analysis of extracts of moss, *Semibarbula orientalis* revealed the presence of carbohydrates, proteins, phenols, phytosterols, flavonoids and terpenoids (Table 1). The availability of specific phytochemicals in a plant can be attributed to its specific pharmacological actions. Plants defend themselves against microbes by various defense mechanisms including the production of antimicrobial proteins and peptides [18].The phenolic compounds account for antioxidant activities in plants [19] and associated with reduced risk of heart diseases [20]. Terpenoids have been reported to possess antibiotic, antihelmintic, insecticidal and antiseptic properties [21].

Using Gas Chromatography-Mass Spectrometry (GC-MS) technique compounds like monoterpenoids, sesquiterpenoids, diterpenoids, sterols, flavonoids, phenolic compounds, and fatty acids can be found out [22].The application of the technique is oriented towards the identification of compounds in a complex mixture on the basis of their molecular mass. Thus for further identification and quantification of bioactive compounds, Gas Chromatography Mass Spectrometry (GC-MS) technique was carried out in the methanolic extract of the plant. All the phytoconstituents reported from the methanolic extract were given in Table 2 and chromatogram for the same has been given in Fig. 1. The GC-MS analysis revealed that the plant is rich in the fatty acids and their derivatives. The major compound identified with highest peak area (43.63 %) was n-Hexadecanoic acid at retention time 20.59 minutes and lowest peak area (0.06%) recorded was of Tridecanoic acid, methyl ester at retention time 17.91 minutes. The GC-MS analysis revealed that the plant is rich in the fatty acids and their derivatives. n-Hexadecanoic acid have been reported to possess antioxidant, anti-inflammatory, hypocholesterolemic, 5-α reductase inhibitor, antiandrogenic [21], antibacterial and antifungal properties [23]. Cis-Vaccenic acid act as a potent antioxidant and

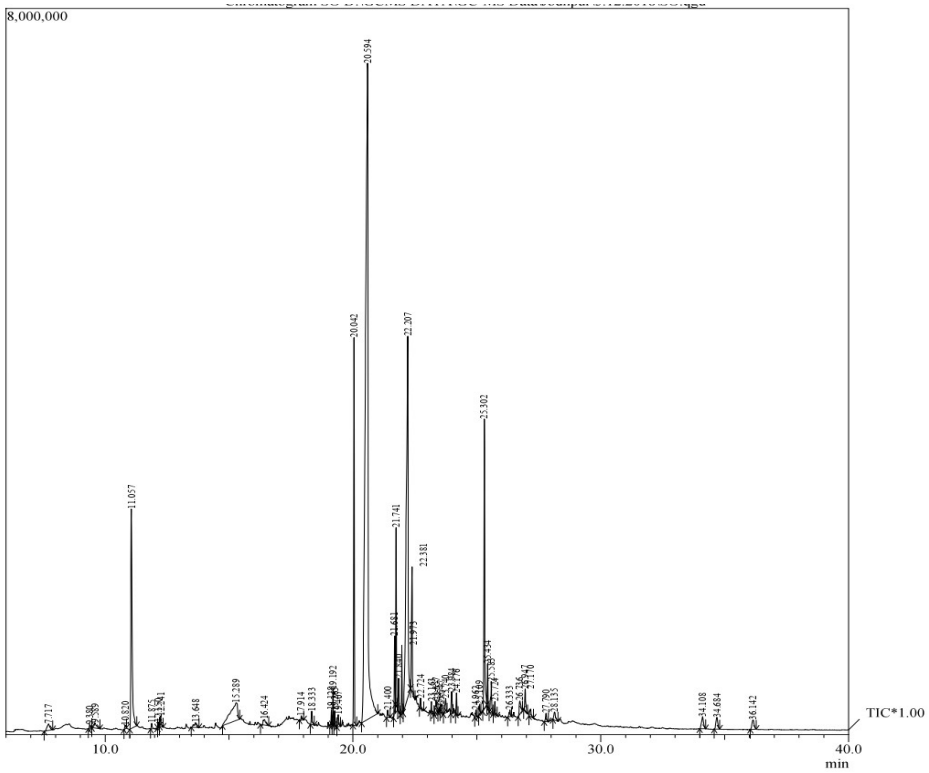

Fig.1. GC- MS Chromatogram of methanolic extract of *Semibarbula orientalis*

Table1. Preliminary phytochemical screening of *Semibarbula orientalis* in different solvents

S.No	Phytochemical components	Test	Aqueous extract	Ethanolic extract	Methanolic Extract
1	Carbohydrates	Molisch's test	+	++	++
		Fehling's test	+	++	++
2	Proteins and Amino Acids	Ninhydrin test	+	++	++
		Xanthoproteic test	+	++	++
3	Alkaloids	Dragendrof's test	-	-	-
		Wagner's test	-	-	-
4	Phenols	Ferric chloride test	+	++	++
		Lead acetate test	-	+	++
5	Flavonoids	Shinoda test	-	+	++
		Alkaline reagent test	-	+	++
6	Terpenoids	Salkowski test	+	++	++
7	Phytosterol	Liberman Burchard's test	+	+	++
8	Glycosides	Keller-Kilani test	-	-	-
		NaOH test	-	-	-
9	Saponin	Froth test	-	-	-
		Olive oil test	-	-	-
10	Olis and fats	Spot test	-	+	++

(+) = phytoconstituents present, (-) = phytoconstituents absent

Table 2. Phytochemical compounds identified in methanolic extract of S*emibarbula orientalis* by GC-MS analysis

S. No.	R. Time	Compound Name	Molecular formula	Molecular weight	Area%
1	7.717	Hexanoic acid	$C_6H_{12}O_2$	116	0.41
2	9.380	Phenol, 4-Methoxy-	$C_7H_8O_2$	124	0.08
3	9.589	Diazene, bis(1,1-dimethylethyl)-	$C_8H_{18}N_2$	142	0.28
4	10.820	Octanoic Acid	$C_8H_{16}O_2$	144	0.10
5	11.057	Azulene	$C_{10}H_8$	128	6.77
6	11.875	4-Oxononanal	$C_9H_{16}O_2$	156	0.08
7	12.150	1,3-Cyclopentanedione, 4-Butyl-	$C_9H_{14}O_2$	154	0.09
8	12..241	Nonanoic acid	$C_9H_{18}O_2$	158	0.26
9	13.648	1-Nonanol, 4,8-dimethyl-	$C_{11}H_{24}O$	172	0.24
10	15.289	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	151	3.10
11	16.424	-			0.30
12	17.914	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	0.06
13	18.333	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.31
14	19.128	Neophytadiene	$C_{20}H_{38}$	278	0.22

S. No.	R. Time	Compound Name	Molecular formula	Molecular weight	Area%
15	19.192	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.50
16	19.263	2-Pentadecanol, 6,10,14-Trimethyl-	$C_{18}H_{38}O$	270	0.23
17	19.407	Didodecyl phthalat	$C_{32}H_{54}O_4$	502	0.35
18	20.042	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	5.69
19	20.594	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	43.63
20	21.400	Heptadecanoic Acid	$C_{17}H_{34}O_2$	270	0.17
21	21.681	9,12-Octadecadienoic Acid (Z,Z)-, Methyl ester	$C_{19}H_{34}O_2$	294	1.08
22	21.741	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	2.46
23	21.840	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	296	0.57
24	21.973	Methyl stearate	$C_{19}H_{38}O_2$	298	1.05
25	22.207	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	16.03
26	22.381	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.65
27	22.724	Oleic Acid	$C_{18}H_{34}O_2$	282	0.17
28	23.163	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	0.21
29	23.357	Oxiraneoctanoic acid, 3-octyl-, methyl ester	$C_{19}H_{36}O_3$	312	0.44
30	23.475	-			0.09
31	23.547	8,11,14-Docosatrienoic acid, methyl ester	$C_{23}H_{40}O_2$	348	0.20
32	23.740	Tetradecanoic acid, 12-methyl-,methyl ester	$C_{16}H_{32}O_2$	256	0.40
33	23.984	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	0.47
34	24.176	Hexadecanoic acid, hexyl ester	$C_{22}H_{44}O_2$	340	0.32
35	24.962	Hexadecanoic acid, heptyl ester	$C_{23}H_{46}O_2$	354	0.12
36	25.109	1-Heneicosanol	$C_{21}H_{44}O$	312	0.20
37	25.302	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	5.43
38	25.434	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	0.61
39	25.583	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	0.75
40	25.724	Hexadecanoic acid, n.-octyl ester	$C_{24}H_{48}O_2$	368	0.23
41	26.333	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	0.19
42	26.736	9-Octadecenal, (Z)-	$C_{18}H_{34}O$	266	0.52
43	26.947	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358	0.53
44	27.170	9-octadecenoic acid, 2,2,2-trifluoroethyl ester	$C_{20}H_{35}F_3O_2$	364	0.26
45	27.790	Squalene	$C_{30}H_{50}$	410	0.20
46	28.135	-			0.33
47	34.108	Ergost-5-en-3-ol, (3.beta.)-	$C_{28}H_{48}O$	400	0.58
48	34.684	Stigmasterol	$C_{29}H_{48}O$	412	0.54
49	36.142	Stigmast-5-en-3-OL, (3.beta.)-	$C_{29}H_{50}O$	414	0.49

Table 3. Major phytochemical compounds identified in methanolic extract of *Semibarbula orientalis* and their biological activities

S. No.	Compound name	Peak Area %	Compound class	Biological activity	References
1.	n-Hexadecanoic acid	43.63	Fatty acid derivative	Antioxidant, anti-inflammatory, 5-α reductase inhibitor, hypercholestrolemic, antiandrogenic, antibacterial, and antifungal.	[23], [21]
2.	Cis-Vaccenic acid	16.03	Fatty acid	Anti-inflammatory and antioxidant	[24]
3.	Azulene	6.77	Aromatic hydrocarbon	Antiinflammatory, hormetic ,uv-protective, antibacterial, and antispasmodic	[32], [33]
4.	Hexadecanoic acid, methyl ester	5.69	Fatty acid ester	Antioxidant, antibacterial , hypocholesterolemic, pesticide, and haemolytic 5-α-reductase inhibitory properties	[25], [26]
5.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	5.43	Fatty acid ester	Antioxidant, anti-inflammatory, and antihelmentic.	[28]
6.	1,3-Propanediol,2-(hydroxymethyl)-2-nitro-	3.10	Glycerol compound	Bacteriostat and microbicidal	[34]
7.	9,12-Octadecadienoyl chloride, (z,z)	2.46	Fatty acid derivative	Antisecretory,contraceptive, antitubercular, and antispermatogenic	[25]
8.	Octadecanoic acid	2.65	Fatty acid	Antibacterial and antifungal	[29]
9.	9,12-Octadecadienoic acid (z,z)-,methyl ester	1.08	Fatty acid methyl ester	Antifungal, antioxidant, and anticancer	[30], [31]
10.	Methyl stearate	1.05	Fatty acid methyl ester	Antidiarrheal, cytotoxic, and antiproliferative	[35], [36]

anti-inflammatory compound [24]. Hexadecanoic acid methyl ester, reported to have antioxidant, hypocholesterolemic, pesticide and haemolytic 5-α-reductase inhibitor [25,26], antibacterial and antifungal [27]. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester is a good antioxidant, anti-inflammatory and antihelmentic [28]. 9,12-Octadecadienoyl chloride, a linoleoyl chloride has reported to possess antisecretory, antispermatogenic, antitubercular, choleretic, contraceptive properties [25]. 9-Octadecenoic acid, possesses anti-inflammatory, antitumour, immunostimulatory, anti-leucotriene-D4, antialopecic, 5-α-reductase inhibitory, anemiagenic, antiandrogenic, lipoxygenase inhibitory, and hypocholesterolemic properties [26]. Octadecanoic acid possesses antibacterial and antifungal actions [29]. 9, 12 Octadecadienoic acid (z, z)-, methyl ester have been reported to possess antioxidant, antifungal [30] and anticancerous property [31]. Bioactivity of some significant compounds with their chemical nature and bioactivity listed in Table 3.

4. CONCLUSION

The preliminary phytochemical screening and GC-MS analysis of *Semibarbula orientalis* showed the presence of various types of high value bioactive compounds from different chemical groups such as fatty acids and their derivatives, glycerol compound, triterpene, phenols and phytosterols with important medicinal properties and other uses in food industries as flavouring and colouring agents. Further, studies are needed to isolate active principle of the extract as well as to elucidate their extract mechanism of action in various disorders to prove these claims. This is the first report of the identification of active constituents from this plant and hence proves its therapeutic potential.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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