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# GC-MS analysis of Biologically active compounds from *Riccia melanosopra* Kashyap: A Bryophyte

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**Abstract**

Present study aims to evaluate the presence of bioactive compounds of methanolic extract of whole plant of *Riccia melanospora* by Gas Chromatography-Mass Spectrometry (GC-MS) which are important to pharmaceutical as well as food industries. The Preliminary phytochemical screening of *R. melanospora* 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. Preliminary phytochemical analysis revealed the presence of carbohydrates, proteins, phenols, sterols, flavonoids and terpenoids where as GC-MS analysis of methanolic extract of whole plant showed the presence of 38 bioactive phytoconstituents which include mainly N-Methyl-3-piperidine carboxamide, Azulene, Hexadecanoic acid metyl ester, ), n-Hexadecanoic acid, 9,12,15-Octadeca-trienoic acid, (Z,Z,Z)-, 7-Tetradecenal,(z)-, 6,6-Dimethyl-1,5-diazabicyclo[3.1.0] hexane, 9,12-Octadecadienoic acid, methyl ester, and Methyl stearate etc. These phytochemicals are medicinally important to cure various types of human ailments.

Bryophytes are amphibian plants, which are found in almost all kinds of habitats worldwide, from desert to humid rainforest, from hot tropical area to the cold Arctic and from sea level to alpine peaks[6]. Bryophytes are rarely consumed by insects and herbivores, not only because bryophytes have low caloric content but also for the diversity of "chemical weapons" they produce[17, 31]. In general, bryophytes show high tolerance against various biotic and abiotic stresses[20,23].

Bryophytes are the store house of bioactive components and therefore are used throughout the World as drugs to cure various diseases[8]. They have been proven to be rich sources of antimicrobials and attempts to find potent, nontoxic, broad-spectrum active molecules have been widely undertaken.

Among the bryophytes liverworts are rich sources of lipophilic terpenoids and aromatic compounds, several of which show interesting biological activities[3, 4]. Liverwort extracts with antimicrobial effects are even sold commercially[14]. Liverworts contain a large amount of mono-, sesqui- and diterpenoids, and aromatic compounds. In particular, they contain pinguisane-type sesquiterpenoids, sacculatane-type diterpenoids and bis (bi-benzyl) aromatic compounds which have not been found in higher plants[5]. Although several liverworts have been used as medicinal plants[15], only 10% of the liverworts have yet been studied systematically[2].

The cosmopolitan genus *Riccia*, family Ricciaceae (Hepaticae), is a thalloid liverwort. Thallus dichotomously branched, usually forming rosettes on moist ground, with a distinct median furrow Fig. 1. Traditionally the species of Riccia used to treat ringworm and to cure cuts and wounds[24]. But the genus is negligibly investigated for its bioactive phytochemicals. With this background the present study was aimed to identify the bioactive compounds present in methanolic extract of *Riccia melanospora* by preliminary phytochemical screening and GC-MS analysis which are important for pharmaceutical as well as food industries.

*Sample collection and extract preparation:*

Plant materials were collected 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 at room temperature in shaded place. Dried thalli were then coarsely powdered with the help of a mortar and pestle.

Dried powder (20 g) was soaked in 100 ml of HPLC Grade methanol (1:10 w/v) in a beaker for 48 hours with occasional stirring. The extract was filtered with sterile muslin cloth followed by Whatmann filter paper No. 1. Further the 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 bottles at 4$^{o}$C till further use[11].

*Preliminary phytochemical screening:*

Preliminary phytochemical screening of methanolic crude extract of *Riccia melanospora* was carried out using standard methods[13,18] to evaluate presence or absence of major primary and secondary metabolites such as Carbohydrates, Protein, alkaloid, steroid, phenol, Glycosides, terpenoids and flavonoids etc.

*GC-MS analysis :*

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. 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. Identification of compounds 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.

Preliminary phytochemical screening reveals the presence of primary and secondary metabolites. Results of preliminary phytochemicals screening are shown in table-1.

The GC-MS chromatogram of methanolic extract of *R. melanospora* revealed the presence of 38 phyto chemicals, which includes alkaloids, terpens, Fatty acids, phenols, flavonoids, and essential oils *etc*. Among these phytochemicals N-Methyl-3-piperidinecarboxamide (40.01%) exibits the highest peak percentage followed by Azulene (11.03%), Hexadecanoic acid, metyl ester (10.44), n-Hexadecanoic acid (9.41), 9,12,15-Octadeca-trienoic acid, (Z,Z,Z)- (5.51), 7-Tetradecenal,(z)- (5.07), 6,6-Dimethyl-1,5-diazabicyclo[3.1.0] hexane (4.00), 9,12-Octadecadienoic acid, methyl ester (1.90), Methyl stearate (1.25). Lowest peak with 0.05% was reported for 10-Heptadecen-8-ynoic acid, methyl ester, (E)-. All the phytoconstituents reported in GC-MS analysis were given in Table 2 and chromatogram for the same has been given in Fig. 2.

Natural products are potential sources for the development of new drugs to use in treatments[30]. The presence of many significant secondary metabolites shows the potential of this plant for various therapeutical and pharmaceutical applications. The present analysis reveals that the plant contains fatty acids in abundance along with piperidine alkaloids and terpenes. These secondary metabolites help the plants to cope up with the biotic and abiotic stresses[21]. Alkaloids are generally toxic to man and many of them have shown physiological activities; hence they are widely used in medicine[18]. Phytol is an acyclic diterpene alcohol that can be used as precursor of synthetic forms of vitamin E and vitamin K. It also reported to have anti-microbial, anti-cancer, anti-inflammatory and diuretic properties[22].

The high fatty acid content found in *R. melanospora* indicated that fatty acids are the common constituent of bryophytes[28, 34]. Several studies have also reported that many bryophytes contain a high abundance of polyunsaturated fatty acids (PUFA)[7]. Saruwatari *et al.*[32] indicated that the physiological function of certain polyunsaturated fatty acids, such as linolenic acid and eicosapentaenoic acid, might

Fig.1. *Riccia melanospora*. Field photograph.

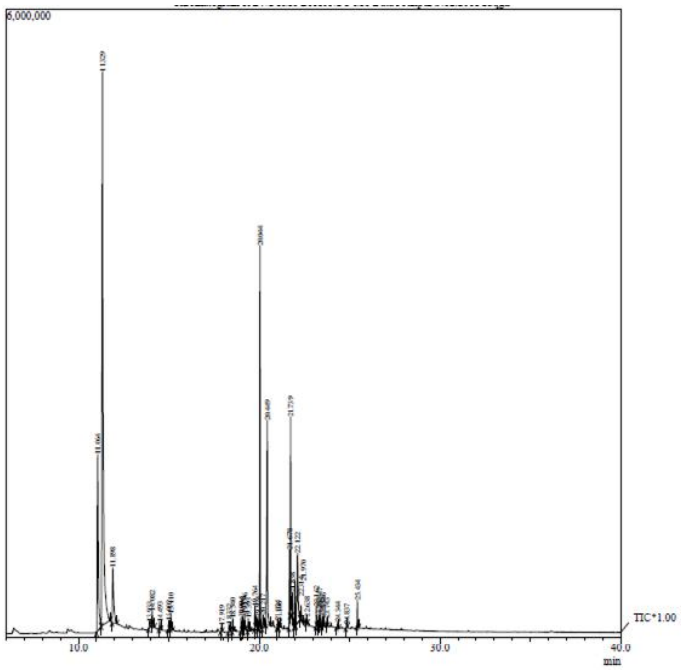


Fig. 2. GC- MS Chromatogram of methanolic extract of *Riccia melanospora*.

Table-1. Preliminary phytochemical screening of *Riccia melanospora*

| S.No | Phytochemical components | Test | 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.

be involved in the freezing-tolerant phenomenon. However, the dominant fatty acids and their derivatives detected in our samples are among the saturated and monounsaturated fatty acids, it showed that the ecological condition such as altitude, substrate and microclimate have an important role in metabolic production in bryophytes. Besides coping up with environmental stress fatty acids and derivatives are also reported to have various bioactivities. n-Hexadecanoic acid have been reported to possess anti-inflammatory, hypocholesterolemic, antioxidant, 5-α reductase inhibitor, antiandrogenic[12], antibacterial and antifungal properties[1]. Hexadecanoic acid methyl ester, reported to have antioxidant, hypocholesterolemic, pesticide and haemolytic 5-α-reductase inhibitor[9, 27], antibacterial and antifungal[10]. 9, 12 Octadecadienoic acid (z, z)-, methyl ester have been reported to possess antifungal, antioxidant[29] and anticancerous property[35]. 9-Octadecenoic acid possesses anti-inflammatory, antitumor, immunostimulatory, antialopecic, anemiagenic, antiandrogenic, 5-α-reductase inhibitory, lipoxygenase inhibitory, and hypocholesterolemic properties [9]. Bioactivity of some significant compounds with their chemical nature is listed in Table-3.

Table-2. Phytochemical compounds identified in methanolic extract of *Riccia melanospora* by GC-MS analysis

| S. No. | R. Time | Compound Name | Molecular formula | Molecular weight | Area% |
|---|---|---|---|---|---|
| 1. | 11.064 | Azulene | $C_{10}H_8$ | 128 | 11.03 |
| 2. | 11.329 | N-Methyl-3-piperidinecarboxamide | $C_7H_{14}N_{20}$ | 142 | 40.01 |
| 3. | 11.898 | 6,6-Dimethyl-1,5-diazabicyclo [3.1.0]hexane | $C_6H_{14}N_2$ | 112 | 4.00 |
| 4. | 13.933 | 4(1H)-Pyrimidinone, 1-methyl- | $C_5H_6N_{20}$ | 110 | 0.46 |
| 5. | 14.082 | 2H-Azepin-2-one, hexahydro-1-methyl- | $C_7H_{13}NO$ | 127 | 0.69 |
| 6. | 14.493 | Piperidine, 3-isopropyl | $C_8H_{17}N$ | 127 | 0.29 |
| 7. | 15.002 | 5-Amino-1H-1,2,4-triazole-3-carbohydrazide | $C_3H_6N_6O$ | 142 | 0.34 |
| 8. | 15.110 | 5-Amino-1H-1,2,4-triazole-3-carbohydrazide | $C_3H_6N_6O$ | 142 | 0.60 |
| 9. | 17.919 | Tridecanoic acid, methyl ester | $C_{14}H_{28}O_2$ | 228 | 0.18 |
| 10. | 18.332 | 9-Octadecenoic acid (z)- | $C_{18}H_{34}O_2$ | 282 | 0.32 |
| 11. | 18.540 | 1-Tridecanol | $C_{13}H_{28}O$ | 200 | 0.36 |
| 12. | 19.004 | Tetradecanoic acid, 12-methyl-, methyl ester, (s)- | $C_{16}H_{32}O_2$ | 256 | 0.42 |
| 13. | 19.092 | 9-Octadecenoic acid (z)- | $C_{18}H_{34}O_2$ | 282 | 0.59 |
| 14. | 19.196 | 2-Pentadecanone, 6,10,14-trimethyl | $C_{18}H_{36}O$ | 268 | 0.29 |
| 15. | 19.393 | 9-Octadecenoic acid (z)- | $C_{18}H_{34}O_2$ | 282 | 0.59 |
| 16. | 19.764 | 9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z) | $C_{19}H_{32}O_2$ | 292 | 0.63 |
| 17. | 19.924 | Cyclopropanedodecanoic acid, 2-octyl-, methyl ester | $C_{24}H_{46}O_2$ | 366 | 0.27 |
| 18. | 20.044 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 270 | 10.44 |
| 19. | 20.217 | | $C_{14}H_{26}O$ | 210 | 0.68 |
| 20. | 20.449 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 9.41 |
| 21. | 21.024 | Palmitic acid | $C_{16}H_{32}O_2$ | 256 | 0.19 |
| 22. | 21.106 | 9-Octadecenoic acid (z)- | $C_{18}H_{34}O_2$ | 282 | 0.18 |
| 23. | 21.678 | 9,12-Octadecadienoic acid, | $C_{19}H_{34}O_2$ | 294 | 1.90 |

| | | | | | |
|---|---|---|---|---|---|
| | | methyl ester | | | |
| 24. | 21.739 | 9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z) | $C_{19}H_{32}O_2$ | 292 | 5.51 |
| 25. | 21.838 | Phytol | $C_{20}H_{40}O$ | 296 | 0.58 |
| 26. | 21.970 | Methyl stearate | $C_{19}H_{38}O_2$ | 298 | 1.25 |
| 27. | 22.122 | 7-Tetradecenal, (z)- | $C_{14}H_{26}O$ | 210 | 5.07 |
| 28. | 22.314 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 0.71 |
| 29. | 22.638 | Heptadecane | $C_{17}H_{36}$ | 240 | 0.15 |
| 30. | 23.162 | 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-z) | $C_{21}H_{34}O_2$ | 318 | 0.54 |
| 31. | 23.283 | Methyl 5,11,14-eicosatrienoate | $C_{21}H_{36}O_2$ | 320 | 0.21 |
| 32. | 23.347 | Methyl(z)-5,11,14,17eicosatetraenoate | $C_{21}H_{34}O_2$ | 318 | 0.45 |
| 33. | 23.485 | 9,12-Octadecadienoic acid, methyl ester | $C_{19}H_{34}O_2$ | 294 | 0.22 |
| 34. | 23.546 | 11,14,17-Eicosatrienoic acid, methyl ester | $C_{21}H_{36}O_2$ | 320 | 0.25 |
| 35. | 23.743 | 2-Ethylhexyl (2E)-3-(4-Methoxyphenyl)-2-Propenoate | $C_{18}H_{26}O_3$ | 290 | 0.14 |
| 36. | 24.344 | Nonadecane | $C_{19}H_{40}$ | 268 | 0.17 |
| 37. | 24.837 | 10-Heptadecen-8-ynoic acid, methyl ester, (E)- | $C_{18}H_{30}O_2$ | 278 | 0.05 |
| 38. | 25.434 | Di-n-octyl phthalate | $C_{24}H_{38}O_4$ | 390 | 0.82 |

Table-3. Major phytochemical compounds identified in methanolic extract of *Riccia melanospora* and their biological activities

| S. No. | Compound name | Compound class | Biological activity | Refe-rences |
|---|---|---|---|---|
| 1. | Azulene | Aromatic hydrocarbon | Antiinflammatory, antibacterial, hormetic, uv-protective, and antispasmodic | [16], [33] |
| 2. | Hexadecanoic acid, methyl ester | Fatty acid ester | antioxidant, antibacterial, hypocholestero-lemic, pesticide and haemolytic 5-α-reductase inhibitor [25,26]. | [9], [27] |
| 3. | n-Hexadec-anoic acid | Fatty acid | Antioxidant, anti-inflammatory, 5-α reductase inhibitor, hypercholestrolemic, antiandrogenic, antibacterial, and antifungal. | [1], [12] |

| | | | | |
|---|---|---|---|---|
| 4. | 9,12,15-Octadecatri-enoic acid, methylester, (z,z,z)- | Fatty acid ester | Antifungal activity | [29], [35] |
| 5. | 7-Tetradecenal,(z) | Aldehyde | Pesticide | [12] |
| 6. | 9,12-Octadecadie-noic acid , methyl ester | Fatty acid ester | Antioxdant, antifungal, and anticancerous properties. | [29],[35] |
| 7. | Methyl stearate | Fatty acid ester | Antidiarrheal, cytotoxic, and antiproliferative | [25], [26] |
| 8. | 5-Amino-1H-1,2,4-triazole-3-carbohydrazide | Heterocyclic compound | Synthesis of energetic salts | [36] |
| 9. | Phytol | Diterpene | Antimicrobial, anticancer, anti-inflammatory properties | [22] |
| 10. | 9-Octadecenoic acid (z)- | Fatty acid | anti-inflammatory, antitumour, immunostim-ulatory, anti-leucotriene-D4, antialopecic, 5-α-reductase inhibitory, anemiagenic, antiandrogenic, lipoxygenase inhibitory, and hypocholesterolemic properties | [9] |

The preliminary phytochemical screening and GC-MS analysis of *Riccia melanospora* showed the presence of various types of high value bioactive compounds from different chemical groups such as fatty acids and their derivatives, alkaloids, triterpene, and phenols with important medicinal properties and other uses in food industries. 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 liverwort and hence proves its therapeutic potential.

*Conflict of interests :*

Authors confirm that there is no conflict of interest.

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