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# GC-MS Analysis of Bioactive Compounds Present in the Aqueous Extract of the Fern Azolla Pinnata

**B. Thiripurasundari[1], E. Padmini[2]**

[1]Research and Development Centre, Bharathiar University, Coimbatore 641046, Tamilnadu, India.
Corresponding Author Email: *sudhamphil2003[at]gmail.com*

[2]Former Principal, Presidency College, Chennai 600005, Tamilnadu, India

**Abstract:** ***Azolla pinnata (Azolla sp.) is an aquatic fern consisting of a short, branched, floating stem, bearing roots which hang down in the water. The present study was aimed to investigate and determine the biologically active compounds present in the aqueous extract of azolla pinnata by GC-MS analysis. GC-MS analysis of aqueous extract of Azolla was performed using the GCMS-TQ8040 NX Triple Quadrupole Gas Chromatograph Mass Spectrometer from Shimadzu Scientific Instruments. The chromatogram revealed the presence of various biologically active compounds from the aqueous extract of azolla using gas chromatography – mass spectroscopy of different types of high and low molecular weight chemical entities. These compounds are considered biologically and pharmacologically important. It may be concluded that the bioactive compounds present in Azolla pinnata serve as the basis for identifying the various biological activities of Azolla and also warrants further biological and pharmacological studies.***

**Keywords:** Azolla pinnata, GC-MS analysis, Aqueous extract, Biological compounds, Biological activity & Pharmacological activity

## 1. Introduction

Azolla, is a free-floating water ferns symbiosis with nitrogen fixing cyanobacteria *Anabaena azollae*. Azolla (Azolla sp.) is an aquatic fern consisting of a short, branched, floating stem, bearing roots which hang down in the water. The leaves are alternately arranged, each consisting of thick aerial dorsal lobe containing green chlorophyll and a slightly larger thin, colorless, floating ventral lobe. Under some conditions, an anthocyanin pigment gives the fern a reddish-brown color. Plant diameter ranges from 1-2.5 cm for small species such as *Azolla pinnata*, to 15 cm or more for *Azolla nilotica*. Azolla plants are triangular or polygonal in shape, and float on the surface of the water, individually or in mats. They give the appearance of a dark green to reddish carpet, except *Azolla nilotica* that does not produce the red anthocyanin pigment. The most remarkable characteristic of azolla is its symbiotic relationship with the nitrogen-fixing blue-green alga (cyanobacterium) *Anabaena azollae*. The fern provides nutrients and a protective cavity in each leaf to Anabaena colonies in exchange for fixed atmospheric nitrogen and possibly other growth-promoting substances (Lumpkin *et al.*, 1980). It is a branched free floating aquatic fern, available mainly on moist soils, ditches, marshy ponds and rice fields, and is widely distributed in the tropical regions of India, Thailand, Korea, Philippines, Brazil and West Africa.

The aquatic fern Azolla is also reported to be an excellent bio fertilizer and green manure in the paddy field. In addition, now a day's azolla (either fresh or dried) is used as a feed ingredient for ruminants and non-ruminants type of livestock. Besides its utilization as a bio fertilizer and livestock feed, azolla the green gold mine of the nature is also used as medicine, water purifier, and human food and for production of biogas. Ability of Azolla-Anabaena system to fix atmospheric nitrogen at faster rates makes it an outstanding agronomic choice for the cultivation of rice under tropical conditions (Yadav *et al.*, 2014). It is considered to be ecofriendly, nontoxic and utmost preferred fertilizer resource for household gardening and organic farming.

**Figure A:** *Azolla Pinnata*

It acts as a natural source of diverse nutrients, especially N, increases the availability of other nutrients, plays a significant role in suppression of weed, strengthens soil organic matter, and enhances the efficiency of the inorganic fertilizers through maintaining the suitable soil pH condition (Thapa and Poudel, 2021). *Azolla* grows well in water habitats with relatively low nitrogen and phosphorus conditions, it has greater efficiency to accumulate nutrients more than its requirement and thus soil or crops benefit after the decomposition of *Azolla* also. Coherently studies reported that the presence of nutrients in the saline habitat, especially phosphate and nitrate, plays a major role in salt tolerance of the plants (Okusanya and Ungar, 1984, Okusanya and Fawole, 1985) if *Azolla* used as green manure in the agricultural field (Mishra and Singh, 2006, Singh *et al.*, 2008a).

Azolla pays a remarkable contribution to the nitrogen economy and the fertility of the nitrogen deficient soil. Masood *et al.*, (2013) explained the salinity tolerance property of *Azolla pinnata* by assessing the activity and modulation of antioxidants under salinity condition. It is exhibiting its own property of salinity tolerance; however, its

property in the cultivated field under saline condition is not explored so far. Hence, use of salt tolerant Azolla strain(s) in saline water can create an interesting new perspective to reduce toxicity caused by the salinity in the field condition.

Hence the present work aimed to investigate and characterize bioactive compounds present in the fern *Azolla* pinnata aqueous extract.

## 2. Materials and Methods

**Cultivation of *Azolla***

The fresh plants of Azolla was collected at Tamilnadu Fisheries University, Madhavaram, Chennai-600-051.The plant authentication was done at Siddha Central Research Institute, Ministry of AYUSH, Government of India, Arumbakkam, Chennai - 600 106. .*Azolla* was cultivated in plastic trays. When plants reached to full cover in a tray then it was harvested, shades dried and used for the preparation of *Azolla* extract.

**GCMS analysis of aqueous extract of *Azolla***

The GC-MS analysis of azolla extract was done at Tamilnadu Test House, Chennai 600 077.GC-MS analysis of aqueous extract of *Azolla* was performed using the GCMS-TQ8040 NX Triple Quadrupole Gas Chromatograph Mass Spectrometer from Shimadzu Scientific Instruments. The injector was kept at 270 °C, split ratio at 2 and high-pressure injection at 200 kPa for 0.50 min. After the high-pressure injection period, the fiber was retracted and removed from the injector. The chemical profile of the resulting extracts was performed by injecting 1 µL of the sample volume on the aforementioned GC–MS, equipped with a split less injector and cross-bonded fused column (30 m × 0.25 mm, 0.25 µm film thickness) to low polarity phases. The oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The identification of components was based on NIST libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST) attached to the GC-MS instrument and the results obtained

## 3. Results

The chromatogram of the GC-MS analysis of Azolla pinnata aqueous extract is depicted in figure 1.

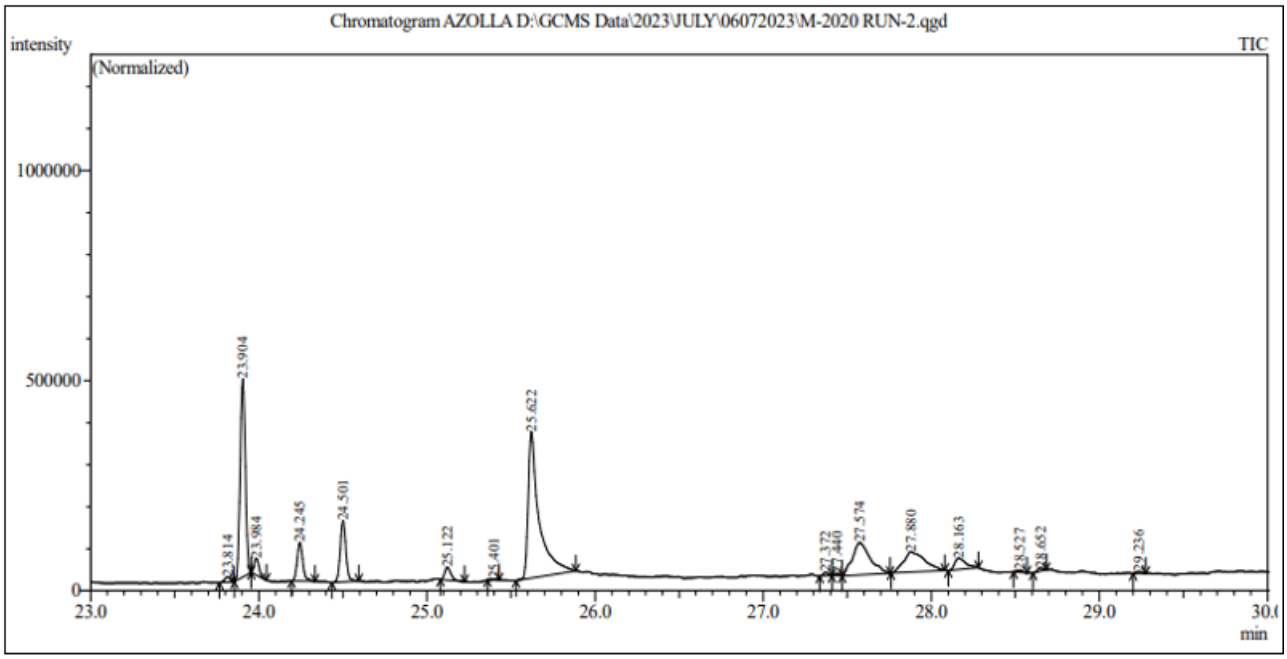


**Figure 1:** GCMS analysis of aqueous extract of Azolla pinnata

Peak Report TIC

| Peak# | R.Time | Area | Area% | Height | Height% | A/H | Name |
|---|---|---|---|---|---|---|---|
| 1 | 23.814 | 69744 | 0.45 | 30464 | 0.74 | 2.29 | 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- |
| 2 | 23.904 | 2549199 | 16.61 | 1194073 | 29.1 | 2.13 | Neophytadiene |
| 3 | 23.984 | 201648 | 1.31 | 101274 | 2.47 | 1.99 | Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester |
| 4 | 24.245 | 539606 | 3.52 | 232811 | 5.67 | 2.32 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol |
| 5 | 24.501 | 861152 | 5.61 | 370052 | 9.02 | 2.33 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol |
| 6 | 25.122 | 202302 | 1.32 | 77174 | 1.88 | 2.62 | Hexadecanoic acid, methyl ester |
| 7 | 25.401 | 10338 | 0.07 | 6650 | 0.16 | 1.55 | Isophytol |
| 8 | 25.622 | 3991858 | 26.01 | 882170 | 21.5 | 4.53 | l-(+)-Ascorbic acid 2,6-dihexadecanoate |
| 9 | 27.372 | 42609 | 0.28 | 20197 | 0.49 | 2.11 | 7-Hexadecyn-1-ol |
| 10 | 27.44 | 24094 | 0.16 | 13670 | 0.33 | 1.76 | 6-Octadecenoic acid, methyl ester, (Z)- |
| 11 | 27.574 | 1415257 | 9.22 | 192916 | 4.7 | 7.34 | Phytol |
| 12 | 27.88 | 1065065 | 6.94 | 123518 | 3.01 | 8.62 | Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate |
| 13 | 28.163 | 306325 | 2 | 68381 | 1.67 | 4.48 | Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester |
| 14 | 28.527 | 27846 | 0.18 | 12957 | 0.32 | 2.15 | Tetracosamethyl-cyclododecasiloxane |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 15 | 28.652 | 46622 | 0.3 | 17835 | 0.43 | 2.61 | trans-2-Hexadecenoic acid |
| 16 | 29.236 | 22338 | 0.15 | 10341 | 0.25 | 2.16 | Arachidamide, N-ethyl- |
| 17 | 30.225 | 43296 | 0.28 | 11967 | 0.29 | 3.62 | Cycloheptanone, 3-butyl- |
| 18 | 30.319 | 17585 | 0.11 | 9867 | 0.24 | 1.78 | 4,8,12,16-Tetramethylheptadecan-4-olide |
| 19 | 31.199 | 47389 | 0.31 | 19775 | 0.48 | 2.4 | Henicosanal |
| 20 | 31.498 | 67994 | 0.44 | 31038 | 0.76 | 2.19 | Tetracosamethyl-cyclododecasiloxane |
| 21 | 31.646 | 19860 | 0.13 | 10077 | 0.25 | 1.97 | 3-Hydroxypropyl palmitate, TMS derivative |
| 22 | 32.279 | 502636 | 3.28 | 122626 | 2.99 | 4.1 | Heptanoic acid, docosyl ester |
| 23 | 32.851 | 85155 | 0.55 | 36234 | 0.88 | 2.35 | Tetracosamethyl-cyclododecasiloxane |
| 24 | 33.323 | 26743 | 0.17 | 13788 | 0.34 | 1.94 | Oxirane, heptadecyl- |
| 25 | 33.506 | 7921 | 0.05 | 4739 | 0.12 | 1.67 | 2-Pentene, 3-(chloroethylboryl)-2-(chlorodimethylsilyl)-, (E)- |
| 26 | 33.576 | 12663 | 0.08 | 6495 | 0.16 | 1.95 | Dihydroniloticin, 2Me derivative |
| 27 | 33.946 | 24776 | 0.16 | 12773 | 0.31 | 1.94 | Eicosane |
| 28 | 34.005 | 9530 | 0.06 | 5221 | 0.13 | 1.83 | Ethanone, 1-(2-tert-butyl-2-methylcyclopropyl)-, semicarbazone |
| 29 | 34.247 | 66561 | 0.43 | 31403 | 0.77 | 2.12 | Eicosanoic acid, methyl ester |
| 30 | 34.325 | 21509 | 0.14 | 9077 | 0.22 | 2.37 | Eicosanal- |
| 31 | 34.679 | 127448 | 0.83 | 35252 | 0.86 | 3.62 | 1-Dimethyl(3-chloropropyl)silyloxyoctadecane |
| 32 | 35.142 | 51420 | 0.34 | 19599 | 0.48 | 2.62 | 2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetracosatetraen-10,15-diol |
| 33 | 35.2 | 9527 | 0.06 | 5075 | 0.12 | 1.88 | 3-Hydroxy-2-methylbenzoic acid, 2TMS |
| 34 | 35.255 | 7040 | 0.05 | 4714 | 0.11 | 1.49 | [1,1'-Bicyclohexyl]-4-carboxylic acid, 4'-butyl-, 4-pentylcyclohexyl ester |
| 35 | 35.493 | 2250260 | 14.66 | 207964 | 5.07 | 10.82 | .gamma.-Sitosterol |
| 36 | 36.125 | 8085 | 0.05 | 4594 | 0.11 | 1.76 | 1,4-Methanoazulene, 7-bromodecahydro-4,8,8-trimethyl-9-methylene- |
| 37 | 37.11 | 67583 | 0.44 | 22122 | 0.54 | 3.06 | Tetracosamethyl-cyclododecasiloxane |
| 38 | 37.627 | 97633 | 0.64 | 24727 | 0.6 | 3.95 | 1,3,2-Dioxaborolane, 4,4'-(1,3-propanediyl)bis[2-ethyl- |
| 39 | 38.795 | 10073 | 0.07 | 4587 | 0.11 | 2.2 | 2,3,5,6-Tetrafluoro-4-methoxybenzoic acid, TMS |
| 40 | 38.91 | 385900 | 2.51 | 95066 | 2.32 | 4.06 | Cholesta-4,6-dien-3-ol, (3.beta.)- |

GCMS analysis of aqueous extract of Azolla revealed the presence of various phytochemicals and they are represented in Table 1.

**Table 1:** Components of Azolla and their Properties

| S.No | Compounds | Properties |
|---|---|---|
| 1 | 2-Hexadecene, 3,7,11,15-tetramethyl-, [R- [R*, R*-(E)]] | It widens blood arteries to prevent blood clotting and aids in the formation of red blood cells.<br>It facilitates the body's absorption of vitamin K<br>Product of degradation of chlorophyll<br>Used in production of synthetic form of vitamin E and vitamin K1 |
| 2 | Neophytadiene | Anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite.<br>Larvicidal- insecticidal activity |
| 3 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | anticancer, anti-inflammatory and antimicrobial, antioxidant activities |
| 4 | Hexadecanoic acid methyl ester | Antioxidant, hypocholesterolemic, nematicide, pesticide, antimicrobial, Antifungal |
| 5 | Isophytol | Isophytol is a terpenoid alcohol that is used as a fragrance and as an intermediate in the production of vitamin E and K<br>Showed no acute toxicity towards fish and algae<br>Toxic for microorganisms and for a common soil |
| 6 | 7-Hexadecyn-1-ol | Important diterpene<br>Component of all plants such as chlorophyll and vitamin E and K |
| 7 | 6-Octadecenoic acid, methyl ester | Anticancer and insect repellant |
| 8 | Phytol | Phytol serves as a precursor for the synthesis of different chloroplast lipids. It can be converted into fatty acid phytyl esters (FAPEs), which accumulate in the plastoglobules of chloroplasts during stress<br>Antioxidant property<br>Prevent lipid peroxidation |
| 9 | Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate | Anti-tumor |
| 10 | Tetracosamethyl-cyclododecasiloxane | Hepatoprotective, antispasmodic, anti-rheumatic |
| 11 | Henicosanal | Antioxidant, Antimicrobial |
| 12 | Eicosanoid | Anti-inflammatory, analgesic, and antipyretic effects.<br>Antifungal compound |
| 13 | gamma.-Sitosterol | Anti-hyperglycemic activity |
| 14 | Tetracosamethyl-cyclododecasiloxane | Hepatoprotective activity and antispasmodic, anti-rheumatic, anti-soporific baths, insecticides for mosquito control, appetizing agent, to combat indigestion, stomach pain, nausea and infection of the gall bladder |
| 15 | Cholesta-4, 6-dien-3-ol, (3.beta.)- | Anti-inflammatory and antifungal compound. |

## 4. Discussion

In the context of GCMS analysis, various components were reported and found to impart diverse beneficial properties (Table 1). 2-Hexadecene, 3,7,11,15-tetramethyl [R-[R*,R*-(E)]], Neophytadiene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid methyl ester, Isophytol, 7-Hexadecyn-1-ol, 6-Octadecenoic acid, methyl ester, Phytol, Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, Tetracosamethyl-cyclododecasiloxane, Henicosanal, Eicosanoid, Gamma.-Sitosterol, Cholesta-4,6-dien-3-ol, (3.beta.) were observed. Most of the bioactive components are found to exhibit antioxidant, anti-inflammatory, antimicrobial, insecticidal, anticancer, hypocholesterolemic, hepatoprotective, antispasmodic, analgesic, antipyretic properties. Similarly, various bioactive components were reported for acetone and methanol extracts of Azolla pinnata in the research study by Rajiv Raju and his coworkers, 2018. In the same study it was also revealed that acetone extract was found to be more effective than methanol extract. Larvicidal property was also tested for methanol and acetone extracts of *Azolla pinnata* and found to be significant for methanol extract than acetone extract. From the results it may be concluded that the bioactive compounds present in Azolla pinnata serve as the basis for identifying the various biological activities of Azolla,

## 5. Conclusion

The study revealed the presence of various biologically active compounds from the aqueous extract of azolla using gas chromatography – mass spectroscopy of different types of high and low molecular weight chemical entities. These compounds are considered biologically and pharmacologically important. It may be concluded that the bioactive compounds present in Azolla pinnata serve as the basis for identifying the various biological activities of Azolla,

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- Family: Azollaceae (Salvinaceae)
- Genus: Azolla
- Sub genus: Eu- Azolla
- Class: Pteridophyta
- Order: Salvinales
- Family: Azollaceae (Salvinaceae )
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