Advanced Phytochemical Characterization of *Riccia fluitans L.* Through GC–MS and LC–MS Approaches

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Abstract

Liverworts are a unique group of bryophytes that have oil bodies inside their cells and can make broad structurally different secondary metabolites that could be useful in medicine. Still, many species of liverworts have not been studied enough to learn about their chemical makeup. This study aimed to analyze and characterize the phytochemical constituents in the methanolic extract of *Riccia fluitans L.* using GC–MS and liquid chromatography–mass spectrometry (LC–MS) techniques. GC–MS analysis found six volatile compounds, while LC–MS analysis found fifty-six non-volatile phytochemicals. The identified constituents were categorized into various chemical classifications, including phenolic compounds, flavonoids, terpenoids, fatty acid derivatives, and other related bioactive molecules. Various studies show that several compounds possess pharmacological activities including antioxidant, antiviral, antimicrobials, and anti-inflammatory properties. Indeed, the presence of many chemically diverse bioactive compounds found in *R. fluitans* proves its medicinal properties. Results from this research not only verified that the liverwort under investigation is rich in many phytochemicals, but the data is also significant as fundamental chemical information and environmentally sustainable which may be very usable in the next research studies. In conclusion, the results obtained suggest that *R. fluitans* might be a good natural source for the research of phytopharmaceuticals. In addition, these results provided the basis for future bioactivity-guided isolations and pharmacological analyses regarding its active compounds.

Keywords: *Riccia fluitans L.,* Phytochemical profiling, Mass spectrometry, GC–MS, LC–MS, Natural products

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1. Introduction

Bryophytes are simple plants that live on land, and their existence dates back to the Ordovician period, about 470 million years ago [1]. Bryophytes are essential for life on land. Bryophytes are recognized for their bioactive compounds, which are useful in medicine, but their potentials are yet to be maximized [2]. Bryophytes are plant types that are useful in traditional medicines practiced in various countries. Isolating and purifying these plant medicines is essential for enhancing their use in medicine [3]. We ought to conduct a complete pharmacognostic analysis to establish what these bioactive molecules are capable of Over 2,200 chemical compounds are believed to exist in bryophytes [4], but this figure is still on the increase. There are various phytocompounds present in bryophytes, but terpenoids (mono-, sesqui-, and diterpenoids), flavonoids, bis-bibenzyls (which are exclusive to liverworts), and various lipids are the principal ones [5]. There are three main groups of bryophytes: liverworts, mosses, and hornworts. The liverworts are unique compared to all other plants due to unique oil-containing cells and the presence of many bioactive chemical compounds like terpenoids, acetogenins, quinones, phenylpropanoids, and flavonoids [6]. Bryophytes have long been employed for ethnomedical purposes by various cultures worldwide, such as those from China, North America, and India. This highlights their significance to traditional medicine [7].

The *Riccia fluitans* is an amphibious liverwort and it is very good in aquatic and terrestrial habitats. It is an excellent model organism for analyzing transitions of plants from water to land [8]. The characteristics exhibited by *Riccia fluitans* include shape modification, which involves modifications in vegetative growth, air pore production, and production of rhizoids. It is now feasible to analyze adaptive molecular processes, facilitating aquatic plant transition to land [9]. The

organism is distributed on almost all continents and has been known to create aggregated and buoyant interwoven underwater vegetation that floats on ponds or sluggish streams. It also thrives as an underwater vegetation [10]. The *Riccia fluitans* is applicable in 'The Green Liver System' used for cleaning up contaminated water bodies in an ecologically friendly approach [11].

Burritt (2010) examined the role of polyamines (PAs) in protecting *Riccia fluitans* against oxidative stresses induced by the toxic chemical phenanthrene (PHEN). This helped to reveal *Riccia fluitans* as a potentially important source of antioxidants for use in food, medicines, and drugs [9]. Further, the ether extract of *Riccia fluitans* has been demonstrated to be capable of killing cancer cells in various cell lines [12]. Chojnacka's (2007) study examined the potential use of *Riccia fluitans* in the preparation of biological minerals, and how *Riccia fluitans*, being a liverwort, can be used in numerous ways [13]. The separated substances of *R. fluitans* display a broad spectrum of biological properties, including antibacterial, antifungic, anti-inflammatory, antioxidant, and cytotoxic properties. They possess varying biological properties, including fragrances, tastes, and some being allergenic, and thus could be used effectively as medicines. Among its several properties, it inhibitors DNA polymerase, cyclooxygenase, lipoxygenase, and various proteases, and also inhibits insects from feeding. LCMS and GC-MS are excellent, rapid, and precise techniques used for detecting numerous compounds, including alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters, and amino acids [14-17].

We want to learn more about the chemical makeup of *Riccia fluitans* through untargeted organic compound analysis using GC-MS and LC-MS. Many reviews of the literature shows that there aren't many scientific reports on the phytochemical makeup of *R. fluitans*. So, the goal of this study is to find and describe the chemical parts that make up the thallus of *R. fluitans* using advanced chromatographic methods.

2. Materials and methods

2.1. Plant Collection and Authentication

The plant specimens (Figure 1) were collected in April 2024 from the vicinity of Machutar, Satara District, Maharashtra, India (17.889265° N, 73.725714° E). The collected specimens were taxonomically identified as *Riccia fluitans* by Dr. Manju C. Nair, Associate Professor, Department of Botany, University of Calicut, Thenhipalam, Kerala, India. A voucher specimen of the authenticated plant material has been deposited in the Calicut University Herbarium (CALI), University of Calicut, Kerala, India, for future reference.

Figure 1: Collection site of *Riccia fluitans* at Machutar, Satara District, Maharashtra, India.

2.2. Extract Preparation

Carefully, the thallus of *Riccia fluitans* was harvested from its substrate and washed with distilled water to eliminate adhering soil, dust particles, and associated taxa. After cleaning, the plant material, including the rhizoid, was air-dried at ambient temperature, making sure of minimum exposure to sunlight to avoid degradation of bioactive aromatic compounds. The dried material was pulverized into a fine powder for subsequent extraction.

For the extraction technique, the powdered form of the plant was subjected to maceration with methanol using a mortar and pestle. The mixture was then agitated in an orbital shaker (Metrex-100C) at 37 °C and 150 rpm for a period of 48 hours. The collected supernatant was concentrated under reduced pressure using a rotary evaporator at 40 °C to remove methanol and refrigerated at 4 °C for further use. The obtained extract was analysed using LC-MS and GC-MS to determine the bioactive compounds with volatile properties in the plant extracts.

2.3. Chemicals and Reagents

All chemicals and reagents used in the present investigation were of analytical grade unless otherwise specified. Methanol (HPLC grade, ≥99.9% purity) used for extraction and chromatographic analysis was procured from Merck Life Science Pvt. Ltd., Mumbai, India. Chloroform, glacial acetic acid, concentrated hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), ferric chloride ($FeCl_3$), magnesium turnings, sodium hydroxide (NaOH), copper sulfate ($CuSO_4$), lead acetate, acetone, Fehling's solution A and B, and Hager's reagent were obtained from Loba Chemie Pvt. Ltd., Mumbai, India.

For GC–MS analysis, high-purity helium gas (99.999%) was used as the carrier gas. For LC–MS analysis, LC–MS grade acetonitrile, water (Milli-Q system, 18.2 MΩ·cm), and formic acid were used as mobile phase components.

2.4. Preliminary Phytochemical screening of the Crude Extract [18-21]

Alkaloids (Hager's test): Sample treated with Hager's reagent; a yellow precipitate shows the presence of alkaloids.
Flavonoids (Shinoda test): Extract mixed with magnesium turnings and conc. HCl; pink/red color indicates flavonoids.
Glycosides (Keller–Killiani test): Extract treated with glacial acetic acid, $FeCl_3$, and conc. H_2SO_4; brown ring indicates glycosides.
Phenols (Ferric chloride test): Extract treated with $FeCl_3$; blue/green color indicates phenols.
Proteins (Biuret test): Extract treated with NaOH & $CuSO_4$; violet color indicates proteins.
Reducing sugars (Fehling's test): Extract heated with Fehling's A & B; brick-red precipitate indicates reducing sugars.
Resins: Extract treated with acetone and water; turbidity indicates resins.
Saponins (Froth test): Extract shaken with water; stable froth indicates saponins.
Tannins (Lead acetate test): Sample treated with lead acetate; white precipitate indicates tannins.
Terpenoids (Salkowski test): Extract mixed with CCl_4 and conc. H_2SO_4; reddish-brown coloration indicates terpenoids.

2.5. Phytochemical Characterization of the Crude Extract

2.5.1. GC-MS Analysis

Performed analysis of the crude extract of *Riccia fluitans* using Agilent single quadrupole GC-MS (model 7890GC-5977B MS). The analysis includes: Column temperature at the initial 5 minutes is 40 °C, then increases to 120 °C at the rate of 3 °C/min and holds for two minutes. The next stage includes increase of the temperature up to 250 °C at the rate of 8 °C/min and holding for 10 minutes. Split ratio 20:1, the injector is held at 200 °C, and the injection volume is 1 µL. The experiment duration is one hour, the carrier gas is helium which flow rate is 1 mL/min, using electron ionization (EI) at 70 eV to produce mass spectra from m/z 10 to 300. Comparison of obtained mass spectra with NIST library and correlation of the GC retention times to the standards made it possible to determine the chromatographic profile of the sample.

2.5.2. Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

The LC-MS analysis of the plant extract was carried out on an Agilent 1260 Infinity II HPLC system combined with an Agilent Q-ToF G6540B mass spectrometer. Separation occurred on an Agilent Eclipse XDB-C18 column (3 × 150 mm, 3.5 µm) maintained at 40 °C. Mobile Phase A consisted of 0.1% formic acid in water, and Mobile Phase B consisted of 0.1% formic acid in acetonitrile, both flowing at a flow rate of 0.3 mL/min. Gradient elution was initiated with 95% A and 5% B for two minutes. This was then ramped linearly from 5% A and 95% B over 25 minutes, held for 3 min, and returned to initial conditions at 28.1 min, which was held until 30 min. Mass spectrometry was performed in a mass range from m/z 100 to 1700, with a positive and negative ion mode. The ion source settings were optimized as follows: nebulizer gas temperature was 300 °C, the sheath gas temperature was 350 °C, the drying gas flow was 8 L/ min, nebulizer pressure was 35 psig, sheath gas flow was 11 L/min, capillary voltage was 3500 V, and nozzle voltage was 1000 V.

3. Results

3.1. Extract Yield

From 10 g of dried powdered thallus of *Riccia fluitans*, 0.350 g of crude methanolic extract was obtained after solvent removal using a rotary evaporator. The percentage yield of the extract was calculated as 3.5%. This yield reflects the extraction efficiency of methanol in recovering polar and moderately polar phytoconstituents from the plant material.

3.2. Preliminary Phytochemical analysis:

Table 1. Phytochemical profile of *R. fluitans* using Methanol extract.

Phytochemical constituents	Result (+/–)
Alkaloids	+
Flavonoids	+
Glycosides	-
Phenols	+
Protein	+
Reducing sugar	-
Resins	-
Saponins	-
Tannin	+
Terpenoids	-

Preliminary phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, phenols, proteins and tannins while glycosides, reducing sugars, resins, saponins and terpenoids were absent (Table 1). Phenolic compounds and flavonoids indicate antioxidant and anti-inflammatory activities, while tannins indicate antimicrobial and astringent activity. These findings provide an initial indication of the biochemical characteristics of the extract, which can form the basis for more pharmacological investigations.

3.2 Phytochemical Characterization Using GC–MS

Phytochemical compounds identification and measurements were influenced by the following parameters: peak size, compound separation time,

weight, and chemical formula. GC-MS (Gas Chromatography-Mass Spectrometry) is an advanced method of analysis that brings together the capabilities of both gas chromatography and mass spectrometry in one process.

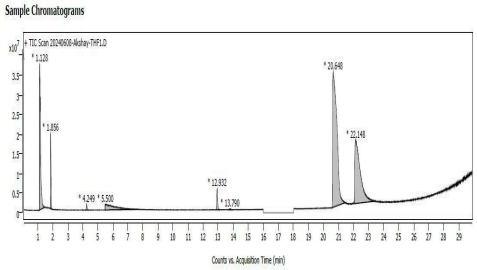

Figure 2, Illustrates the chromatogram of the methanolic sample of *Riccia fluitans* obtained using GC–MS.

Table 2. Identification of phytocomponents of methanolic extract *Riccia fluitans* using GC-MS analysis.

Sr. No.	Area %	RT	Compound name	Molecular formula	Molecular weight g/mol.
1	7.58	1.856	(2-Aziridinylethyl) amine	$C_4H_{10}N_2$	86.14
2	0.74	4.249	Ethylene	C_2H_4	28.05
3	3.38	12.932	Cyclopropane	C_3H_6	42.08
4	0.25	13.790	Nickel tetracarbonyl	$Ni(CO)_4$	170.73
5	14.12	20.580	Furan	C_4H_4O	68.07
6	61.77	22.148	Isopropyl Alcohol	C_3H_8O	60.01

3.3 Phytochemical Characterization Using LC–MS

The LC–MS analysis of the methanolic extract of *Riccia fluitans* showed that the species possesses an array of phytocomponents, some of which are of biological significance.

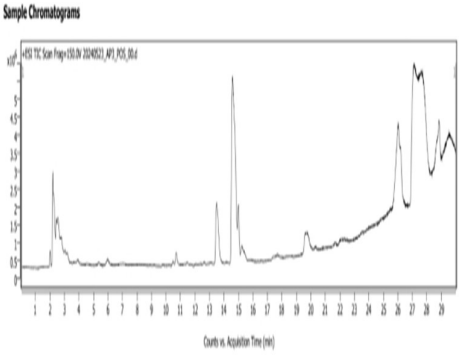

Figure 3, Depicts the LC–MS chromatogram of the methanolic sample of *Riccia fluitans*.

Table 3. LC-MS spectra of the methanolic extract of *Riccia fluitans*, depicting the identified phytochemicals and their respective ionization profiles. Presents the identified phytocomponents in the methanolic extract of selected plant through LC–MS analysis.

Sr. No.	RT (min)	Compound name	Molecular Formula	Molecular Weight g/mol
1	2.015	N-Acetylserine	$C_5H_9NO_4$	2.015
2	2.217	Deazaflavin	$C_{11}H_7N_3O_2$	213.0531
3	2.271	4-Methoxybenzyl O-(2-sulfoglucoside)	$C_{14}H_{20}O_{10}S$	380.0759
4	2.330	Ismine	$C_{15}H_{15}NO_3$	257.1049
5	2.360	4-Amino-2-methylenebutanoic acid	$C_5H_9NO_2$	115.0639
6	2.380	Isoamyl nitrite	$C_5H_{11}NO_2$	117.0795
7	2.493	Squamolone	$C_5H_8N_2O_2$	128.0590
8	2.634	Triethylamine	$C_6H_{15}N$	101.1208
9	2.910	Brugine	$C_{12}H_{19}NO_2S_2$	273.0869
10	2.979	Ectocarpen	$C_{11}H_{16}$	148.1249
11	3.203	Dinex	$C_{12}H_{14}N_2O_5$	266.0902
12	3.255	Fenothiocarb	$C_{13}H_{19}NO_2S$	253.1144
13	3.918	1-(5-Acetyl-2-hydroxyphenyl)	$C_{13}H_{16}O_3$	220.1099

		-3- methyl-1-butanone		
14	6.053	Eremopetasidione	$C_{14}H_{20}O_3$	236.1418
15	7.470	Pymetrozine	$C_{10}H_{11}N_5O$	217.0969
16	11.082	Lysyl-Phenylalanine	$C_{15}H_{23}N_3O_3$	293.1739
17	11.470	Curcumin II	$C_{22}H_{22}O_5$	366.1462
18	11.615	Phenylalanyl-Tryptophan	$C_{20}H_{21}N_3O_3$	351.1577
19	12.064	HC Toxin	$C_{21}H_{32}N_4O_6$	436.2324
20	12.376	Citronellyl betasophoroside	$C_{22}H_{40}O_{11}$	480.2590
21	12.635	6-Benzylaminopurine	$C_{12}H_{11}N_5$	225.1021
22	12.759	Lignans	$C_{22}H_{22}O_8$	414.1329
23	13.014	19-Methoxypomolic acid 3-arabinoside	$C_{36}H_{58}O_8$	618.4134
24	13.126	Scutigeral	$C_{23}H_{32}O_4$	372.2293
25	13.823	Goldinodox	$C_{44}H_{62}N_2O_{12}$	810.4273
26	14.144	3,4,5-Trimethoxycinnamic acid	$C_{12}H_{14}O_5$	238.0837
27	14.146	Metabutethamine	$C_{13}H_{20}N_2O_2$	236.1518
28	14.183	3'-N-Acetyl-4'-O-(9-octadecenoyl)fusarochromanone	$C_{35}H_{54}N_2O_6$	598.3993
29	15.277	Thermozeaxanthin-13	$C_{59}H_{90}O_8$	926.6634
30	15.523	1-Hydroxyacorenone	$C_{15}H_{22}O_3$	250.1563
31	16.311	Caulophylline	$C_{12}H_{16}N_2O$	204.1254
32	16.377	Coriandrone A	$C_{16}H_{20}O_5$	292.1310
33	16.540	7-Hydroxyisoflavone	$C_{15}H_{10}O_3$	238.0636
34	16.795	Pteroside B	$C_{20}H_{28}O_7$	380.1850
35	17.571	Helinorbisabone	$C_{14}H_{18}O_4$	250.1200
36	17.831	Picein	$C_{14}H_{18}O_7$	298.1053
37	19.669	Metoprolol	$C_{15}H_{25}NO_3$	267.1833
38	19.767	Urate-3-ribonucleoside	$C_{10}H_{12}N_4O_7$	300.0704
39	20.041	8-Methyldihydrochelerythrine	$C_{22}H_{21}NO_4$	363.1474
40	20.121	(22E, 24x)-Ergosta4,6,8,22-tetraen-3-one	$C_{28}H_{40}O$	392.3078
41	20.336	Kanzonol O	$C_{22}H_{22}O_6$	398.1166
42	21.133	Suberenone	$C_{14}H_{12}O_4$	244.0733
43	21.477	Picrasin C	$C_{23}H_{34}O_7$	422.2313
44	21.945	Picrasin F	$C_{22}H_{30}O_8$	422.1960
45	22.312	Quizalofop-P-tefuryl	$C_{22}H_{21}ClN_2O_5$	428.1144
46	22.523	Terbucarb	$C_{17}H_{27}NO_2$	277.2040
47	22.599	Neoarctin B	$C_{42}H_{46}O_{12}$	742.3001
48	23.388	Capsiate	$C_{18}H_{26}O_4$	306.1830
49	24.793	Corchorosol A	$C_{29}H_{44}O_9$	536.3009
50	25.989	Alangimarckine	$C_{29}H_{37}N_3O_3$	475.2849
51	26.060	Methicillin	$C_{17}H_{20}N_2O_6S$	380.1057
52	26.724	Brevianamide B	$C_{21}H_{23}N_3O_3$	365.1733
53	26.811	Methandriol dipropionate	$C_{26}H_{40}O_4$	416.2936
54	28.736	Fungichromin	$C_{35}H_{58}O_{12}$	670.3931
55	29.515	Tridihexethyl	$C_{21}H_{36}NO$	318.2797
56	29.518	7,8-Dihydrovomifoliol 9-[rhamnosyl-(1->6)-glucoside]	$C_{25}H_{42}O_{12}$	534.2689

In this research, bioactive compounds in *Riccia fluitans* were successfully analyzed through the application of GC-MS and LC-MS methods. The research uncovered many phytochemicals which may be useful for medical purposes and thus provides more insight on the therapeutic importance of *Riccia fluitans*. This is a good base for future pharmacological research.

4. Discussion

From the first phytochemical testing of the methanol extract, it was found to have flavonoids, phenolic compounds, proteins, and tannins as the only compounds in it whereas there were no traces of alkaloids, glycosides, reducing sugars, resins, saponins, and terpenoids in it [22]. From the

methanol extract, there is a high percentage of flavonoids and phenolic compounds, which clearly shows its potential to act as an environment friendly natural source of antioxidant materials that help in protecting free radicals while controlling inflammation in the body [23]. The environmental health condition takes importance from this aspect in the sense that free radicals and pollutants cause inflammation in the body [24]. Plant-based antioxidants can be used as an environmentally sustainable substitute for chemical synthetic compounds in nature.

The extract contains tannins which scientists recognize for their antimicrobial and astringent characteristics that show potential to create environmentally friendly antimicrobial solutions. The use of natural tannin-based products which contain actual tannins instead of artificial antimicrobials will decrease the amount of enduring chemical contaminants which enter soil and water systems [25]. The tannins play a dual role as active ingredients in products that are developed for the purpose of environmental protection and agricultural advancement [26]. The value of the extract is enhanced by the detection of protein, which confirms its nutritional value and functional benefits, thus making the presence of renewable natural sources evident to yield different bioresources. In summary, the phytochemical profile of the extract makes it applicable in the development of medicine and nutraceuticals that are environmentally sustainable. The absence of any toxic or environmentally resistant phytochemical group makes it even more suitable for development of biodegradable plant formulations.

The GC-MS study of *Riccia fluitans* was found to contain a variety of bioactive compounds. These compounds have been identified based upon their retention time, weight, and relative peak area values in a chromatographic diagram (Table 2). The compounds tend to show varied properties related to different biological activities such as their capability to act as antiviral, antimicrobial, antifungal, insecticidal, anti-inflammatory, analgesic, anticancer, and stress-regulatory agents. Some compounds affect cell growth and response to stress, while other compounds tend to show a vast number of properties related to human health. Some compounds tend to show properties related to their toxicity rather than their beneficial uses with respect to human welfare [27-32].

Analysis using the LC-MS showed in (Table 3), that there were varying bioactive compounds with antimicrobial, antioxidant, anti-inflammatory, anticancer, and metabolic regulation functions. Curcumin II, deazaflavin [33], ismine, and squamolone are essential for combating cancer [34], while methicillin, lysyl-phenylalanine, and picrasin C are essential for combating bacteria. Thermozeaxanthin-13, picein, and kanzonol O are believed to combat inflammation and free radicals [35]. Various biological functions presented by the extract reveal its prospective use in several therapeutic areas. In past researches, the focus was primarily on the detection of specific key compounds within liverworts, namely sesquiterpenes, lipids, and aromatic compounds, using only one research technique for the discovery of bioactive compounds, typically using techniques like the GC-MS technique for analysis [36]. In our research, however, many more compounds were discovered, and this was primarily made possible by the simultaneous use of the GC-MS and LC-MS techniques. This made the discovery of many new compounds with much prospective use in medications easier, including curcumin II, lignans, and furan compounds [37]. Numerous studies have demonstrated the pharmacological potential of liverworts due to their diverse bioactive metabolites. GC–MS analyses of *Plagiochasma intermedium* [38] and *Asterella wallichiana* [38] identified several compounds with pharmaceutical significance. Similarly, *Asterella* species have been reported to exhibit antibacterial, antifungal, antioxidant, anticancer, α-amylase inhibitory, and α-glucosidase inhibitory activities [39].

The originality of this scientific research is embedded in combining both GC-MS and LC-MS techniques in identifying a vast range of phytochemicals. The application of this technique will significantly impart a better understanding of the nature of phytochemistry found in *Riccia fluitans*. It is a fact that this organism holds immense value in acting as a source of bioactive compounds with potential uses in medicine.

5. Conclusion:

Phytochemical analysis of the extract revealed that it has an array of chemical components, such as flavonoids, phenols, tannins, and proteins. All these components are characterized by their antioxidant, anti-microbial, and anti-inflammatory qualities, among others. GC–MS and LC–MS/MS analyses have helped to reveal the presence of various bioactive substances, which are known to be active from the point of view of pharmacology. Antimicrobial, antifungal, antioxidant, and anticancer properties have been found among the listed substances. It means that the combination of various bioactive substances in the extract might help the latter increase its effectiveness.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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