



Qualitative phytochemical analysis and GC-MS analysis of free floating aquatic fern *Salvinia molesta*

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Abstract

The present investigation was carried out to find out the Phytochemical constituents of the plant *salvinia molesta* in five different solvents. Phytochemical tests disclosed the presence of Tannins, Saponins, Flavanoids, Carbohydrate, Sterols, Alkaloids, Coumarin, Anthocyanin, Phenol, Cardiac glycosides, Proteins, Quinones, Amino acid, Fats and oil. The flavanoids, alkaloids, tannins, steroids and saponins are prime pharmaceutical parameters that are accountable for ethnomedicinal values were present in different proportions. The GC-MS analysis of *salvinia molesta* leaves disclosed the presence of 103 Phytochemical compounds. The first molecular compound identified with minimum R.T. was 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one with 9.579 min and last molecular compound identified with maximum R.T. was Vitamin E with 51.1 min.

Based on the results this study also exhibit that this obtrusive aquatic weed which is environmentally, sparsely and anthropologically destructive in the world could also have the ability to make drugs to work out many health related problems.

Keywords: GC-MS, Plant extracts, phytochemical screening, *Salvinia molesta*, Water fern

Introduction

Medicinal plants have been used from the time immemorial by the human beings due to their restorative potential values and have become foremost constituents of health maintenance system. The latest ethnobotanical, pharmaceutical, and biological exploration discloses the therapeutic and phytochemical property of pteridophytes which have beneficial potential utilization for health and industry sector. However many species of pteridophytes are still to be discovered for their therapeutic ability for future use and to recognize new active principles from them.

The ferns play an important role in traditional medicine. In the year 1935 Kirthikar have reported 27 species of ferns having numerous medicinal uses^[1]. Nadkarni in the year 1954 have reported 11 species and Chopra have reported 44 species of ferns having many medicinal uses^[2, 3]. Nayar in the year 1959 have reported 29 medicinal ferns^[4]. Singh in the year 1999 have reported 160 species of beneficial pteridophytes in India based on their phytochemical, pharmaceutical, and ethnobotanical studies in a recent collection^[5].

Salvinia molesta is a free floating aquatic fern belonging to the family salvinaceae, is one of the world's largest aquatic weed whose exponential growth had catastrophic socio-economic impacts in some parts of Asia, Brazil, Philippines, Sri Lanka, Australia, Africa. *Salvinia* which is also known as Giant *salvinia*, African Pyle, butterfly weed, kariba weed and aquarium water moss is a fresh water free floating aquatic fern^[6]. Under appropriate conditions this free floating aquatic fern can become a destructive aquatic annoyance disrupting indigenous species as well as mankind activities by swiftly covering immense areas^[7]. Thick mats of giant *salvinia* impede with the cultivation of rice, fishing nets and agitate entry of water for human beings, wildlife and animal husbandry^[8], restoration, irrigation, transportation, generation of hydroelectricity and controlling of flood are also obstructed^[9].

Till now no folkloric medicinal use of *salvinia molesta* has been described, but it has an ability to used it as a fodder for ruminants and for making paper alone or in combination with rice-straw or making low grade papers such as newspaper, tissue paper, craft paper which are used for packing^[10].

Materials and Methods

1. Sample collection- Fresh and healthy leaves of *salvinia molesta* was collected from the pond of B.A.U Kanke Road, Ranchi.
2. Solvents used - The plant extract were prepared by using six different organic solvents. The solvents are Acetone, Benzene, Methanol, Petroleum ether, Distilled water.
3. Preparation of Plant Extract- Fresh leaves of *salvinia molesta* was collected .The leaves were washed with running tap water 2-3 times to remove all the dust particles and impurities. After washing it with running tap water wash it with distilled water also. The leaves sample were dried at normal room temperature for 3weeks until and unless it is completely dried. The dried leaves sample was grinded through mortar and pestle to make a powdered form.

The grind sample was individually mixed with five different solvent which are in the ratio of 1:10. After mixing the plant sample in different solvent shake the solution and keep the solution in dark for 48-72hrs. After 48hrs the sample was filtered through whatman filter paper in a dried and clean beaker. The filtrate which was obtained was kept in a room temperature for complete dry. The weight of the beaker having filtrate and the weight of the empty beaker was measured to calculate the difference.



Fig 1: Leaves of *Salvinia molesta*



Fig 2: Drying of leaves

Qualitative Phytochemical Analysis

Following standard methods⁽¹¹⁻¹³⁾ were used for the screening of phytochemical compounds of leaf extract of *salvinia molesta*. Five different solvents were used for phytochemical screenings which were Acetone, Benzene, Methanol, Petroleum ether, and Distilled water to identify the secondary metabolites groups such as Carbohydrate, sterols, alkaloids, coumarin, anthocyanin, quinones, flavanoids, tannins, phenol, cardiac glycosides, proteins, saponins, amino acids,

fats and oil.

1. Test for carbohydrate

- a) Molish Test: - 1ml of plant extract, 1ml of Molish reagent and 1ml of conc.H₂SO₄ were added. Brick reddish colour indicates the presence of carbohydrate.
- b) Benedict's Test:- 1ml of plant extract was added to 1ml of Benedict's reagent, and the mixture was heated in a boiling water bath for 2 min. Red colour precipitate indicates the presence of sugar.

2. Test for Sterols

- a) Salkowski Test: - 1 ml of plant extract, 2ml of chloroform was mixed and 3ml of conc. H₂SO₄ was added carefully into it. Presence of reddish brown indicates the presence of sterols.

3. Test for Alkaloids

- a) Dragendroff's Test: 1 ml of plant extract was taken and add 1ml of HCL into it. Then add Dragendroff's reagent to this acidic medium. Orange or red precipitate indicates the presence of alkaloids.
- b) Mayer's Test: A few ml of plant extract was taken and add few drops of Mayer's reagent into it by the side of the test tube. A cream or white precipitate indicates the presence of alkaloids.
- c) Wagner's Test: Plant extract was taken and add wagner's reagent into it. Reddish brown precipitate indicates the presence of alkaloids.

4. Test for coumarin

- a) Sodium Hydroxide Test: 1 ml of plant extract and 1ml of 10% sodium hydroxide was added into it. Yellow colour indicates the presence of coumarin.

5. Test for Anthocyanin

- a) Sulphuric acid Test: In 1 ml of plant extract add 1ml of conc H₂SO₄ into it. Yellowish orange colour indicates the presence of anthocyanin.

6. Test for Quinones

- a) Hydrochloric acid Test: In 1ml of plant extract add 1ml of conc. HCL. Yellow colour indicates the presence of quinones.

7. Test for Flavanoids

- a) Ammonia Test: In 1ml of plant extract, 2ml of 10% ammonia solution and 1ml of conc. H₂SO₄ were added. Yellow colour indicates the presence of flavanoids.
- b) Alkaline Reagent Test: In 1 ml of plant extract add 1ml of 10% NaOH. Yellow fluorescence colour indicates the presence of flavanoids.
- c) Shinoda's Test: In 1ml of plant extract, a small piece of magnesium ribbon was added after that 3-4 drops of conc. HCl were added. Change in the colour of red into pink indicates the presence of flavanoids.

8. Test for Tannins

- a) Fecl₃ Test: A small quantity of plant extract was mixed with water and heated it in water bath for 10min. Then the mixture was filtered and ferric chloride was added to the filtrate. Dark green colour indicates the presence of tannins.
- b) Lead Acetate Test: 1 ml of plant extract add lead acetate solution into it. Appearance of white precipitates confirms the

presence of tannins.

9. Test for Phenol

a) Potassium Dichromate Test: - In 1ml of plant extract add 1ml of 10% of potassium dichromate. Red colour indicates the presence of phenol.

b) Ellagic Test (FeCl₃ Test):- In 1ml of plant extract add 1ml, of 5% ferric chloride into it. Dark green/ reddish brown/ violet/ purple/ blue indicate the presence of phenol.

10. Test for Cardiac Glycosides

a) Keller- killiani Test: In 1ml of plant extract, add 1ml of glacial acetic acid, after that add few drops of 5% FeCl₃ and Conc. H₂SO₄ into it. Reddish brown/ blue green colour indicates the presence of Glycosides.

b) Glycoside Test: In 1ml of plant extract, 2ml of chloroform and 1ml of 10% ammonia solution were added. Pink colour indicates the presence of glycosides.

c) Legal Test: In 1 ml of plant extract add 1ml of pyridine and 1ml of sodium nitroprusside into it. Colour changes from pink to red indicate the presence of glycosides.

11. Test for Proteins

a) Biuret Test: In 1ml of plant extract add 1ml of 40% of sodium hydroxide into it after that add few drops of copper sulphate solution slowly into the sided of the test tube. Violet/ pink colour indicates the presence of proteins.

12. Test for Saponin

a) Foam Test: In 1ml of plant extract add 1ml of distilled water into it and shake it vigorously. Formation of foam indicates the presence of saponin.

13. Test for Amino Acids

a) Xanthoprotein Test: In 1ml of plant extract add 1ml of conc. HNO₃(white precipitate is formed), then it is heated for 2-3 minutes and then cooled it down. After cooling add 1ml of 20% NaOH into it. Orange colour indicates the presence of Amino acids.

b) Ninhydrin Test: 1 ml of plant extract was taken and add 3 drops of 5% of ninhydrin solution into it and heated it in a boiling water bath for 10 minutes. Purple colour or bluish colour indicates the presence of Amino acids.

Table 1: Phytochemical analysis of *salvinia molesta* in five different solvent

Serial no.	Phytochemical Test	Plant extract					Colour indication
		Ace	Ben	Met	P.E.	D.W.	
1	Carbohydrate						
a.	Molish Test	++	+	+++	+++	+	Brick Red
b.	Benedict's Test	++	+	-	+	-	Red/ Brown
2	Sterols						
a.	Salkowski Test	+	-	+	+++	+	Brownish Red
3	Alkaloids						
a.	Dragendorff's Test	-	+	+	-	-	Reddish Brown
b.	Mayer's Test	++	+++	++	-	-	Creamy/ white ppt.
c.	Wagner's Test	++	+	+	-	+	Yellow/ Brown ppt.
4	Coumarin						
a.	NaOH Test	+	+	-	+	+	Yellow
5	Anthocyanin						
a.	H ₂ SO ₄ Test	-	-	-	-	-	Yellowish orange
6	Quinones						
a.	Hcl Test	++	-	+	+	+	Yellow/ Red
7	Flavanoids						
a.	Ammonia Test	++	-	-	+	+	Yellow
b.	Alkaline Reagent Test	+	+	-	+	++	Yellow fluorescent
c.	Shinoda Test	+++	++	-	+	+	Crimson Red/ Pink
8	Tannins						
a.	FeCl ₃ Test	+	+++	++	+	-	Brownish green/ Blue black
b.	Lead acetate Test	++	++	+++	+	+	White ppt.
9	Phenol						
a.	Potassium Dichromate	-	+++	-	+	+++	Dark colour
b.	Ellagic Test	++	+++	++	+	++	Dark green/ Reddish brown/ Blue
10	Cardiac Glycosides						
a.	Keller killiani Test	+	+	-	+	+	Brown/Reddish brown/ Blue green
b.	Glycoside Test	+	-	-	+	-	Pink
c.	Legal Test	-	+	-	-	+	Pink/Red
11	Proteins						
a.	Biuret Test	-	+	-	-	+	Violet/ Pink
12	Saponin						
a.	Foam Test	-	+++	++	-	++	
13	Amino Acid						
a.	Xanthoprotein Test	+	-	+	-	+	Orange/ Reddish orange
b.	Ninhydrin Test	+	-	+	+	-	Purple / Bluish
14	Fats and oil						
a.	Solubility Test	+++	-	-	+	-	

(+) Present (-) Absent.

Ace = Acetone, Ben = Benzene, Met = Methanol, P.E. = Petroleum ether, D.W. = Distilled water.



Fig 3

GC-MS Analysis of *Salvinia molesta*Table 2: Major components present in Methanol extract of *salvinia molesta* and their biological activity

Peak	Retention Time	Area%	Height%	A/H	Compound Name	Biological Activity
1.	9.57	0.11	0.22	2.1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	Antioxidant
2.	15.25	0.11	0.13	3.68	Benzoic acid	Antimicrobial, food preservative
3.	17.62	0.19	0.20	3.88	Benzeneacetic acid	Hyperammonaemia
4.	21.14	0.06	0.04	5.65	Pipradrol	Antidepressant, obesity, attention deficit, hyperactivity disorder and narcolepsy
5.	27.79	0.14	0.20	2.95	Dodecanoic acid	Antimicrobial, treatment of bronchitis, gonorrhoea
6.	31.658	1.01	1.54	2.70	Tetradecanoic acid	Antitumor activity, anti-asthmatics and throat disorders
7.	33.089	1.98	4.61	1.77	Neophytadiene	Anti-inflammatory, Antioxidant, cardio protective properties
8.	34.20	0.37	0.83	1.85	Hexadecanoic acid, methyl ester	Antioxidant, Antibacterial
9.	34.33	0.17	0.38	1.84	n-Hexadecanoic acid	Antibacterial, Antifungal
10.	34.38	0.18	0.26	2.82	Oleic Acid	Antioxidant, preventing heart disease
11.	36.95	1.80	2.22	3.34	Phytol	Antimicrobial, allergic disorders
12.	37.34	1.07	1.17	3.78	9,12-Octadecadienoic acid (Z,Z)-	Anti-inflammatory, Antitumor activity, joint disorders, wound healing
13.	37.97	4.82	4.27	4.65	Octadecanoic acid	Antimicrobial
14.	38.45	0.11	0.14	3.12	1-Nonadecene	Antifungal
15.	42.70	0.25	0.64	1.61	Palmitoyl chloride	Antioxidant,
16.	42.77	0.10	0.17	2.36	2-Ethylbutyric acid, eicosyl ester	Antioxidant,
17.	42.93	5.70	11.22	2.09	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antioxidant, Hemolytic, Pesticide
18.	44.65	0.39	0.49	3.29	Octadecanoic acid, 2,3-dihydroxypropyl ester	Anticancerous, Antimicrobial
19.	50.98	0.52	0.46	4.61	Cholesterol	Antimicrobial, regulating cell function
20.	51.15	1.48	1.32	4.62	Vitamin E	Antioxidant

12. Test for Fats and oils

a) Solubility Test: Plant extract sample was dissolved in ether, ethanol, chloroform and checked it for solubility.

GC-MS Analysis

The GC-MS analysis of *salvinia molesta* leaves disclosed the presence of 103 Phytochemical compounds. The recognition of these Phytochemical compounds was confirmed by on the basis of their Retention time (R.T.), Compound name (C.N.), Area %, Height%, Area/Height (A/H).

The first molecular compound identified with minimum R.T. was 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one with 9.579 min and last molecular compound identified with maximum R.T. was Vitamin E with 51.1min.

Results and Discussion

Preliminary phytochemical studies

The phytochemical analysis of the five different solvent extracts was tested for 14 phytochemicals. The process of phytochemical analysis was carried out by using standard protocols. Phytochemical screening of *salvinia molesta* leaf extracts of different solvents is Acetone, Benzene, Methanol, Petroleum ether and Distilled water confirmed the presence of important bioactive components with different qualitative range as high concentration, medium concentration, and low concentration and absent.

Carbohydrate, Tannins, Phenol are present in all five solvent extracts. Coumarin, Flavanoids, Cardiac glycosides were present in four solvent extracts except methanol. Sterols and Quinines were present in all the four solvent extract except benzene. Anthocyanin were absent in all the five different solvent extracts. Alkaloids were present in four solvent extracts except petroleum ether. Proteins were present in two solvent extracts except acetone, methanol, and petroleum ether. Saponins were present in three solvent extracts except acetone, petroleum ether. Amino acids were present in four solvent extracts except benzene. Fats and oils were present in two solvent extracts except benzene, methanol and distilled water.

The GC-MS analysis of *salvinia molesta* leaves disclose the presence of 20 important bioactive compounds that provide the medicinal quality of the plant. The recognition of these bioactive compounds was confirmed based on the peak area, retention time, Area%, Height%, A/H, compound name. The first molecular compound identified with minimum R.T. was 2, 4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one with 9.579 min and last molecular compound identified with maximum R.T. was Vitamin E with 51.1 min.

Conclusion

Plants are mainly rich source of secondary metabolites. These secondary metabolites have diversity of structural arrangements and properties^[14]. Information available about green plants illustrate a source of effective chemotherapeutants, as these are more systemic, non-toxic and easily biodegradable^[15]. For this reason scientists all over the world have started screening plants for searching new antimicrobial agents. Flavanoids are water soluble antioxidants and free radical scavengers, which prevents cell damage and have strong anticancer activity^[16]. Tannins are also called as antimicrobial agents^[17]. The growth of many bacteria, fungi, viruses, yeasts were hinder by these tannins. Tannins have astringent and wound healing property in them. Saponins have the property of precipitating and coagulating

RBCs.

Although *salvinia molesta* is considered as an aquatic weed but this present study validates the phytochemical analysis so it opens up a new pathway to assess its medicinal value, of their pharmacological drugs. Thus, the present study showed that the *salvinia molesta* a fresh water aquatic weed, which is considered as an aquatic threaten to many countries can be effectively used as a curative agent for many infectious diseases at a low cost and in a safer manner.

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Conflict of interest statement

The authors report no conflict of interest.

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