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Fluorescent study, preliminary phytochemistry and HPTLC profiling of *Salvinia molesta* D. S. mitchell

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Abstract
Salvinia molesta D. S. Mitchell is one of the most invasive alien aquatic plant species commonly known as giant salvinia or Kariba weed. The present study is focused on fluorescent study, preliminary phytochemistry, and HPTLC profiling of ethanolic leaf extract of *S. molesta*, so that some possible out-way could be drawn to use this weed for the benefit of mankind. In the fluorescent pharmacognostic study, the leaf extract showed distinct colors with routine chemicals and reagents of the laboratory under normal sunlight and under UV light; it could be used as a marker test for powder to identify adulteration. Further, the preliminary phytochemistry revealed that the plant is rich in major phytochemicals. The HPTLC profile showed that the ethanolic extract showed the presence of some phenolic compounds like ferulic acid, vanillic acid, caffeic acid and flavonoids which are possible compounds that provide specific bioactivities to the plant and the plant could be further explored for related drug formulation.

Keywords: Fluorescence study, phytochemistry, HPTLC, *Salvinia molesta*

Introduction

The plant under study, *Salvinia molesta* D. S. Mitchell, has been identified as a troublesome invasive aquatic plant and known as the world's most invasive alien species in the Global Invasive Species Database since 2013. *Salvinia molesta*, commonly known as giant *Salvinia* or Kariba weed, is a floating fern belonging to the family of Salviniaceae. It was native of Southern Africa and spread across the globe through various types of introduction patterns and established itself as an alien invasive aquatic plant species.

Some biologists did a comprehensive study to explore the phytochemical composition, antioxidant potential, and antibacterial properties of *S. molesta*. Salleh *et al*., (2023) [14] reported that the plant is rich in phenolics, flavonoids, tannins, alkaloids, and saponins. Earlier similar report was given by Gaya *et al*., (2016) [5] and Al-Maliki *et al*., (2017). In their study, Gini and Jothi, 2018 [6], isolated the active fraction of the leaf extracts of *S. molesta* using column chromatography, showed the presence of phenolic compounds, and also demonstrated that the plant possesses significant pharmacological activities (Gini and Jothi, 2018) [6].

Though *S. molesta* is a troublesome invasive plant, the ability of *Salvinia* species to bioaccumulate certain metals makes it potentially useful for waste management and effluent treatment. The phytochemical investigation of its allied species, the *S. natans* showed that it consists of 96% of amino compounds, such as γ-amino butyric acid, asparginine, and glutamine (Lahdesmaki, 1968) [11]. Few scientists have explored the utility of *S. molesta* in phytoremediation and wastewater treatment (Israa *et al*., 2011 and Hauwa *et al*., 2021) [9, 8]. An understanding of the phytochemistry of *Salvinia* plants can help control their invasive growth, and promote their utilization for useful purposes. Therefore, the present study was planned to investigate the preliminary phytochemistry and HPTLC profile of *S. molesta* to understand which bioactive chemicals are present in this plant.

Material and Methods

The plant *Salvinia molesta* D. S. Mitchell was collected from the small perennial lakes of Akola regions (MS) India in December 2022. The plant was taxonomically identified using the flora of Maharashtra State (Karthikeyan and Singh, 2000). Further, the plant specimen was submitted to the herbarium of the Department of Botany, Shri Dr. R. G. Rathod Arts and Science College, Murtizapur District-Akola (MS). Later, the leaves were shade-dried and made into powder. The powdered material was processed further for fluorescent analysis, preliminary phytochemical analysis and HPTLC profiling. The fluorescent analysis was done using the protocol of (Gandhi *et al*., 2022) [4], the preliminary phytochemistry was done using the standard protocols (Harborne, 1998 and Jasutkar *et al*., 2018) [7, 10]. The powdered material was sent to the Qualichem Laboratory Nagpur (MS) for HPTLC profiling.

Results and Discussion

The plant *Salvinia molesta* D. S. Mitchell was selected for the study. The plant material is preserved for the pharmacognostic study of the leaf. The powdered sample of plant material was subjected for the preliminary phytochemical analysis using three solvents, distilled water, ethanol, and chloroform. Also, the powder sample is use for the HPTLC test. The various observations done and results obtained are as follows.

Fluorescent analysis of leaf powder of *S. molesta*

Pharmacognostic and fluorescent analysis was done to authenticate the crude powder. This was done using routine laboratory chemical reactions with powdered material and its appearance under normal sunlight and UV- light. In the present investigation, the powder as such appeared light green under normal sunlight, while under UV- light is showed dark green coloration. With concentrated HCL, it appeared light green but after exposing UV light, it turned light yellow. With 50% HCL, the powder appeared colorless in normal sunlight, while showing a light yellow color under

UV. When mixed with H_2SO_4, the powder gives brown coloration but when observed under UV light, the color changes to blackish brown. But with 50% H2SO4, it turns dull green in normal sunlight and when observed under UV-light, it showed a light brown color. In the case of NaOH solution, when mixed with powder, it appeared light yellow under normal sunlight but the same in UV -light was colorless. With 5% ferric chloride, the mixture appeared pale yellow and turned light yellow under UV. With nitric acid, the powder showed a light yellow color under sunlight but the color changed to blackish green when observed under UV- light (Table 1).

Table 1: Fluorescent analysis of leave powder of *S. molesta*

Sr. No.	Test (Powder)	Sunlight	UV-light
1	Powder such as	Light green	Dark green
2	Powder + HCL	Light green	Light yellow
3	Powder + 50% HCL	Colourless	Light yellow
4	Powder + H2SO4	Brown	Blackish brown
5	Powder + 50% H2SO4	Dull green	Light brown
6	Powder + NaOH solution	Light yellow	Colourless
7	Powder + Ferric Chloride5%	Pale yellow	Light yellow
8	Powder + Nitric acid 50%	Light yellow	Blackish green

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of *Salvinia molesta* leaves is presented in Table 2. The qualitative tests were carried out for 09 major phytochemicals including alkaloids, phenolics, flavonoids, steroids, quinones, glycosides, saponins, and tannins. It was noted that most phytoconstituents are present in chloroform extracts. The extract of distilled water showed the presence of phenolics and tannins. The qualitative tests of ethanolic extract for terpenoids, phenolics, flavonoids, steroids and Tannins. In the chloroform extract, only flavonoids, phenolics, saponin, and tannin (Table 2). Thus, it indicates that among the selected solvents, chloroform is the most suitable solvent to obtain the most number of phytochemicals from the crude plant powder. The experimental proof is given in photoplate-1.

Table 2: Preliminary phytochemical tests of *Salvinia molesta*

Sr. No.	Test	Distilled water	Ethanol	Chloroform
1	Alkaloids	-	-	-
2	Flavonoids	-	+	+
3	Terpenoids	-	+	-
4	Quinones	-	-	-
5	Steroids	-	+	-
6	Phenol	+	+	+
7	Glycosides	-	-	-
8	Saponin	-	-	+
9	Tannin	+	+	+

Note: the results are average of triplicate analysis

HPTLC Profiling of *Salvinia molesta* Chloroform leaf extract

The HPTLC analysis facility was availed from Qualichem Laboratories Pvt. Ltd. Nagpur. The analysis of done using a glass tank chamber (10 x 10 cm) with a solvent system Toluene: Ethyl Acetate: Formic acid (5:4:0.2). The solvent front position 70.00 mm, dryer used- Oven with temperature 60oC and time 5 minutes. The detector used was CAMAG TLC Scanner "Scanner_171005" S/N 171005 (2.01.02) with a scanning speed of 20mm/S and data resolution of 100mm/step. The analysis was done on two wavelengths 254 nm and 366 nm.

HPTLC analysis of ethanolic *S. molesta* leaf extract on 254 nm

At 254 nm, the HPTLC chromatogram showed 15 different peaks (Fig. 1) and visualized the same number of bands on the TLC visualizer (Fig. 2). The details of different peaks are presented in table-3 that includes, including peak number, start rf, maximum rf, the height of peak and peak area, etc. This information is necessary to identify the respective compounds giving that peak or band.

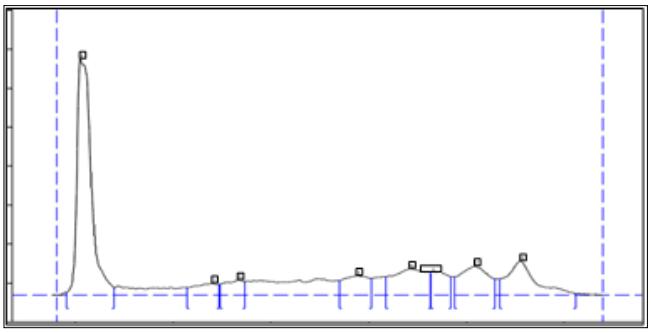

Fig 1: HPTLC chromatogram of *S. molesta* leaf extract at 254 nm

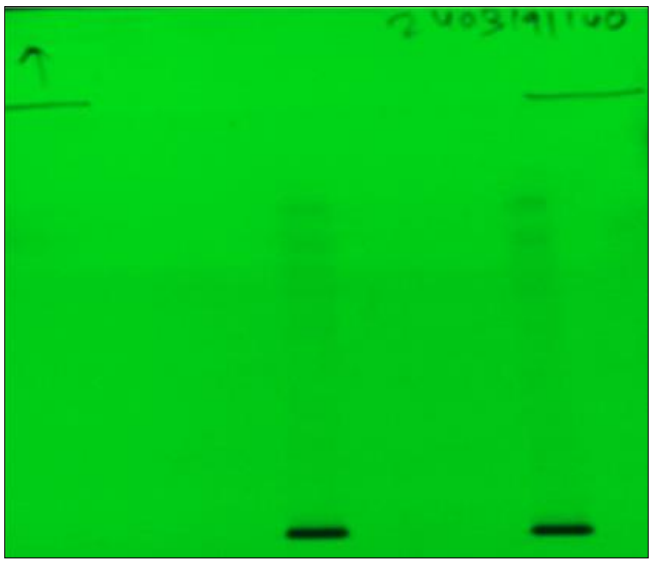

Fig 2: HPTLC Bands for *S. molesta* leaf extract at 254 nm

Table 3: Details of HPTLC chromatogram of ethanolic leaf extract of *S. molesta* at 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	7.4	0.01	606.1	59.66	0.08	19.5	12680.8	44.75	Un-identified
2	0.23	19.8	0.28	30.5	3.00	0.30	27.7	1088.5	3.84	Flavonoid
3	0.30	28.1	0.33	39.2	3.86	0.35	35.1	1135.2	4.01	Un-identified
4	0.54	38.2	0.58	50.8	5.00	0.61	43.4	1875.7	6.62	Caffeic Acid
5	0.64	47.9	0.69	68.4	6.74	0.73	57.6	3364.7	11.87	Un-identified

6	0.73	57.4	0.74	58.8	5.79	0.77	46.9	1433.3	5.06	Vanillic acid
7	0.78	47.9	0.82	74.6	7.35	0.86	41.8	3104.7	10.96	Un-identified
8	0.87	42.2	0.91	87.5	8.61	1.03	4.9	3653.8	12.89	Ferulic acid

From the chromatogram of leaf extract of *S. molesta*, which showed 8 peaks at 254 nm, 04 peaks were identified. These were peak numbers 2 4, 6, and 8 having maximum Rf values 0.28, 0.58, 0.74 and 0.91. These compounds were identified as flavonoid, caffeic acid, vanillic acid and Ferulic acid.

HPTLC analysis of P. guajava leaf extract on 366 nm

At 366 nm, the HPTLC chromatogram showed 5 different peaks (Fig. 4) and visualized same number of bands on the TLC visualizer (Fig. 5). The details of different peaks are presented in table-4 that includes, including peak number, start Rf, maximum Rf, height of peak and peak area etc. This information is necessary to identify the respective compounds giving that peak or band.

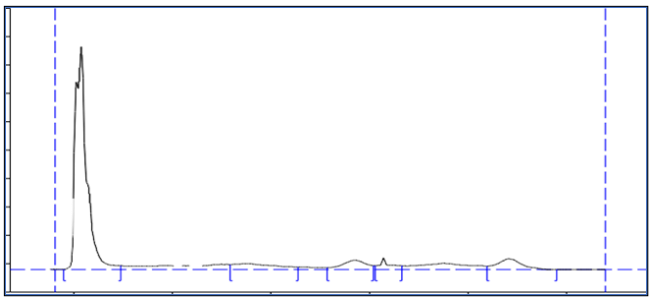

Fig 3: HPTLC chromatogram of *S. molesta* leaf extract at 366 nm

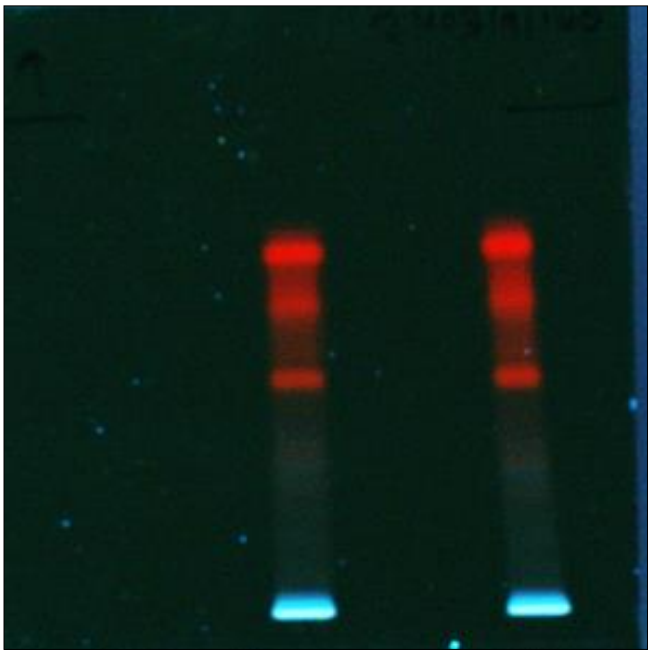

Fig 4: HPTLC band visualization of *S. molesta* leaf extract at 366

Table 4: Details of HPTLC chromatogram of *S. molesta* leaf extract at 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	0.1	0.02	760.7	72.02	0.10	12.7	13403.7	51.75	unknown
2	0.40	20.5	0.41	21.3	2.01	0.46	12.8	639.3	2.47	unknown
3	0.51	11.1	0.58	56.1	5.31	0.61	25.3	2034.5	7.86	Caffeic acid
4	0.67	26.8	0.75	59.2	5.60	0.82	44.5	4010.5	15.48	Vanillic acid
5	0.82	44.7	0.88	159.0	15.05	0.97	1.8	5811.6	22.44	Flavonoid

From the chromatogram of leaf extract of *S. molesta*, which showed 5 peaks at 366 nm, 05 peaks were identified. These were peak numbers 3, 4and 5 having maximum Rf values 0.58, 0.75, and 0.88. These compounds were identified as caffeic acid, vanillic acid and flavonoid.

A major study conducted in *S. molesta* by Li *et al*. (2013) using bioactivity-guided fractionation of ethanol extract yielded 50 compounds, including 17 abietane diterpenes nine phenolics (Chaudhary *et al*., 2008) [3], five fatty acids, five triterpenes, four apocarotenoids, two acyclic sesquiterpenoids, two monoterpenes, two jasmonates, two steroids and two coumarins. Another study has shown that naringenin was the major phenolic compound present in acetone: methanol (1:1) extract of *S. molesta* which was identified and quantified by HPLC followed by myricetin along with rutin, epicatechin, catechin, quercetin, kaempferol, and vanillin. These compounds were also found to have free radical scavenging potential (Panda *et al*., 2014 and Gini and Jyoti, 2018) [13, 6]. Salleh *et al*., (2023) [14] reported that the plant is rich in phenolics, flavonoids, tannins, alkaloids, and saponins. Santhosh *et al*., (2022) [15] and Al-Knani *et al*., (2023) showed that allied species *S. cucullata* and *S. natans* have potential antimicrobial and antioxidant activities. These are some reports which supports the present study.

The present study showed the plant is very rich in phytochemicals and has various phenolic compounds like ferulic acid, vanillic acid, and other flavonoids. Therefore, it can be concluded that this plant is one of the plausible natural antioxidants the phenolic compounds could be used

as a lead candidate for synthesizing antioxidant drugs which can be useful for the treatment of many oxidative stress-related diseases.

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