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Phytochemical,Antibacterial and GC MS analysis of a floating fern *Salvinia molesta D.S.Mitchell (*1972)

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Abstract: The present study was performed to screen significant bioactive compounds in *Salvinia molesta D.S.Mitchell* using five different solvents and also to elucidate the antibacterial activity of the leaf extract in various concentration to determine the inhibitiory efficacy against selected pathogens *viz., Pseudomonas aeruginosa, Aeromonas hydrophila ,Escherichia coli, Bacillus cereus, Bacillus subtilis, and Staphylococcus aureus.* The concentrated leaf extract in acetone was separated and analysed by Column chromatography and the fraction with highest antioxidant activity was subjected to Gas chromatography-Mass spectrum analysis (GC MS) to elucidate the active components present in the leaf extracts.Phytochemical screening of the leaf extracts in different solvents revealed the presence of significant components. The antibacterial activity of ethanolic extract of *Salvinia molesta* showed highest activity against *Pseudomonas aeruginosa* followed by *Aeromonas hydrophila* and moderate to least activity against other pathogens. The GC MS analysis confirmed the presence of bioactive components such as Apiol, Hexadeconoic acid, pentadecanoic acid and octadecatriene etc, which are highly responsible for antibacterial and antioxidant activity of the extract. Thus *salvinia moletsa* can be used as an complete therapeutic agent since it possess significant activities ranging from antibacterial to immunomodulatory.
Key words: *Salvinia molesta,* phytochemical screening, antibacterial activity, GC-MS.

Introduction

Medicinal plants have been used by human beings since ages due to their therapeutic potential values and has become an important component of health care system.India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world(1).A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also will be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies(2).The therapeutic benefits are generally traced to specific plant compounds; but are specifically due to the active constituents of the plants(3). Phytochemicals are natural bioactive compound found in plants and fibers which act as a defense system against diseases and more accurately, to protect against diseases (4).The most important of these bioactive constituents of plants are Alkaloids, Tannins, Flavonoids ,Cardiac glycosides, Steroids and Saponins. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties.

Microorganisms are the causative agents of almost all kinds of acute and chronic diseases. Plants based antibacterials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antibacterials. The use of plant extracts with known antibacterial properties, can be of great significance in therapeutic treatments. Although hundreds of plant species have been tested for antibacterial properties, the vast majority of them have not been adequately evaluated(5).

Free radicals cause the oxidation of biomolecules which leads to cell injury and death. Reactive oxygen species (ROS) markedly alter the physical, chemical, and immunological properties of superoxide dismutase (SOD), which further exacerbates oxidative damage in cells. This has raised the possibility that antioxidants could act as prophylactic agents(6). Therefore, there has been a growing interest in research concerning alternative antioxidant active compounds, including plant extracts and essential oils that are relatively less damaging to living beings health and environment.

Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids (7) and alkaloids (8).In this present study *Salvinia molesta* ,a floating fern belonging to the family Salviniaceae also known as giant *Salvinia*, water fern, or kariba weed was tested for the presence of phytochemicals and the antibacterial activity exhibited by ethanolic extract against selected pathogens. Also GC MS study was performed for the best antioxidant fraction of the *Salvinia molesta* leaf extract to elucidate the possible medicinal propreties of the species.

Materials and Methods

Collection of *Salvinia molesta*

The fresh *Salvinia molesta* whole plant was collected from lakes at Kalyiyakkavilai Kanyakumari district and the leaves alone were separated ,drained and allowed to air dry.

Preparation of *Salvinia molesta* powdered sample

The collected leaves were cleaned and cut into small pieces before being dried under shade at room temperature.The dried material were ground to fine powder using a mechanical blender and passed through 24 mesh sieve.The powdered sample was further used to make different extraction.

Preparation of the plant extract

Plant extracts were prepared by standard methods[9].One gram of dried leaf powder of *Salvinia molesta* leaf materials were extracted with 20 ml ethanol(75%), acetone, chloroform, aqueous and petroleum ether (Merck, extra pure) for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No.1 filter paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotator at 40 °C then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18 °C until use.

Phytochemical screening of *Salvinia molesta*

The phytochemical screening of leaf extracts were assessed by standard methods (9-11). Phytochemical screening was carried out on the leaf extracts using five different solvents viz., aqueous, ethanol, acetone,chloroform and petroleum ether to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids.

GC-MS (Gas Chromatography-Mass Spectrometry) analysis

The plant powder was extracted with methanol and analyzed using GC-MS analyzer. The data were obtained on an Elite-1(100% Dimethyl poly siloxane) column (30 0.25mm 1µmdf). Helium (99.999%) was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 2µl of ethanol solution

of the sample was injected into the column with the injector temperature at 250ºC.GC oven temperature started at 110ºC and holding for 2min and it was raised to 200ºC at the rate of 10ºC/min, without holding. Holding was allowed at 280ºC for 9 min with program rate of 5ºC/min.The injector and detector temperatures were set at 250ºC and 280ºC respectively. Ion source temperature was maintained at 200ºC. The mass spectrum of compounds in samples was obtained by electron ionization at 70 eV and the detector was operated in scan mode from 45-450amu (atomic mass units). A scan interval of 0.5seconds and fragments from 45 to 450 Da was maintained. The total running time was 36minutes.

Growth and Maintenance of Test Microorganism for Antibacterial Studies:

Bacterial cultures of *Pseudomonas aeruginosa, Aeromonas hydrophila ,Escherichia coli, Bacillus cereus, Bacillus subtilis, and Staphylococcus aureus* were maintained on Nutrient Agar(NA) slants at 4°C.For further study ,cultures have been grown in Nutrient Broth (NB) for 24hrs as overnight cultures.

Preparation of inoculums

The pure cultures of bacteria were grown on nutrient agar slants and incubated at 37°C for 24hrs. Nutrient broth and the slants were stored at 4°C and maintained in active state by regular sub-culturing for further use.

Disc diffusion Method

The antibacterial assay of ethanolic extracts of *Salvinia molesta* leaves was performed by Disc diffusion method(12).The Nutrient agar media (20ml) was poured into sterilized petri dishes and left to solidify at room temperature. The overnight bacterial cultures have been spread plated on these petridishes using sterile L rod. Different concentration (10, 20 and 30 mg/ml) of the concentrated ethanolic *salvinia molesta* extracts was tested for its antimicrobial strains against selected pathogens.The bacterial cultures were grown in Mueller Hinton Agar and Mueller Hinton broth (Himedia).The filter paper discs were placed equidistantly on inoculated media and diffusion of solution was allowed to occur for 30 minutes at room temperature. Plates were incubated at 37°C for 24 hours. The average zone of inhibition was recorded. Sterile distilled water and Ethanol were maintained as control. The diameters of the inhibition zones were measured in mm.

Results

Phytochemical screening of *Salvinia molesta* leaf extracts using five different solvents *viz.,* aqueous, ethanol, acetone, chloroform and petroleum ether, confirmed the presence of significant bioactive components with varying qualitative range as positive, strong positive and negative values(Table 1).Antibacterial activity assay performed by disc diffusion method against selected pathogens showed that among five different solvents ethanolic extracts of *Salvinia molesta* exhibited maximum activity against test pathogens. It was recorded that the maximum zone of inhibition by ethanolic extract of *salvinia molesta* was against *P.aeruginosa* with 28 mm at 30mg/ml concentration, followed by 26 mm for *A.hydrophilla* at 30mg/ml.Following this range, a moderate activity against *B.cereus* and *S.aureus* with 27 mm and 19mm respectively were recorded and a least activity against *E.coli* of 13 mm *and B.Subtilis* of 10 mm was exhibited at 30 mg/ml concentration (Table 2). The GC-MS analysis of *salvinia molesta* leaves revealed the presence of seven important bioactive compound that could contribute the medicinal quality of the plant The identification of this bioactive compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage (Table 3) and Fig (1) & (2).The first compound identified with less retention time (13.9 min) was Apiol, whereas Heptadecanoic acid, 16-methyl ester was the last compound which took longest retention time (19.09min) to identify. The components identified through GC-MS analysis showed many biological activities relevant to this study (Table 3).

Table 1. Phytochemical screening from leaf extracts of *Salvinia molesta*

Phytochemical	Different solvents used for extract preparation				
	Aqueous	Ethanol	Chloroform	Acetone	Petroleum ether
Tannins	-	++	+	+	+
Saponins	++	+	-	+	-
Quinones	+	++	+	++	+
Terpenoids	+	+	+	++	+
Steroids	+	++	+	++	+
Flavonoids	+	++	+	++	+
Phenol	++	++	+	++	+
Alkaloids	+	+	-	++	-

Key : + = positive, ++ = strong positive , - = negative

Table:2 Zone of inhibition by *Salvinia molesta* ethanolic extract in varying concentration(mm)

Bacterial Isolates	Ethanolic extract concentration(mg/ml)		
	10mg	20mg	30mg
P.aeruginosa	10.22 ±0.12	14.18 ±0.33	28.45 ±0.32
A.hydrophila	10.13 ±0.14	18.12 ±0.14	26.12 ±0.51
B.cereus	9 .16±0.17	12.12 ±0.12	25.14 ±0.12
S.aureus	6.33 ±0.18	8 .12±0.33	19.13 ±0.64
E.coli	7.12 ±0.14	9.13 ±0.54	13.12 ±0.46
B.subtilis	6 .17±0.13	8.12 ±0.62	10.12 ±0.51

Table 3: GC-MS analysis of leaf extract of *Salvinia molesta*

S.No	R/T	Compound	Molecular Formula	Peak Area %	Biological Activity
1	13.921	Apiol	$C_{12}H_{14}O_4$	12.39	Antioxidant Antiseptic, Antifungal
2	17.160	Hexadecanoic acid, methyl ester	C17H34O2	49.53	Antioxidant, Antibacterial
3	18.846	Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	30.91	Antimicrobial
4	19.091	Heptadecanoic acid,16-methyl-, methyl ester	$C_{19}H_{38}O_2$	7.17	Antioxidant

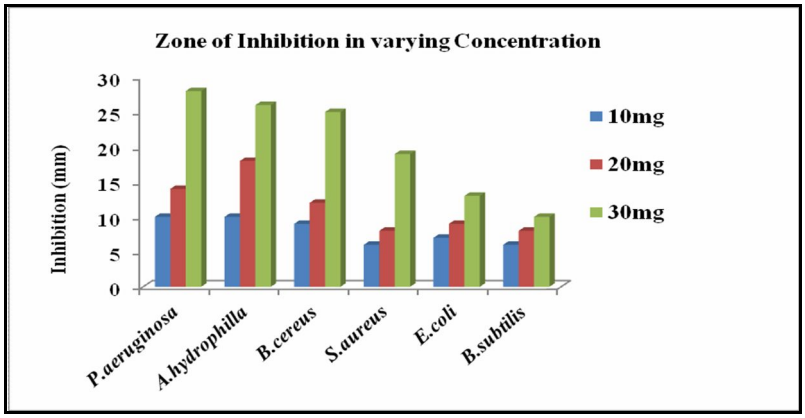

Fig 1: Antibacterial activity of ethanolic extract of *Salvinia molesta*

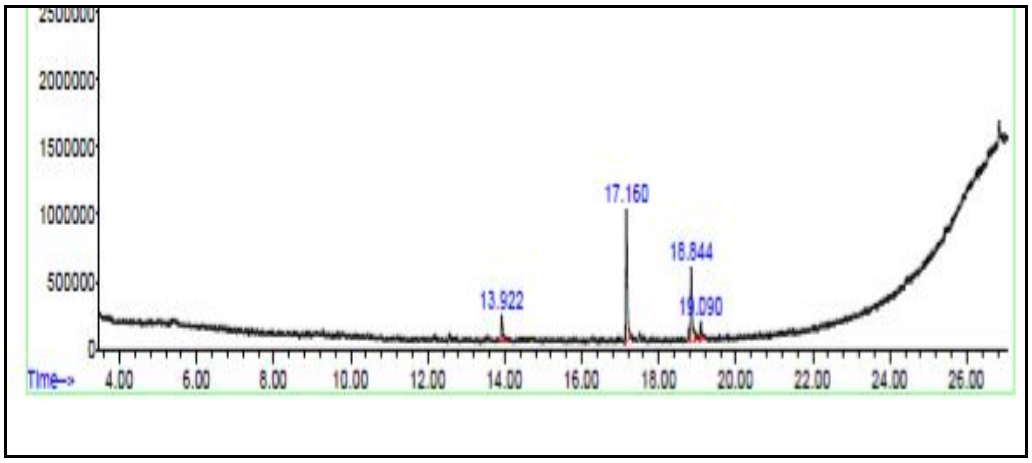

Fig 2.GC MS analysis of leaf extract of *Salvinia molesta*

Discussion

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties(13). Reports available on green plants represent a reservoir of effective chemotherapeutants, these are non-phytotoxic, more systemic and easily biodegradable (14).For these reasons, medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. Since, the main concern of the general public and science is in finding new natural and therapeutically active agents; scientists all over the globe have started screening plants for searching new antimicrobial agents(15).Flavonoids are known to be synthesized by plants in response to microbial infection. Hence it should not be surprising that they have been found to be effective as antibacterial substances against a wide array of infectious agents(16).Tannins (commonly referred to as tannic acid) are also known as antimicrobial agents. They are water-soluble polyphenols and precipitated proteins present in many plant foods and have been reported to prevent the development of microorganisms by precipitating microbial protein. The growth of many fungi, yeasts, bacteria, and viruses were inhibited by this Tannins.(17).In this present study *Salvinia molesta* was found to possess significant secondary metabolites that were responsible for its potent antibacterial activity against selected pathogens. Antimicrobial activity results obtained in the present study revealed that the tested extracts possess potential antibacterial activity against *P.aureginosa and A.hydrophila* than other selected pathogens. The results also showed that increase in concentration of the extract increased the zone of inhibition. The range of zone of inhibition by ethanolic extracts against pathogens were in the following order of higher to lower,*viz., Pseudomonas aeruginosa ,Aeromonas hydrophila, Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*. The GC MS results of *Salvinia molesta* also confirmed the presence of major components highly responsible for its antioxidant, antibacterial, antiseptic and immunostimulant properties. Thus the present study has proven that *Salvinia molesta* a fresh water weed, considered as an aquatic menace worldwide can be successfully used as an therapeutic agent on processing to cure many emerging infectious diseases at a cheaper and safer manner.

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