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Research Article

ANTIOXIDANT ACTIVITY, TOTAL PHENOL, FLAVONOID, ALKALOID, TANNIN, AND SAPONIN CONTENTS OF LEAF EXTRACTS OF *SALVINIA MOLESTA* D. S. MITCHELL (1972)

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ABSTRACT

Objective: The main aim of the study was to screen the leaf extracts of *Salvinia molesta* D. S. Mitchell, a fresh water weed to evaluate the antioxidant activity and also to quantify total phenol, flavonoid, alkaloid, tannin, and saponin contents to find possible sources for future novel antioxidants in food and pharmaceutical formulations.

Methods: Qualitative and quantitative analyses of significant phytochemicals were performed by standard methods. The antioxidant activity was evaluated using extracts of aqueous, ethanol, methanol, chloroform, and petroleum ether by the diphenyl-2-picrylhydrazyl assay. Butylated hydroxytoluene, gallic acid (GA), and quercetin (Q) were taken as standard.

Results: Among the five different solvents, the maximum antioxidant activity of *S. molesta* was found in the ethanolic extract (90.3%) followed by other solvents. Total phenolic content measured by Folin–Ciocalteau method was 9.84 mg GA equivalents/g and the total flavonoid contents as measured by aluminum chloride method was 10.89 mg quercetin equivalents (QE)/g. Alkaloids, tannins, and saponins were measured by standard methods and found in significantly high ranges exhibiting a rich source of phytochemical constituents ensuring the plant as a useful therapeutic agent.

Conclusion: *S. molesta* a fast growing fresh water weed also abundantly available in nature possess significant antioxidant activity and hence can be used as a potent therapeutic agent.

Keywords: *Salvinia molesta*, Antioxidant activity, Total phenol, Flavonoid, Alkaloid, Tannin, and Saponin.

INTRODUCTION

Medicinal plants play a pivotal role in the health care of ancient and modern cultures [1]. Natural plant products have been used for therapeutic purposes since time immemorial, and their use is of greater demand nowadays. Majority of the users rely on herbal medicines for health care because the other treatment options are a more expensive and they are often thought to be more associated with serious side effects [2]. Therefore, there is continuous and urgent need to discover new therapeutic compounds with diverse chemical structures and also novel mechanism of action is required for new and emerging infectious diseases [3]. Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide, and free radicals, plays a crucial role in the development of various ailments such as immunodepression, diabetes mellitus, ageing, dementia, carcinoma, and Parkinson's disease [4]. Many natural herbs contain antioxidant compounds which protects the cells against the damaging effects of ROS. Though our body is safeguarded by the natural antioxidant defense, there is always a demand for antioxidants from external natural source. In addition, secondary metabolites, such as phenolic compounds, flavonoids, alkaloids, and tannins, are widely distributed in plants and are reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic effect, etc. [5]. Aquatic weeds are those unabated plants which grow and complete their life cycle in water and cause harm to the aquatic environment directly. The menace of aquatic weed is reaching alarming problems in many parts of the world, but it is particularly severe in tropical countries [6]. Aquatic weed populations often reach nuisance proportions and interfere with beneficial uses of natural waters. A few of them are consumed by local people but many remain unutilized and go to waste [7]. Though these aquatic weeds are a disturbing factor to the environment they possess significant unexplored medicinal properties which may serve as a potent therapeutic agent for many emerging diseases. Furthermore, there is a growing interest all over the world to discover the untapped medicinal properties of aquatic weeds. *Salvinia molesta* D. S. Mitchell (Table 1), a free-floating aquatic fern, is one of the world's largest aquatic weed whose explosive growth had devastating socio-economic impacts in parts of Africa, Sri Lanka, Asia, Philippines, and Australia. *S. molesta*, commonly known as giant *Salvinia* or Kariba weed belongs to the family *Salviniaceae*, a fresh water fern [8]. It is a free floating plant that does not attach to the soil, but instead remains buoyant on the surface of a body of water. *S. molesta* prefers to grow in slow-moving waters such as those found in lakes, ponds. Thus, this particular plant species *S. molesta* D. S. Mitchell available in abundant can be utilized to generate novel medicinal compounds to cure emerging diseases. Hence, the present study was aimed at exploring the positive medicinal values of *S. molesta* by evaluating the antioxidant activity, relative content of total phenol, flavonoids, alkaloids, tannins, and saponins in leaf extracts of *S. molesta* procured from fresh water lakes of Kalyiyakkavilai, Kanyakumari, Tamil Nadu, India.

METHODS

Collection of *S. molesta*

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

Table 1: Taxonomy of plant

Kingdom	*Plantae*
Division	*Pteridophyta*
Class	*Polypodiopsida*
Order	*Salviniales*
Family	*Salviniaceae*
Genus	*Salvinia*
Species	*S. molesta*
Binomial name	*S. molesta D. Mitchell*

S. molesta: Salvinia molesta

Preparation of *S. 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 the different extraction.

Preparation of the plant extract
Plant extracts were prepared by standard methods [9]. 1 g of dried leaf powder of *S. molesta* leaf materials was extracted with 20 ml ethanol (75%), acetone, chloroform, aqueous and petroleum ether (Merck, extra pure) for 1 minutes 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.

Qualitative analysis of antioxidant activity of *S. molesta*
The antioxidant activity of leaf extracts of *S. molesta* was determined by standard method [10]. 50 µl of leaf extracts of *S. molesta* were taken in the microtiter plate. 100 µl of 0.1% methanolic diphenyl-2-picryl hydrazyl (DPPH) was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive, respectively, (Table 2). The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of free radical scavenging activity of *S. molesta*
The antioxidant activity were determined using DPPH (Sigma-Aldrich) as a free radical.100µl of leaf extracts were mixed with 2.7 ml of methanol and then 200 µl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of a blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control [11]. Subsequently, at every 5 minutes interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% butylated hydroxytoluene (BHT).

Free radical scavenging activity was calculated by the following formula:

$$\text{DPPH radical} - \text{scavenging in \%} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

Estimation of total phenol content
Total phenolic content in the leaf extracts of *S. molesta* was determined by the Folin–Ciocalteau colorimetric method [12]. For analysis, 0.5 ml of aliquot of the sample was added to 0.5 ml of Folin–Ciocalteau reagent (0.5 N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (2%) was added, and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-visible spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Table 2: Qualitative antioxidant activity of *S. molesta*

Extractions	*S. molesta*
BHT (standard)	+++
Aqueous	+
Ethanol	+++
Acetone	++
Chloroform	Semi positive
Petroleum ether	Semi positive

BHT: Butylated hydroxy toluene, *S. molesta: Salvinia molesta*

Estimation of total flavonoid content
Total flavonoid content in leaf extracts of *S. molesta* was determined by aluminum chloride colorimetric method [13]. 0.5 ml of leaf extracts of *S. molesta* at a concentration of 1 mg/ml were taken, and the volume was made up to 3 ml with methanol. Then, 0.1 ml $AlCl_3$ (10%), 0.1 ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of sample.

Estimation of total tannin content
Tannins content in leaf extracts of *S. molesta* was estimated using standard method [14]. The leaf extracts (1 ml) were mixed with Folin–Ciocalteau's reagent (0.5 mL) followed by the addition of saturated sodium carbonate (Na_2CO_3) solution (1 mL) and distilled water (8 mL). The reaction mixture was allowed to stand for 30 minutes at room temperature. The supernatant was obtained by centrifugation, and absorbance was recorded at 725 nm using a UV-visible spectrophotometer. Different concentrations of standard tannic acid were prepared, and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as µg tannic acid equivalent (TAE) a gram of the sample.

Estimation of saponin content
The determination of total saponin was done by standard method [15] with minor modifications. 1 g of powdered leaf was added to 100 ml of 20% aqueous ethanol and kept in a flask on stirrer for half hour and then heated over a for 4 hrs at 45°C with mixing. The mixture was filtered by using Whatman filter paper no 1 and the residue again extracted with another 100 ml of 25% aqueous ethanol. The combined extracts were concentrated using rotary evaporator in 40°C to gets 40 ml approximately. The concentrate was transferred into separator funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was kept and then re-extracted with 30 ml n-butanol was added. The n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated. After evaporation, the samples were dried in the oven at 40°C to a constant weight and the saponin content was calculated.

Determination of total alkaloids
The quantification of alkaloids determinations was performed by standard methods [16]. 100 ml of 10% acetic acid in ethanol was added to 1 g of leaf extracts of *S. molesta*, and then the extracts were covered and allowed to stand for 4 hrs. After that the extracts were filtered and concentrated on a water bath to 25 ml of its original volume. The droplets of concentrated ammonium hydroxide were added to the extract until the whole solution was allowed to settle, and then the precipitates were washed with dilute ammonium hydroxide and then filtered using Whattman filter paper. The residue was dried in the oven at 40°C and weighed.

The alkaloid content was determined using the following formula:

Percentage of alkaloid = (final weight of the sample/initial weight of the extract) × 100

RESULTS

All the five different solvents *viz*., methanol (80%), ethanol (75%), petroleum ether, chloroform, and aqueous extract of *S. molesta* leaves showed significant antioxidant activity when estimated for free radical scavenging activity using DPPH assay (Fig. 1). Among five different solvent extracts the ethanolic leaf extract recorded the most effective DPPH radical scavenging activity (90.3%) with BHT as control. When *S. molesta* leaf extract was quantified by standard methods, the leaf

Table 3: Quantification of major phytochemicals using different solvent leaf extracts of *S. molesta*

S. No	Solvent extractions	Phytoconstituents				
		Phenol (mg GAE/g)	Flavonoid (mg QE/g)	Alkaloid (mg/g)	Tannin (mg TAE/g)	Saponin (mg/g)
1	Aqueous	90.3	6.14	81.0	5.6	37
2	Ethanol	98.4	10.89	90.8	12.5	42
3	Acetone	86.9	7.11	73.4	8.3	33
4	Chloroform	53.4	5.6	65.4	5.2	26
5	Petroleum ether	58.4	7.1	63.1	4.2	24

S. molesta: Salvinia molesta

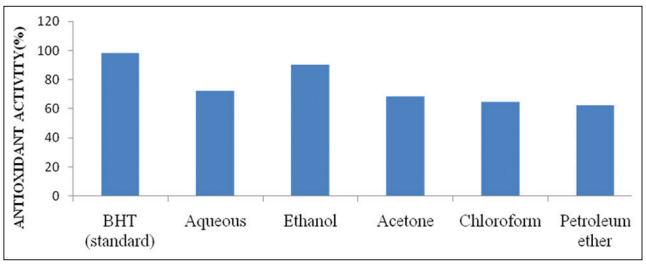

Fig. 1: Antioxidant activity of different solvent extracts of *Salvinia molesta*

extract of *S. molesta* exhibited total phenolics content of 9.84 mg GAE/g. Total flavonoid contents measured by aluminum chloride method was 10.89 mg QE/g. Furthermore, leaf extract of *S. molesta* showed the highest alkaloid range of 90.8%. Tannins levels were evaluated as 12.5 TAE/g. Total saponin content was evaluated as 42 mg/g (Table 3). The present study on analysis of antioxidant activity and phytochemical quantification showed significant effects and hence confirmed the therapeutical value of *S. molesta.*

DISCUSSION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is well-known that plant produces these chemicals to protect themselves but recent research demonstrate that they can also protect humans and other living forms from diseases. Phytochemical studies have attracted the attention of plant scientists due to the development of innovative techniques. These techniques played a significant role in the search for additional resources of raw material for the pharmaceutical industry [17]. Phenolic compounds are considered as important secondary metabolites and these phytochemical compounds derived from phenylalanine, and tyrosine occurs ubiquitously in plants and is diversified [18]. Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability. Plant materials rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food [19]. Flavonoids are naturally occurring secondary metabolite in plants and are thought to have positive effects on human health. Studies on flavonoid derivatives have shown a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities [20,21]. Flavonoids have been shown to be highly effective scavengers of the most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases [22]. Tannins were found to be responsible for high immunomodulatory activity in previous studies [23], and *S. molesta* showed a significant presence of tannins. Alkaloids have a wide range of pharmacological activities including anticancer and antibacterial activities and hence responsible for many healing properties in natural medicine. Being widely distributed amongst plants, saponins have long been regarded as phytochemical material to protect plant against pathogens. Therefore, it is no doubt that saponins function as potential medicinal candidates [24]. The present study revealed the presence of significant levels of antioxidant activity, total phenol, total flavonoid, tannins, alkaloids, and saponin content in leaf extract of *S. molesta.*

CONCLUSION

Plants are a source of a large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, and antimicrobials. A large number of the plants are claimed to possess antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of medicinal plants. Extraction of the bioactive plant constituents has always been a challenging task for the researchers. In this present study, the active constituent of *S. molesta* D. S. Mitchell, a fast growing fresh water weed was studied, and the positive aspect of the plant was discovered which was known as an aquactic menace and subjected to eradication in western countries. Thus, *S. molesta* an abundantly available aquatic weed is a promising therapeutic agent which can be beneficially utilized for their medicinal properties to cure and solve various emerging diseases.

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