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Original Research Article

Qualitative Phytochemical Screening of *Sida rhombifolia* Linn.

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Abstract	Keywords
The aim of this study is to determine the phytochemical constituents of *Sida rhombifolia*. Phytochemistry helps in standardizing the herbal preparations and possibly relates the constituents to their medicinal/pharmacological uses. Dried material was grounded to coarse powder and stored in airtight container followed by the extraction with ethanol. The dry powder of sample was observed under U.V. light to evaluate the fluorescence. Chemical tests were performed on ethanol extract. The quantitative studies revealed that *Sida rhombifolia* possessed alkaloids, carbohydrates, glycosides, saponins, phytosterols, proteins, flavonoids and lignin. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.	Fluorescence analysis Phytochemical screening *Sida rhombifolia*

Introduction

Traditional medicinal practice has been known for centuries in many parts of the world. Herbal medicines are gaining interest because of their cost effective and eco-friendly attributes. Traditional knowledge regarding of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future. Plant and plant extracts have formed important position in modern medicine, due to their chemical and medicinal contents found in the natural form. The secondary metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities .The use of higher plants and their extracts to treat infections is an age-old practice. Medicinal plants have been an integral part of the ethno botanical aspects of the people. The modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening. Thus, plants remain the major source of medicinal compounds. UNESCO (1998) estimated that 20,000 plant species are used for medicinal purposes.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization (WHO, 1998), medicinal plants would be the best source to obtain a variety of drugs and more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical

substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds.

The phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants. In the present study an attempt has been made the laboratory evaluations to assess the analytical and phytochemical screening of *Sida rhombifolia* Linn. and possibly relate the constituents to their medicinal/pharmacological uses. Phytochemical studies of the plant are necessary for standardization, which helps in understanding the significance of phytoconstituents in terms of observed activities. Phytochemical screening also helps in standardizing the herbal preparations.

Botanical description

Sida rhombifolia Linn. is a perennial or sometimes annual plant in the Family Malvaceae, native to the New World tropics and subtropics. Other common names include Paddy's lucerne, jelly leaf. Synonyms include *Malva rhombifolia*. It is used in Ayurvedic medicine, where it is known as kurumthotti. The stems are erect to sprawling and branched, growing 50 to 120 centimeters in height, with the lower sections being woody. The dark green, diamond-shaped leaves are arranged alternately along the stem, 4 to 8 centimeters long, with petioles that are less than a third of the length of the leaves. They are paler below, with short, grayish hairs. The apical half of the leaves has toothed or serrated margins while the remainder of the leaves is entire. The petioles have small spiny stipules at their bases.

The moderately delicate flowers occur singly on flower stalks that arise from the area between the stems and leaf petioles. They consist of five petals that are 4 to 8 millimeters long, creamy to orange-yellow in color, and may be somewhat reddish in the center. Each of the five overlapping petals is asymmetric, having a long lobe on one side. The stamens unite in a short column. The fruit is a ribbed capsule, which breaks up into 8 to 10 segments. The plant blooms throughout the year. This species is usually confined to waste ground, such as roadsides and rocky areas.

Systematic position of *Sida rhombifolia* Linn. as per Bentham and Hooker classification is given below:

Class : Dicotyledons
Sub class : Polypetalae
Series : Thalamiflorae
Order : Malvales
Family : Malvaceae
Genus : *Sida*
Species : *rhombifolia* Linn.

Materials and methods

Collection and identification of plant materials

Areal parts of *Sida rhombifolia* Linn. for the present study were collected during the month of July-August 2013 from Tiruchirappalli district, Tamil Nadu and care was taken to select healthy plants. The identity of the plant specimens was confirmed by the use of local Floras. The botanical identify was authenticated in the Department of Botany, Jamal Mohamed College, Tiruchirappalli. The collected plant materials were washed thoroughly with tap water and dried under shade for ten days. Dried material was grounded to coarse powder and stored in airtight container. It was then extracted with ethanol. The dry powder of sample was observed under U.V. light to evaluate the fluorescence. Chemical tests were performed on ethanol extract.

Qualitative phytochemical study

Preparation of ethanol extract: About 600 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrate thus obtained was concentrated using a rotary evaporator to get the extract.

Phytochemical screening

Phytochemical screening means to investigate the plant material in terms of its active constituents. The plant extract was subjected to qualitative tests for the identification of the phytoconstituents present in it viz, alkaloids, carbohydrates, glycosides, phytosterols, fixed oils and fats, phenolic compounds & tannins, proteins and free amino acids, flavonoids, lignins and saponins

(Harborne , 1998; Sofowora , 1982; Trease and Evans , 1989; Chase and Pratt , 1949).

Test for phenolic compounds: Two to three drops of 1% ferric chloride (FeCl3) solution was added into 2ml of extract. Phenolic compounds produce a deep violet colour with ferric ions.

*Test for flavonoids - Shinoda Test:*To the alcoholic¬ solution of the extract a few fragments of magnesium ribbon were added. To this concentrated hydrochloric acid (HCl) was added drop wise. Magenta colour was produced after few minutes which are the characteristic reaction of flavonoid.

Test for tannins - ferric chloride test: Water extract¬ was treated with 15 % ferric chloride test solution. The resultant colour was noted. A blue colour indicates condensed tannins; a green colour indicated hydrolysable tannins.

Test for saponins: The extract was diluted with 20ml¬ of distilled water and it was agitated on a graduated cylinder for 15 min. the presence of saponins was indicated by the formation of 1cm layer of foam.

Test for Alkaloids - Mayer's test: The Extract was¬ dissolved in chloroform. The chloroform was evaporated and the residue was acidified and added few drops of Mayer's reagent (Potassium Mercuric Iodide). Alkaloids are precipitated by Mayer's reagent to give a cream coloured precipitate.

Wagner's Test: The extract was dissolved in¬ chloroform. The chloroform layer was evaporated, to the residue was acidified and added few drops of Wagner's reagent (Iodine in Potassium Iodide). Orange precipitate indicates alkaloids.

*Test for the carbohydrate:*The aqueous extract 5 ml was treated with the reagent of the starch (iodine). Any shift to blue violet indicates the presence of starch.

*Test for Glycosides - Fehling's Test for reducing¬ sugars (In Glycosides):*The extract was re-dissolved in water on the water bath. To 2 ml of the solution, in the test tube was added, 1ml each of Fehling's solutions A and B. The mixture was shaken and heated in a water bath for 10min. The colour obtained was recorded. A brick-red precipitate indicates reducing sugar.

Test for proteins - Xanthoproteic Test: Extract was treated with few drops of Concentrated HNO3. Formation of yellow colour indicates the presence of proteins.

Test for Phytosterols - Liebermann-Burchard's Test: One gram of the extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride boiled and cooled, concentrated sulphuric acid was added through the sides of the test tube. The formation of brown coloured ring at the junction of two liquids confirmed the presence of steroids.

Test for fixed oil and fats: Press the extract in between¬ the two filter papers, a permanent stain indicates the presence of fixed oil. Extract was treated with few drops of 0.5N potassium hydroxide and few drops of phenolphthalein and heat, formation of soap indicates the presence of fixed oil and fats.

Fluorescence analysis

Fluorescence analysis of the plant powder was observed in daylight and UV light (254nm) in a UV chamber (Kokoshi et al., 1958; Harborne, 1973) after treating with different chemical reagents is reported (Table 1). It can be as a diagnostic tool for testing the adulterations.

Quantitative study of phytochemicals

Determination of alkaloid (Bohm and Kocipai Abyazan, 1994)*:* Five gram of the sample was¬ weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Determination of flavonoid (Obadoni and Ochuko, 2001)*:* Ten gram of the plant¬ sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Determination of saponin (Zafar and Lalwani, 1989)*:* Twenty gram of each grounded sample was put into a conical flask and 100cm3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel, added20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.

Determination of glycosides: There were two tests performed for the determination of glycosides as detailed below.

Test 1: Extract of 200mg of the sample was taken warmed in a test tube with 5ml of diluted 10% sulphuric acid on the water bath at 1000 c for 2 min. Centrifuged; pipette off the supernatant. The acid extract was neutralized with 5% solution of NaOH. Added 0.1 ml of Fehling's solution A and then Fehling's solution B until alkaline (tested with pH paper) and heated on the water bath for 2 min. Noted the quality of red precipitate formed and compared with that formed in Test 2.

Test 2: Extract of 200mg of the sample was taken an added 5ml of water instead of Sulphuric acid. After boiling added a volume of water equivalent to the volume of NaOH used in the Test 1, step 2 and above. Added 0.1 of Fehling's solution B until alkaline (tested with pH paper) and heated on the water bath for 2 min. Note the quantity of red precipitate formed (Test 2). Compared the quality of the precipitate formed in test 2 that formed in Test 1. The precipitate in Test 1 was greater than that in Test 2. That indicated the presence of glycoside in the crude drug (Rai et al., 2008).

Determination of lignins: Two grams of the oven-dry material passed through an 80-mesh sieve were extracted in a Soxhlet apparatus with 200ml, 95% alcohol for 4 h. The extracted residue was transferred to a round-bottomed flask of 300ml capacity and extracted with 150ml boiling water under a reflux for one hour. The contents of the flask are then filtered and residue was transferred back to the flask and hydrolyzed with 150ml 5% H for one hour. The hydrolyzed product was then collected in a weighed sintered glass crucible after being washed free of acid. The residue is weighed and well powdered. 0-2g lots were weighed out into 1000 ml beakers and treated with 20ml 72% H_2SO4. The powder was well mixed with the acid and allowed to stand overnight, at a temperature varying between 20 and 220 (this was the range between 4p.m and 8a.m. the following morning). The following morning the contents of the beakers were made up to 800ml. with water and boiled for 2 h, the volume being maintained by occasional addition of water. Leave the beakers overnight to settle down the Lignin's, thereby facilitating filtration. The filtration was done under suction in crucibles with Whatmann filter paper. After drying, the precipitates were weighed and ignited and the lignin's calculated on an ash-free basis.

Determination of phytostroids: One gram of powdered dried sample was extracted 3 times using a vortex mixture (15min) with 7.5 ml chloroform. All the extracts were combined and evaporated to dryness. This chloroform extract contains free sterols and terpernoids. The residue was hydrolyzed with 2N HCl in methanol (2h, 75-80°C), neutralized with 10N NaOH, then diluted with 25ml water, and then the steroidal alkaloids and saponins were extracted 3 times with 10ml chloroform. The chloroform phase was collected and evaporated to dryness. The amounts of phytosteroids were weighed.

Determination of fixed oil

Transfer a 50g of the air dried, crushed drug to an extraction thimble, extract with Solvent ether in a continuous extraction apparatus (Soxhlet extractor) for 6 h. Filter the extract quantitatively into a tarred evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105º C to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

The result of fluorescence analysis, qualitative and quantitative analysis of *Sida rhombifolia* have been presented and discussed herewith. Fluorescence analysis of drug showed in Table 1 is an important parameter in detecting adulteration or improper handling of drugs. It can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application.

Table 1. Fluorescence analysis of *Sida rhombifolia* in day light and in UV light.

S. No.	Chemical test	Day light	UV light
1	Sample	Yellow	Dark yellow
2	Powder + 1N aq. NaOH	Yellow	Green
3	Powder+ 1N alc. NaOH	Yellowish green	Dark green
4	Powder + 1N HCl	Greenish yellow	Dark green
5	Powder + 50% HNO3	Light green	Green
6	Powder + 50% H2SO4	Light yellow	Green
7	Powder + Methanol	Green	Dark green
8	Powder + NH3	White	Green
9	Powder + I 2	Reddish orange	Yellowish brown
10	Powder + FeCl3	Brownish orange	Dark brown

Results from the phytochemical screening test in Table 2 indicated the presence of phenolic compounds, flavanoid, phytosterol, alkaloids, glycoside and saponin in the aerial parts of the *Sida rhombifolia*. Tannins and volatile oils were found to be absent.

Table 2. Phytochemical screening of the ethanolic extract of *Sida rhombifolia.*

S. No.	Phytochemical constituents	Presence/ absence
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Saponins	+
5	Tannins	–
6	Phytosterols	+
7	Proteins	+
8	Flavonoids	+
9	Lignin	+
10	Volatile oils	–
11	Fixed oils and fats	+
'+' present; '–' absent.		

The phenolic compounds are considered as being a major group to the number of the secondary metabolites that contributes to the antioxidant activity of the plant. The presence of phenolic compounds in the plant indicates that this plant may have the ability as an anti-microbial agent (Shivhare et.al 2010). It has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides and alkaloids. These phytoconstituents may be responsible for the many pharmacological actions of the plant like wound healing (Sharmila, 2007) cholesterol lowering (Delanty and Dichter, 2000) and antidiabetic activity. Phytosterols are an important breakthrough in the human fight against high cholesterol. It has been known that plant steroids and flavonoids are antioxidants. These antioxidants are compounds that reduce the formation of free radicals or react with and neutralize them thus potentially protecting the cell from oxidative damage. The results of quantitative phytochemical evaluation of *Sida rhombifolia* are shown in Table 3.The results revealed the presence of alkaloids, glycosides, saponins, phytosteroids, flavonoids, lignin and fixed oils.

Table 3. Quantitative phytochemical evaluation of *Sida rhombifolia.*

S. No.	Phytochemical constituent	Content
1	Total alkaloids (mg/kg)	1.78
2	Glycosides (mg/kg)	0.16
3	Saponins (mg/kg)	0.15
4	Phytosteroids (mg/kg)	0.02
5	Total flavonods (mg/kg)	0.84
6	Lignin (mg/kg)	0.05
7	Fixed oils (µg/l)	0.19

On the other hand, the macronutrients; proteins, carbohydrate and reducing sugar are involved in the energy giving and body building function of this plant.

Conclusion

The results obtained from the phytochemical analysis of the areal part of *Sida rhombifolia* showed the presence of alkaloids, steroids, flavonoids, Phytosterols, lignins, proteins, carbohydrates, reducing sugars, fats and oil. This justifies the use of *Sida cordifolia* in the treatment of many ailments including arthritis, diabetic mellitus, carcinoma, heart disease and asthma. Non-nutrient phytochemicals act as antioxidant against dangerous free radicals in the body system (Ranjani, 2015).The present study suggest that the identified phytochemical compounds may be the bioactive constituents which are medicinally valuable. Therefore, the extracts from this plant could be seen as a good source for useful drugs and further work is in progress to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

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