Phytochemical study on medicinal plant – *Sida cordifolia* Linn

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Abstract— *Medicinal plants form the major source of drugs in all the traditional systems of medicine practiced in Sri Lanka, India viz., Siddha, Ayurveda, Unani and Homeopathy. There is a growing importance in medicinal plants and traditional health systems providing health care for a wider population across the globe, especially, in the developing countries. Sida cordifolia Linn is belonging to the family Malvaceae. Text states that the Sida cordifolia has analgesic antispasmodic anti-inflammatory, hypoglycemic and hepatoprotective activities. This species is found in tropical and subtropical regions of Sri Lanka and India. The aim of this study is to determine the phytochemical constituents of Sida cordifolia. Phytochemistry helps in standardizing the herbal preparations and possibly relate the constituents to their medicinal/ pharmacological uses. The matured areal parts of Sida cordifolia were collected during the month of July-august 2012 from Jaffna, Sri Lanka.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. Sida cordifolia showed the presence of alkaloids, glycosides, phytosterol, flavonoids, lignins, protein and saponins. The quantitative studies revealed that Sida cordifolia possessed alkaloids (1.99mg/kg), flavonoids (0.92mg/kg), lignin (0.08mg/kg), glycosides (0.19mg/kg), saponins (0.17mg/kg), phytosterols (0.02mg/kg), fixed oils (0.18 µg/lit). Phytochemicals act in numerous ways to assist the human body in combating disease and health problems. 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. The Sida cordifolia studied here can be seen as a potential source of useful drug. It is also justify the traditional medical uses and the claims about the therapeutic values of this plant as curative agent. Further to this the isolation, identification, characterization and elucidation of the structure of the bioactive compounds of Sida cordifolia would be obtained with a view to obtain useful chemotherapeutic agent.*

Keywords— **Phytochemical, *Sida cordifolia*, Medicinal plants**

I. INTRODUCTION

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 (Narendra et al., 2013). Medicinal plants form the major source of drugs in all the traditional systems of medicine practiced in Sri Lanka, India viz., Siddha, Ayurveda, Unani and Homeopathy. There is a growing importance in medicinal plants and traditional health systems providing health care for a wider population across the globe, especially, in the developing countries. The World Health Organization (WHO) currently encourages, recommends and promotes traditional remedies in health care programs as they are easily available at low cost, comparatively safe and are culturally acceptable (WHO 1998). Every culture has depended on the healing power of herbs.

Traditional medical texts states that the *Sida cordifolia* posses analgesic, antispasmodic anti-inflammatory, hypoglycemic and hepato-protective activities (Anilkumar 2010., Indian Pharmacopoeia 1996). This plant is used in asthma, nasal congession, skin disease, uninary diseases, obesity, cardiac diseases, bleeding haemorrhoids and preparation of anelgesic massage oils (Nadkarni 1976). Kirtikar and Basu stated that *Sida cordifolia* is used in Arrhythmia, hemiplegia, sciatica, neuritis, neuralgia, epilepsy, rheumatism, anorexia, fatigue, impotence, spermatorrhea, gonorrhea, cystitis, leucorrhea, urinary frequency, diabetes, diarrhoea, dysentery, hemorrhoids, chronic fever (Kirtikar and Basu 1980).

This species is found in tropical and subtropical regions of Sri Lanka and India. It grows as wasteland weed. It is also known as the "Bala" in Hindi and Sanskrit (Nayar *et al*. 1956).

The aim of this study is to determine the phytochemical constituents of S*ida cordifolia* 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. Phytochemistry also helps in standardizing the herbal preparations.

Sida cordifolia Linn (Figure 1) belongs to the family Malvaceae. Found throughout the tropical and sub-tropical plains of India and Sri Lanka.

Fig 1. *Sida cordifolia*

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae
Order	Malvales
Family	Malvaceae – Mallow family
Genus	Sida L. – fanpetals
Species	Sida cordifolia L.

English : Country mallow,

Sanskrit : Bala

Tamil : Thuththi

Sinhala : Bala, babila

Botanical Description

Plant- an erect, perennial shrub, upto 1m. tall. Stem- Ascending, terete or sulcate, softly villious and densely stellate-pubescent all over. Leaves- Ovate or ovate-oblong, obtuse or subacute at apex. Flowers- Yellow, peduncles, axillary, jointed much above the panicles, upper flowers nearly sessile and fasciculate towards the tip of the branches forming subspicate inflorescence. Fruits- Subdiscoid, 6-8 mm across, mericarps 10, 3 sided. Seeds- Trigonous, glabours, tufted-pubescent near the hilum. Flowering & Fruiting Time - August-November in Jaffna conditions.

II. MATERIALS AND METHODS

Collection and Identification of plant materials

Areal parts of Sida cordifolia for the proposed study were collected during the month of July-August 2012 from Jaffna district, Sri Lanka and care was taken to select healthy plants. The identity of the plant specimens was confirmed by the use of local Floras (Kirthikar and Basu, 1965). The botanical identify was authenticated by a botanist, Department of Botany, University of Jaffna. 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.

A. Phytochemical study

1) 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.

2) 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 phyto-constituents present in it viz, alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats, phenolic compounds & tannins, proteins and free amino acids, flavonoids, lignins and saponins (Kokate, 1994, Harborne, 1998, Sofowora et al.1982, Trease and Evans, 1989).

Test for Phenolic compounds- Two to three drops of 1% ferric chloride ($FeCl_3$) 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 5ml 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 2ml 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 HNO_3. 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.

B. Fluorescence Analysis

Fluorescence analysis of the plant powder was observed in daylight and UV light (254nm) in a UV chamber (Chase Pratt, 1949, Kokoshi et al. 1958) after treating with different chemical reagents is reported (Table 1). It can be as a diagnostic tool for testing the adulterations.

C. Quantitative study of Phytochemicals

1) Determination of Alkaloid - Harborne (1973): 5 g 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.

2) Determination of Flavonoid - Bohm and Kocipai Abyazan (1994): 10 g 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 No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

3) Determination of Saponin - Obadoni and Ochuko, (2001): 20 g of each grounded sample was put into a conical flask and $100cm^3$ 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 separatory 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.

4) Determination of Glycosides:

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 100^0c for 2 minutes. 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 minutes. 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 minutes. 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 (Zafar R, Lalwani).

5) Determination of Lignins: 2gms of the oven-dry material passed through an 80-mesh sieve were extracted in a Soxhlet apparatus with 200ml, 95% alcohol for 4 hours. The extracted residue was transferred to a round-bottomed flask of 300ml capacity and extracted with 150ml boiling

water under a reflux for 1 hour. The contents of the flask are then filtered and residue was transferred back to the flask and hydrolyzed with 150ml. 5% H 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% H2SO4.

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 hours, 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 Gooch crucibles with No. 42 Whatmann filter paper. After drying, the precipitates were weighed and ignited and the lignin's calculated on an ash-free basis.

6) Determination of Phytostroids: 1g 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 hydrolysed with 2N HCl in methanol (2hr, 75-80^0 c), neutralized with 10N NaOH, then diluted with 25ml water, and then the steroidal alkaloids and sapogenins were extracted 3 times with 10ml chloroform. The chloroform phase was collected and evaporated to dryness. The amounts of phytosteroids were weighed.

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

III. RESULTS AND DISCUSSION

The result of Fluorescence analysis, Qualitative and quantitative analyses of *Sida cordifolia* are presented and discussed below.

When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study (Rai et *al*. 2008).

Fluorescence analysis of drug 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.

Serial No	Chemical test	*Sida cordifolia* Day light	*Sida cordifolia* 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	Green
5.	Powder + 50% HNO_3	Light green	Dark green
6.	Powder + 50% H_2SO_4	light yellow	Green
7.	Powder + Methanol	Green	Blackish green
8.	Powder + NH_3	White	Green
9.	Powder + I_2	Reddish orange	Yellowish brown
10.	Powder + $FeCl_3$	Brownish orange	Dark brown

Table1. Fluorescence analysis of *Sida cordifolia*

Serial No	Constituents	*Sida cordifolia* ethanolic extract
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4	Saponins	+
5.	Tannins	-
6.	Phytosterols	+
7.	Proteins	+
8.	Flavonoids	+
9.	Lignin	+
10.	Volatile oils	-
11.	Fixed oil and fats	+

Table 2. Phytochemical Screening of the ethanolic extract of *Sida cordifolia*

Result from the phytochemical screening test in Table 2 indicated the presence of phenolic compounds, flavanoid, phytosterol, alkaloids, glycoside and saponin in the areal parts of the *Sida cordifolia*. 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 (Harisharanraj *et al*. 2009).

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 (Shivhare et al., 2010) cholesterol lowering

(Sharmila *et al*. 2007) and antidiabetic activity. Phytosterols are an important breakthrough in the human fight against high cholesterol. It has been known that plant steroids, flavonoids are antioxidents. These antioxidents are compounds that reduce the formation of free radicals or react with and neutralize them thus potentially protecting the cell from oxidative damage (Delanty, 2000).

Serial No	Parameters	*Sida cordifolia*
1.	Total Alkaloids (mg/kg)	1.99
2.	Glycosides (mg/kg)	0.19
3.	Saponins (mg/kg)	0.17
4.	Phytosterols (mg/kg)	0.02
5.	Total Flavonoids (mg/kg)	0.92
6.	Lignin (mg/kg)	0.08
7.	Fixed oil (µg/lit)	0.18

Table 3. Quantitative phytochemical evaluation of *Sida cordifolia*

Serial No	Constituents	*Sida cordifolia* ethanolic extract
1.	Carbohydrates	+
2.	Reducing Sugar	+
3.	Protein	+
4.	Fixed oil and fats	+

Table 4. Results of the Macronutrient analysis of Sida Cordifolia

The results of the micronutrient analysis showed the presence of carbohydrate, fats, protein, reducing sugar and oil were present (Table 4).

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

IV. CONCLUSION

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

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