

Quantitative and Qualitative identification of Phytochemical Constituents of *Sida* *rhombifolia* leaves extract

Anooj E.S, Amrutha T.M, M.Charumathy, Lekshmi Gangadhar

Abstract: *Anti-inflammatory, analgesic, antimicrobial, anti-arthritic, antibacterial, antispasmodic, hypoglycaemic and hepatoprotective characteristics of Sida rhombifolia are included in the Malvaceae family. Most of the plant belongs to the Malvaceae family, as they comprise a number of phytochemicals and biological compounds, are potential sources of different medications. This is study on Sida rhombifolia leaf extract's tophytochemical constituents. Phytochemical screening results in herbal standardization and preparation and may relate the components to their medicinal / pharmacological uses. The qualitative phytochemical analysis has shown that the extract is positive for saponins, flavonoids, alkaloids, phenols and same extract is negative for carbohydrate, tannins, glycosides, cardiac glycosides, terpenoids, coumarins, steroid & phytosteroids, phlobutanins, anthraquinones. Quantitative analysis of phytochemicals includes the estimation of flavonoid, tannin and total content of phenol. The result suggest that the Sida rhombifolia leaves extract consist plenty of phytochemicals beneficial in alternative medical and pharmaceutical industries.*

Index Terms: *Sida rhombifolia, Malvaceae, Phytochemicals, Phytochemical analysis.*

I. INTRODUCTION

Head

One of the two hundred species in the *Sida* genus is *Sida rhombifolia* (Figure 1). It was discovered in warm and humid areas and dispersed all over the tropics [1]. *Sida rhombifolia*

Revised Manuscript Received on July 05, 2019.

Dr. M. Charumathy, ²Assistant Professor, Department of Biochemistry, Marudhar Kesari Jain College For Women, Vaniyambadi -635751, Tamilnadu, India, sarumathym09@gmail.com

Amrutha T.M, Department of Nanotechnology, Noorul Islam Centre for Higher Education, Kumaracoil, Kanya kumari District. Tamilnadu, India

Anooj E.S, Lekshmi Gangadhar, Xcellogen Biotech INDIA Pvt Ltd, Nagercoil, Tamilnadu, India, xcellogenbiotech@gmail.com

belongs to the Malvaceae native to the new world tropics and climatic zone. It's usually referred to as arrow leaf *Sida*, jellyleaf etc. *S. Rhombifolia* might be somewhat erect woody, variable yearly or perpetual bush concerning 1.5 meters high with harsh branches and symmetrical hairs. Leaves region unit frightfully factor in get along to 5mm by 18mm, short petiole, rhomboid-lanceolate to pointed, unpleasant towards the most astounding, whole towards the base. The blossoming and develop inside the plant start from september to december. Blossoms are yellow or white, axillary, lone or two by two. The leaves are decreased on the blooming branches. The natural products are discouraged, globose, schizocarpic, encased inside the curl, isolating into one-seeded indehiscent unit. Seeds are dark and wash [2].



Kingdom: Plantae
Division: Tracheophyta
Class: Mangnaliopsida
Order: Malvales
Family: Malvaceae
Genus: *Sida*
Species: *Sida rhombifolia*
Linn.

Figure1: *Sida rhombifolia*

Sida rhombifolia is asserted for the treatment of various diseases such as rheumatism, seminal weakness and diarrhoea [3]. *Sida rhombifolia* is far used for treating ulcers, inflammation, swellings and ant nociceptive [4]. *S. rhombifolia* is thought for its great choice of medicative uses. This plant's infusion is used locally to treat skin illnesses and infected wounds [5]. The roots are used to treat rheumatism and abdomen illnesses, digestive drawback, infectious disease, chickenpox, blood purification and tiredness [6] headache and migraine headache (fruits), fever, infection with gum, inflammation, tonsillitis, cuts, injuries and ophthalmia [7].

The infusion of dried leaf of *S. rhombifolia* is used for diabetes, chest pain and



Quantitative and Qualitative identification of Phytochemical Constituents of *Sida rhombifolia* leaves extract

diarrhoea on oral administration. *S. rhombifolia* showed many pharmacological operations, including antibacterial ones, antifungal, anti-arthritis, anti-inflammatory, anti-diarrheal, anti-diabetic, wound healing, hypotensive, nephrotoxicity, anti-malarial, antioxidant, hepatoprotective [8]. The World Health Organization (WHO) presently inspires, recommends and encourages traditional remedies in health care plans as they're simply obtainable at low value, relatively harmless and traditionally common [9].

The plant growths leads to the production various chemical compounds are called phytochemicals and refer as "secondary metabolites" that consist, flavonoids, alkaloids, coumarins, tannins, phenols, phlobatannins, anthraquinones, terpenoids, cardiac glycosides, steroid and phytosteroid [10]. It is known that the phytochemical compounds identified have medicinal significance. Alkaloids have been recorded as potent poisons, for instance, and many alkaloids from medicinal plants demonstrate biological activities such as antispasmodic and pharmacological impacts antimalarial, antimicrobial cytotoxicity, anti-inflammatory [11]. Similarly, steroids derived from plants are known to have cardiotoxic impacts and also have antibacterial and insecticide properties [12]. Because of their well-known biological activities, they are very often used in drugs. According to research, tannins have antibacterial actions, antitumor and antiviral [13]. Other phytochemicals called respiratory glycosides were handled for congestive heart failure and cardiac arrhythm. [14].

The medical uses *S. rhombifolia* lies within the bioactive components, that shows diverse physiological properties, could be detected through phytochemical analyses [15]. Therefore, the aim of this study to identify qualitative and quantitative photochemistry from the leaf extract of *Sida rhombifolia*. Phytochemical studies help in standardization of herbal preparation and understanding the importance of phytoconstituents in terms of their medical uses.

II. MATERIALS AND METHODS

2.1 Sample Preparation:

The blossoming plant of *Sida rhombifolia* leaves were gathered from street sides and around the Vellore locale, Tamil Nadu during December, 2018. The sample was authenticated from botanical survey of India. Thoroughly washed the leaves with tap water, after which washed with refined water. Air dried and grounded to coarse powder at that stage and placed away in an impermeable compartment.

2.2 Sequential Extraction:

Sida rhombifolia leaves about (100gm) of sample and 300ml of hexane for extraction kept for 24 hours.

The sample was filtered after extraction using Whatmann's filter paper. The filtered thus obtained was cooled and concentrated to dryness. Then dry leaf powder (100gm) was placed in 300ml of ethyl acetate for added 24 hours in maceration. After 24 hours mixed and then filtered thus obtained was cooled and concentrated to dryness. Then dry powder of leaves in (100gm) was placed 300ml of ethanol for 24 hours maceration. After 24 hours mixed and filtered thus obtained to cool. Then the extraction mixture was filtered and red concentrated of distilling unit. Then its residues were weighed and kept in bottle to use phytochemical and other biological screening methods. Then collected to filtrate.

2.3 Qualitative Phytochemical Tests:

2.3.1 Test for carbohydrate:

Added to 2ml of leaf extract 1ml of Molisch reagent and a few drops of concentrated sulphuric acid, appearance of purple or reddish shading demonstrates the presence of carbohydrate. Fehling A 1ml and Fehling B 1ml boiled for 10 minutes, presence of reddish darker (brown) shading.

2.3.2 Test for tannins:

Added to 1ml of leaf extract and 2ml of 5% ferric chloride lead to the appearance of dark greenish or dull blue shows the conformation of tannins.

2.3.3 Test for saponins:

In a graduated flask, 2ml of distilled water was added to 2ml of leaf extract and shaken for 15 minutes. Appearance of 1cm layer of a lather shows the conformation of saponins.

2.3.4 Test for flavonoids:

Added 1ml of 2N sodium hydroxide to 2ml of leaf extract. Yellow shading appearance shows the existence of flavonoids.

2.3.5 Test for alkaloids:

Added to 2ml of leaf extract 2ml of concentrated HCl. At that point, few drops of Mayer's reagent were added, the appearance of green or white precipitate shows the alkaloid conformation.

2.3.6 Test for quinones:

Added 1ml of concentrated sulphuric acid to 1ml of leaf extract. A red color appearance indicates the existence of quinones.

2.3.7 Test for phenol:

Mixed with 1ml of leaf extract and a few drops of phenol picric acid reagent followed by a few drops of 15% sodium carbonate. Blue (or) green appearance.

2.3.8 Test for glycosides:



Adding 3ml of chloroform and 10% ammonia solution to 2ml of leaf extract contributes to the formation of pink colour shows appearance of glycosides..

2.3.9 Test for cardiac glycosides:

Added 2ml of glacial acetic acid and a few drops of 5% ferric chloride to 0.5ml of leaf extract. This was layered with 1ml of concentrated sulphuric acid and dark colored ring growth at the interface suggests the existence of heart glycosides.

2.3.10 Test for terphenoids:

2ml of chloroform was added to 0.5ml of leaf extract, then concentrated sulphuric acid was closely added to the red brown color formation at the interface indicating terphenoid existence.

2.3.11 Test for anthraquinones:

Added 1ml of leaf extract and few drops of 10% of ammonia solution. The appearance of pink color precipitate shows the presence of anthraquinones.

2.3.12 Test for coumarins:

Added 1ml of leaf extract and 1ml of 10% of sodium hydroxide leads to yellow colour formation suggests presence of coumarin.

2.3.13 Test for steroid and phytosteroid:

Added to 1ml of leaf extract and equal volume of chloroform then exposed with few drops of concentrated sulphuric acid leading to brown ring formation shows the existence of steroids and bluish brown ring appearance suggests the existence of phytosteroids.

2.3.14 Test for phlobatannins:

Added 1ml of leaf extract and few drops of 2% of HCL suggests the existence of phlobatannins, leading to the appearance of red color precipitate.

2.4. Quantitative Phytochemical Tests:

2.4.1 Determination of flavonoid content:

Total flavonoid content was determined in the extract (ethyl acetate) Using this technique mentioned by Sakanaka (2005). Aluminum chloride technique used quercetin as normal determined the flavonoid content.

The extract and quercetin were prepared in ethyl acetate (10mg / ml). 0.1ml of extract was blended in test tubes with 0.9 ml of distilled water accompanied by an addition of 75µl of 5% sodium nitrate.

Added 10µl of 10% aluminum oxide solution after 6 minutes and mixture was allowed to stand for 5 minutes. After 0.5ml

of 1 M sodium hydroxide was added to the response mixture. The reaction mixture was carried to 2.5ml distilled water and blended well. The absorbance was immediately evaluated by the spectrometer at 510 nm. A calibration curve was obtained using distinct quercetin concentration and the sample equivalence (QE) was represented in the extract's µg / mg.

2.4.2 Determination of tannin content

Folin-ciocalteu technique was used to determine the content of tannin extract. 0.1ml of 1 mg leaf extract added to the test tube. Then added 7.5ml of distilled water and 0.5ml of folin-ciocalteu reagent, 1ml of 35% Na₂CO₃. It is diluted up to 10ml with distilled water. Then shaken well and held for 30 minutes at room temperature. Added a collection of standard tannic acid solution (20, 40, 60, 80, 100)(µg/ml). Standard solution absorbance calculated with respect to absorbance of blank at 725 nm with UV visible spectrometer and the concentration of tannin measured in mg of extract tannic acid equivalence (TE) µg / mg.

2.4.3 Determination of total phenol content:

The concentration of phenolic content in the extract compound was found by the calorimetric technique of folin-ciocalteu and calculated as standard from the calibration curve attained with gallic acid (10mg/10ml).

Different test tubes were taken from the standard solution 20-100µl and added. Leaf extract was added to separate test tubes at concentrated 10mg / ml and then folin-ciocalteu dilution (1:10) of 5ml was added and mixed thoroughly. 0.7 M sodium carbonate was added in 4ml quantity followed by mixing and 30 minutes incubation. The absorbance was measured at 765 nm UV visible spectrometer resulting in sample gallic acid equivalence (GE) µg / mg extract.

III. RESULT AND DISCUSSION

3.1 Qualitative phytochemical test:

In addition, the dried powdered sample was taken to qualitative testing according to conventional operation for the identification of phytochemical constituents.

Quantitative and Qualitative identification of Phytochemical Constituents of *Sida rhombifolia* leaves extract



Figure 1: The preliminary phytochemical investigation shows the presence of phytoconstituent such as the saponins, flavonoids, alkaloids, phenols was subjected to qualitative analytical test are present plant extract.

Phytochemical screening of crude hexane, ethylacetate and ethanol extracts from *S. rhombifolia* leaf samples disclosed the existence of certain secondary metabolites such as tannins, saponins, carbohydrates, alkaloids, quinones, flavonoids, glycosides, terpenoids, heart glycosides phenols, coumarins, phytosteroid, anthraquinones, phlobutanins, as shown in Table 1. *Sida rhombifolia* (L.) leaves have been subjected to the plant's qualitative analytical test.

Flavonoids, steroids, glycosides and alkaloids have been detected to be the most effective principles in plant. These phytoconstituents can be liable for many the pharmacological activities such as antidiabetic, cholesterol lowering and wound healing.

3.2 Quantitative analysis of *Sida rhombifolia*:

In addition that, dried powder sample was exposed to quantitative tests for the identified of phytochemical components according to standard procedure. The primary phytochemical investigation showed the occurrence of phytoconstituents flavonoid, tannin and phenolic content. The leaves of *Sida rhombifolia* was subjected to quantitative analytical tests for the plant.

3.2.1 Flavonoids estimation:

Flavonoids are very important because they assist the human body combat disease. Flavonoids' capacity to behave as powerful antioxidants relies on their molecular structures, hydroxyl group position, and further characteristics in their biochemical structure. As their glycoside they are discovered abundantly in plants [35]

Table 1: Phytochemical screening of the leaf extract of *Sida rhombifolia*

Sl. No	Test	Hexane	Ethyl acetate	Ethanol
1.	Carbohydrate	No	No	No
2.	Tannins	No	No	No
3.	Saponins	Yes	Yes	No
4.	Flavonoids	Yes	No	Yes
5.	Alkaloids	Yes	Yes	No
6.	Quinones	No	No	No
7.	Glycosides	No	No	No
8.	Cardiac glycosides	No	No	No
9.	Terpenoids	No	No	No
10.	Phenols	Yes	Yes	Yes
11.	Coumarins	Yes	No	Yes
12.	Steroids & Phytosteroids	No	Yes	No
13.	Phlobatanins	No	No	No
14.	Anthraquinones	No	No	No

Table 2: Concentration of flavonoids content in *Sida rhombifolia* (L.)



Quercetin calibration	Absorbance
Hexane	2.57%
Ethanol	16.85%

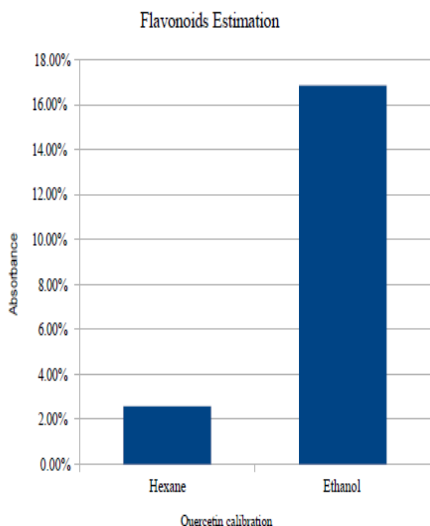


Figure 2: The primary phytochemical analysis of flavonoids in the leaf extract of *Sida rhombifolia* (L.)

The result clearly indicate that the flavonoid content in the leaf extract of *Sida rhombifolia* (L.) was found to be more in ethanolic extract (16.85%) when compared to the hexane(2.57%)

3.2.2. Phenol Estimation

The phenolic compounds are regarded the number of second ary metabolites that contribute to the plant's antioxidant activity as a significant group. The existence of phenolic compounds in the plant shows that as an anti-microbial agent this plant may have the ability. Plant polyphenols are the important group of complexes that act as free radical scavengers or main antioxidants, so determining phenolic content in plant extract is justifiable

Polyphenolic compounds, including their functional derivatives, have an aromatic benzene ring with replaced hydroxyl groups. These can absorb free radicals and metal ions that can catalyze the formation of ROS that promotes lipid peroxidation.

Table 3: Concentration of phenolic content in *Sidarhombifolia*(L)

Gallic acid calibration	Absorbance
Hexane	5.8%
Ethylacetate	13.8%
Ethanol	24.8

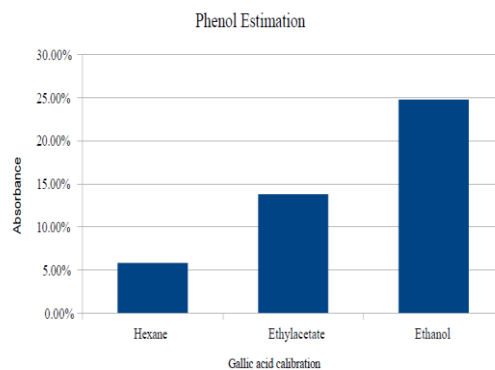


Figure 3: The primary phytochemical analysis of phenol in the leaf extract of *Sida rhombifolia* (L.)

The result clearly indicate that the phenolic content in the leaf extract of *Sida rhombifolia* (L.) was found to be more in ethanolic extract (24.8%) when compared to the hexane(5.8%) and ethyl acetate(13.8%)

IV. CONCLUSION

Sida rhombifolia (L.) encompasses a long history as a meditative plantwith numerous therapeutic applications. Thisresearch was aimed to analyze the effect of *Sida rhombifolia* (L.) (Family, Malvaceae) leaf extract. The qualitative phytochemical analysis has shown that the extract is positive for saponins, flavonoids, alkaloids, phenols, and same extract negative for carbohydrates, tannins, glycosides, cardiac glycosides, terpenoids, coumarins, steroids & phytosteroids, phlobutannins, anthraquinones. In this research, the recognized phytochemical compounds may be the medically precious bioactive components. Therefore *Sida rhombifolia* leaf extract could be considered as a good source of helpful drugs and further work on isolating, purifying and characterizing these phytochemical constituents. Thus, the above research concludes that its therapeutic properties may be due to the occurrence in this plant of certain flavanoid and phenolic compounds and other phytochemicals.

REFERENCES

1. Maberley DJ, Pignatti-Wikus E, Riedl-Dorn C. Ferdinand Bauer's field drawings of endemic Western Australian plants made at King George Sound and Lucky Bay, December 1801–January 1802.



Quantitative and Qualitative identification of Phytochemical Constituents of *Sida rhombifolia* leaves extract

- I. Rendiconti Lincei. 2000 Jun 1;11(2):69.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol. iii: 1968-1969. Lalit Mohan Basu. 1984;40.
3. Rainsford KD, Whitehouse MW. Anti-inflammatory/anti-pyretic salicylic acid esters with low gastric ulcerogenic activity. Agents and actions. 1980 Nov 1;10(5):451-6.
4. Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS, Israf DA. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. Fitoterapia. 2008 Dec 1;79(7-8):557-61.
5. Maheshwari JK, Singh KK, Saha S. Ethnomedicinal uses of plants by Tharus in Kheri district. UP, pg. 1980:318-37.
6. Woldeyes S, Adane L, Tariku Y, Muleta D, Begashaw T. Evaluation of antibacterial activities of compounds isolated from *Sida rhombifolia* Linn. Malvaceae). Nat Prod Chem Res. 2012;1(101):2.
7. Singh A, Dubey NK. An ethnobotanical study of medicinal plants in Sonebhadra District of Uttar Pradesh, India with reference to their infection by foliar fungi. Journal of Medicinal Plants Research. 2012 Apr 16;6(14):2727-46.
8. Mishra SH, Chaturvedi SC. Antibacterial and antifungal activity of alkaloid of *Sida rhombifolia* Linn. Indian drugs. 1978.
9. Muanza DN, Kim BW, Euler KL, Williams L. Antibacterial and antifungal activities of nine medicinal plants from Zaire. International Journal of Pharmacognosy. 1994 Jan 1;32(4):337-45.
10. Gupta SR, Nirmal SA, Patil RY, Asane GS. Anti-arthritis activity of various extracts of *Sida rhombifolia* aerial parts. Natural product research. 2009 May 20;23(8):689-95.
11. Dunstan CA, Noreen Y, Serrano G, Cox PA, Perera P, Bohlin L. Evaluation of some Samoan and Peruvian medicinal plants by prostaglandin biosynthesis and rat ear oedema assays. Journal of Ethnopharmacology. 1997 Jun 1;57(1):35-56.
12. Mullick R, Ranjan SR, inventors; General Electric Co, assignee. Method and system for performing image registration. United States patent US 7,995,864. 2011 Aug 9.
13. Dhalwal K, Shinde VM, Singh B, Mahadik KR. Hypoglycemic and hypolipidemic effect of *Sida rhombifolia* ssp. *retusa* in diabetic induced animals. International Journal of Phytomedicine. 2010 Apr 1;2(2).
14. Seshathri K, Thiyagarajan T. Antimicrobial Activity of Chewing Sticks of Jimma ,Ethiopia against. Journal of Phytology. 2011 Jul 18.
15. Mishra SH, Varma KC. Preliminary pharmacological investigations of alkaloid of *Sida rhombifolia* Linn.[India]. Science and Culture. 1979.