Phytochemical Screening of Aqueous Leaf Extract of Sida acuta Burm. F. and its Antibacterial Activity

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ABSTRACT: Sida acuta is one of the Indian medicinal plants which belong to the family Malvaceae. The whole plant is reported to have many biological activities such as anthelmintic, antiemetic, demulcent, diuretic, aphrodisiac, stomachic, diaphoretic, antipyretic and wound healing properties. Therefore main aim of the present study is to evaluate the phytochemical constituents and the antibacterial activity of the aqueous extract of Sida acuta. The preliminary phytochemical screening has shown the presence of alkaloids, steriods, flavonoids, phenols, terpenoids, and cardiac glycosides. The maximum zone of inhibition was observed against Bacillus subtilis and Escherichia coli at the maximum tested concentration. The aqueous leaf extract of S. acuta have shown moderate anti-bacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. From this study, was concluded that, Sida acuta has rich phytochemical compounds, .thus the presence of these secondary metabolites attributed to treat various diseases.

Keywords: Sida acuta, Aqueous extract, Phytoconstituents, Antibacterial activity.

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1. Introduction

Traditional use of medicine is recognized as a way to learn about the potential for future medicines. Plants are tremendous source for the discovery of new products of medicinal value for drug development. Many compounds are secondary metabolites which generally involved in plant adaptation to environmental stress conditions. Today several distinct chemicals derived from plants are important drugs used in one or more countries in the world. Many of the drugs are simple synthetic modifications or copies of the naturally obtained substances. A vast number of natural, plant-based extracts and chemicals proposed to have beneficial effects are present in India.

Phyto is the Greek word for plants; chemical compounds that arise naturally in plants are called phytochemicals. These phytochemical compounds protect themselves against environmental threats (disease, pollution, insects, etc.,). Plants are used throughout the world traditionally for home remedies over the counter drug products and raw substances for the pharmaceutical, cosmetics industries and represent a substantial proportion of the world drug market. It is therefore significant to establish their quality. Phytochemical evaluation is one of the tools for quality assessment, which includes preliminary phytochemical screening. Use of chromatography for standardization of plant products was introduced by World Health Organization (WHO) and is accepted as a strategy for identification and evaluation of the quality of plant medicines [1].

Natural products from microbial sources have been used as the primary source of antibiotics, but with the increasing recognition of plant-based herbal medicines as an alternative form of human health care industry. The screening of medicinal plants for active compounds has become very popular [2]. A more number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Nair and Chanda [3] discussed about the antimicrobial compounds from plants and validate their uses with composition. Thus, these medicinal plants are of extreme importance to the health of individuals and communities. On the other side, the chemical substances in plant produces some physiological action on the individual. The most important of these phytoconstituents of plants are alkaloids, flavonoids, tannins and phenolic compounds [4].

1.1. Sida acuta Burm.f.

Sida acuta Burm.f. (Family of Malvaceae) is an erect perennial shrub found throughout the hotter parts of India and Nepal [5]. It is believed to be originated in Central America and it is considered as a weed in some areas and the whole plant of *Sida acuta* is widely used in traditional medicine [6]. The bark is smooth, greenish, the root is thin, long, cylindrical and very rough; leaves are lance late, nearly glabrous, peduncles equal to the petioles (**Fig. 1**), seeds are smooth and black; the flowers are yellow, solitary or in pairs. In Indian traditional knowledge of medicinal plants, the whole plant is reported to have many biological activities such as anti-microbial, anthelmintic, anti-emetic, demulcent, diuretic, aphrodisiac, stomachic, diaphoretic, anti-pyretic and wound healing properties. The root of *S. acuta* is extensively used as a stomachic, diaphoretic and antipyretic [7]. Scientific research on the leaf of this plant reveals that it possesses many beneficial plant phytochemicals and its leaf extract has a great potential to be used in biological applications.

Fig. 1. Habit of *Sida acuta*

Kingdom : Plantae
Division : Angiosperms
Class : Dicotityledanay
Order : Malvales
Family : Malvaceae
Genus : *Sida*

Species : *acuta*

The plant *S. acuta* is a very common weed which is also useful in ayurveda. It is used in the treatment of malaria, diarrhea, asthma, headache, cold, fever, skin diseases, urinary disease, ulcer, snake bite, facial paralysis, anti-fertility agent and sedative [8-10]. The juice of the leaves is boiled in oil and applied to testicular swellings and in elephantiasis. In the Philliphines, leaves are employed for making poultices for sores and anticancer activity [11].

The present study deals with preliminary phytochemical analysis of the aqueous leaf extract of *S. acuta* and its antibacterial properties.

1.2. Target microorganisms

1.2.1. *Staphylococcus aureus*

S. aureus is a Gram-positive round shaped bacterium. *S. aureus* has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most staph infections are not serious, *S. aureus* can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections [12].

1.2.2. *Bacillus subtilis*

B. subtilis is a Gram-positive round shaped bacterium. *B. subtilis* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally accountable for product recalls due to food contamination [13].

1.2.3. *Escherichia coli*

E. coli is a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts and are occasionally responsible for product recalls due to food contamination. Several different *E. coli* strains can cause diverse intestinal and extraintestinal diseases by means of virulence factors that affect a wide range of cellular processes [14].

1.2.4. *Pseudomonas aeruginosa*

P. aeruginosa is a common Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans and act as a species of considerable medical importance. *P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, it's intrinsically advanced antibiotic resistance mechanisms and its association with serious illnesses, especially hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes. *P. aeruginosa* also cause eye infections in users of extended-wear contact lenses [15].

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

The *Sida acuta* Brum.f plant was collected from Maruthamalai hill, Coimbatore, Tamilnadu, India. The collected plant was identified at Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India with the authentication number BSI/SRC/5/23/2016/TECH/282.

2.2. Preparation of Aqueous Leaf Extract of *S. acuta*

About 10g of fresh *S. acuta* leaf was rinsed thoroughly with running tap water and followed by distilled water in order to remove soil and other bound particles. The leaf extract was prepared by putting 10 g of finely cut leaves in 100 mL of sterile distilled water and then boiled for 10 min at 60 °C. Then, the solution was removed from the heat source and left at room temperature. Following this step, the obtained extract was then filtered through a normal filter paper followed by Whatman No.1 filter paper. The plant extract was kept in refrigerator at 4ºC for further experiments.

2.3. Qualitative Phytochemical Analysis of Aqueous Leaf Extract of *S. acuta*

Phytochemical components of the aqueous leaf extracts of *S. acuta* were screened using methods outlined by Harborne (1984), Evans (1998) and Sofowora (2005). The components analyzed were Alkaloids, Flavonoids, Saponins, Phenol, Tannins, Steroids, Anthroquinone, Terpinoids, Phlobatannins and Cardiac glycosides [16-17].

2.3.1. Test for Alkaloids (Mayer's test): Alkaloid solution produces white yellowish precipitate when few drops of Mayer's reagents are added.

2.3.2. Test for Flavonoids (Shinoda's test): About 2 ml of aqueous solution of the extract was treated with 1 ml of 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

2.3.3. Test for Saponins (Frothing test): About 10ml of the aqueous extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth.
2.3.4. Test for Phenols (Ferric chloride test): About 5 ml extract was added to few drops of neutral 5% ferric chloride solution. A dark green color indicates the presence of phenolic compounds.
2.3.5. Test for Tannins (Ferric Chloride test): One ml of water and 1-2 drops of 0.1% ferric chloride solution was added with 1 ml of aqueous extract of *S. acuta* and the blue color observed indicates the presence of gallic tannins and the green black color indicates the presence of catecholic tannins.
2.3.6. Test for Steroids (sulphuric acid test): Two ml of acetic anhydride was added to 5 ml aqueous extract with 2 ml H_2SO_4. The colour change from violet to green or blue confirms the presence of steroids in sample.
2.3.7. Test for Terpenoids (Salkowski test): About 5 ml of leaf extract was mixed in 2 ml of chloroform, and 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration at the interface showed positive results for the presence of terpenoids.
2.3.8. Test for Cardiac glycosides (Keller-Killani test): About 5 ml of aqueous extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This mixture was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.
2.3.9. Test for Anthroquinone (Bontruger's test): About 3 ml of aqueous extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonical (lower) phase indicates the presence of free anthraquinones.
2.3.10. Test for Phlobatannins: (hydrochloric acid test): Deposition of a red precipitate when 2 ml of extract was boiled with 1 ml of 1% aqueous hydrochloric acid was used as evidence for the presence of phlobatannins.

2.4. Preparation of Bacterial Inoculum

The Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and Gram-negative bacterium *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) were pre-cultured in Nutrient Broth (NB) over night in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min and the cell density was standardized spectrophotometrically (A_{610} nm).

2.5. Anti-bacterial Assay

The anti-bacterial activities of aqueous leaf extract of *S. acuta* was tested against both Gram positive and Gram negative human pathogens by the standard well diffusion method. After solidification, the test human pathogens were seeded into respective medium by spread plate method with 10 µL of 24h old respective bacterial culture at 10^5 cells/mL cultures of bacteria grown in nutrient broth. Three wells of 5 mm diameter were made on pre-incubated nutrient agar plates using gel puncture. Each well was loaded with different volumes such as 20µL, 40µL and 60µL of the prepared aqueous leaf extract of *S. acuta* solution using micropipette. The plates were then incubated at 37°C for 24h. The inhibition zone around the well was measured in millimeter and recorded. Amoxicillin (Hi-Media) was used as the positive controls against test bacterium to compare the efficacy of the test samples.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening of Aqueous Leaf Extract of *Sida acuta*

The preliminary photochemical screening of the aqueous leaf extract of *S. acuta* showed the presence of following various phytoconstituents (**Table 1)**.

The results showed the presence of alkaloids, steriods, flavonoids, phenols, terpenoids, and cardiac glycosides. However, there was absence of tannins, saponins, anthroquinones and phlobatannins. Raimi coworkers [18] also reported that the phytoconstituents such as alkaloids, flavonoids, terpenoids and phenolics were observed in the *S. acuta* leaf sample and in contrast to our study they observed the presence of tannins, saponins. The chloroform and ethanolic extract of *S. acuta* showed the presence of carbohydrates, alkaloids, phytosterols, saponins and fixed oils [10].

The study of Richa coworkers [19] indicated the presence of high amounts of alkaloids, flavonoids, terpenoids and glycosides in methanolic leaf extract of *S. acuta*. Presence of these phytoconstituents in *S. acuta* showed excellent anti-bacterial and anti-malarial properties [20-21].

Table 1: Qualitative analysis of the phytoconstituents

S.No	Phytoconstituents	Aqueous leaf extract of *Sida acuta*
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Saponins	Negative
4	Phenols	Positive
5	Tannins	Negative
6	Steroids	Positive
7	Anthroquinones	Negative
8	Terpenoids	Positive
9	Phlobatannins	Negative
10	Cardiac glycosides	Positive

3.2. Anti-bacterial activity of aqueous leaf extract of *S. acuta*

The anti-bacterial activity of the prepared aqueous leaf extract of *S. acuta* against Gram positive and Gram-negative pathogens investigated using standard Zone of Inhibition (ZOI) method at different concentrations of 20, 40 and 60 µL were shown in Table.2. Significant

anti-bacterial properties were obtained against the tested gram positive and negative bacterial pathogens when compared with positive control. The diameter of inhibition zones increased when there was increase in the concentration of the aqueous leaf extract of *S. acuta* against the test pathogenic bacteria.

Table 2: Anti-bacterial activity of aqueous leaf extract of *S. acuta*

S. No	Bacterial Strains	Zone of Inhibition (mm)			
		Aqueous Leaf Extract of *S. acuta*			Reference Drug
		20 µL	40µL	60µL	
1	*B. subtilis*	08	11	14	09
2	*S. aureus*	07	10	13	07
3	*E. coli*	08	12	15	09
4	*P. aeruginosa*	08	09	11	08

The maximum zone of inhibition was observed against *B. subtilis* and *E. coli* at the maximum tested concentration of 60 µL. The aqueous leaf extract of *S. acuta* have shown moderate anti-bacterial activity against *S. aureus* and relatively less activity against *P. aeruginosa*. Figure 2 showed anti-bacterial activity of aqueous leaf extract of *S. acuta* against all the four tested human pathogens.

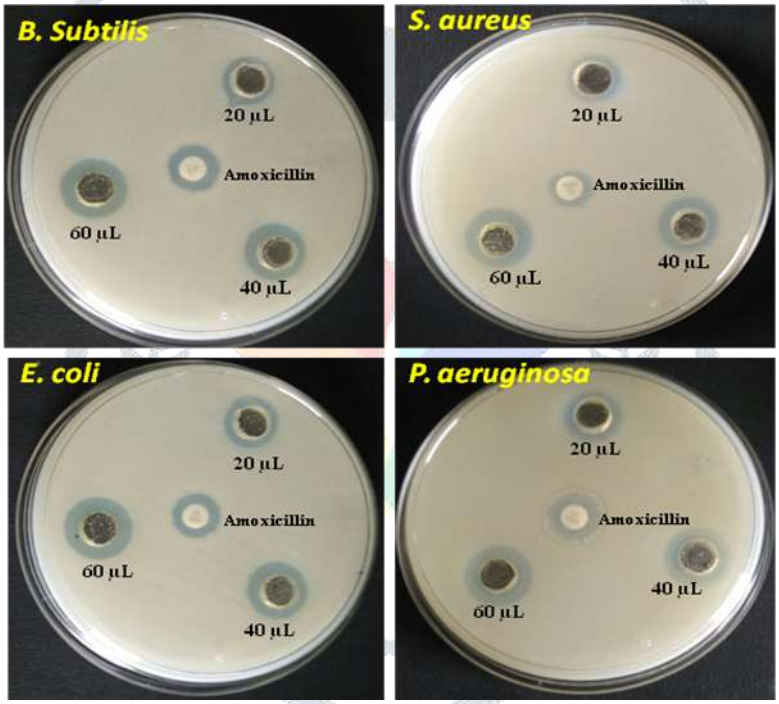

Figure 2: Anti-bacterial Activities of aqueous leaf extract of *S. acuta*

It was evident from the figure 2 that while increasing the aqueous leaf extract of *S. acuta* concentration an increasing zone of inhibition was observed against the growth of human pathogens. However, prepared plant extract was showed better anti-bacterial activity against *B. subtills* and *E. coli.* Biologically active phytoconstituents are present in *S. acuta* leaf extract and its showed excellent anti-bacterial properties against Escherichia coli, Staphylococcus aureus, Proteus mirabilis and Plasmodium falciparum [20-21].The standard drug Amoxicillin also showed anti-bacterial activity against all tested bacterial pathogens. In support to our reports, Damintoti coworkers [22] have also proved the alkaloids had a good antimicrobial activity against the test microorganisms. In the agar-well diffusion assay, highest inhibition zone diameters were recorded with Gram-positive bacteria.

4. Conclusion

In the present study the photochemical components of alkaloids, steriods, flavonoids, phenols, terpenoids, and cardiac glycosides were present in the aqueous extract of *S. acuta*. Biologically safe, eco-friendly and active drug and effective as antibacterial agent. Usually medicinal plants contain numerous phytochemical compounds, which are very much necessary to control the growth of the microorganisms. Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in human beings which may also have lower cost and lesser toxicity. Therefore, based on the results it can be concluded that the aqueous extract of *S. acuta* may hold enormous resource of pharmaceutical properties.

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