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Research Article

PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIMICROBIAL POTENTIAL OF *SENNA ALATA* LINN. LEAVES EXTRACT

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ABSTRACT

Objective: The objective of the present work is to evaluate the presence of phytochemical constituents and antimicrobial activity of different extracts from the leaves of *Senna alata* Linn.

Methods: The serial exhaustive extraction was done with various solvents: Aqueous, chloroforms, ethanol, methanol, acetone, benzene, petroleum ether with increasing polarity using soxhlet apparatus. The phytochemical analysis was done using the standard procedure. Antimicrobial activity was evaluated by disc diffusion method using leaves extract against various human pathogens.

Results: The results revealed that the leaves extracts contain flavonoids, terpenoids, tannins, phlobatannins, saponins, cardiac glycosides, carbohydrate, protein, and anthraquinones in major proportion. The aqueous extract was shown to be more effective against all the organisms followed by ethanol, chloroform, methanol, acetone, benzene, petroleum ether extracts. *Salmonella typhi* (28 mm) and *Bacillus subtilis* (28 mm) were found to be most sensitive organism followed by *Pseudomonas fluorescens* (27 mm) and *Escherichia coli* (27 mm).

Conclusions: It can be concluded that the different extracts of *S. alata* leaves extract contain a broad spectrum of secondary metabolites and also exhibit antimicrobial activity against all the tested microorganisms. Further phytochemical research is needed to identify the active product of *S. alata* may serve as leads in the development of new pharmaceuticals.

Keywords: Phytochemical analysis, Antimicrobial potential, *Senna alata*, Disc diffusion, Human pathogen.

INTRODUCTION

Plant-derived medicines have been part of traditional health care in most in most parts of the world for thousands of years, and there is an increasing interest in plants as sources of agents fight against microbial diseases [1]. The increasing development of drug resistance in human pathogens, as well as the unwanted side effects of some commonly, use antimicrobial agents prompted the search for newer agents with promising effectiveness and safety [2]. The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. Over the past few years, the medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine in view of its lesser side effects compared to allopathic medicine, in addition, the necessity of meeting the requirements of medicine for an increasing human population. An ever increasing global inclination toward herbal medicine for healthcare and their boom in recent years has imposed a great threat to the conservation of natural resources and endangered plant species. Currently, 4000-10,000 medicinal plants are on the endangered species list, and this number is expected to increase [3].

Most of the pharmaceutical industries are highly dependent on the wild population for the supply of raw material for extraction of medicinally important compounds. The genetic diversity of medicinal plants in the world is getting endangered at an alarming rate because of ruinous harvesting practice and over-harvesting for the production of medicines, with little or no regard to the future. Furthermore, extensive destruction of the plant-rich habitat as a result of forest degradation, agriculture encroachments, and urbanization. In modern medicine, plants are used as sources of direct therapeutic agents, as a model for new synthetic compounds and as a taxonomic marker for the elaboration of more complex semi-synthetic chemical compounds [4].

One of the surveys conducted by the WHO reports that more than 80% of the world's population still depends on the traditional medicines for various diseases [5,6]. Forced with the growing resistance of organisms to antibiotics and other drugs, the search for alternatives is urgent [7-9]. Herbal Ayurveda products are used as medicines in the form of either extracts or powder; and, they do have growth inhibitory effect against microbial pathogens. Many scientists have validated the biological activities of plants and their chemical constituents and demonstrated that aqueous and alcoholic extracts of several plants elicit antibacterial activity [10-13].

Plants were evaluated for their antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* which are most common pathogens causing serious infections [14]; and *Escherichia coli* which is an opportunistic pathogen at the site of the cut wound. *S. aureus* and *P. aeruginosa* are most common pathogens which infect the skin [15,16]. *S. aureus* express surface proteins that promote attachment to host proteins that form part of the extracellular matrix on epithelial and endothelial cell surfaces as well as being a component of blood [17,18]. Of the two million nosocomial infections each year, 10% are caused by *P. aeruginosa* [18,19].

In the worldwide as well as in the developing countries, the most human death due to infectious bacterial diseases [20]. The bacterial organisms including Gram-positive and Gram-negative such as different species of *Bacillus*, *Staphylococcus*, *Salmonella,* and *Pseudomonas* are the main source to cause severe infections in humans. Because these organisms have the ability to survive in harsh condition due to their multiple environmental habitats [21].

The methanol extract of *Physalis minima* was reported to possess antibacterial activity when tested by disc diffusion method against Gram-positive and Gram-negative bacteria. The various extract

(benzene, chloroform, hexane, methanol, and petroleum ether) of dried fruits of were tested for their antibacterial activity. Acetone and chloroform extracts showed activity against all the foodborne pathogens tested such as *Bacillus subtilis, E. coil, and Enterococcus faecalis, P. aeruginosa* and *S. aureus* [22,23].

Chloroform, diethyl ether, ethanol, ethyl acetate, and methanol were found to possess antibacterial activity against *Bacillus cereus, B. subtilis, Enterobacter aerogenes, E. coil, P. aeruginosa, P. fluorescens, and S. aureus*. Overall antibacterial assay revealed that ethanolic extract was found to be more effective than the other solvents used [24]. Mature berries of *P. minima* are repeated to possess antibacterial potential against a battery of Gram-positive and Gram-negative bacterial strain when tested by using streak plate, well diffusion, and bioautographic methods [25].

The methanol extract of *Nigella sativa* (Ranunculaceae) for antimicrobial activity against bacteria such as *Aspergillus niger*, *Agrobacterium tumefaciens*, *Bacillus* sp., *Candida* sp., *Corynobacterium pyogens, E. coil, Proteus* sp., *Pseudomonas* sp., *Salmonella* sp., *S. aureus,* and results showed that it was active against all tested microorganisms; however, greater activity was recorded with *Bacillus* sp., hemolytic *E. coil*, *Proteus* sp., *Pseudomonas* sp., and *Salmonella* sp. and the activity of the mistletoe was least toward *S. aureus*. It also had antibacterial toward *A. tumefaciens* and antifungal activities against *A. niger* and *Candida* sp. [26].

Senna alata Linn is an important medicinal plant as well as ornamental flowering plants in the subfamily Caesalpinioideae. It also known as a candelabra bush, empress candle plant, ringworm tree, or "Candle tree." *S. alata* is native to Mexico, and can be found in diverse habitats. It is a large shrub with very thick finely downy branches. It is named for its flower buds which grow in a column and looks like fat yellow candles each complete with flames. It is found commonly in Somalia, Saudi Arabia, some parts of Pakistan and Kutch area of Gujarat. It is largely cultivated in Madurai, Ramanathapuram, Salem, and Tirunelveli, districts of Tamil Nadu for its medicinal values. In the tropics, it grows up to an altitude of 1200 m. It is an invasive species in Austronesia. The shrub stands 3-4 m tall, with leaves 50-80 cm long. The inflorescence looks such as a yellow candle. The fruit shaped like a straight pod is up to 25 cm long. Seed are distributed by water or animals. The seed pods are nearly straight, dark brown or nearly black, about 15 cm long and 15 mm wide. On both sides of the pods, there is a wing that length of the pod. A pod contains 50-60 flattened train angular seeds. The leaves close in the dark. In Sri Lanka, this is use an ingredient of Sinhala traditional medicine. It the Indian system of medicine, namely Ayurveda, Siddha, and Unani, decoctions of the leaves, flowers, bark, and wood are used in skin diseases such as eczema, pruritus, itching, and constipation [27].

S. alata leaves are found to possess anthraquinones [28], flavonoids [29], quinines, and sterols [30]. The constituents of the leaves have been investigated for their laxative [31], antibacterial [32], antifungal [33], anti-inflammatory, and analgesic effects [34,35]. The plant has widely been employed for combating dysentery, helminthic infections and stomach disorders. In Ghana and Nigeria, the decoctions of the fresh leaves, roots, and seeds have been used for the treatment of wound infections, skin diseases, bronchitis, asthma, and ringworm [36]. The leaves have been reported to be useful in the treatment of plant are not only important to the millions of the people to whom convulsions, gonorrhea, heart failure, abdominal pains, edema, and also as a purgative [37]. *S. alata* has been reported to certain Anthraquinones, and methanol fractions were found to be active against *Aspergillus flavor*. Traditional medicine serves as the only opportunity for healthcare but also to those who use plant products for various purposes in this daily lives and also as a source for new pharmaceuticals. In this study, the different extracts of *S. alata* were investigated for antibacterial activity.

METHODS

Chemical and media

All the solvents and chemicals used were of GR grade and were obtained from Merck India. The nutrient agar was obtained from Hi-media (Mumbai, India). Streptomycin and tetracycline were used as the reference antibiotics.

Collection of plants

S. alata plant was collected from the Ponmalai, Tiruchirappalli. Fresh plant material was washed under running tap water; air dried and then homogenized to fine powder. The powder was stored in airtight container at −20°C until further use.

Methods of extract preparation

The dried plant material of 1 kg was extracted with 2 L of ethanol in a soxhlet apparatus for 72 hrs at 50°C. After the extraction, the solvent was removed with the help of rotator evaporator. The same process was carried out to get aqueous, chloroform, methanol, acetone, benzene, and petroleum ether extracts.

Tested microorganisms

Bacterial cultures play a major role in the research of the antibacterial activities. *S. aureus, Staphylococcus epidermidis, Klebsiella pneumonia, E. coli, Alcaligenes* sp., *E. faecalis, Salmonella typhi, P. aeruginosa, B. subtilis, Pseudomonas fluorescens, and Staphylococcus* sp.

Phytochemical screening

The freshly prepared leave extracts of *S. alata* were qualitatively tested for the presence of chemical constituents. They identified by characteristic color changes and precipitation reactions using standard procedures [38,39].

Antimicrobial assay

The antimicrobial assay was carried out using disc diffusion method [40]. Streptomycin and tetracycline (50 µg/ml each) are used as reference drugs, and the corresponding solvents (ethanol, methanol, chloroform, acetone, benzene, petroleum ether, and

Table 1: Phytochemical screening of leave extract of *S. alata* Linn.

Phytochemical constituents	Ethanol extract	Methanol extract	Chloroform extract	Acetone extract	Benzene extract	Petroleum ether extract	Aqueous extract
Alkaloids	+	+	-	+	+	-	+
Flavonoids	+	+	+	+	-	-	+
Carbohydrates	+	+	+	+	+	-	+
Protein	+	+	-	-	-	+	+
Terpenoids	-	-	-	-	+	-	+
Tannins	+	+	-	+	+	-	+
Saponins	+	-	+	+	+	-	+
Anthraquinones	+	+	+	+	+	+	+
Phlobatannins	-	-	-	-	+	-	+
Cardiac glycosides	+	+	+	+	+	+	+

S. alata: Senna alata

aqueous) are used as positive controls. About 20 ml of nutrient agar medium for bacteria was poured in the sterilized petri dishes and allowed to solidify. The agar medium was spread with 24 hrs cultured 10^8 CFU/ml of microbial strains by a sterilized rod. Discs of 6 mm in diameter were made in the culture medium using sterile cork borers. About 50 µl, 75 µl, and 100 µl of the plants extracts (1 mg/ml) were added to the discs. Different volume impregnated disc were placed on the bacterial swapped plates. Plates were then incubated at 37°C for 24 hrs. Antimicrobial activity was evaluated by measuring the inhibition zone diameters in mm formed around the disc. The assay was carried out in triplicates.

RESULTS

Extraction

The total yield of the extracts obtained after removing the solvents in aqueous - 67g, ethanol - 15 g, methanol - 42 g, chloroform - 33 g, acetone - 43.7 g, benzene - 20 g, and petroleum ether - 55 g.

Phytochemical screening

Phytochemical evaluation of various leaves extracts of *S. alata* was done for the presence of alkaloids, flavonoids, carbohydrate, protein, saponins, terpenoids, tannins, anthraquinones, phlobatannins, cardiac glycosides, and the result is presented in Table 1.

Antimicrobial activity

The antimicrobial activity was examined by disc diffusion method. The aqueous extract of *S. alata* leaves exhibited potent antimicrobial activity toward all the microbes. The zones of inhibition values were presented in Table 2. *S. typhi*, *B. subtilis* were found to be more susceptible toward the aqueous and chloroform extract of leaves with a maximum inhibitory zone (28 mm).

Ethanol extract

The ethanol extract of *S. alata* was a wider spectrum of inhibitory activity on streptomycin. It was slightly sensitive to *S. aureus* (mm). But the extract on the other organisms had hardly revealed their activity, and also these results are represented in Table 2. As the volume increased, the zone of inhibition was also increased. The negative control (disc having only the solvent ethanol) expressed their inability for their antibacterial activity against all the Gram-positive and Gram-negative organisms used in this study.

Methanol extract

The methanol extract of *S. alata* had the wider spectrum of inhibitory activity on streptomycin-resistant *E. coli* when compared to all the other organisms. It was slightly sensitive to *Alcaligenes* sp. and *P. fluorescens* (mm) (Table 3).

Acetone extract

The extract collected by acetone displayed moderate inhibitory activity on *E. coli and S. aureus* (mm). Slight variation in the activity was observed as the volume increases. There was no activity in the negative control given in Table 4.

Chloroform extract

The effect of the extract on *E. faecalis, P. fluorescens, Staphylococcus* sp., *S. epidermis*, and *S. typhi* display almost equal inhibitory activity of the zone ranging from 20 to 30 mm in diameter (Table 5).

Petroleum ether extract

The extract collected by petroleum ether displayed moderate inhibitory on *K. pneumonia, E. coli, B. substilis, P. fluorescens*, slight variations in the inhibitory activity was observed as the volume increases (Table 6).

Benzene extract

All the bacterial strains were susceptible to benzene extract and their inhibition zones from 10 to 27 mm. There was no activity in the negative control given in Table 7.

Table 2: Antimicrobial activity of ethanolic extract of *S. alata* leaves

Tested organisms	Zone of inhibition (mm)			
	Streptomycin	Ethanol extract		
		50 µl	75 µl	100 µl
S. aureus	15	25	25	25
S. epidermidis	13	11	11	13
K. pneumonia	15	19	19	12
E. coli	17	21	23	22
Alcaligenes sp.	15	10	15	18
E. faecalis	14	19	21	25
S. typhi	17	19	19	20
P. aeruginosa	13	11	12	12
B. subtilis	15	15	15	17
P. fluorescens	17	17	19	19
Staphylococcus sp.	18	18	18	19

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. subtilis: Bacillus subtilis, P. fluorescens: Pseudomonas fluorescens, S. alata: Senna alata

Table 3: Antimicrobial activity of methanol extract of *S. alata* leaves

Tested organisms	Zone of inhibition (mm)			
	Streptomycin	Methanol extract		
		50 µl	75 µl	100 µl
S. aureus	14	19	21	25
S. epidermidis	18	18	19	19
K. pneumonia	20	19	20	23
E. coli	13	18	18	21
Alcaligenes sp.	11	21	21	25
E. faecalis	13	16	16	17
S. typhi	15	20	20	25
P. aeruginosa	14	20	20	19
B. subtilis	13	22	22	23
P. fluorescens	15	20	20	25
Staphylococcus sp.	14	17	17	18

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. subtilis: Bacillus subtilis, P. fluorescens: Pseudomonas fluorescens, S. alata: Senna alata

Table 4: Antimicrobial activity of acetone extract of *S. alata* leaves

Tested organisms	Zone of inhibition (mm)			
	Streptomycin	Acetone extract		
		50 µl	75 µl	100 µl
S. aureus	17	11	11	11
S. epidermidis	18	10	11	10
K. pneumonia	17	10	20	22
E. coli	17	22	24	26
Alcaligenes sp.	15	14	22	14
E. faecalis	13	19	24	25
S. typhi	13	21	24	26
P. aeruginosa	18	11	21	23
B. subtilis	18	17	17	18
P. fluorescens	14	21	21	27
Staphylococcus sp.	15	11	14	14

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. subtilis: Bacillus subtilis, P. fluorescens: Pseudomonas fluorescens, S. alata: Senna alata

Table 5: Antimicrobial activity of chloroform extract of *S. alata* leaves

Tested organisms	Zone of inhibition (mm)			
	Streptomycin	Chloroform extract		
		50 µl	75 µl	100 µl
S. aureus	15	20	17	20
S. epidermidis	22	17	20	23
K. pneumonia	16	15	21	18
E. coli	18	19	18	22
Alcaligenes sp.	17	20	19	20
E. faecalis	16	21	25	25
S. typhi	10	17	24	28
P. aeruginosa	11	18	20	20
B. subtilis	15	21	21	24
P. fluorescens	10	20	20	25
Staphylococcus sp.	10	21	21	23

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. subtilis: Bacillus subtilis, P. fluorescens: Pseudomonas fluorescens, S. alata: Senna alata

Table 6: Antimicrobial activity of petroleum ether extract of *S. alata* leaves

Tested organisms	Zone of inhibition (mm)			
	Streptomycin	Petroleum ether extract		
		50 µl	75 µl	100 µl
S. aureus	14	17	21	19
S. epidermidis	20	11	20	20
K. pneumonia	15	16	20	23
E. coli	18	20	21	23
Alcaligenes sp.	13	19	20	20
E. faecalis	15	13	16	16
S. typhi	15	15	19	25
P. aeruginosa	14	23	22	21
B. subtilis	20	20	21	23
P. fluorescens	13	19	22	23
Staphylococcus sp.	20	20	19	24

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. subtilis: Bacillus subtilis, P. fluorescens: Pseudomonas fluorescens, S. alata: Senna alata

Table 7: Antimicrobial activity of benzene extract of *S. alata* leaves

Tested organisms	Zone of inhibition (mm)			
	Streptomycin	Benzene extract		
		50 µl	75 µl	100 µl
S. aureus	13	20	18	21
S. epidermidis	17	20	18	21
K. pneumonia	17	16	20	23
E. coli	15	13	24	27
Alcaligenes sp.	13	12	12	16
E. faecalis	18	13	15	16
S. typhi	13	13	19	24
P. aeruginosa	23	18	18	22
B. subtilis	17	19	20	21
P. fluorescens	18	15	19	23
Staphylococcus sp.	13	18	20	22

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. subtilis: Bacillus subtilis, P. fluorescens: Pseudomonas fluorescens, S. alata: Senna alata

Table 8: Antimicrobial activity of *S. alata* - Aqueous extract leaves

Tested organisms	Zone of inhibition (mm)			
	Streptomycin	Aqueous extract		
		50 µl	75 µl	100 µl
S. aureus	18	21	24	25
S. epidermidis	19	18	18	20
K. pneumonia	15	25	25	26
E. coli	19	23	25	27
Alcaligenes sp.	20	25	24	27
E. faecalis	20	22	21	22
S. typhi	10	22	27	28
P. aeruginosa	16	18	24	24
B. subtilis	15	22	22	28
P. fluorescens	18	24	25	27
Staphylococcus sp.	20	19	22	24

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, B. subtilis: Bacillus subtilis, P. fluorescens: Pseudomonas fluorescens, S. alata: Senna alata

Aqueous extract

Table 8 displayed inhibitory activity was observed the very high ranging of the zone in 27-28 mm. Aqueous extracts showed very significant antimicrobial activity against the tested organisms (Table 8).

DISCUSSION

S. alata leave extract has significant antimicrobial activity against broad spectrum of microorganisms. The antibacterial activity of the extracts against *S. aureus*, *S. epidermidis*, *K. pneumonia*, *E. coli*, *Alcaligenes* sp., *E. faecalis*, *S. typhi*, *P. aeruginosa*, *B. subtilis*, *P. fluorescens*, *and Staphylococcus* sp. were reported for the first time. The microbial studies properties indicating the potential for the discovery and novel drugs from plants. The aqueous extract was shown to be as potent as anthraquinones (zone of inhibition – 28 mm). The order of the antimicrobial efficacy is aqueous water – chloroform – ethanol – petroleum ether – benzene – methanol – acetone. Increase the extract concentration to be increase zone of inhibition (antibacterial activity). The results clearly showed that terpenoids, flanovoids, and anthraquinones which were abundantly found in distilled water, chloroform extracts of *S. alata* leaves.

The present study through light on the antibacterial efficacy of *S. alata* leaves this study offers a valuable source for the discovery of alternatives to the present antibacterial drugs. The study also concludes that *S. alata* leaves this study offers a valuable source for the discovery of alternatives to the present antibacterial drugs. This study also concludes that *S. alata* leaves contain a number of pharmaceutically important phytochemicals such as alkaloids, saponins, flavonoids, terpenoids, tannins, anthraquinones, carbohydrates, and protein.

A further study of the extracts is in progress to isolate, characterize, and elucidate the structure of the bioactive compounds present which were responsible for the potent antimicrobial activity.

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