Antimicrobial activity of Aqueous, Ethanol and Acetone extracts of *Sesbania grandiflora* leaves and its phytochemical characterization

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Abstract

Plants are being highly explored as a major source of medicinal compounds due to the presence of various phytochemical groups. Leaves of *Sesbania grandiflora* was consumed in traditional medicinal system of Ayurveda for numerous harmful syndromes and infections. This present study was explored the various phytochemicals present in the plant leaves of *S. grandiflora.* The qualitative analysis of various phytochemicals was exploited using different solvent systems. The aqueous, 80% ethanol and 70% acetone extraction was carried out in this study. Ethanolic extract shown presence of high amount of Alkaloids, Tannins, Saponins, Glycosides and steroids were confirmed by formation of colour intensity during chemical reactions. All the three extracts were tested for antimicrobial activity against pathogenic micro-organisms especially methicillin resistant *Staphylococcus aureus* and dermatophytes *Candida sp* using Agar well diffusion method. Among these three extracts ethanol extracts shows good antibacterial activity compared with aqueous and acetone extracts. Because of the presence of alkaloids, flavonoids, tannins and steroids ethanol extract shows high antibacterial activity. So these active compounds can be used in the field of medicine as therapeutic agent.

Keywords: *Sesbania grandiflora*, Phytochemicals, antimicrobial activity

Introduction

Herbalism is a traditional medicinal system to eradicate the various diseases using the medicinal plants and plant derived active compounds. In recent years focus on use of non-traditional approaches to treat diseases has been revived worldwide. The extracts from medicinal plants which issued for centuries throughout the world in traditional cures, herbal remedies and ashomeopathic medicine[1]. Approximately 70-80% of world'spopulation depends on traditional medicinalplants. Plants have an almost endless variety of metabolites which is very useful to human beings [2]. The evidence collected till now shows immense potential of medicinal plants used in traditional systems.

Nowadays many bacteria and fungi have been affected the people in several abnormal conditions. Commercial antibacterial and antifungal agents now in use has emerged. The microbes are resistant to these commercial antimicrobial agents while long term using to treatment of diseases.The genus of fungi*Candida* is composed of an extremely heterogeneous group of organisms with *C. albicans* being the most pathogenic species and the predominantetiologic agent of candidiasis. Several of the other lessfrequently encountered *Candida* species, which tend to be less susceptible to the commonly used antifungaldrugs, have recently emerged as significant opportunisticpathogens [3, 4]. Antifungal drug resistance is quickly becoming a major problem due to the increasing emergence of resistant strains. This has resulted in the drastic increase in the incidence of opportunistic and systemic fungal infections witnessed over the last decade [5, 6].Therefore, there is an inevitable and urgent medical need for novel antimicrobials. Plant derived drug serve as a prototype to develop more effective and less toxic medicinesfor many of the diseases.In developing countries, the WHO estimatesthat about three quarters of the population relies on plant basedpreparations used in their traditional medicinal system and as thebasic need for human primary health care [7].Therefore to check this in present work an attempt have been carried out for retesting preliminary phytopharmacological survey of the *Sesbania grandiflora* and characterize its antimicrobial effects against bacteria and opportunistic fungi (*Candida sp*).

*Sesbania grandiflora L.*is an Indian medicinal plant commonly known as Agathi is a widely available plant which belongsto family Fabaceae; it is an open branching tree tall up to 15mand 39cm in diameter[8]. It is cultivated in south or west India in the Ganga valley andin Bengal [9]. Bark, leaves, gums, and flowers have medicinal potential. Dried bark powder is used in cosmetics. An aqueous extract of plant is said to be toxic to cockroaches. The plant used in colic disorder, jaundice, poisoning condition, small-pox, eruptive fever, epilepsy

etc.[10, 11]. Different parts of this plant are used in Siddha system of Indian traditional medicine for the treatment of a wide spectrum of ailments including anemia, bronchitis, fever, headache, ophthalmia, nasal catarrh, inflammation, leprosy, gout and rheumatism.The flowers and young leaves of *S. grandiflora* are edible and are often used to supplement meals. The dried leaves of *S. grandiflora*are used in some countries as a tea which is considered to have antibiotic, anthelmintic, [12, 13] anti – tumor[14] and contraceptive properties. In addition, sesbania is mentioned as a potent antidote for tobacco and smoking-related diseases [15]. In this present study we reported that phytochemical characterization of aqueous, ethanol and acetone extracts of locally available, medicinally valuable plant *S. grandiflora*and also to study its antimicrobial activities.

Materials and methods

Collection of plant leaves

The leaves were collected from vandhavasi area, TN, India. The collected plant leaves were washed with running tape water and distilled water. Washed leaves were shade dried at room temperature for a week and grinded usingmixe and make into fine powder. After they are kept in air tight container and used to solvents extraction. Powdered leaves were subjected for extraction with aqueous, ethanol and Acetone using soxhlet apparatus.

Aqueous extraction

About 10 g of powdered leaves were mixed with 100 ml of sterile double distilled water and incubated on a water bath shaker for 12 h at 40°C. Then the mixture was filtered through Whatman No 1 filter paper, then the supernatant was collected and used for preliminary phytochemical analysis.

Acetone and methanol extraction

A 25gm of powdered leaves were extracted withorganic solvents by using Soxhlet apparatus. These were successively extracted with 80 % ethanol at 60°C for 48 h and 70%acetoneat 55°C for 48 h. The obtained extract was further filtered with Whatman No 1 filter paper and then allowed to evaporation. After evaporation, the sample was in the form of powder (concentrated form) and this form was stored at 4°C untilfurther use. During assay the bioactive compound was diluted by using double distilled water.These extracts used to preliminary different phytochemical screening for the analysis of various phytochemical groups.

Phytochemical screening

The three extracts thus obtained were analyzed to preliminary phytochemical screening following the standard protocols [16-19].

Test for Alkaloids

Presence and absence of alkaloids was tested by Wagner's Test. To the extract (1 ml) add 1 ml of Wagner's reagent prepared by mixing 2 g of iodine and 6 g of potassium iodide in 100 ml distilled water. The formation of reddish brown precipitate was an indication of the presence of alkaloids.

Test for tannins (Ferric chloride test)

To the 5 ml of extract add few drops of 1 % ferric chloride solution and note the color of reaction. Formation of Green color precipitate indicates presence of tannins.

Test for saponins

About 5 ml of diluted extracts were taken in a test tube and shaken vigorously and kept for 5 min. Formation of foamy layer indicates the presence of saponins.

Test for glycosides

About 2 ml of the concentrated leaf extracts taken in a test tube and add a quantity (10 ml of 50% H_2SO_4) was added to. The mixture was heated in a water bath shaker for 15 min. to this mixture add 2 ml of Fehling's solution and then the mixture was boiled. Development of a brick-red precipitate indicated the presence of glycosides in the extracts.

Test for flavonoids

A 2 ml of each extracts were taken in separate test tube add few drops of sodium hydroxide solution. The yellow color was formed and it became turn to colorless while addition of diluted sulfuric acid confirmed the presence of flavonoids.

Test for protein

To the aqueous, ethanol and acetone leaf extracts add 1 ml of Biuret Reagent (40% NaCl& 1% CuSO4). The blue reagentturns into violet in the presence of proteins.

Test for triterpenoids

The extract was treated with tin and thionyl chloride solution and notice the pink color formation indicates presence of triterpenoids.

Test for sugars

About 10 ml of extract were boiled with 3-4 drops ofFehling's A and Bsolutions for 2 minutes in water bath. Formation of red color is theindication of the presence of reducing sugars.

Test for phenol

To the extracts add 3-4 drops of 5 % ferric chloride solution and observed the formation of dark blue or blackish color which may indicates the presence of phenol in the extracts.

Test for steroids

To the leaf extract add few drops of acetic anhydride, warmed and cooled under tap water and add few drops of concentrated sulfuric acid and observe the color charge violet to green color indicates the presence of steroids.

Test for terpenoids

About 5 ml of each leaf extract was taken and add 2 ml of chloroform and 3 ml of concentrated sulfuric acid notice the formation of layer and color. A reddish brown coloration of the interface confirms the presence of terpenoids.

Antimicrobial activity assay

The extract of *S. grandiflora* was tested for antimicrobial activity by agar well diffusion method against pathogenic Gram positive and negative bacteria are *Bacillus subtilis. Klebsiella pneumonia, Klebsiellaplanticola, E. coli, Staphylococcus aureus, Pseudomonas aeruginosa*and *Candida sp.* Different volumes of crude plant extracts were dissolved in distilled water (10 mg/ml). Muller Hinton Agar medium was used to cultivate bacteria. Fresh overnight culture of each strain was swabbed uniformly onto the individuals plates using sterile cotton swabs. 6 wells were made on each Muller Hinton Agar plates with 5 mm in diameter. Then the dilute extracts with different concentrations (25, 50 and 75 µL) were poured into each well on all plates. The commercial drug Ciprofloxacin was maintained as control and incubate for 24 h at 37°C. After incubation the different levels of zonation formed around the well was measured.

Results and Discussion

Screening of phytochemicals constituents

The powdered leaves of Sesbania grandifloraextracted with different solvent. The resultant extract was dried in air until constant weight of the plant extract was obtained. The plant extract was then performed for the phytochemical characteristics to identification of various phytochemicalconstituents.

The plants may be consisting of many chemical constituents like alkaloids, glycosides, carbohydrates, proteins, steroids, tannins, saponins,flavonoids etc. These chemical constituents are called as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of these secondary metabolites in aqueous, ethanol and acetone extracts was subjected to colored reactions of chemical tests.

The preliminary phytochemical analysis of aqueous extract of *S. grandiflora* revealed the presence of alkaloids, tannins, flavonoids, sugars, phenol, terpenoids and proteins and absence of saponins, glycosides, triterpenoids and proteins. Ethanol extract confirmed the presence of alkaloids, tannins, saponins, glycosides, flavonoids, phenol, steroids, terpenoids and proteins and absence of triterpenoids and sugars. Acetone extract revealed the presence of alkaloids, tannins, saponins, glycosides, triterpenoids and terpenoids. The tannins, flavonoids, alkaloids, and steroids were more often present in ethanolic extracts than aqueous and acetone extract was confirmed based on the formation color intensity of reactions.

Various tests have been executed to find out the presence of phytochemical constituents in the different solvent derived leaf extract. The results have shown that each and every phytochemical has the ability to get extracted with different solvents[20]. This might differ according to the polarity of the solvent [21]. Ethanolic leaf extract has shown that it has extracted most of thecompounds and this is confirming thatmethanol is being used as a solvent in Ayurveda centers for extracting bioactive compounds[22]. So that, the polarity of the solvent is the major characteristic of them to be used as a basic for extraction. Our experiment has evaluate clearly that ethanol extract can be used as an active extracting solvent and also the evaporation of ethanol is almost immediately.

Antibacterial activity

Antibacterial activity of leaf extract was carried out to determination of the bacterial inhibiting activity and inhibiting minimal concentration of the extracts. All the extracts were shown significant inhibitory activity on all strains of bacteria. The inhibiting minimal concentrations of the bacteria by the extracts indicate that the extracts generally act with low dose. Ethanol extract was active on strains resistant to methicillin and dermatophytes. Indeed, the inhibited growth of *S. aureus*and *Candida sp*.Antibacterial activity was increased while increasing the concentration of extracts. The minimum inhibitory concentration is 50 µL shown high zone of inhibition.

Among these three extracts ethanol extract shown maximum inhibitory activity due to the presence of alkaloids, tannins, saponins, phenol and steroids. These secondary metabolites responsible for the antimicrobial activity. Tannins are responsible for antimicrobial, astringency [23, 24] and phenol compounds has the biological activity of such as antiapoptosis, antiaging, anticarcinogen, and antiinflammation[25], as well as inhibition of angiogenesis and cell proliferation activities. Saponins have been extensively shows property like precipitating and coagulating red blood cells [26]. The plant has medicinal property due to presence of these phytochemicals.

Conclusion

In the present study simply available plant *Sesbania grandiflora* were selected for the phytochemical screening of aqueous, ethanol and acetone extracts and assessed its antimicrobial activity against pathogenic bacteria. It is observed that ethanol extracts showed a marked presence of alkaloids, flavonoids, saponins, glycosides and steroids. Ethanol extracts shown maximum zone of inhibition on all the organisms especially *Staphylococcus aureus* (18 mm) and *Candida sp* (14 mm) at 75 µL concentration. Maximum antimicrobial activity was observed due to the presence of secondary metabolites. Based on our results, we concluded that ethanol extract of *S. grandiflora*have great potential activity as antimicrobial agent and can be combined with folk medicine and used in the treatment of infectious diseases caused by antibiotics resistant microorganisms.

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Figure 1: Sesbania grandiflora

Table 1:Phytochemical constituents of aqueous, ethanol and acetone extract of *S. grandiflora* leaves

compounds	**Aqueous extract**	**Ethanol extract**	**Acetone extract**
Colour of the extract	Greenish brown	Yellowish green	Yellowish brown
Alkaloids	+	++	+
Tannins	++	++	+
Saponins	-	++	+
Glycosides	-	++	++
Flavonoids	-	+	-
Triterpenoids	-	-	+
Sugars	++	-	-
Phenol	+	+	-
Steroids	-	++	-
Terpenoids	++	+	++
Proteins	+	+	-

+ = present, - = absent, ++ = highly present

Table 2:Antibacterial activity of aqueous extract of *S. grandiflora* leaves at different concentrations

Concentration	**25µL**	**50 µL**	**75 µL**	***Ciprofloxacin***
E.coli	06	07	09	12
Pseudomonas aeruginosa	08	09	11	13
Staphylococcus aureus	09	13	15	12
Klebsiella pneumonia	08	10	12	11
Bacillus subtilis	07	08	10	14
Candida sp	10	12	15	11
Klebsiellaplanticola	06	07	10	13

Table 3:Antibacterial activity of ethanol extract of *S. grandiflora* leaves at different concentrations

Concentration	**25 µL**	**50 µL**	**75 µL**	***Ciprofloxacin***
E.coli	07	09	11	12
Pseudomonas aeruginosa	08	10	13	14
Staphylococcus aureus	12	17	18	15
Klebsiella pneumonia	09	12	14	13
Bacillus subtilus	07	10	13	14
Candida sp	11	13	14	11
Klebsiellaplanticola	06	08	12	12

Table 4:Antibacterial activity of acetone extract of *S. grandiflora* leaves at different concentrations

Concentration	**25 µL**	**50 µL**	**75 µL**	***Ciprofloxacin***
E.coli	07	09	11	12
Pseudomonas aeruginosa	08	10	12	12
Staphylococcus aureus	09	12	16	15
Klebsiella pneumonia	08	09	11	10
Bacillus subtilus	10	11	13	11
Candida sp	10	12	14	13
Klebsiellaplanticola	09	11	12	14