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Phytochemical Analysis of *Sesbania grandiflora* (L) Leaf

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Abstract

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics. *Sesbania grandiflora* (L) is a multipurpose tree with edible flowers and is a source one of the medicinal products. *S. grandiflora* (L) has unique medicinal properties and used as a herbal drug for its antibiotic, anti helmintic, anti-tumour and contraceptive properties. The present study intends to provide an overview of the chemical constituents present in the crude leaf extracts of *S. grandiflora* (L) with special emphasis on their pharmacological actions. Qualitative phytochemical screening was carried out using the crude leaf extracts in four different solvents such as water, acetone, ethanol and methanol. Preliminary phytochemical analysis revealed the presence of eleven compounds such as carbohydrates, tannins, steroids, terpenoids, alkaloids, flavanoids, cardiac glycosides, oils, saponins, coumarins, gum and mucilage. The results suggest that the leaves are a rich source of valuable primary and secondary metabolites exhibiting the antimicrobial activity.

Keywords: *Sesbania grandiflora* (L), phytochemical analysis, Secondary metabolites, Crude leaf extracts, Pharmacology.

Introduction

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte, 1998). Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Sandhya et al., 2006). In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and siddha (Sathyavathi et al., 1987). The study of plants continues principally for the discovery of novel secondary metabolites.

Phytochemistry in the strict sense is the study of phytochemicals or phytonutrients which are non essential nutrients but still have been scientifically confirmed as being important to human health. These are chemicals derived from plants which exhibit a number of protective functions for human consumers. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from bacteria, fungus and other pathogens. Sanskrit writings such as the Rig Veda and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of some plants that are used for medicinal purposes. Phytochemicals are antibiotic properties of plants which have been reported to possess antibacterial, antifungal and anti-inflammatory activities. Thus medicinal plants play an important role in the developing of newer drugs because of their effectiveness, less side effects and relatively low cost when compared with synthetic drugs (Raj et al., 2011). Plant secondary metabolites are broadly divided into three chemically distinct groups (Taiz and Zeiger, 2011) such as Terpenes, Phenolics and nitrogen containing compounds.

Sesbania grandiflora (L) also known as agate or hummingbird tree, is a small tree about 10 m high with unique medicinal properties as all parts of the plant serves as a natural anti-oxidant (Okonogi et al., 2007). The tree thrives under full exposure to sunshine and is extremely frost sensitive. Agathi is native to tropical Asia and is widespread in India, Malaysia, Indonesia and Philippines. It is commonly found in disturbed and agricultural environments including along roadsides, on dikes between rice paddies and in backyard vegetable gardens. The flowers, young leaves and tender pods of the white flowered Agati are edible and are sold in local ethnic markets (Galeano et al., 2003). All parts of *S. grandiflora* (L) are utilized for medicine in Southeastern Asia and India including preparations derived from the roots, bark, gum, leaves, flowers, and fruits (Okonogi et al., 2007). .

Powdered roots of *S. grandiflora* (L) var. coccinea are mixed with water and applied externally as a poultice or rub to rheumatic swellings. The bark is considered as an

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astringent and is utilized for the treatment of smallpox, in Philippines for the treatment of ulcers in the mouth and alimentary canal, in Java for the treatment of thrush and infantile disorders of the stomach and in Cambodia the pounded bark is applied to scabies. The juice of the leaves is considered anthelmintic and tonic and is used to treat worms, biliousness, fever, gout, itchiness and leprosy (Duke, 1983). Malayans apply crushed leaves to sprains and bruises. In Ayurvedic medicine the leaves are utilized for the treatment of epileptic fits and clinical research supports the anticonvulsive activity of Agati leaves. Agati is valued as fodder throughout Indonesia, particularly in dry season for feeding of cattle and goats.

In recent years, secondary plant metabolites / phytochemicals, previously with unknown pharmacological activities, have been extensively investigated, as a source one of medicinal agents (Krishnaraju et al., 2005). These can be derived from any part of the plant such as bark, leaves, flowers, seeds etc (Cragg, 2004). Knowledge of the chemical constituents of the plant is desirable since such information will be of great value for the synthesis of complex chemical substances. Thus it is anticipated that phytochemicals with adequate antibiotic efficiency can be used against microbial infections (Balandrin et al., 1985). Moreover, the increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio (1996), lwu et al., (1999)). The present study thus aims in exploring the phytochemical constituents of the leaf extracts of *S. grandiflora* (L).

Materials and Methods

Collection of plant material

The fully matured fresh leaves of *S. grandiflora* (L) were collected from kudapanakunnu area and were identified in the department of botany, Mahatma Gandhi College, Trivandrum. The leaves were washed thoroughly, shade dried and finely powered. The dried powdered leaves were extracted with four different solvents such as water, acetone, methanol and ethanol. For aqueous extraction, ten grams of the powdered leaves was mixed with 100mL distilled water, boiled for two hours and filtered. Whereas acetone, methanolic and ethanolic extracts were prepared by mixing ten grams of powdered leaf samples with 100mL of each solvent separately in mechanical shaker for 48 hours at room temperature. Extracts were filtered, concentrated, dried and stored in the refrigerator at 4°C for further use.

Phytochemical Analysis

The prepared plant extracts were analyzed for the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, fixed oils, steroids, terpenoids, tannins, flavonoids, gum and mucilages (Raaman, 2006).The presence of different phytochemicals extracted in different solvents was confirmed by standard protocols.

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

Test for carbohydrates

a) Molisch Test

To 2mL of molisch reagent 2mL of extracts were added and shaken well. To this 2mL of concentrated sulphuric acid was added through the sides of the test tube. Appearance of a reddish violet ring at the junction of the two layers indicates the presence of carbohydrates.

b) Fehling's Test

To the extracts added equal amount of Fehling's reagent, mixed well and heated gently. Formation of a brick red precipitate indicates the presence of reducing sugars.

Test for Tannins

To the extracts were added a few drops of 10% ferric chloride solution. Appearance of a Green or blue color indicate the presence of tannins.

Test for Steroids

Leaf extracts were mixed with 1mL of chloroform and later 2-3 drops of con.H_2SO_4 was added. Appearance of a pink or red color indicates the presence of steroids.

Test for Terpenoids

Salkowiski test

5mL of the extracts were mixed with 2mL of chloroform and 3mL of con.H_2SO_4 solution. A reddish–brown color at the Interphase indicate the presence of terpenoids.

Test for alkaloids

Mayer's test

To the extracts, 1% hydrochloric acid, 6 drops of Mayer's reagent and Drangendroffs reagent were added. Appearance of an organic precipitate indicates the presence of alkaloids in the sample.

Detection of Flavanoids

The extracts were treated with con.H_2SO_4 and observed for a yellowish orange color for the presence.

Test for protein

a) Biuret Test

1mL of 40% NaCl and 2 drops of 1% $CuSO_4$ were added to the leaf extracts. Appearance of a Violet colour confirms the presence of proteins.

b) Xanthoprotein Test

To the leaf extracts 20% NaOH were added and the formation of an orange colour confirms the presence of proteins which is characteristic for ammonia formation.

Test for Cardiac Glycosides

Keller-killani Test

5mL of the extracts were treated with 2mL of glacial acetic acid containing 2-3 drops of Ferric chloride solution and

1ml of con.H_2SO_4 acid solution. A green ring initially appears which first turns to violet and then to brown at the inter phase indicates the presence of cardiac glycosides.

Test for fixed oils

2 drops of extracts were pressed between two filter papers. Appearance of an oil strain on the filter paper indicates the presence of fixed oils.

Test for saponins

Foam Test

2mL of the extracts were diluted with 20mL of distilled water, shaken vigorously and was observed for a stable persistent froth.

Table 1. Phytochemical analysis of crude leaf extracts of *S. grandiflora* (L).

SOLVENTS	M	A	E	W
TEST				
Detection of carbohydrates				
Molischs test	-	-	+	+
Fehlings test	-	-	-	+
Test for tannins	-	-	-	+
Test for steroids	+	-	-	+
Test for terpenoids				
Solkowiski test	-	-	-	+
Detection of Alkaloids				
Mayer's Test	+	+	+	+
Detection of Flavanoids	-	-	-	+
Test for protein				
Biuret test	-	-	-	-
Xanthoprotein	-	-	-	-
Test for cardiac glycosides				
keller killani test	+	-	-	+
Test for fixed oils	-	-	-	+
Test for saponins				
Foam test	-	+	-	+
Test for phenolic compounds				
FeCl3 test	-	-	-	-
Detection of coumarins	+	+	-	-
Test for amino acids				
Ninhydrin test	-	-	-	-
Gum and mucilage	-	-	-	+

*M-Methanol,*A-Acetone,*E-Ethanol,*W-Water; +: Present, - : Absent

Test for phenolic compounds
Ferric chloride test
2mL of diluted extracts were treated with dil.$FeCl_3$ solution. Appearance of a violet color indicates the presence of phenol like compounds.

Detection of coumarins
To the test solution were added a few drops of alcoholic sodium hydroxide solution. Appearance of an intense yellow colour on addition of concentrated HCl indicates the presence of coumarins.

Test for amino acids
Ninhydrin test
Two drops of ninhydrin solution (10mg of ninhydrin in 200mL of acetone) were added to 2mL of aqueous filtrates. A characteristic purple colour indicates the presence of amino acids.

Test for Gum and mucilage
To 100mL of each extract added 10mL of distilled water. To this 25mL of absolute alcohol was added with constant stirring. Cloudy precipitate indicates the presence of gum and mucilage.

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Test for Gum and mucilage
To 100mL of each extract added 10mL of distilled water. To this 25mL of absolute alcohol was added with constant stirring. Cloudy precipitate indicates the presence of gum and mucilage.

Results and Discussion

In recent years, secondary plant metabolites are extensively investigated as a source of medicinal agents. It has been accepted that natural compounds play an important role in health care. Plant-derived substances have recently been of great interest owing to their versatile application. Plants are the richest bio-resource of traditional system of medicine, food supplements, folk medicine and pharmaceutical intermediates. It is well documented that the presence of these chemicals is responsible for various medicinal properties by various researchers. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. The present study was conducted to analyze the phytochemical analysis of the crude leaf extracts of *Sesbania grandiflora* (L).

The powdered leaf extracts of *S. grandiflora* (L) have been screened for phytochemical constituents in four different solvents such as methanol, acetone, ethanol and water. The preliminary phytochemical analysis revealed the presence of eleven compounds such as carbohydrates, tannins, steroids, terpenoids, alkaloids, flavanoids, cardiac glycosides, oils, saponins, coumarins, gum and mucilage (Table 1). Phytochemical studies of all the four different extracts conclude that methanol and aqueous extracts of leaf samples had more positive results for glycosides, steroids, cardiac glycosides, coumarins and alkaloids.

Phytochemical analysis of methanolic leaf extract of *S. grandiflora* (L) was analyzed for compounds such as steroids, alkaloids, glycosides and absence of carbohydrates, tannins, terpenoids, flavanoids, protein, oil, saponin, phenolic compounds, coumarins, amino acids, gum and mucilage etc. (Table 1). With acetone and ethanolic solvents, only alkaloids, saponins, coumarins and carbohydrates were detected. Traditionally saponins have been extensively used as detergents, pesticides as well as mollucicides, in addition to their industrial application such as foaming, surface active agents etc. And also found to have beneficial health effects (Arunasalam et al., 2004).

Aqueous extract has showed the presence of compounds such as carbohydrates, tannins, Steroids, terpenoids, alkaloids, flavanoids, cardiac glycosides, oils, saponins, gum and mucilage. The role of tannin is to protect from predation also pesticides and in plant growth regulation. Previous studies by various other workers prove that flavanoids provide health benefits through cell signalling pathways and antioxidant effects.

Conclusion

Medicinal plants were the potent source of human health due to the active phytochemical compounds that is responsible for its various pharmacological activities. On the basis of the results obtained, the present study conclude that the leaves of *S. grandiflora* (L) are rich in phytochemical constituent even though the phytochemical screening of the leaf extracts of samples showed variation in their phytochemical constituents with the presence and or absence of some components. Most components were present in aqueous and methanolic extracts of the leaves. The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, saponins, phenols and flavanoids were believed to exhibit the antibiotic properties of *S. grandiflora* (L) leaves.

The present study highlights the possible use of *S. grandiflora* (L) leaf extracts as a source of antioxidants and as antibacterial agents that can be used to prevent enteric diseases. The study reveals that the results of extraction yield, total phenolic and flavonoid compounds and bioactivity tests varied depending upon the type of solvent being used. The leaves of *S. grandiflora* (L) contain a considerable quantity of phenolic - flavonoid compounds which were considered to be the major contributor for their antioxidant and antibacterial activities. Hence it can be concluded that the leaves of *S. grandiflora* (L) would direct to the establishment of some compounds that could be used to invent new and more potent anti microbial drugs of natural origin. Therefore future research should be addressed on the application of using *S. grandiflora* (L) leaves as natural remedier and to protect against infectious diseases.

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