ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Physico-Chemical and Phytochemical Investigation of Plant *Sesbania sesban.*

Kumar Sandeep*, Bajwa Baljinder Singh and Kumar Narinder

Lala Lajpat Rai College of Pharmacy, Moga-Fzr G T Road, Near P.S Sadar, Moga - 142001, Punjab, India.

ABSTRACT

The genus Sesbania sesban contains about 50 spieces, the majority of which are annuals. The greatest spieces diversity occurs in Africa with 33 spieces. The spieces *Sesbania sesban* belongs to sub-family Papilionoidea of the family Leguminosae. It is a small perennial tree with woody stems, yellow flowers and linear pods. *Sesbania sesban* is very common throughout Africa and in asian countries like India, Malaysia, Indonesia and Phillipines. Campesterol, β-sitosterol, Cyanidine, Delphinidin glycosides, α-Keto glutaric, Oxaloacetic and pyruvic acids, Oleanolic acid, saponins, Palmitic acid, Stearic acid, Oleic acid, Linoleic acid and Linolenic acid are reported in Whole plant. Cyanidin and Delphinidin glycosides, Flavonols are reported in flowers. The plant has been reported to possess various activities such as anti-inflammatory activity, antinociceptive activity, antidiabetic activity, antifertility activity and antioxidant activity. The physicochemical parameters such as ash value, acid insoluble ash, loss on drying, alcohol soluble extractive value and water soluble extractive value were determined. Further fluorescence analysis was done. The extracts of *Sesbania sesban* stem were prepared and phytochemical screening of extracts were done. The Phytochemical study showed that chloroform, methanol, ethanol and aqueous extracts gave positive tests for alkaloids, proteins, flavonoids and phytosterols. Methanol and ethanol extracts were found to contain phenolic compounds. Aqueous extract gave postive test for saponins. Methanol, ethanol and aqueous extract gave positive test for carbohydrates.

Keywords: Physicochemical, phytochemical, alkaloids, flavnoids.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Unlike modern allopathic drugs which are single active components that target one specific pathway, herbal medicines work in a way that depends on an orchestral approach. A plant contains a multitude of different molecules that act synergistically on targeted elements of the complex cellular pathway [1,2,4]. Medicinal plants have been source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions [3].The use of herbal medicines becoming popular due to toxicity and side-effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications [5]. Now a days in pharmaceuticals herbal medicines gained more interest. These herbal medicines comprises of active ingredients from different parts of plants such as roots, stems, rhizomes, leaves, seeds and fruits. The active constituents so obtained are crude in nature. Medicinal properties of plants are due many active compounds like alkaloids, glycosides, saponins, terpenoids, lactones, phenols and flavnoids. Ayurvedic medicines are now a day's not only used by Indian peoples but also in developed countries like USA, Canada, Japan, China etc. Most of facility for the ayurvedic or herbal medicines are manufactured and implemented in India and China [6]. *Sesbania sesban* is very common throughout Africa and in asian countries like India, Malaysia, Indonesia and Phillipines. In India these crops have had a long history of agricultural use and as a source of forage. One of the major advantages of perennial *Sesbania sesban* spieces over other forage trees and shrubs is their rapid growth rates. *Sesbania sesban* grows well in the sub-tropics and is most suitable for altitudes between 200-500m. *Sesbania sesban* is outstanding in its ability to tolerate water-loging and is ideally suited to seasonally water-logged environments. The flowers of *Sesbania sesban* have been reported to have Cyanidin and Delphinidin glycosides, Flavonols [7]. The seeds of *Sesbania sesban* have been reported to have Saponins, Palmitic acid, Stearic acid, Lignoceric acid, Oleic acid, Linoleic acid and Linolenic acid. [8]. The leaves of *Sesbania sesban* have been reported to have Kaempferol [9].

Table 1: Synonyms of *Sesbania sesban*

Hindi	Jayanti
Kannada	Jeenangi
Oriya	Thaitimul
Marathi	Shewarie
Telgu	Samintha
Sanskrit	Jayantika

Table 2: Taxonomical Classification of *Sesbania sesban*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	Sesbania
Spieces	Sesbania sesban

ISSN: 0975-8585

MATERIALS AND METHODS

Plant Material

Dried stem portion of *Sesbania sesban* was collected from S V University, Tirupati, Andhra Pradesh.and botanical Authentication of *Sesbania sesban* has been obtained from Dr. K. Madhava Chetty, S V University, Tirupati, Andhra Pradesh.

Solvents Used

Petroleum ether (60-80°C) , Chloroform and Methanol were employed for extraction of plant material using soxhlet apparatus and finally the drug was boiled with distilled water. Dimethyl sulphoxide ,was used as solvent for dissolving different extracts. It is colourless liquid with boiling point 189°C. It is miscible with water, chloroform, acetone, alcohol and petroleum ether.

Chemicals Used

Sodium hydroxide, Chloral hydrate, Copper sulphate, Ferric chloride, Sulphuric acid, Iodine, Lead acetate, Magnesium, Potassium iodide, Potassium mercuric iodide, Picric acid, Mercuric chloride, Nitric acid, Gelatin, Sodium chloride, α-napthol,, sodium nitropruside, pyridine, were used for phytochemical screening of the plant extracts.

Physico-Chemical Evaluation

Ash values

The ash values, following ignition of medicinal plant materials is determined by thee different methods, which measures total ash, acid insoluble ash and water-soluble ash (WHO., 1998).

Total ash

It is designed to measure the total amount of material remaining after ignition. This includes both “physiological ash”, which is derived from the plant tissue itself and “non-physiological” ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant material.

Method

The finely ground and dried plant material was accurately weighed (2g) and placed in a previously ignited and tarred crucible (silica). The material was spread in an even layer and ignited by gradually increasing the heat to 500-600°C until it is white, indicating absence of carbon. It was then cooled in a desiccators and weighed. If carbon-free ash cannot be obtained in this manner, the crucible was cooled and moistened the residue with about 2mL of water or a saturated solution of ammonium nitrate R. Dried on a water bath, then on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable

desiccator for 30 min, and weighed without delay. The percentage of ash with reference to air dried drug was calculated.

Acid-insoluble ash: It is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Method

To the crucible containing the total ash, 25 mL of hydrochloric acid (~70g/L) was added. It was covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a vacuum desiccator for 30 min, and weighed without delay. The percentage of ash with reference to air dried drug was calculated.

Water-soluble ash: It is the difference in weight between total ash and the residue left after treatment of total ash with water.

Method

To the crucible containing the total ash, 25 mL of water was added and boiled for 5 min. The insoluble matter was collected in a sintered glass crucible or on an ashless filter paper. Washed with hot water and ignited in a crucible for 15 min. at a temperature not exceeding 450°C, and the ash obtained was weighed. The weight of this residue was subtracted from the weight of total ash. The percentage of ash with reference to air dried drug was calculated

Fluoresence Analysis

A small quantity of dried and finely powdered stem of *Sesbania sesban* was placed on a grease free clean microscopic slide and added 1-2 drops of freshly prepared reagent solution, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 m) ultraviolet radiations. The colours observed by application of different reagents in different radiations were recorded (WHO. 1998).

Preparation of extracts

The stems of plant was dried under shade and coarsely powdered. Five hundred gram powder material was subjected to successive. The solvents used were petroleum ether (60-80°C), chloroform, hexane, methanol, ethanol and then distilled water. Soxhlet extraction was carried out by these solvents in an increasing order of their polarity for not less than 48 hours. After each extraction the powdered material was dried in air at room temperature. Finally, marc was digested with distilled water for 24 hours or more to obtain

aqueous extract. Each extract was concentrated in vaccum using Rotatory evaporator.Extracts were weighed subsequently and the percentage yields were calculated of each extract obtained individually in terms of the air dried weigh of plant material.

Phytochemical screening

Test for Carbohydrates

Molisch test: To 2-3 mL of extract, few drops of 95% α-napthol solution in alcohol were added. Conc. H_2SO_4 was added from sides of the test tube. Appearance of a red brown ring at the junction of the liquids revealed the presence of carbohydrates.
Fehling's solution test: One mL of Fehling's A and Fehling's B solutions were mixed and boiled for one minute. Equal volume of extract was added and heated on boiling water bath for 5-10 min. Appearance of first a yellow and then brick red precipitates revealed the presence of carbohydrates.
Benedict's solution test: Equal volume of Benedict's reagent and extract were mixed in test tube and heated on boiling water bath for 5 min. Appearance of red solution revealed the presence of carbohydrates.

Tests for Proteins

Biuret test: To 3 mL of aqueous extract 4% NaOH and few drops of 1% $CuSO_4$ solution were added. Appearance of violet or pink colour revealed the presence of proteins.
Test with Millon's reagent: To 3 mL of extract, 5 mL Millon's reagent was added. Appearance of white precipitates initially which turned to red when heated revealed the presence of proteins.
Xanthoproteic test: To 3mL of extract 1mL conc. H_2SO_4 was added. Appearance of white precipitates which turned yellow on heating and orange on addition NH_4OH revealed the presence of proteins
Ninhydrin solution test: To 3 mL of extract, 3 drops of 5% Ninhydrin solution was added and heated in boiling water bath for 10 min. Appeareance of purple or bluish colour revealed the presence of proteins.

Test for Alkaloids

Extract was dried and treated with few drops of dilute HCl. Filtered and subjected the filtrate to no of tests.

Dragendorff's test: To 2-3 mL of filtrate few drops of Dragendorff's reagent were added. Appearance of orange brown precipitates revealed the presence of alkaloids.
Mayer's test: To 2-3 mL filtrate, few drops of Mayer's reagent were added. Appearance of cream coloured precipitates revealed the presence of alkaloids.
Hager's test: To 2-3 mL filtrate, few drops of Hager's reagent were added. Appearance of yellow colour precipitates revealed the presence of alkaloids.
Wagner's test: To 2-3 mL filtrate few drops of Wagner's reagent were added. Appearance of reddish brown precipitates revealed the presence of alkaloids.

ISSN: 0975-8585

Test for Flavonoids

Shinoda's test: A small quantity of test residue was dissolved in 5 mL ethanol (95% w/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. Appearance of pink, crimson or magenta colour within a minute or two, revealed the presence of flavonoids.
Lead acetate solution test: To small quantity of extract, lead acetate solution was added. Appearance of yellow coloured precipitates revealed the presence of flavonoids.

Test for Phenolic compounds

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagents.

Ferric chloride test: A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Appearance of dark green or deep blue colour revealed the presence of phenolic compounds.
Lead acetate test: A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Appearance of white precipitate revealed the presence of phenolic compounds.
Potassium dichomate test: If on an addition of a solution of potassium dichomate in test filtrate, appearance of dark colour revealed the presence of phenolic compounds.

Test for Saponins

Foam test: A few mg of the test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. Appearance of stable, characteristic honeycomb like forth indicates presence of saponins.

RESULTS AND DISCUSSION

Table 3: Physico-Chemical Studies

S.NO.	PHYSICAL PARAMETERS	% VALUE OBTAINED
1	Total ash	5.70
2	Acid insoluble ash	1.35
3	Loss on drying	9.15
4	Alcohol soluble extractive value	6.25
5	Water soluble extractive value	9.75

ISSN: 0975-8585

Table 4: Fluoresence Analysis

Drug name	Solvent used	Colour under visible light	Colour under long UV (365 nm)	Colour under short UV (254 nm)
S. sesban	Water	Yellow	Yellow	Yellow
S. sesban	Concentrated nitric acid	Green	Yellow	Yellowish Green
S. sesban	Concentrated ammonia	Lemon Yellow	Brown	Yellow
S. sesban	Concentrated hydrochloric acid	Yellow	Yellow	Dark Yellow
S. sesban	Concentrated sulphuric acid	Brown	Brownish Black	Blue
S. sesban	Ethanol	Yellow	Yellow	Light Brown
S. sesban	Iodine	Yellow	Yellow	Brown

Table 5: Phytochemical Screening

S. no	Phytochemical constituents	Chloroform extract of *S. sesban* leaves	Methanol extract of *S. sesban* leaves	Ethanol extract of *S. sesban* leaves	Water extract of *S. sesban* leaves
1.	Alkaloids				
	1. Mayer's reagent	++	++	+	+
	2. Hager's reagent	++	++	+	+
	3. Wagner's reagent	++	++	+	+
	4. Dragendorff's reagent	++	++	+	+
2.	Phenolic compounds and Tanins				
	1. $Fecl_3$				
	2. Lead acetate test	-	+	+	-
	3. Bromine water test	-	+	+	-
		-	+	+	-
3.	Saponin				
	1. Frothing test	-	-	-	+
4.	Carbohydrates				
	1. Molisch test	-	+	+	+
	2. Fehling's solution A	-	+	+	+
	2. Fehling's solution B	-	+	+	+
5.	Protein and Amino acids				
	1. Millon's test	+	+	+	+
	2. Biuret test	+	+	+	+
	3. Ninhydrin test	+	+	+	+
6	Flavonoids test				
	1. Alkaline reagent test	+	+	+	+
	2. Shinoda test	+	+	+	+
7	Phytosterols test				
	1. Liebermann's test	+	+	+	+
	2. Libermann Burchard test	+	+	+	+

+ = present, - = absent

ISSN: 0975-8585

DISCUSSION

The physico-chemical parameters (ash values, extractive values), preliminary phytochemical screening was done. The total ash value was 5.70%, acid insoluble ash was 1.35%, alcohol soluble extractive value was 6.25%, water soluble extractive value was 9.75% and loss on drying was 9.15%.

The Phytochemical study showed that chloroform, methanol, ethanol and aqueous extracts gave positive tests for alkaloids, proteins, flavonoids and phytosterols. Methanol and ethanol extracts were found to contain phenolic compounds. Aqueous extract gave postive test for saponins. Methanol, ethanol and aqueous extract gave positive test for carbohydrates.

CONCLUSION

The present study was designed to study the physico-chemical and phytochemical Investigation of stem of plant *Sesbania sesban.* The physiochemical parameters like ash values, loss on drying and fluorescence analysis were also determined following WHO guidelines and their results were noted. The soxhlet extraction of powdered stem was carried out for preparation of various extracts with the solvents in increasing order of polarity *viz* pet ether, chloroform, methanol, ethanol and water. The physical appearance and percentage yield of various extracts were noted. The phytochemical screening of extracts was carried out and it revealed the presence of alkaloids, flavonoids, proteins and phytosterols chloroform, methanol, ethanol and aqueous extracts. Phenolic compounds were present in methanol and ethanol extracts. Carbohydrates were observed in methanol, ethanol and aqueous extracts. Saponins were observed in aqueous extract. So, it is concluded that the various activities of plant Sesbania sesban may be due to the presence of phytoconstituents such as alkaloids and flavnoids.

REFERENCES

[1] Sarkar DN, Azad-ud-doula Prodhan AKM. Indian J Agric Res 2001;35(4):211-218.
[2] Aslan M, Orhan DD, Orhan N, Sezik E, Yesilada E. J Ethnopharmacol 2007;109(1):54-59.
[3] Durmowicz AG, Stenmak KR. Pediatr Rev 1999;20:91-101.
[4] Arif T, Bhosale JD, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R. J Asian Nat Prod Res 2009; 11(7):621-638.
[5] Dasilva EJ. EJB 1999; 2(2):57-70.
[6] Meena AK, Bansal P and Kumar S. Asian J Trad Med 2009;4:152-70.
[7] Kathiresh M, Suganya P Devi, Saravanakumar M. IJPSR 2011;3(2):1307-1312.
[8] Dande PR, Talekar VS, Chakraborthy GS. Int J Res Pharm Sci 2010:1(3):296-299.
[9] Sayed NH. Pharmize 1991:46(12):898.
[10] World Health Organization. 2002. Traditional Medicine Strategy. World Health Organization Geneva, England.