J. Res. Educ. Indian Med., Jan. - March, 2009

PHARMACOGNOSTIC EVALUATION OF THE LEAVES OF *SESBANIA SESBAN* (L.)

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Abstract: In Ayurveda, the leaves of *S. sesban* are purgative, maturant demulcent, used in all pains and inflammation and is credited with anthelmintic properties. Scientific parameters are not yet available to identify the true plant material and to ensure its quality. Therefore the present work has been undertaken to establish the necessary pharmacognostic standards for evaluating the plant material. Various parameters like morphology, microscopy, physico-chemical constants and phytochemical profiles of the leaves were studied and the salient diagnostic features are documented.

Keywords: *Sesbania sesban;* Pharmacognostic, Microscopy, Physico-chemical constants, Phytochemical profiles.

Introduction

Sesbania sesban (Leguminosae), commonly known as 'Egyptian sesban' is a short-lived shrub up to 8 m tall, with pinnately compound leaves. The origins of *S. sesban* are unclear but it is widely distributed and cultivated throughout tropical Africa and Asia **(Humperys LR, 1995).** According to ethno medicinal studies the poultice of leaves of *S.sesban* promotes suppuration of boils and abscesses and absorption of inflammatory rheumatic swellings. (The Wealth of India, 2003). Juice of fresh leaves is credited with anthelmintic properties. **(The Wealth of India, 2003)**. In the folk-lore remedies the juice of leaves is used in scorpion stings also. *S.sesban* leaves are found to have clinical application in Vicharchika (a skin disease like Eczema.) The paste of *S.sesban* leaves also showed excellent results (73.33%) in eczema treatment. Phenolic acid guaiacyl and coumaryl lignins are reported to be investigated from cell walls and leaves. **(Hans *et al.*, 2000)** Triterpenes were found in a study carried out on leaves of *S.sesban.* **(Tokyao U., 2000).** A phytochemical investigation of the leaves of the plant *S.sesban* reported to contain chikusetsu saponin IV, Ilexoside VIII, Labloside A and Kaikosaponin. No scientific parameters are available to identify the true plant material and to ensure its quality. Therefore the present work has been undertaken to establish the various pharmacognostic and phytochemical parameters, which could serve as a measure of authentication and quality control for commercial samples of the crude drug. In addition the detailed microscopy of the leaves has also been studied and documented.

Materials and Methods

Plant Material:

The plant material was collected from the Sangamner region of the Ahmednager district, Maharashtra, in the month of September 2005 in the morning. The collection site is geographically located on altitudes and latitudes, 76.16^0 x 20.4^0 on the geographical map of India. The plant was authenticated by Dr. D.A. Patil, Reader, Dept. of Botany, S.S.V.P.S.College, Dhule, Maharashtra, India and a voucher specimen (No. RCP/04) was submitted in Pharmacognosy Dept. of R.C.Patel College of Pharmacy.

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Microscopic Characteristics of Powder Drug

The microscopic characteristics of leaf powder were carried out in order to reveal various diagnostic characters of the leaves **(Khandelwal KR, 2005).** The plant specimens were stained with various stains and the Microphotographs of the sections were made using Olympus BX 40 microscope attached with Olympus DP12 digital camera.

Physico-Chemical Constants

Physico-chemical constants such as the percentage of total ash, acid-insoluble ash, water-soluble ash, water and alcohol soluble extractives and loss on drying (LOD) were calculated based upon standard procedures**(Indian Pharmacopoeia, 1996)**.

Micro Chemical Investigation/ Histochemistry

Micro-chemical tests and testing of behaviour of specific reagent towards plant drug tissue was carried out. The plant drug tissue was subjected to various reagents and based upon the inference, the histological zones were estimated. **(Johansen DA, 1940; Singh V.K 2002)**

Fluorescence Analysis

The fluorescence analysis of the powdered leaves was done by placing dry powdered leaves on a slide and treating with several drops of specified reagents. The observation of the developed colour was done within one minute in order to avoid drying and resultant colour change. (Evans WC. 1997; Brain KR *et al.*1975). For few tests, the sample of powdered drug after being placed on a slide and being treated with several drops of specified reagent was allowed to dry completely. Then the dried specimen was mounted in nitrocellulose, allowed to dry and observed under UV lamp.

Elemental Analysis

Ash of drug material was prepared and 50% $^{v}/_{v}$ sulphuric acid was added to it. The treated ash was kept for 1 hr. and then filtered. Tests were performed on the filtrate and the results were noted. **(Harborne JB 1973)**

Phytochemical Screening

For preliminary phytochemical studies 65 g of powdered material was extracted in a Soxhlet apparatus with petroleum ether (60^0-80^0C), chloroform, methanol and water successively, obtained extracts were dried and weighed. The presence of various phytoconstituents viz. steroids and terpenoids (Leibermann Burchard test), alkaloids (Dragendroffs test), tannins and phenolics (Ferric chloride test), flavonoids (Shinoda test), Sugars (Fehling solution test), amino acids (Ninhydrin test), etc. was detected by usual methods prescribed in standard texts **(Houghton PJ, 1998; Peach K, 1955; Khandelwal. 2005)**

Results

Sesbania sesban is a short-lived shrub or small tree up to 8 m tall. Its leaves are pinnately compound, 2-18 cm long with 6-27 pairs of linear oblong leaflets (26 x 5 mm). **(Figure 1)**

Microscopic Characteristics of Powdered Drug:

The various diagnostic characters of the leaf powder are depicted in **(Figure 2)**. Microscopical Characteristics of Powdered Drug reveal the presence of trichomes, Epidermal Cells**,** Lignified cells and Palisade cells.

***Epidermal Cells*:** The Epidermal Cells are straight walled and polygonal in shape. Polygonal thin walled parenchymatous cells with wavy and anticlinal walls are also observed. The stomata are parasitic in form surrounded by irregular number of cells

Trichomes: The covering trichomes are uniseriate, multicellular wavy and with blunt apex. Lignified cells stained with Phluroglucinol and conc. HCl. are also shown.

***Palisade Cells*:** Palisade cells with spongy parenchyma and with epidermal cells were observed in the powdered drug sample.

Figure 1: Photograph of *Sesbania sesban* (L.) Merr. Leaves and Flowers**.**

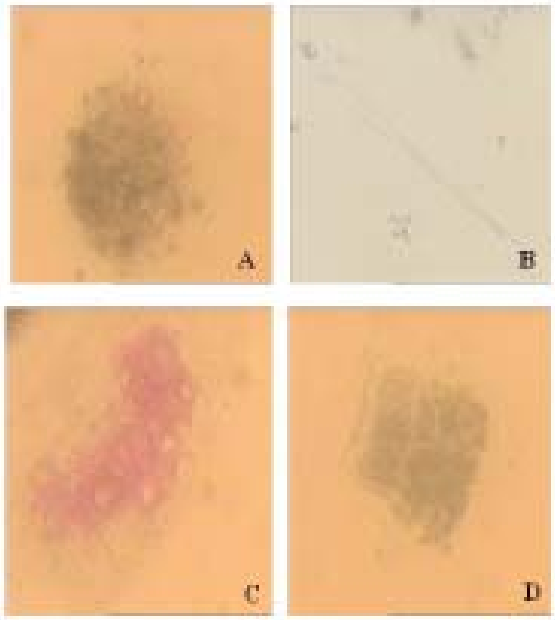

Figure 2 : Microscopical Details of Leaves of *S.sesban.* (A) Epidermal cells, (B) Covering Trichomes, (C) Lignified cells: Lignified, (D) Palisade Cells.

Physical constants Determination

The observed loss on drying for *Sesbania sesban* (L.) Merr. leaves was 14.2 % **w/w**. The Total ash, Acid insoluble ash, Water soluble ash and Sulphated ash were 10.5 %, 1.0%, 4.5% and 2.4% respectively . The values obtained for various ash values being determined are given in **Table 1.**

Table 1. Results of Ash Values

Sr. No.	Type of ash	Results (%)
1.	Total ash	10.5 %
2.	Acid insoluble ash	1.0%
3.	Water soluble ash	4.5%
4.	Sulphated ash	2.4%

Micro Chemical Investigation/ Histochemistry

Observation and result pertaining to micro-chemical tests and behaviour of specific reagent towards plant tissue are presented in **Table .2.** In the histochemical analysis the micro chemical tests showed the presence of Midrib, vascular bundle and pith.

Table 2. Results of micro-chemical tests/histochemistry of *Sesbania sesban* (L.)Merr. Leaves

Reagent	Test For	Inference	Histological Zone
Phloroglucinol + hydrochloric acid	Lignin	+	Vascular bundles and midrib region
Aniline sulphate + Sulphuric acid	Lignin	+	Vascular bundle and midrib region
Weak iodine solution	Starch	_	_
Sudan III solution	Oil globules	_	_
Aq. $FeCl_3$ Solution	Tannins & phenolics	+	Midrib, Vascular bundle and pith.
Dragendroff's reagent	Alkaloids	-	-
Libermann-Burchardlt reagent	Steroids	+	Midrib, Vascular bundle and pith.
Millon's reagent	Proteins	+	Midrib and vascular bundle

Table 3. Results of Fluorescence analysis of powdered leaves.

Sr. No.	Sample	Colour in Day light	Colour in UV	
			Long Wave length	Short Wave length
1	Powder is as such.	Light green	Greenish	Dark brown
2	Powder + nitrocellulose in amyl acetate	Green	Pinkish Brown	Brown
3	Powder + Sodium hydroxide in methanol	Green	Brown	Dark brown
4	Powder + Sodium hydroxide in methanol + nitrocellulose in amyl acetate	Light green	Dark brown	Dark brown
5	Powder +Sodium hydroxide in water	Green	Greenish brown	Green
6	Powder + Sodium hydroxide in water + nitrocellulose in amyl acetate	Light green	Green	Pinkish Brown
7	Powder +1N Hydrochloric acid	Dark Green	Brown	Dark Brown
8	Powder +1N hydrochloric acid+ nitrocellulose in amyl acetate	Whitish grey	Dark brown	Brown
9	Powder + 50%nitric acid	Orange	Red	Green
10	Powder + 50%sulphuric acid	Dark brown	Yellow	White

Table 4. Results of Qualitative Tests for Determination of Inorganic Elements

Sr. No.	Inorganic Constituents	Results of the Tests
1	Test for calcium	Positive
2	Tests for iron	Negative
3	Tests for magnesium	Negative
4	Tests for potassium	Positive
5	Tests for sodium	Positive
6	Tests for carbonate	Negative
7	Tests for Sulphate	Negative
8	Tests for phosphate	Positive
9	Tests for chloride	Negative
10	Tests for nitrate	Positive

Table 5. Extractive values of various extracts.

Extracts	Colour	Extractive value
Petroleum ether	Yellowish brown	4.3% w/w
Dichloromethane	Brown	3.1% w/w
Acetone	Dark brown	1.9% w/w
Chloroform	Brownish black	1.3% w/w
Methanol	Brown	15.4 % w/w
Aqueous	Greenish brown	6.1%w/w

Fluorescence Analysis

The results of fluorescence analysis are given in **Table .3.** The colour emitted by the powdered leaves in the above tests were designated in terms of the three primary colors, (red, yellow and blue) three secondary colors (orange, green, purple) and brown, which is a mixture of three primary colors.

Elemental Analysis

The elemental analysis of ash of powder of leaves of *S. sesban* reveal the presence of calcium, potassium, nitrates and phosphates. The results of elemental analysis are given in **Table .4.**

Phytochemical Investigation

Successive extraction and cold maceration of the leaf powder was carried out and methanolic extract was found to give a maximum extractive

Table 6. Results of Phytochemical Investigation of *Sesbania sesban* (L.) Merr. Leaves

Sr. No.	Chemical Tests	Pet.ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract
1.	**Acidic compounds**	+	+	+	+
2.	**Tests for Alkaloids:**				
	a) Dragendorff's Test	-	-	-	-
	b) Mayer's Test	-	-	-	-
	c) Wagner's Test	-	-	-	-
	d) Hager's Test	-	-	-	-
	e) Tannic acid test	-	-	-	-
3.	**Test for amino acids**				
	a) Million's Test	-	-	-	+
	b) Ninhydrin Test	-	-	-	+
4.	**Test for Carbohydrates**				
	a) Molisch's Test	-	-	-	+
	b) Barford's Test	-	-	-	+
	c) Selivanoffs test	-	-	-	-
	d) Test for pentoses	-	-	-	-
	e) Osazone formation	-	-	-	-
5.	**Test for Flavonoids**				
	a) Shinoda Test	-	-	-	-
	b)Alkaline Reagent Test	-	-	-	+
	c)Zinc-HCl Test	-	-	-	-
6.	**Test for glycosides**				
I)	**General test**				
	i) Tests for specific glycosides	-	-	+	+
	ii) Tests for Anthraquinone glycosides	-	-	-	-
	a) Borntrager's test	-	-	-	-
	b) Modified Borntrager's	-	-	-	-
	c) Test for hydroxy anthraquinones	-	-	-	-
II)	**Tests for cardiac glycosides**				
	a) Kedde's test	-	-	-	-
	b) Keller-Killiani Test	-	-	-	-
	c) Raymond's number	-	-	-	-
	d) Baljet's Test	-	-	-	-
	e) Legal's Test	-	-	-	-
III)	**Tests for coumarins glycosides**	-	-	-	-
IV)	**Cynogentic glycosides**	-	-	-	-
V)	**Saponin glycosides**				
	a) Froth test	-	-	-	-
7.	**Tests for inulin**	-	-	+	+
8.	**Tests for Lignin**	-	-	+	+
9.	**Tests for Mucilage**	-	-	-	-
10.	**Tests for tannins**				
	a) Ferric-Chloride Test	-	-	-	+
	b) Gelatin test	-	-	-	-
11.	**Tests for proteins**				
	a) Heat test	-	-	+	+
	b) Biuret Test	-	-	+	+
	c) Xanthoproteic test	-	-	+	+
12.	**Test for starch**	-	-	+	+
13.	**Tests for steroids and triterpenoids**				
	a) Libermann Burchard Test	-	-	+	+
	b) Salkowski test	-	-	+	+
	c) Sulfur powder test	-	-	+	+

yield of 15.4 % $^{w/w}$. In the phytochemical tests the Pet .ether and Chloroform extract revealed the presence of Triterpenoids and Steroids. Methanolic and Aqueous extract, showed the presence of Carbohydrates, vitamins, Amino acids, proteins, tannins, saponin glycosides and steroids. The extractive values and results of individuals tests for various phytoconstituents are stated in **Table 5 and 6** respectively.

Conclusion

Sesbania sesban is currently being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in a herbal pharmacopoeia, pharmacognostic parameters and standards must be established. Any crude drug which is claimed to be *Sesbania sesban* but whose characters significantly deviate from the accepted standard above can be checked for being either contaminated or adulterated. The Phytochemical investigation of the leaf extracts reveal the presence of Tannins, Triterpenoids, Steroids and Glycosides. Hence the investigation, may serve in providing preliminary basis for investigation of the potential of leaves of *S.sesban* as antioxidant, anti-inflammatory, and hypolipidemic agent.

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