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Short Communication

Histological study, phytochemical screening and TLC studies of *Sesbania grandiflora*

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Abstract

Sesbania grandiflora (S. grandiflora), folklore medicinal plant is used in various parts of world. Leaves were used as alexeteric, for epilepsy, nyctalopia, gout, anthelmintic, itch, leprosy and ophthalmia. The plant possesses various therapeutic properties such as antiphlogistic, mildly laxative, anthelmintic, odontalgic properties and antirheumatic. The flowers were used as aperitif and refrigerant, for bronchitis, biliousness (abhorrence), gout, nyctalopia (night blindness), ozoena and quartan fever. Roots and barks were used in inflammation and as astringent respectively. In the present investigation phytochemical screening, histological examination and thin layer chromatographic identification of bark and extracts has been studied extensively and provided diagnostic key to spot adulterant. It is concluded that our research data helps to isolate and characterize the different phytochemicals responsible for medicinal potential of the S. grandiflora as anticancer, antiinflammatory, antibacterial, anxiolytic and anticonvulsant.

Keywords: *Sesbania grandiflora*; Morphology; Microscopy; Physiochemical; Phytochemical; TLC.

Introduction

Sesbania grandiflora (*S. grandiflora*) commonly called as Agati (in Hindi) belonging to Fabaceae family. It is native to many Asian countries and grows well in settled areas at low and medium altitude.*S. grandiflora* possesses many medicinal properties as anticancer, chemo preventive and hepatoprotective. It is also reported to have anxiolytic, anticonvulsive[1], antioxidant, antiurolithiatic[2] and hypolipidemic properties[3]. In addition, it is reported as a strong antidote for tobacco and smoking-related disorders[4]. Different phytoconstituents such as steroids, terpenoids [5], flavonoids and isoflavonoids as isovestitol; medicarpin and sativan were isolated and reported previously[6]. In this study the attempt was made to establish the phytochemical screening, histological examination and TLC identification of bark and its extracts respectively.

Material and methods

Identification and authentication of plant: The bark of *S. grandiflora* was collected locally from Nagpur. The plants were collected locally and taxonomic authentication was done by the Botany Department, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The above plant materials were dried under the shade.

The herbarium sheets of plant specimen was prepared and deposited for future reference (Herbarium voucher specimen number 9580).

Morphological and microscopical evaluation: The bark of *S. grandiflora* were procured and examined for various organoleptic properties. These studies include parameters such as taste, odour, shape, margin, venation, size, surface and apex. The microscopical study of *S. grandiflora* was done with the help of Motic Image plus 2.0 microscope. The air-dried plant material was then, pulverized into a coarse powder and used for research work.

Determination of Physicochemical constants: Physicochemical constants of *S. grandiflora* barks were determined to elicit water soluble ash, total ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value as per the method described in Pharmacopoeias and reported previously[7].

Preparation of extract: Bark was dried and milled to a coarse powder. One kg of fresh plant material was grounded and defatted with petroleum ether. It was extracted subsequently with chloroform, ethyl acetate, acetone and methanol in a Soxhlet apparatus followed by maceration with 50% methanol (hydroalcoholic) for 7 days.

The organic solvents were evaporated using rotary vacuum evaporator to yield chloroform extract (CHSG), ethyl acetate extract (EASG), acetone extract (ACSG), methanolic extract (MESG) and 50 % methanolic or hydroalcoholic extract (HASG) respectively. Phytochemical and TLC studies of these extracts were undertaken.

Phytochemical Screening: The Phytochemical screening of the extracts were assessed to detect the presence of different phytoconstituents such as alkaloids, flavanoids, saponins, triterpenoids, steroids, carbohydrate, tannin, coumarins, phenols, carboxylic acid, amino acid and proteins by performing chemical tests described previously[8,9].

TLC of extracts: One gram of extracts were dissolved in methanol, filtered and utilised for TLC studies. About Six µl of extracts of *S. grandiflora* in 6 mm of bandwidth were applied on aluminium plates pre-coated with silica gel G F254 using CAMAG Linomat 4 (Muttenz, Switzerland) TLC applicator[10]. The plate was developed in different solvent system in CAMAG twin trough chamber. Visualisation and documentation of developed plates were done in short and long UV using CAMAG Photo documentation unit[11].

Results and discussion

Morphological and microscopical evaluation: The bark of *S. grandiflora* was investigated for different organoleptic properties. The size, shape, taste, colour and odour was observed.

The following morphological and microscopical characters were observed in the plant.

Morphology of *S. grandiflora bark:* The morphological studies revealed that the bark of *S. grandiflora* varying in size and thickness, odour is faint and taste is acrid. The bark pieces were curved or channelled, 1 to 10cm in length, 1 to 3cm in width and 5 to 8mm in thickness. The outer surface of the bark showed the presence of longitudinal wrinkles with transverse cracks, rough in nature, dark reddish brown in colour, while the inner surface was striated and varying in colour from slightly yellowish to cream. Bark possesses outer short and inner fibrous fracture.

Microscopical features of *S. grandiflora bark:* The microscopical study of *S. grandiflora bark* was done with the help of Motic Image plus 2.0 microscope.The transverse section of the bark of *S. grandiflora* contains 4- 12 layers of tangentially elongated cork cells with brownish matter. Phellogen showed the presence of two layers of thin walled tangentially elongated cells. Cortex contains wide parenchymatous cells interspersed with stone cells. Few non lignified pericyclic fibres are also present. Secondary phloem showed the presence of phloem parenchyma, sieve tubes and companion cells. Round stone cells separated by medullary rays encircled by a sheath of parenchyma are also present. Radially arranged medullary rays from the centre to the cortex through the phloem, three to five cells in width are also present (Figure-1).

Determination of Physicochemical constants: The residue remaining after ignition of the crude drug is designated as ash. The ash and acid insoluble ash value was found to be 9.23% and 2.23% respectively. It varies within definite limits as per the nature of soils. Sometime it also includes inorganic matter which may deliberately add as adulteration. The total ash mainly consists of phosphates, silicates, carbonates, and silica. The acid insoluble ash is insoluble in dilute hydrochloric acid. A higher limit of acid-insoluble ash reflects contamination with the earthy material. The alcohol soluble and water soluble extractive value was found to be 2.41% and 2.81% respectively indicating the presence of polar phytoconstituents[12]. The extractive values are indicative of approximate quantity of their chemical constituents, these values are used to determine the superiority of drugs since many times, drugs are identified as of substandard value due to either improper collection or inappropriate storage.

Extraction of plant material: The air dried plant material was first defatted with petroleum ether and then extracted successively with solvents of increasing polarity viz. chloroform, ethyl acetate, acetone, methanol and hydro-ethanol (1:1) to obtain the polarity based phytoconstituents. The appearance and yield of different extracts were mentioned in Table-1. The maximum yield was found as 10.78% and 11.44% in MESG and HASG extracts respectively. These findings are in accordance with the results of extractive value determination. The extracts obtained are indicates the approximate values of chemical constituents, which obtained from specific amount of air dried plant[13].

Preliminary phytochemical screening of extracts: Preliminary phytochemical screening of extracts was carried out to reveal the presence of different secondary metabolites. Petroleum ether extract (PESG) and CASG extract revealed the presence of steroids and triterpenoids. Flavonoids, tannins and proteins were found in EASG and ACSG extract respectively. Whereas MESG and HASG extracts showed the presence of flavonoids, carbohydrates, saponins, proteins and tannins respectively (Table-2).

TLC studies of extracts: TLC studies were carried out to estimate number and type of phytoconstituents present in extract. Chromatography method is extensively used for the separation, isolation, identification and quantification of components in a mixture[14]. Components of the mixture are separated with the help of the stationary and mobile phase. Different solvent systems were prepared and tried for all the extract and fractions. The best resolution in solvent system was considered as optimised, valid and useful.

The satisfactory resolution was obtained in the mobile phase mentioned in Table-3 and photo documentation was shown in Figure-2. However, presence of constituents was confirmed by spraying different reagents on TLC plates.

Conclusion

In the present work, pharmacognostic characterization, determination of their physiochemical parameters, phytochemical screening and TLC studies of the crude extracts of plants *S. grandiflora* was studied. The selected plant was authenticated and the macroscopic studies were performed to establish their identity and purity. The microscopic study was carried out to determine basic cellular composition of the leaf petiole, stem and the type of stomata etc. These microscopic characteristics of particular species were treated as standard for identification of the plant species. Physicochemical studies were carried out as per Ayurvedic and Indian Pharmacopoeia (I.P., 1996) such as ash value, acid insoluble ash values and extractive values. The important phytoconstituents were present as depicted in phytochemical screening which are well known for their medicinal potentials which was further characterized by TLC. It is concluded that our research data helps to isolate and characterize the different phytochemicals responsible for medicinal potential of the *S. grandiflora* as anti cancer anti inflammatory, anti bacterial, anxiolytic and anticonvulsant.

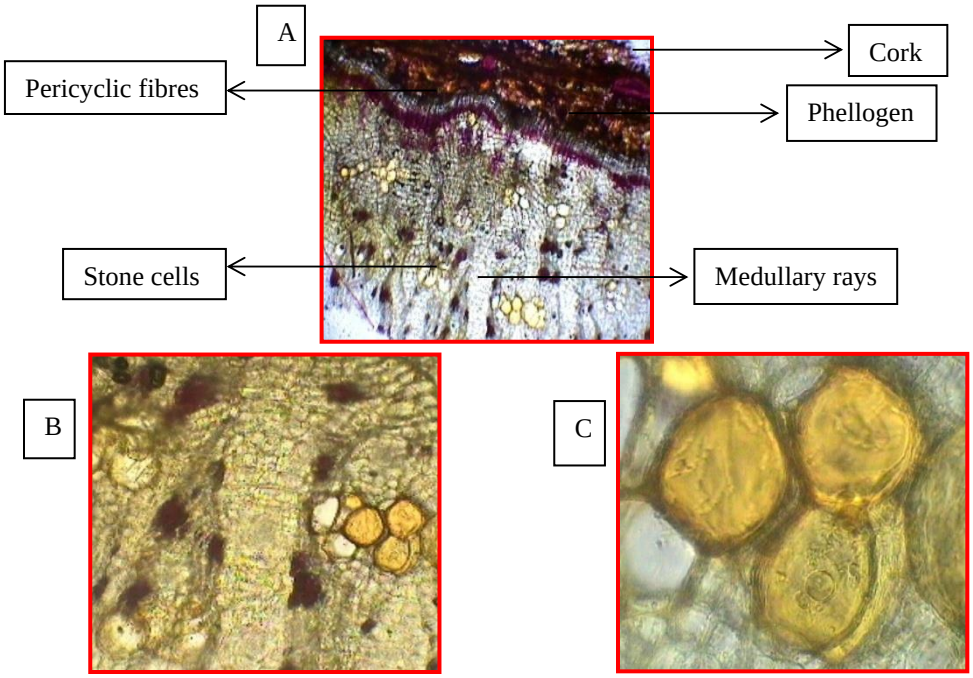

Figure-1: Microscopical features of *Sesbania grandiflora* bark (A) Transverse section of *Sesbania grandiflora* bark; (B) Enlarged view of the *Sesbania grandiflora* bark section showing Medullary rays. (C) Enlarged view of the *Sesbania grandiflora* bark section showing Stone cells.

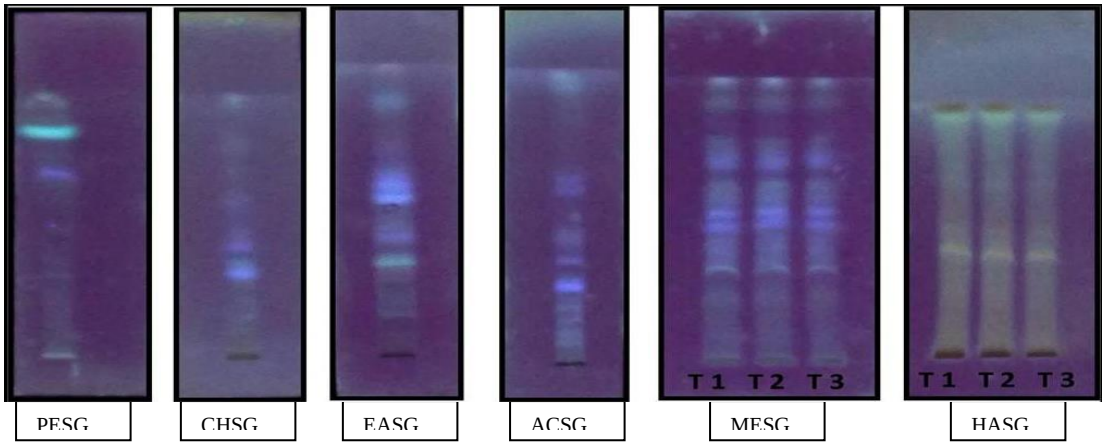

Figure-2: TLC Photo documentation of PESG, Petroleum ether extract of *S. grandiflora;* CHSG, chloroform extract of *S. grandiflora;* EASG, ethyl acetate extract of *S. grandiflora*; ACSG, acetone extract of *S. grandiflora;* MESG, methanolic extract of *S. grandiflora*; HASG, hydroalcoholic extract of *S. grandiflora* observed under long UV at 366 nm.

Table-1: Yield of extracts obtained from successive extraction of aerial parts of *Sesbania grandiflora.*

Plant	Type of Extract	Appearance/ State	Yield (% w/w)
Sesbania grandiflora bark	Petroleum ether (PESG)	Dark Brown/Semisolid	2.7
	Chloroform (CHSG)	Dark Brown-black/ Semisolid	2.6
	Ethyl acetate (EASG)	Dark Brown-black/ Semisolid	2.5
	Acetone (ACSG)	Dark Brown-black/ Semisolid	4.54
	Methanolic (MESG)	Dark Brown-black/ Semisolid	10.78
	Hydroalcoholic (HASG)	Dark Brown-black/ Semisolid	11.44

PESG, Petroleum ether extract of *S. grandiflora;* CHSG, chloroform extract of *S. grandiflora;*EASG, ethyl acetate extract of *S. grandiflora*; ACSG, acetone extract of *S. grandiflora;* MESG, methanolic extract of *S. grandiflora*; HASG, hydroalcoholic extract of *S. grandiflora*

Table 2: Preliminary phytochemical screening of extracts of aerial parts of *Sesbania grandiflora*

Chemical tests	*Sesbania grandiflora bark extracts*					
	Name of the extracts					
	PESG	CHSG	EASG	ACSG	MESG	HASG
Proteins & Amino acid	-	-	-	+	+	+
Carbohydrate	-	-	-	-	+	+
Sterol	+	+	-	-	-	-
Terpenoids	+	+	-	-	-	-
Saponin	-	-	-	-	+	+
Flavonoid	-	-	+	+	+	+
Alkaloid	-	-	-	-	-	-
Tannin	-	-	+	+	+	+

+ indicates present and – indicates absent. PESG, Petroleum ether extract of *S. grandiflora;* CHSG, chloroform extract of *S. grandiflora;*EASG, ethyl acetate extract of *S. grandiflora*; ACSG, acetone extract of *S. grandiflora;* MESG, methanolic extract of *S. grandiflora*; HASG, hydroalcoholic extract of *S. grandiflora*

Table 3: Mobile phase for TLC studies of extracts of *Sesbania grandiflora plant*

Test extract	Solvent system	Number of Bands
PESG	Chloroform (100 %)	07
CHSG	Toluene: Methanol(9.5:0.5)	10
EASG	Toluene: Methanol: Triethylamine (8.5:1:0.5)	10
ACSG	Toluene: Methanol: Triethylamine (8:1.5:0.5)	09
MESG	Toluene: Methanol: Triethylamine (7.5:2:0.5)	09
HASG	Toluene: Methanol: Triethylamine (7:2.5:0.5)	10

PESG, Petroleum ether extract of *S. grandiflora;* CHSG, chloroform extract of *S. grandiflora;* EASG, ethyl acetate extract of *S. grandiflora*; ACSG, acetone extract of *S. grandiflora;* MESG, methanolic extract of *S. grandiflora*; HASG, hydroalcoholic extract of *S. grandiflora*

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