Phytochemical and HPTLC Studies of *Sesbania Grandiflora L.*

Ms. Trupti.B. Shevante[1], Suresh K.Dev[2]. Suresh L Jadhav[3]

[1] *Research Scholar, Pacific University, Udaipur, Rajasthan, India.*
[2]*Professor, Pacific University, Udaipur, Rajasthan, India.*
[3] *Principal, Vishal Institute of Pharmaceutical Education and Research, Pune, Maharshtra,India.*

Abstract

The plant *Sesbania grandiflora (L).Pers* or Agathi or Agasthya, belonging to family Fabeaceace, has great medicinal value in Indian traditional medicine for treatment of wide range of diseases that goes as a principal ingredient in preparation of Ayurveda medicinal formulations. It is used anthelmintic, diuretic, laxative, antigout antipruritus, antiepilepsy,skin disorders. The present study deals with the detail study of pharmacognostic, phytochemical and physiochemical investigation on fresh leaves and powder of *Sesbania grandiflora* L. Physiochemical investigation revealed loss on drying (2.98± 0.03%), total ash(8.13± 0.51),water soluble ash (1.83± 0.34),acid insoluble ash(1.67± 0.36),alcohol soluble extractive (10.23± 0.89),water soluble extractive (4.07± 0.31). Phytochemical analysis was performed to confirm the presence of various functional groups. The HPTLC technique of alcoholic extract showed the presence of six and seven spots at 254 nm and 366 nm. The study done will provide relevant data used for proper identification and authentication of used herbal plant.

Keywords: Agasthya, Ayurvedic, pharmacognostical, physicochemical evaluation, HPTLC.

Correspondence Author:- Ms. Trupti.B. Shevante, Research Scholar, Pacific University, Udaipur, Rajasthan, India. Email Id:-lataashevante@gmail.com,Mobile No:-7385966025

DOI: 10.48047/ecb/2023.12.Si10.00375

Introduction

Ancient humans used herbal medicinal plants as a form of traditional medicine. Today, the majorities of people in the globe rely on or utilize largely herbal remedies to cure a variety of disorders. Different medical systems use the entire plant as well as various plant sections as medicinal agents. The *Sesbania grandiflor* (L) has therapeutic medical significance and is used as a medication in various dose forms in whole or in separate parts [1].it is straight cylindrical with soft wooded, sparsely branched with 8-10 m height, about 22-26 cm diameter tree. Leaves are linear, oblong 15–30 pair's leaflets with 15-30 cm long. Racemes with 2-4 white or pink flowers are 2-3 cm long. All parts have medicinal. [2, 3] Due to its unique medicinal properties it is used as a herbal drug for its antibiotic, anthelmintic, anti-tumour and contraceptive , natural anti-oxidant, anthelmintic property and is used to treat worms, biliousness, fever, gout, itchiness, and leprosy.[4] Additionally in the Siddha system of Indian traditional medicine, this plant is used to cure a variety of illnesses, such as anemia, bronchitis, fever, headaches, ocular problems, nasal congestion, inflammation, leprosy, gout, and rheumatism. S. grandiflora is also cited as a strong remedy for illnesses brought on by smoking and tobacco use. Sesbania's varied sections are utilized in the

treatment of numerous illnesses and disorders. [5] This effort sought to establish the pharmacognostical study, phytochemical screening, and HPTLC identification of leaves and its extract.

Materials and Methods

Identification and Authentication of plant.

Fresh leaves of *Sesbania Grandiflora* was collected during the month of January to February from the local area like Ale, Junnar, Dist-Pune, Maharashtra. The voucher herbarium specimens along with voucher crude drug is preserved at Agharkar Research Institute, Pune and was identified and authenticated by Dr.R.K.Choudhary Scientist, Agharkar Research Institute, Pune.

Methods;-

Morphological and microscopical evaluation

The leave of *S. grandiflora* was 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 Swift Ives camera lucida microscope. The air-dried plant material was then, pulverized into a coarse powder and used for research work. [5]

Determination of Physicochemical constants: Physicochemical constants of S. grandiflora leaves were determined water soluble ash, total ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value as per the method described in Pharmacopoeias.[6]

Ultraviolet screening of leaf powder of *Sesbania grandiflora*

The fluorescence study of powder with different reagents under ordinary day light and uv light (long & short) shows distinct characteristics fluorescence.[6]

Preparation of extracts:*Sesbania grandiflora* leaves were cleaned under running water and dried in the shade for seven days. Dried leaves were mechanically crushed to a coarse powder, sieved, and stored at room temperature in an airtight container. The extraction method was chosen based on the presence of active ingredients in the medicine. By using the soxhlet extraction method, dried powder (500 g) was extracted with Acetone, ethanol, methanol and distilled water. The extracts were dried by distilling the solvent at low temperatures with a rotary evaporator. The extracts were kept in a refrigerator at 4^0 C. [7,8]

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.[9,10]

HPTLC analysis of extract

HPTLC analysis of methanolic extract of leaves of Sesbania Grandiflora was done by lane analysis. HPTLC analysis was done to access presence of components. **[11, 12, 13, 14]**

TLC instrumentation and conditions

Sample Preparation: Sample Dissolved in methanol & incubated over night for 24 hrs to 48 hrs. followed by concentrating the sample by Rotary evaporator method.

Sample loading About 5 µl of extract of Sesbania Grandiflora is diluted with methanol and standard solution of Quercetin and Gallic acid were loaded as 6.0 mm 60F 254 TLC plate with use of Hamilton Syringe.

Scanning

TLC developed was dried to evaporate solvent and then placed in photo documentation chamber and images were captured at 254nm and 366nm.

Band Size: 5mm

Analysis Type: Lane Analysis

Seperation Technique: Ascending

Test : methanolic extract of Sesbania Grandiflora

Standards: Quercetin, Gallic acid

Mobile Phase: Toluene; Ethyl Acetate; Formic Acid (5:4:1)

Results and Discussion

Macroscopic determination

It is straight cylindrical with soft wooded, sparsely branched with 8-10 m height, about 22-26 cm diameter tree. Leaves are compound linear, oblong numerous pairs leaflets arranged oppositely with 15-30 cm long. Single leaflet is 2-4 cm long while in mature compound leaf there are approximately 25-30 oppositely paired leaflet and 10-15 mm. Racemes with 2-4 white or pink flowers are 2-3 cm long. Pods are Slender, falcate or straight, and 30–45 cm (12–18 in) long, with a thick suture and approximately 30 seeds 8 mm (0.3 in)
in size. [Figure 1 and 2]

Figure 1: Flowering twings with leaflet of *S.Grandiflora*

Figure 2: Pods of *S.Grandiflora*

Microscopic Characters

Microscopy of *S. grandiflora* indicates dorsiventral, single layered upper and lower epidermises with thick cuticle. 2-3 layers of narrowly arranged angular collenchymatous cells below upper epidermis and 1-4 layers of closely arranged round bottom flask necked parenchymatous cells. Midrib region consists of collateral, conjoint vascular bundle closed with metaxylem facing towards the lower region and protoxylem facing towards the upper region

Lower region of the leaflet consists of 2-3 layers of angular collenchymas followed by 1-3 layers of parenchyma. About 1-4 layers of collenchymatous cells are arranged near the vascular bundle region.Laminar region shows single layered upper and lower epidermis, 2 layered palisade parenchyma and loosely arranged spongy parenchyma cells.the cells are compactly arranged long and tubular with chloroplast.(Figure 3). Powder Microscopy reveals the presence of vessels,trichomes,calcium oxalte crystals, parenchymatous cell(Figure 4,5,6).

Figure 3 - T.S of Leaflet showing upper and lower epidermis of *S.Grandiflora*

Figure 4: Powder microscopy showing trichomes and vessels

Figure 5 Cellular structure of leaf with thin walled parenchymatous cell

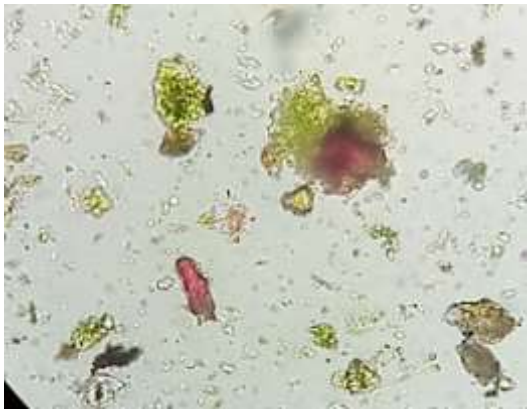

Figure 6 Powder microscopy showing calcium oxalte crystals

Physical Analysis of leaf powder of *Sesbania grandiflora*

Various physical analyses are done for various parameters like foreign organic matter,loss on drying,ash content, extractive values.

Table 1: Physiochemical determination of *Sesbania grandiflora* leaf powder

Sr no	Evaluation Parameters	Results (gm%w/w)
1	Foreign Organic matter	0.93 ±0.12
2	LOD 110^{0}C	2.98± 0.03
	Ash Values	
3	Ash Content	8.13±0.51
4	Water soluble ash	1.83±0.34
5	Acid insoluble Ash	1.67±0.36
	Extractive Values	
6	Alcohol soluble extractive	10.23±0.89
7	Water soluble extractive	4.07±0.31

Ultraviolet screening of leaf powder of *Sesbania grandiflora*

The fluorescence study of powder with different reagents under ordinary day light and uv light (long & short) shows distinct characteristics fluorescence.

Table 2: UV fluorescence studies of leaf powder of *Sesbania grandiflora*

Powder + reagent`	Ordinary light	UV short wave (254nm)	UV long wave (365nm)
Only Powder	Green	Green	Fluorescent Green
Powder + 1n NaOH	Green	Dark green	Yellowish Brown
Powder +CH_3COOH	Dark brown	Dark green	Orange
Powder +50% KOH	Green	Deep green	Orange
Powder +50%HNO_3	Brown	Green	brown

Powder +50%H_2SO_4	**Blue**	**Greenish blue**	**Light green**
Powder +water	**Green**	**Green**	**Yellow**

Extraction of leaves of *Sesbania Grandiflora*

The % extraction yield of in aquous,Ethanol,Acetone and Methanol are 12.6% w/w,11.50%w/w,3.5%w/w and 17.8% w/w respectively. Figure 7 indicates that % w/w yield of leaves extract is higher in methanolic extract.

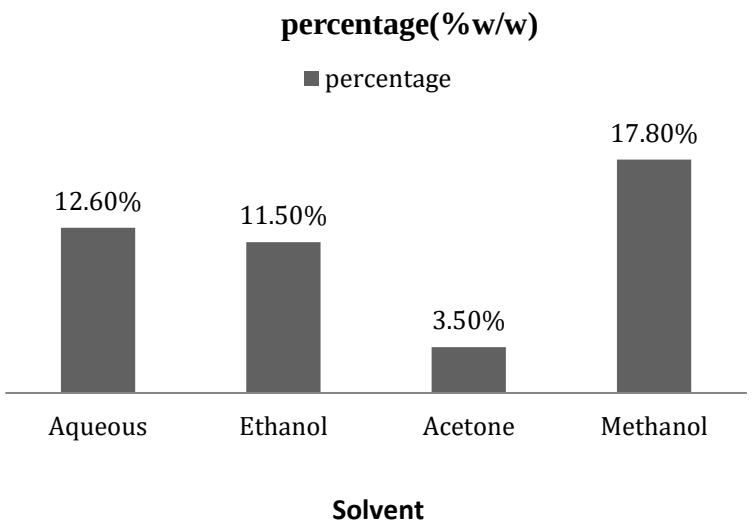

Figure 7: Extraction yield percentage in different solvents

Phytochemical Screening of *S.grandiflora L.*

The phytochemical study of methanolic leaf extracts of S.grandiflora was investigated for compounds like alkaloids,glycosides,flavonoids,saponins,steroids and tannins and found most of them are present.

Table 3: Phytochemical screening of *S.Grandiflora L.*leaves extract

Solvent / Test	Aqueous	Ethanol	Methanol	Acetone
Detection of Carbohydrates				
Molisch's test	-	+	+	+
Fehling's test	+	-	+	-
Detection of Alkaloids				

Mayer's test	+	-	+	+
Wagner's test	+	+	+	+
Other				
Steroids	-	+	+	-
Glycosides	-	-	+	+
Killer killani test	-	-	+	-
Saponins	-	+	+	+
Flavanoids	-	-	+	-
Tannins	+	+	+	+
Triterpenoids	-	-	+	+
Xanthoprotein test	+	-	-	-
Biuret test	+	-	-	+

Table 3 reveals that methanolic extracts shows the presence of most numbers of components and clearly indicates clearly that methanol can be used as a principle extracting solvent and also evaporation of methanol is easy.

HPTLC analysis of Methanolic extract

Phytochemical profiling and quantification of quercetin and garlic acid in the methanolic extract of leaves of *Sesbania Grandiflora* was studied and results were obtained in the form chromatograms depicted in figures 8 and 9. Chromatograms from standards and test samples were obtained by CAMAG TLC scanner III at short (254 nm) (fig. 8) and long (366 nm) (fig.9) wavelength.

Table 4: HPTLC details of *S.Grandiflora L.*leaves extract

Band number	**Rf Value**		**Assigned substances**
	254 nm	**366 nm**	
1	0.504, 0.449, 0.336	0.538, 0.476, 0.429,0.165	Methanolic extract of Sesbania Grandiflora leaves

2	0.322	0.185	Gallic acid
3	0.438	0.511,0.453	Quercetin
4	0.468	0.458	Flavanoids

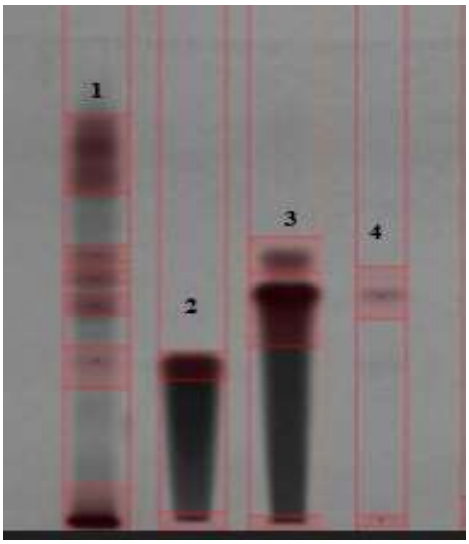

Figure 8-image of TLC plate at 254nm

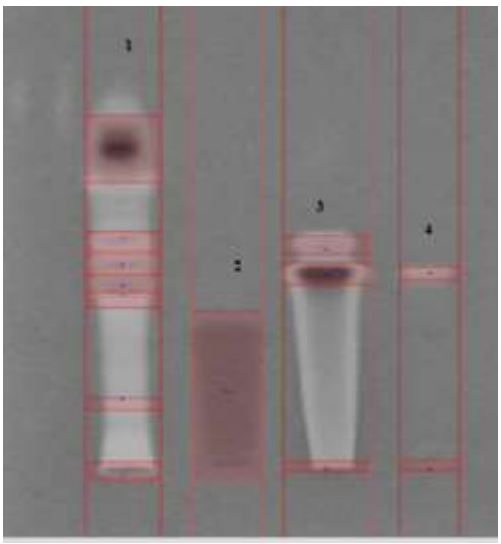

Figure 8-image of TLC plate at 366nm

Figure 8 and 9 indicates the presence of gallic acid and quercetin in the methanolic extract of leaves of Sesbania grandiflora.

Conclusion

The current study advances science by making pioneering preliminary findings regarding physicochemical properties, the presence of useful constituents through HPTLC, microscopic diagnostic characteristics through powder microscopy, and sufficient scientific material to initiate future studies As HPTLC phytochemical profiling reveals the presence of several bioactive compounds like Quercetin and gallic acid in the methanolic extract of Sesbania Grandiflora reveals that can be further explored up to their identification and future application in pharmacological treatment.

Future Scope

The current study may contribute to research pioneering preliminary study with respect to pharmacognostical physiochemical, phytochemical and advanced parameters like HPTLC so that benefits of Agasthya reaches out their therapeutic values.

Acknowledgement

The author is thankful to Dr.Suresh K Dev for giving me proper guidance .I also sincerely thanks to College management and Dr.Suresh L Jadhav for providing technical facilities and assistance required for this work.

References

1. M Janani A Aruna A review on neutraceutical value of sesbania grandiflora (agati) World J Pharm Res20176780416.
2. The Wealth of India, Raw Materials. *C.S.I.R. (Council of Scientific and Industrial Research)* 1999;Vol. 9:295.
3. Yadav, P., Harisha, C. R., & Prajapati, P. K. (2010). Pharmacognostical and physicochemical evaluation of Agasti leaf. *International journal of Ayurveda research*, *1*(4), 231–236. https://doi.org/10.4103/0974-7788.76787
4. Aa, Arun & Pb, Karthikeyan & Pattiyappan, Sagadevan & Ra, Umamaheswari & Peo, Rex. (2014). Phytochemical screening of Sesbania grandiflora (Linn). International Journal of Biosciences and Nanosciences. 1. 2014.
5. Wallis TE. 15th ed. London: Churchill Publications; 1985. Textbook of Pharmacognosy; pp. 572–82
6. Vol. 5. New Delhi: Government of India Publication; 2016. Ayurvedic Pharmacopoeia of India. Part 1,2.1.3-2.1.9
7. *Veeresh P Veerapur, Pramod P Desai, S Vijayakumar. Pharmacognostic and Preliminary Phytochemical Screening of Sesbania grandiflora root. Res. J. Pharmacognosy and Phytochem. 2018; 10(4): 285-290. doi: 10.5958/0975-4385.2018.00046.8*
8. *Amit Roy, Dayananda Bhoumik, Ram Kumar Sahu, Jaya Dwivedi. Phytochemical Screening and Antioxidant Activity of Sesbania grandiflora Leaves Extracts. Asian J. Res. Pharm. Sci. 4(1): Jan.-Mar. 2014; Page 16-21.*

9. Richardson PM and Harborne JB. (1990). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Second Edition. Brittonia., 42(2), 115. doi:10.2307/2807624
10. Deepthi, K., Renjith, P.K., Shameem, K. et al. Phytochemical screening of leaves and flower extracts of Sesbania grandiflora (L.) Pers. and its antimicrobial activity against fish pathogens. Vegetos (2022).
11. A.N, A. (2011). Phytochemical and TLC Studies of Ethanolic Extract of Sesbania grandiflora (Fabaceae).
12. Avalaskar A.N., Itankar .P.R , Joshi V.S , Agrawal.M., Vyas J.(2011) Phytochemical and TLC Studies of Ethanolic Extract of Sesbania grandiflora (Fabaceae).International Journal of PharmTech Research CODEN (USA): IJPRIF ISSN : 0974-4304 Vol.3, No.3,pp 1346-1349.
13. Vaidevi Sethuraman, Kumar Janakiraman, Venkateshwaran Krishnaswami, Subramanian Natesan & Ruckmani Kandasamy (2021) Combinatorial analysis of quercetin and resveratrol by HPTLC in Sesbania grandiflora/ phyto-based nanoformulations, Natural Product Research, 35:13, 2243-2248, DOI: 10.1080/14786419.2019.1662012