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MICROSCOPIC, PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF SESBANIA GRANDIFLORA LEAVES

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ABSTRACT
Objective: *Sesbania grandiflora* is a short-lived and fast-growing edible ornamental plant belonging to the Fabaceae family. Due to its unique therapeutic characteristics such as anti-inflammatory, antitumor, neuroprotective antioxidant, etc., it is utilized as an herbal medication in the Indian traditional medical system to treat various diseases. Therefore, the objective of the current investigation was to offer certain beneficial information regarding the standardization and identification of *S. grandiflora* leaf, which may be helpful in terms of its validity, purity, and quality. **Methods:** The micro- and macroscopical study of fresh and dried leaves of Sesbania grandiflora was investigated. Physicochemical parameters were performed according to WHO-recommended parameters. The dried leaves were powdered and extracted with different solvents in a Soxhlet apparatus. The concentrated extract was further used for physiochemical and phytochemical studies. **Result:** The fresh leaves of *S. grandiflora* were examined for their organoleptic characteristics. The leaves are regular compound and pari-pinnate with an average length of 15–30 cm long with green color. The transverse section of the leaf demonstrated the presence of spongy and palisade type of mesophyll cells. Stomata are anxiolytic and anisocytic stomata. Furthermore, Powder microscopy revealed the presence of simple epidermal hairs, dark yellowish-brown tannin fragments, light yellowish resinoids, oil globules, mucilage cells, and spiral vessels. Phytochemical screening revealed the presence of triterpenes, glycosides, tannins, flavonoids, gallic acid, biotin, and rutin. **Conclusion:** This study adds to the body of knowledge regarding the standardization and identification of the subject matter and facilitates future research on the Ayurvedic medical system.

INTRODUCTION

The medicinal herbs or natural remedies do not cause any imminent risk to human health and the environment [1]. Since ancient times, medicinal plants and their metabolites have been used as a reliable source of medicine to treat a wide range of injuries and illnesses [2]. Due to their natural origin, herbal remedies are always safer, more affordable, and have fewer adverse effects than modern medication. Most medicinal plants

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are also utilized as a food source for nutritional purposes [3]. In several Asian countries, such as China, Nepal, India, Pakistan, Sri Lanka, Bhutan, and Indonesia, medicinal plants play a significant role in the healthcare system [4]. The primary factors contributing to the widespread use of herbal medicine are safety and cost-effectiveness [5]. According to a WHO report, roughly 80% of the world's population depends on herbal medicine to meet their fundamental medical needs due to its higher cultural acceptability and minimal side effects [6].

Furthermore, the market for herbal remedies is growing daily. Hence, several prominent healthcare companies actively research plant-based compounds for possible medical applications [7]. In India, 7,000 -7,500 medicinal plants cure various diseases [8]. In addition to secondary metabolites, medicinal plants contain numerous phytomolecules; some of these phytomolecules are toxic [9]. Therefore, to reduce the prevalence of toxicity, the quality control of herbal medications is essential to ensure the drug's efficacy and safety. *S. grandiflora*, also known as Agati in Hindi and da hua tian jing in Chinese belongs to the Fabaceae family and is cultivated as an ornamental plant all over India and Yunnan in China [10]. Xishuangbanna in China is considered the birthplace of ethnobotany, where several edible plants, such as *S. grandiflora*, are utilized by the local tribes for food and medicine purposes [11]. It is a fast-growing, loosely branched multipurpose plant that can grow up to 8 to 15 m [12]. Due to its unique therapeutic characteristics, it is utilized as a herbal medication in traditional Indian medical systems as well as in traditional Chinese medicine system to treat various acute and chronic conditions such as depression, fungal infection, diabetes, inflammation, etc.[13-14].

In addition it is also used for the removal of toxins from the body and nourishment of liver [15]. The Leaves of *S. grandiflora* are dark green, arranged in a pinnate pattern, and have a slightly bitter, acrid, and astringent taste [16]. The leaf of *S. grandiflora* contains abundant fibers, protein, vitamins, carbohydrates, fats, and minerals, and due to its high nutritional content, the plant's leaves, petals, and seeds can all be eaten [17]. The newly sprouted leaves are tasty and frequently used as a salad in a meal. The dried leaves are thought to have strong antibacterial, anticancer, and contraceptive effects [18]. Flowers are reportedly used as an emollient, astringent, and antipyretic property [19]. Additionally, flowers can treat intermittent fevers, running nose, inflammation of the mucous membrane inside the nose, and liver and spleen disorders [20]. The bark juice is used in dyspepsia and diarrhea. In the conventional medical systems, several portions of this plant are used to treat a variety of ailments, such as headaches, smallpox, sore throat, stomatitis, cough, cold, indigestion, jaundice, and excessive body heat [13].

The leaves of *S. grandiflora* contain many secondary metabolites, such as flavanoids, glycosides, saponin, triterpenoid, tannin, pectin, and grandiflorol. In light of all of this, the objective of the present study was to offer certain beneficial information regarding the standardization and identification of *S. grandiflora* leaf, which may be helpful in terms of its validity, purity, and quality.

MATERIALS AND METHODS

Collection of plant material:

The leaves of *S. grandiflora* were collected in February 2022 from the local area of Mathura, Uttar Pradesh, and the plant has been identified and authenticated by the Chief Scientist of CSIR-National Institute of Science Communication and Policy Research New Delhi. The Voucher specimens of the leaf were deposited (NISePR/RHMD/Consult2022/4023-24) at the herbarium for future reference. The fresh leaves of *S. grandiflora* were washed with tap running water and then dried at room temperature 17-20°C for 25 days. Free-hand sectioning was used to perform the histochemical and pharmacognostic studies of *S. grandiflora*. The powdered leaves were used for microscopical physicochemical evaluation, fluorescence, and chemo-microscopy analysis.

Drugs and Chemicals

Petroleum ether, Chloroform, Ethanol, Hydrochloric acid, Glycerine, Pholoroglucinol, and all other chemicals used in the current investigation were analytical grades.

Organoleptic evaluation

The leaves of *S. grandiflora* were assessed morphologically for organoleptic properties, including color, aroma, taste, shape, and texture, per the WHO guidelines [21].

Microscopical evaluation: The fresh leaves of *S. grandiflora* and powdered drug were subjected to a histochemical and microscopic examination using the procedure outlined by Kokate and Khandelwal. The Cilika BT-E phase contrast microscope was used for the microscopic evaluation [22].

Physicochemical evaluation and Fluorescence analysis

The shade-dried leaves were crushed in a mixer grinder to get a fine powder and pass through #40 number sieves. The powder was then analyzed for extractive value, Loss on drying, swelling index, and ash value (total, water-soluble, and acid-insoluble), determined according to the World Health Organization (WHO) guidelines. Thiex et al. and fluorescence analysis by Singh et al. [23-25] at visible light, in short, UV light (254 nm) and long UV light (365 nm) using ultraviolet lamps.

Chemo-microscopy analysis:

Chemo-microscopic analysis was performed according to the procedure described by Trease and Evans to evaluate the presence or absence of calcium carbonate, calcium oxalate crystals, lignin, protein, fats/oil, and starch grains in *S. grandiflora* leaves [25].

Preparation of Extract

The dried powder of *Sesbania grandiflora* leaves was successfully extracted using the soxhlet apparatus with different organic solvents (Petroleum ether, Chloroform, and Hydroalcoholic) in an increasing polarity order. Initially, the powdered material was defatted with petroleum ether (60-80°C), and then the defatted residue was later on extracted with Chloroform (61.15 ℃) and finally with 70% ethanol in distilled water (70:30) for 72 h. Each extract was concentrated under reduced pressure using a rotary evaporator. The excess solvent was recovered, and the dried extract was stored in a tight container for further study. The obtained extract was then calculated in terms of % w/v and stored in an airtight container.

Preliminary phytochemical evaluation

The standard procedure described by Trease and Evans, and Kokate was used to determine the presence of secondary metabolites in various extracts of *S. grandiflora* [26-27].

RESULTS

The crude extracts of *S. grandiflora* leaves were fractionated using successive polar extraction techniques to determine the presence of various active components. Solvents based on polarity, petroleum ether, Chloroform, Ethanol, and Hydroalcoholics were employed to extract material from low-polar to high-polar sequences. The principal phytoconstituents in each fraction were determined using the standard protocol mentioned by Trease and Evans, and Kokate.

Organoleptic evaluation

S. grandiflora is a fast-growing edible medicinal plant. The leaves are regular compound and pari-pinnate with an average length of 15–30 cm long. A single leaflet might be linear, oblong, lanceolate, or setaceous deciduous, measuring 3-4 cm in length and 10–15 mm in width. A mature compound typically has 12–20 pairs of oblong, rounded leaflets. The upper surface of the leaf showed a green color. The young leaves showed lighter green. However, mature leaves showed dark green. Results are tabulated in Table 1.

Table 1: Organoleptic characteristic of *S. grandiflora*

Part	Color	Taste	Size	Shape	Smell	Texture
Leaf	Light green	Bitter	15–30 cm long	Oblong to elliptical	Characteristic	Coarse
Bark	Light gray	Bitter	4–15 m tall	Corky and deeply furrowed	Characteristic	Light gray, corky, and deeply furrowed
Fruit	Green beans	Bitter	30–45 cm long	Linear to slightly curved pod	Characteristic	Smooth velvety
Flower	White, red or pink	Bitter	1.5-10 cm long	Butterfly-shaped flowers	Characteristic	Pendulous inflorescence

Microscopical and Histochemical evaluation*:*

The transverse section of the leaf showed a single layer of barrel-shaped cells with a thick cuticle over the upper epidermis; however, the lower epidermis has stomata in a single layer and is compactly packed. Two different types of mesophyll cells (spongy and palisade) were observed under the microscope, and palisade cells were arranged in two layers under the epidermis. However, the spongy parenchyma cells were observed to be spherical and arranged loosely (fig-1). The stomata are anxiolytic in nature in the lower epidermis, whereas the upper epidermis has both anisocytic and normocytic stomata (fig-2). The trichomes are of unicellular covering with a conical bulbous base and thick-walled (fig-3). There is a sizable vascular bundle in the midrib, and every vascular bundle is conjoint, collateral,

and closed with a parenchymatous bundle sheath (fig-4-5). Furthermore, the powder microscopy of the leaf demonstrated the presence of simple epidermal hairs, dark yellowish-brown tannin fragments, light yellowish resinoids, oil globules, mucilage cells, spiral vessels, and epidermal cells observed (fig-6) under the microscope Cilika BT- E Phase contrast with 40x magnification and the section was stained by the staining reagent 'Safranin.'

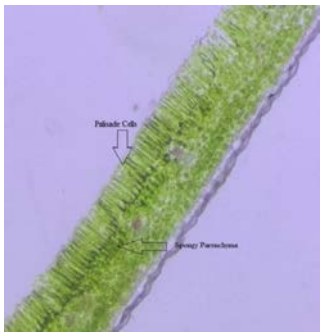

Figure 1: Palisade cells with spongy parenchyma

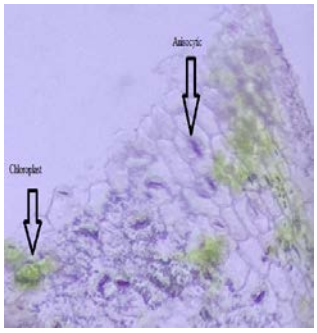

Figure 2: Lower and upper Leaf peel with anisocytic stomata with Chloroplast

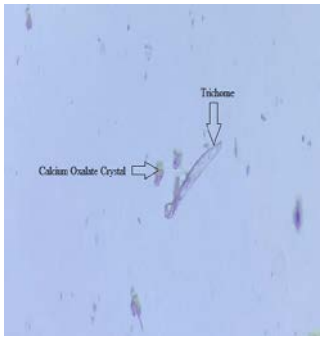

Figure 3: Conical based trichome with prismatic calcium oxalate crystal

Figure 4: Vascular bundle present in the midrib

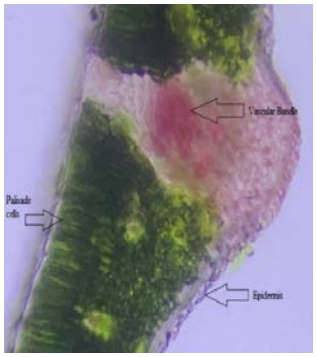

Figure 5: TS of leaflet showing epidermis along with palisade cell and vascular bundles

Figure 6: Powder microscopy of leaf showing the presence of spiral vessels, oil globules mucilage cells with tannin contents

Physicochemical evaluation

S. grandiflora leaves were examined for physicochemical characteristics, such as moisture content, extractive value, ash value, and foaming index. The outcomes of the evaluated parameters are summarized in Table 2, such as Loss on drying of 0.84%, percentage of moisture content of 1.6% w/w, swelling index of 5 ml, and foaming index of 111.11 ml, respectively. Furthermore, the alcohol-soluble and Hydro alcoholic-soluble extractive values were 22.3%w/w and 32.4%w/w. However, the total ash and acid insoluble ash values were 7%w/w and 1.0%w/w, respectively. The fluorescence analysis is depicted in Table 3

Table 2: Physicochemical analysis of *S. grandiflora leaves*

SNo	Parameter studied	Observed value
1	Loss on drying	0.84%
2	Alcohol-soluble extractive	22.3% w/w
3	Hydroalcoholic soluble extractive	32.4% w/w
4	Total ash	7% w/w
5	Acid insoluble ash	1.0% w/w
6	Water soluble ash	3.5% w/w

Table 3: Fluorescence studies of leaf powder of *S. grandiflora*

Solvent	Visible light	254 nm	366 nm
Powder	Gray-green	Gray-green	Fluorescent Green
In methanol and NaOH	Dark green	Blackish dark green	Blackish green
Ethanol and NaOH	Gray-green	Dark green	Greenish orange
Powder +50%H_2SO_4	Blue	Greenish blue	Light green
Picric acid	Yellowish	Light yellowish green	Orange color

Chemo-microscopy analysis

Table 4 summarizes the results of the chemo-microscopic study. The study outcomes demonstrated the presence of cellulose, tannins, calcium carbonate, fat and fatty oils, protein, lignin, and starch in the leaves of *S. grandiflora*.

Phytochemical study: Table 5 summarizes the results of the Phytochemical study of different extracts of *S. grandiflora* leaf. The study outcomes demonstrated the presence of various secondary metabolites in the leaves of *S. grandiflora*.

Table 4: Chemo-microscopic characteristics of the leaf of *S. grandiflora.*

Constituents	Reagents	Observation	Inference
Cellulose	N/50 Iodine+80%H_2SO_4	Dark black coloration	Strong positive
Tannins	70% methanol+$FeCl_3$(Dilute)+ More $FeCl_3$(Dilute) dropwise	Yellowish brown suspension	Weak positive
Calcium carbonate	Acetic acid+ 50%H_2SO_4	Effervescence light brown	Weak positive
Fat & fatty oils	Sudan III	Light brown suspension	Weak positive
Protein	Few drops of ninhydrin+	Light yellow	Moderate positive
Lignins	Few drops of phloroglucinol+ drop of HCl	Reddish brown	Moderate positive
Starch	Few drops of N/50 iodine	Brown, black coloration	Strong positive

Table 5: Phytochemical screening of Hydroalcoholic extract of *S. grandiflora leaves*

SNo.	Phytoconstituents	Identification Test	Water	Ethanol	Hydroalcoholic
1.	Alkaloids	1.Mayer's test 2.Wagner's test	+ +	- +	+ +
2.	Glycosides	Borntrager's test	-	-	+
3.	Steroids	Salkowoski's test	-	+	+
4.	Saponin	Foam test	-	+	+
5.	Flavanoid	Lead acetate test	-	+	+
6.	Tannin	FeCl3 test	+	+	+
7.	Carbohydrates	1.Molisch test 2.Fehling test	- +	+ -	+ +
8.	Amino acid and Protein	1.Xanthoprotein test 2.Biuret test	- -	- +	- +

DISCUSSION

The current investigation demonstrated the morphological characteristics such as color, taste, size, shape, odor, and texture, as well as physiochemical, microscopic, and histochemical studies of *S. grandiflora* leaves. Further, fluorescence analysis of *S. grandiflora* powdered drugs was also carried which validated the authenticity of *S. grandiflora*. The phytochemical analysis of *S. grandiflora*. *S. grandiflora* leaves revealed the presence of various secondary metabolites such as alkaloids, glycosides, flavonoids, etc. Therefore, it can be presumed that these are the standard parameters for the standardization of *S. grandiflora* leaves.

Standardizing herbal medicine is essential to confirm the drug's identity, purity, quality, and efficacy. Authentication is the initial stage of establishing the starting material. Therefore, the standardization of herbal remedies has increased rapidly in the past few decades [28]. Morphological evaluation, such as color, texture, size, odor, taste, and so on, is helpful in the identification of herbal drugs. The morphological assessment of *S. grandiflora* showed that the leaves were green in color, compound, pinnate, had a flat surface, and alternately arranged. In addition to a bitter taste, the leaves had a characteristic odor.

It is important to remember that substitutes may closely look like the original material; therefore, the quantitative identification of closely associated substituent's present in the raw drug material is essential and can be identified by optical microscopy [29]. Although there are, several sophisticated approaches are available for the assessment of crude drugs. However, microscopic approaches are still one of the most cost-effective and simple methods to identify the precise identity of the source of material [30]. Moreover, microscopic studies are considered a crucial pharmacognostic criterion in compiling modern monographs [31]. The microscopic and powder research of *S. grandiflora* revealed several distinctive anatomical features such as mesophyll cells, anisocytic stomata, parenchymatous bundle sheath, epidermal hairs, etc. Furthermore, evaluating

physicochemical parameters can be a valuable tool for determining the quality and purity of crude drugs. The extractive values of the drugs provide a rough idea about their chemical constituents in a particular solvent, which may help decide the appropriate extraction solvents [32]. Current investigation revealed that hydroalcoholic soluble extractive has the highest extractive followed by an alcohol-soluble extractive value. This shows that the concentration of alcohol and water soluble is higher than the alcohol-soluble components. The extractive values of *S. grandiflora* were within the range of pharmacopeia standards. Therefore, in the future, it can be used as a reference for identifying and assessing the overall quality and purity of *S. grandiflora* leaves. Ash values serve as vital quantitative parameters to assess the quality, identity, and purity of crude drugs [33]. An excessive ash value is a sign of adulteration and contamination of the drug. The ash values of the current investigation were based on earlier findings of Nandi et al. [34], and the total ash value was 7%. Furthermore**,** the portion of the overall ash that dissolves in water is called water-soluble ash. The water-soluble ash value was found to be 3.5%. However, the acid-insoluble ash was reported to be 1%. The chemo-microscopic analyses revealed the presence of cellulose, tannins, calcium carbonate, fat & fatty oils, protein, reddish-brown color lignin, and starch.

Fluorescence analysis is a valuable method for identifying and standardizing herbal medicine. Several phytochemical constituents of the plant material exhibit fluorescence [35]. When a drug sample exhibits fluorescent properties, it demonstrates that it contains specific phytoconstituents that show fluorescence either in the visible spectrum or when exposed to ultraviolet light. In the present investigation, various organic solvents were used to assess the fluorescence analysis of dried powder. The result of the fluorescence analysis is tabulated in Table 3.

Furthermore, the active phytoconstituents present in plants are responsible for their biological activity [37]. Therefore, it is crucial to standardize medicinal plants according to their phytochemical aspects to support their therapeutic action in experiments. The preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, phenolics, tannins, flavonoids, etc. Polyphenols such as flavonoids and tannins act as natural antioxidants and fight against several diseases such as cardiovascular disease, cancer, and neurological disorders [38] and regular consumption of flavonoids can decrease the risk of heart failure. Additionally, flavonoids minimize oxidative stress and inflammation by regulating several pathways, including MAPK, extracellular signal-regulated kinase, phosphoinositide 3 kinase (PI3K)/Akt, and protein kinase-related pathways.

CONCLUSION

To ensure the quality, authenticity, and purity of herbal drugs, physico-chemical standards are important. Therefore, the standardization of herbal drugs is important to provide a good quality of medication. The methodology used in the current investigation plays a significant role in identifying and collecting authentic drugs and eliminating counterfeited drugs. Therefore, it can be concluded that the parameters studied in the present work may be used for quality evaluation and standardization of crude drugs to achieve genuine and standard drugs for therapeutic purposes. Additionally, this Information may be helpful in the preparation of the monograph. Furthermore, radioactive contamination and determination of heavy metals and pesticide residue can be calculated in the future to confirm the safety and efficacy of the drug. Additionally, chemical fingerprinting and DNA barcoding can be done to validate the authenticity of the plant.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

All authors contributed equally to this work. Sharad Sharma performed the experimental work and prepared the manuscript. Bhupesh Chander Semwal developed the concept, analyzed the results, and corrected the manuscript. Avijit Mazumder checked the manuscript for grammar and plagiarism.

REFERENCES

[1] Sharma M, Mandloi AK, Govind P, Sahni YP.Antimicrobial activity of some medicinal plants against fish pathogens, *Int Res J Pharm*, **3(4),** 28–30 (2012).

[2] Mintah SO, Asafo-Agyei T, Archer MA, Junior PA, Boamah D, Kumadoh D, Appiah A, Ocloo A, Boakye YD, Agyare C. Medicinal plants for treatment of prevalent diseases. *Pharmacognosy-medicinal plants*, **17**, 1-9 (2019).

[3] Ramalingum N, Mahomoodally MF.The therapeutic potential of medicinal foods. Advances in *Pharmacological and Pharmaceutical Sciences*,**1**,354264(2014).

[4] Astutik S,Pretzsch J,Ndzifon Kimengsi J.Asian medicinal plants' production and utilization potentials: A review. *Sustainability*, **3**, 11(19), 5483 (2019).

[5] Patel DK. Therapeutic role of columbianadin in human disorders: Medicinal importance, biological properties and analytical aspects, *Pharmacol. Res. - Mod. Chin. Med,* **1(6),** 100212 (2023).

[6] Boakye MK, Pietersen, DW, Kotzé A, Dalton DL, Jansen R. Knowledge and uses of African pangolins as a source of traditional medicine in Ghana, *PLoS One.,* **10(1),** 0117199 (2015).

[7] Sumbul S, Ahmad MA, Asif M, Akhtar M, Saud I. Physicochemical and phytochemical standardization of berries of Myrtus communis Linn, *Journal of Pharmacy and Bio Allied Sciences*, **4(4),** 322-326 (2012).

[8] Singh PP, Jha S, Irchhaiya R, Fatima A, Agarwal P. *International Journal of Pharmaceutical Sciences and Research,* **3(4),** 1001-1004 (2012).

[9] Malik S, Kaur K, Prasad S, Jha NK, Kumar V. A perspective review on medicinal plant resources for their antimutagenic potentials, *Environmental Science and Pollution Research*, **29(41),** 62014-29 (2022).

[10] Deepthi K, Renjith PK, Habeeb Rahman K, Chandramohanakumar N. A comprehensive review of Sesbania grandiflora (L.) Pers: traditional uses, phytochemistry and pharmacological properties. *Vegetos*, **37(1),** 31-40 (2023).

[11] Pei SJ. Preliminary study of ethnobotany in xishuang banna, people's Republic of China *J. Ethnopharmacol*, **13(2),** 121-37 (1985).

[12] Deepthi K, Renjith PK, Shameem K, Habeeb Rahman K, Chandramohanakumar N. Phytochemical screening of leaves and flower extracts of Sesbania grandiflora (L.) Pers. and its antimicrobial activity against fish pathogens. *Vegetos*, **36**, 626–33 (2023).

[13] Arfan N, Julie A, Mohiuddin A, Khan S, Labu Z.Medicinal properties of the sesbania grandiflora leaves. *Ibnosina J. Med. Biomed. Sci,* **8(6),** 271-277 (2016).

[14] Neethu SK, Dhanyaraj FS. Phytochemical analysis and antimicrobial activities of *Sesbania grandiflora* (L) leaf extracts, *Int. J. Pharm. Sci.,* **36(1),** 144-148 (2016).

[15] Zhang Q, Cheng Z, Fan Y, Zhang D, Wang M, Zhang J, Sommano S. *et al.* Ethnobotanical study on edible flowers in Xishuangbanna, China. *J. Ethnobiol. Ethnomed,* **19(1),** 43 (2023).

[16] Sharma S, Semwal B, Mazumder A. Evaluation of toxicity studies of sesbania grandiflora leaves extracts in wistar albino rats. *Journal of Applied Pharmaceutical Research*, **12(1),** 42-51 (2024).

[17] Semwal BC, Verma M, Murti Y, Yadav HN. Neuroprotective activity of *Sesbania grandifolara* seeds extract against celecoxib induced amnesia in mice. *Pharmacogn. J.,* **10(4),** 747–52 (2018).

[18] Pajaniradje S, Mohankumar K, Pamidimukkala R, Subramanian S, Rajagopalan R. Antiproliferative and apoptotic effects of Sesbania grandiflora leaves in human cancer cells. *Biomed Res Int*, **10**, 474953 (2014).

[19] Mohiuddin AK. Medicinal and therapeutic values of Sesbania grandiflora. *J Pharm Sci Exp Pharmacol*, **3**, 81-6 (2019).

[20] Arthanari S, Periyasamy P.Phenolic composition, antioxidant and anti-fibrotic effects of Sesbania grandiflora L. (Agastya) – An edible medicinal plant. *AYU An Int. Q. J. Res. Ayurveda,* **41(4),** 242–9 (2020).

[21] World Health Organization. Quality control methods for medicinal plant materials. *World Health Organization*, (1998).

[22] Singh S, Semwal BC, Upadhaya PK. Pharmacognostic study of Sphaeranthus indicus Linn.: A Review. *Pharmacognosy Journal*, **11(6**), 1376-1385 (2019).

[23] Thiex N, Novotny L, Crawford A. Determination of ash in animal feed: AOAC official method 942.05. *J AOAC Int* **95**, 1392–397 (2012).

[24] Singh S, Semwal BC, Neeli GS. Microscopic and physicochemical evaluation of leaves of Sphaeranthus indicus Linn. Pharmacognosy Journal, **9(1),** 21-26 (2017).

[25] Prasanth DS, Rao AS, Yejella RP. Assessment of pharmacognostic, phytochemical and physicochemical standards of Aralia racemosa (L.) root. *Ind J Pharm Edu Res*, **50(3)**, 225-30 (2016).

[26] Evans WC.Trease and evans pharmacognosy.*15th ed. London: Saunders Ltd*,545-547(2003).

[27] Bhairam M, Roy A, Bahadur S, Banafar A, Turkane D. Standardization of herbal medicines-an overview. *J. Appl. Pharm. Res.*, **1**, 14–21 (2013).

[28] Gagan S, Amit C, Uttam SB, Sandeep R, Prabh SS, Ravi KD.Pharmacognostic standardization of leaves of Melaleuca Leucadendron. *Phcog J.,* **5(4),** 143-8 (2013).

[29] Muyumba NW, Mutombo SC, Sheridan H, Nachtergael A, Duez P. Quality control of herbal drugs and preparations: The methods of analysis, their relevance and applications. *Talanta* **1(4),** 100070-11 (2021).

[30] Sunil KN, Sangeetha B, Suchitra P, Ravishankar B, Yashoverma B.. Pharmacognosy and quality characterization of fruits of *Ingudi–Balanites aegyptica. IJNPR,* **7(1),** 40-50 (2016).

[31] Upadhyay P, Joshi BC, Sundriyal A, Mukhija M. Pharmacognostic standardization and physicochemical evaluation of Caesalpinia crista L. root for quality control assessment. *Journal of Nature and Science of Medicine,* **2(3),** 135-40 (2019).

[32] Kripa KG, Sangeetha R, Chamundeeswari D. Pharmacognostical and physicochemical evaluation of the plant *Leucas aspera*. *Asian Journal of Pharmaceutical and Clinical Research*, **9**, 263-8 (2016).

[33] Feenna OP, Estella OU, Obianuju PO, Ifeanyi NF, Obodike EC. Pharmacognostic and phytochemical studies of leaves of Psydrax horizontalis Schum. and Thonn (Rubiaceae). *Pharmacognosy Journal*, **12(3),** 541-550 (2020).

[34] Nandi MK, Garabadu D, Singh TD, Singh VP. Physicochemical and phytochemical standardization of fruit of Sesbania grandiflora. *Der Pharmacia Lettre,* **8(5),** 297-304 (2016).

[35] Alam F, Saqib QN. Pharmacognostic study and development of quality control parameters for fruit, bark and leaf of Zanthoxylum armatum (Rutaceae). *Ancient Science of Life,* **34(3),** 147-55 (2015).