

Preliminary phytochemical, pharmacognostic and physicochemical evaluation of leaf of *Gomphrena serrata*

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ABSTRACT

Background and aims: To analyze the pharmacognostic characteristics and physicochemical parameters of the leaves of *Gomphrena serrata* (*G. serrata*).

Methods: Microscopic characters and powder analysis had been carried out with the help of a microscope. The physicochemical properties such as loss on drying, total ash value, acid insoluble ash value, water soluble ash value, extractive values and fluorescence of *G. serrata* had been performed.

Results: Macroscopically, the leaves are simple, elliptical in shape, rounded or obtuse apex with an entire margin. Microscopically, the leaf showed the presence of epidermal cells with uniseriate multicellular covering trichomes and anomocytic stomata, followed by 1-2 layered collenchymatous cells and 10-15 numbered conjoint, collateral closed vascular bundles are some of the diagnostic characteristics observed from an anatomical study. Powder microscopy of leaf revealed the presence of uniseriate multicellular covering trichomes, lignified xylem vessels, epidermis with anomocytic stomata and parenchyma cells. The investigations also included leaf surface data i.e., quantitative leaf microscopy and fluorescence analysis. Physicochemical parameters such as loss on drying, extractive values and ash values were also determined. Preliminary phytochemical screening showed the presence of flavonoids, alkaloids, tannins, steroids, carbohydrates, glycosides, amino acids and proteins.

Conclusion: The morphological, microscopical and physicochemical parameter results provided in this paper may be utilized as a basis for the preparation of a monograph on *G. serrata* leaves.

Keywords: Pharmacognostic, *Gomphrena serrata*, Anomocytic stomata, Lignified xylem vessels, Phytochemical and Physicochemical analysis.

Original article

INTRODUCTION

Medicinal plants tend to be playing a crucial role in conventional medicines for remedy of different health problems. On the

other hand a vital barrier, that has obstructed the promotion in the utilization of alternative medicines in the developed nations, is no

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proof of documentation and lack of stringent quality control measures. There is also a requirement for the records of all the research work meted out on conventional medicines by means of documentation. With this particular problem, it has become very essential to make assurance regarding the standardization of the plant and its parts to be utilized as a medicine. In the process of standardization, we can make use of various techniques and methodology to attain our objective in a step wise manner e.g. pharmacognostic and phytochemical studies. These methods and procedures are useful in identification and standardization of the plant material. Proper characterization and quality assurance of beginning material is an important step to make sure reproducible quality of herbal medicine to help us to rationalize its safety and efficacy. For this reason, we have carried out pharmacognostic studies of *Gomphrena serrata* belongs to family Amaranthaceae.¹ This kind of study will not only assist in authentication but also assures reproducibility of herbal products in marketing.²

In the current study, we are emphasizing our investigation on one of the commonly available plant in India i.e., *Gomphrena serrata*, belongs to family Amaranthaceae. The family Amaranthaceae contains nearly 60-70 exotic species. The genus *Gomphrena*, with around 138 species, some of the important species include *G. boliviana*, *G. celosioides*, *G. globosa*, *G. haenkeana*, *G. macrocephala*, *G. martiana*, *G. meyeniana*, *G. perennis* and *G. pulchella*.³ All parts of this plant are widely used as a folklore medicine for the treatment of various ailments by the Indian traditional healer. Traditionally, the plant is utilized in the remedy of bronchial asthma, diarrhea, hay fever, pains, tonic, carminative, diabetes, dermatitis and piles.⁴⁻⁷

G. serrata is annual, ascending or erect herbs, up to 40 cm tall; branches clothed with white, shaggy hairs; leaves obovate-lanceolate,

2-4 X 1-1.5 cm, glabrescent above, long white shaggy hair below, obtusely apiculate, base cuneate; flowers white with yellow tinge in axillary and terminal compressed, cylindrical spikes; utricles enclosed hardened perianth; seeds brown, shiny.⁸

Phytochemical constituents have been separated from the genus *Gomphrena* i.e., oleuropein³, stigmasterol, β -sitosterol, isochavicolonic acid, campesterol, betalain, friedelin, 3-epi-friedelinol, allantoin and chrysoeriol-7-O- β -D-glucoside.⁹

Ethnomedicinally, the genus *Gomphrena* has been documented various pharmacological activities including antimicrobial,¹⁰ anticancer, antimalarial and analgesic.¹¹⁻¹³

Although the plant has been extensively used for its traditional value, but pharmacognostic, phytochemical and pharmacological account remains unexplored. Therefore, the current investigation had been carried out to study the morphological, microscopical, physicochemical and phytochemical characteristics of leaves of *G. serrata* with the purpose of contributing to the establishment of monograph.^{14,15}

METHODS

The plant obtained from tirupati, chittoor district of Andhra Pradesh, India during the month of December 2016 and authenticated by Dr. K. Madhava chetty, Taxonomist at Sri venkateswara University tirupati, India. Voucher specimen No. 1864 was deposited at the herbarium for future reference. One portion of the leaf is preserved in formalin: Acetic acid: Alcohol mixture for histological studies and the remaining portion was shade dried, powdered and sieved through 20 mesh and kept in an air tight container for future use.

All analytical grade chemicals were utilized in this study were procured from E. Merck, Germany. absolute alcohol, phloroglucinol, acetic acid, chloral hydrate,

H₂SO₄, NaOH, HNO₃, FeCl₃, distilled water, Conc. HCl and chloroform.

Organoleptic evaluation of *G. serrata* leaves has been carried out in accordance with the colour, size, odour, shape and taste as per WHO quality control methods of herbal medicine.¹⁶

Microscopic studies had been done by preparing thin hand section of the leaf with the help of sharp cutting edge of the blade. Then, it was cleared with chloral hydrate solution, stained with phloroglucinol-hydrochloric acid (1:1) and mounted in glycerin.

The powder microscopy was carried out in accordance with the procedure described in Khandelwal.¹⁷

The quantitative examinations including stomatal number, stomatal index, vein islet number and vein termination number were studied using standard method.²

The powdered material had been extracted with various solvents according to its polarity i.e., chloroform, methanol and water. 5 g leaf powder was extracted with 20 ml of the respective solvent by maceration at room temperature for 24 hours. Then, it was filtered through whatmann filter paper and collect the filtrate, concentrated with roto-evaporator. Then, the extracts had been subjected to preliminary phytochemical screening according to standard methods.^{17,18}

Physicochemical parameters such as ash value, moisture content and extractive values were determined according to the procedures mentioned in WHO quality control methods for herbal materials.¹⁶

Various reagents were utilized to check the fluorescence activity. In this, 0.1 g of leaf powder was blended with 1.5 ml of respective reagent (Table 4). The mixture was placed on slide for a minute and observed under visible light, short ultra-violet light (254 nm) and long ultraviolet light (365 nm).¹⁹

RESULTS

The morphological characteristics of *G. serrata* leaves were described in Figure 1 and Table 1.



Figure 1: Organoleptic characteristics of whole Plant of *Gomphrena serrata*

Table 1: Morphological Characteristics of Leaf of *Gomphrena serrata*

Characters	Observation
Colour	Green
Odour	Characteristic
Taste	Characteristic
Texture	Smooth
Length	3 cm

The transverse section of leaf passing through midrib is convexly protruding at the lower side slightly with more prominent ridged on the upper side,

showed uniseriate epidermal cells on both surfaces of the leaf, which was covered by thick cuticle. The epidermis is composed of rectangular shaped cells and contains an anomocytic type of stomata. There are uniseriate multicellular covering trichomes on the adaxial and abaxial surface of epidermal cells, relatively more on abaxial surface. The epidermal cells followed by 1-2 layered collenchymatous cells beneath upper epidermis and 2-3 layered collenchymatous cells above lower

epidermal cells in the midrib region. The cells of collenchyma were thick walled and round in shape showing small intercellular spaces, followed by broad parenchymatous ground cells with intercellular spaces. Conjoint, collateral closed vascular bundles 4-5 were present in the ground tissue. The phloem consists of companion cells and sieve tubes and xylem consists of spiral annular thickened vessels, tracheids, fibres and xylem parenchyma (Figure 2).

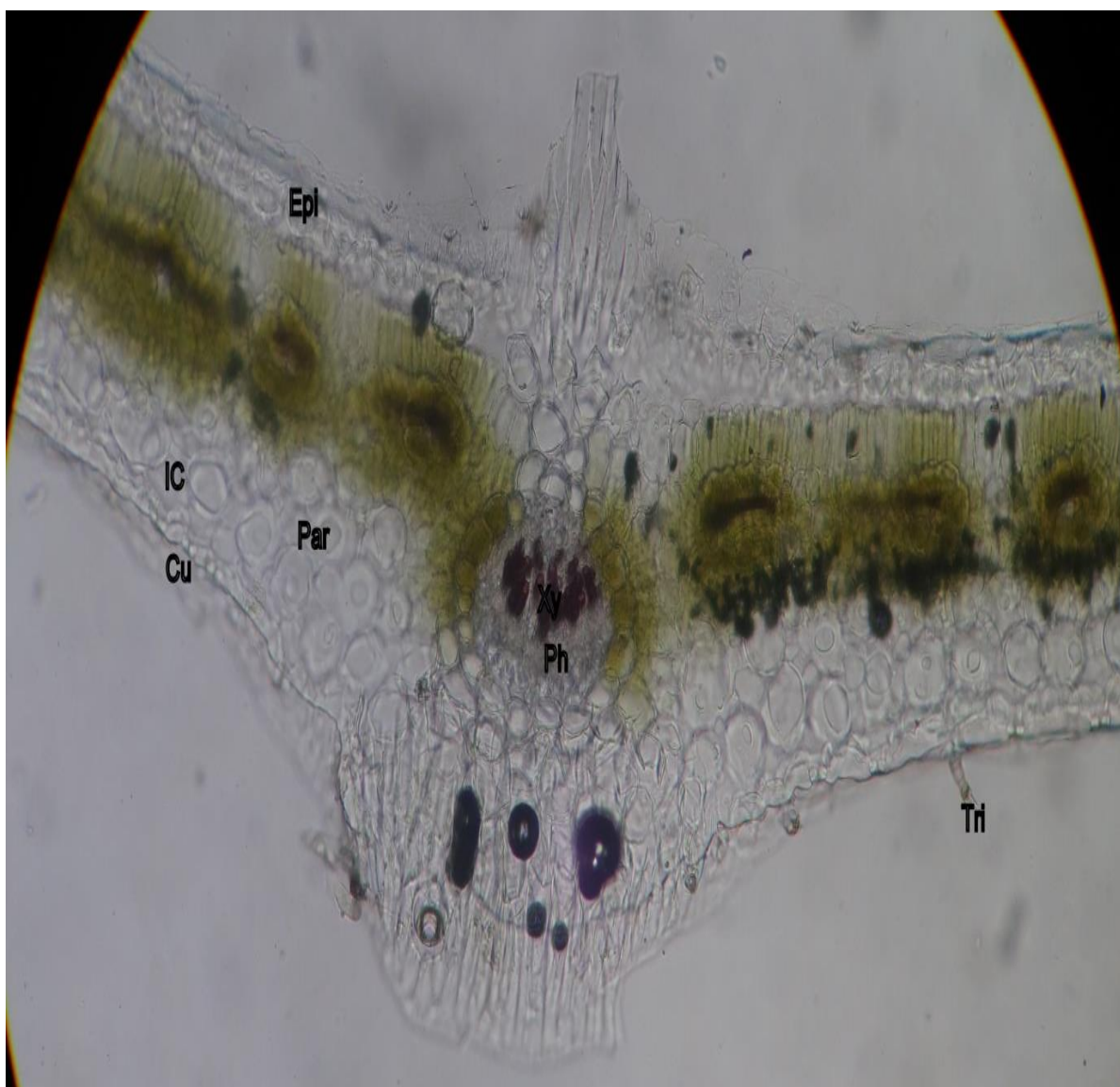


Figure 2: Transverse section of leaf midrib portion of *Gomphrena serrata*; Cu: Cuticle; Epi: Epidermis; IC: Intercellular spaces; Par: Parenchyma; Xy: xylem and Ph: Phloem

Circular shaped petiole was observed in T.S, showing a layer of thickly walled epidermis with uniseriate multicellular covering trichomes. Followed by 3-5 layers of collenchymatous cells were present beneath the epidermal layer. Various sized

parenchymatous cells from the ground tissue with intercellular spaces. Vascular bundles are open, bicolateral and arranged in a ring, which was present at the center of the petiole and the nature is similar to that of the leaf (Figure 3).

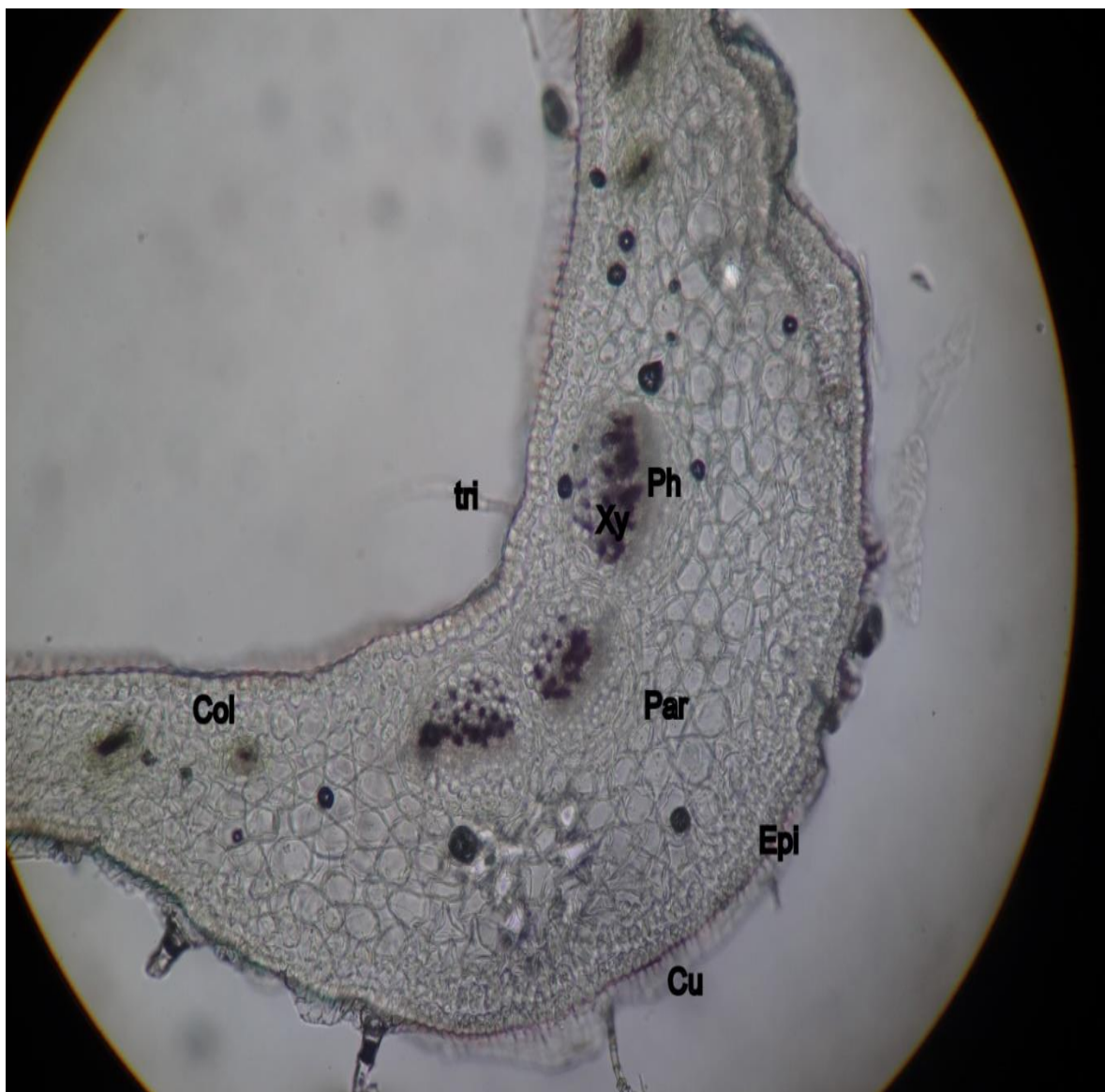


Figure 3: Transverse section of petiole of *Gomphrena serrata*; Tri: Trichomes; Col: Collenchyma; Cu: Cuticle; Epi: Epidermis; Par: Parenchyma; Xy: Xylem and Ph: Phloem

The crude powder of leaf was green in colour with characteristic odour and taste. Microscopic study of the powder showed

revealed different characters such as anomocytic stomata, covering trichomes, xylem vessels and parenchyma cells (Figure 4).

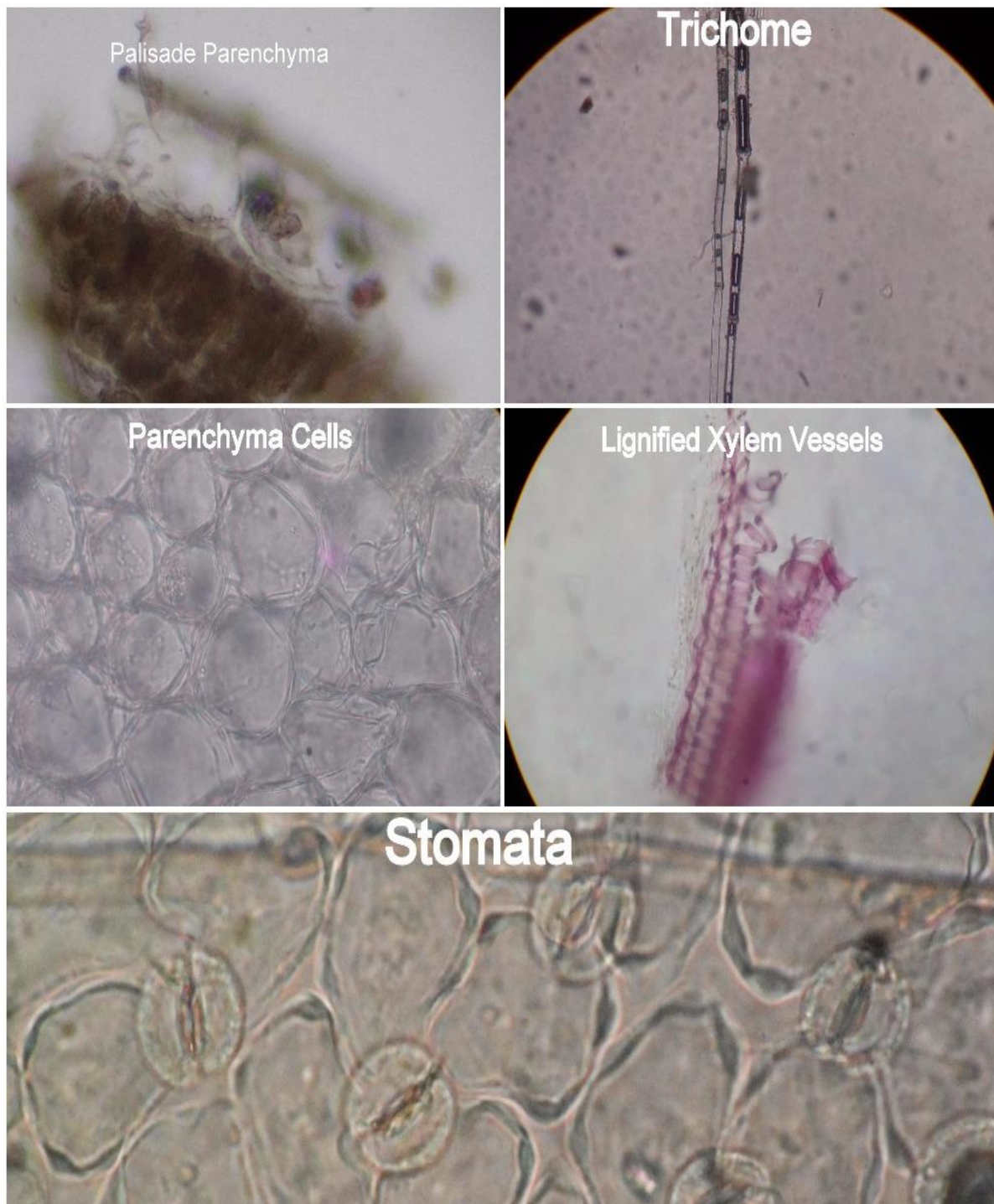


Figure 4: Powder microscopy of leaf of *Gomphrena serrata*

Leaf venation was reticulate with 2-3 pairs of alternate lateral veins. Vein islet number is 14 ± 6.2 and vein termination number is 16 ± 2.5 . The stomatal number and

stomatal index for lower epidermis is 26.5 ± 7.2 and 30 per sq. mm respectively, for upper epidermis 12.6 ± 8.7 and 33.33 per sq. mm respectively.

The results of qualitative phytochemical analysis of crude powder of *G. serrata* leaf are shown in Table 2.

The results attained from various determinations of physicochemical analysis are produced in Table 3.

Table 2: Preliminary Phytochemical analysis of *Gomphrena serrata* leaf

Phytoconstituents	Method	Aqueous extract	Methanolic extract	Chloroform extract	Pet. ether extract
Flavonoids	Shinoda test	+	+	-	-
	Zn. Hydrochloride test	+	+	-	-
	Lead acetate test	+	+	-	-
Volatile oil	Stain test	-	-	-	-
Alkaloids	Wagner test	+	+	+	-
	Hager's test	+	+	+	-
Tannins and phenols	FeCl ₃ test	+	+	-	-
	Potassium dichromate test	+	+	-	-
Saponins	Foaming test	+	+	-	-
Steroids	Salkowski test	+	+	+	+
Carbohydrates	Molish test	+	+	-	-
Acid compounds	Litmus test	+	+	-	-
Glycoside	Keller-Killani test	+	+	-	-
Amino acids	Ninhydrin test	+	+	-	-
Proteins	Biuret	+	+	-	-

+: Present; -: Absent

Table 3: Physicochemical parameters of leaf powder of *Gomphrena serrata*

Parameters	Values%w/w
Moisture content (Loss on drying)	5.2±0.25
Total ash	5.23±0.22
Acid insoluble ash	1.43±0.06
Water soluble ash	1.92±0.32
Petroleum ether soluble extractive value	0.56±0.05
Chloroform soluble extractive value	1.69±0.13
Ethyl acetate soluble extractive value	3.65±0.04
Alcohol soluble extractive value	8.92±0.02
Water soluble extractive value	11.22±0.05

Fluorescence analysis of leaf powder was performed out after treating with different solvents. Fluorescence was observed at 254

and 365 nm comparing its change of colour in the visible light. The observations presented in Table 4 shows the variation in colour.

Table 4: Fluorescence analysis of *Gomphrena serrata* leaf powder

Solvent used	Visible light	UV light	
		At short (254nm)	At Long (365nm)
Distilled water	Green	Black	Black
Methanol	Greyish white	Greenish black	Greenish black
1N HCl	Green	Black	Black
50% HNO ₃	Green	Greenish white	Blue
FeCl ₃	Orange	Dark blue	Black
CHCl ₃	Pale green	Buff	Black
Picric acid	Yellowish green	Dark blue	Black
Ethyl acetate	Green	Buff	Greenish black

DISCUSSION

Indian systems of medicine utilize majority of the crude drugs which are of plant origin. It is important that standards need to be set down to control and check the identity of the plant and confirm its quality before use. Hence a detailed pharmacognostic assessment is extremely an important prerequisite. In accordance with World Health Organization (WHO) the organoleptic and histological description of a medicinal plant could be the first step towards establishing its identity and purity and should be performed before to any tests tend to be undertaken.²⁰

G. serrata, extensively utilized in conventional medicines has tremendous therapeutical potential due to its various biological activities. The prominent diagnostic characteristics of leaf were uniseriate multicellular covering trichomes, anomocytic stomata, lignified xylem vessels and parenchymatous cells. These characters can be utilized for standardization of drugs as well as useful for preparation of plant monograph and also reduces the possibilities of adulteration, when the drug is available in the powdered form studies of physicochemical parameters can serve as an important source to judge the purity and quality of crude drugs. Ash values are utilized to establish the quality and purity

of the crude drug. It implies the existence of various impurities like carbonate, oxalate and silicate. The water soluble ash is water soluble part of total ash employed to calculate the amount of inorganic substances found in the drugs. The acid insoluble ash comprises mostly silica and indicates contamination with earthy matter. The moisture content of drugs might be at minimum level in order to suppress the growth of microorganisms like bacteria, yeast or fungi during storage.

The extractive values are helpful to judge the chemical constituents present in the crude drug and also assist in the evaluation of particular constituents soluble in a specific solvent. Total ash and acid insoluble ash are essential indices to illustrate the quality and purity of the herbal medicine. Total ash consists of physiological ash, which is derived from plant tissue itself, and nonphysiological ash that is usually from atmosphere contaminations includes sand and soil. Total ash content alone is not adequate to indicate the quality of herbal medicine, because the plant materials usually contain a significant level of physiological ash, calcium oxalate in particular. Therefore, the acid insoluble ash content is another index to indicate the quality of herbal medicine.²¹⁻²³ The phytochemical

analysis of extracts viz., petroleum ether, chloroform, methanol and water were analyzed and it indicates the presence of tannins, flavonoids, steroids, glycosides, volatile oil, amino acids, proteins, and alkaloids.

CONCLUSION

Standardization of herbal drugs is very much crucial because they are produced from heterogeneous sources which could result in variations. These kinds of variations can cause spurious results in various pharmacological and phytochemical studies. *Gomphrena serrata* leaves are recognized for many therapeutical properties. Therefore, the current study might be beneficial to supplement information in respective to its identification, authentication, and standardization; no such information is available for the same till date.

CONFLICT OF INTEREST

Authors have declared that no conflicts of interest exist.

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