ABSTRACT

Objectives: *Gomphrena serrata* (Amaranthaceae) has been utilized for many ailments in the conventional system ethnomedicinally; most significantly against bronchial asthma, diarrhea, hay fever, pains, tonic, carminative, diabetes, dermatitis, and piles. The key challenge experienced in the standardization of herbal drugs is the correct identification of the plant source. Thus, setting up quality control parameters by means of pharmacognostic and phytochemical analysis which assures the purity, safety, and efficiency of *G. serrata* is necessary. The current research was conducted to assess the pharmacognostic characteristics including macroscopic, microscopic, phytochemical and physicochemical parameters of the root of *G. serrata*.

Methods: Micro, as well as macroscopic characteristics was investigated. Physicochemical parameters had been done by implementing WHO suggested parameters; preliminary phytochemical and fluorescent evaluation of root was executed for appropriate identification and standardization.

Results: The color, shape, size, odor, and surface characteristics were reported from the root and powdered root material of *G. serrata*. Light microscope images of cross section and powdered root revealed the presence of lignified xylem fibers, xylem vessels, cork cells and parenchyma cells. Phytochemical testing confirmed the presence of alkaloids, carbohydrates, saponins, tannins, proteins, amino acids, phytosterols and flavonoids. Physicochemical parameters such as moisture content, ash value, extractive value and fluorescent behavior of root powder have also been established.

Conclusion: The current research is useful in order to supplement the information with regard to its standardization, identification and in carrying out further investigation in Ayurvedic system of medicine.

Keywords: *Gomphrena serrata*, lignified xylem vessels, pharmacognostic, phytochemical analysis.

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 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 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 stepwise 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. This kind of study will not only assist in authentication but also assures reproducibility of herbal products in marketing.

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. celosioide*, *G. globosa*, *G. haenkeana*, *G. macrocephala*, *G. martiana*, *G. meyeniana*, *G. perennis* and *G. pulchella*. 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.\textsuperscript{4,7} \textit{G. serrata} is annual, ascending or erect herbs, up to 40 cm tall; branches clothed with white, shaggy hairs; leaves are obovate-lanceolate, 2-4X1.5 cm, glabrescent above, long white shaggy hair below, obtusely apiculate and the base is cuneate; flowers are white with yellow tinge in axillary and terminal compressed, cylindrical spikes; uricles enclosed hardened perianth; seeds are brown and shiny.\textsuperscript{5} Phytochemical constituents have been separated from the genus \textit{Gomphrena} i.e., oleuropein,\textsuperscript{8} stigmasterol, β-sitosterol, isochavicinic acid, campesterol, betalain, friedelin, 3-epi-friedelinol, allantoin, and chrysoeriol–7-O-β-D-glucoside. Ethnomedicinally, the genus \textit{Gomphrena} has been documented various pharmacological activities including antimicrobial,\textsuperscript{10} anticauses,\textsuperscript{11} antimalarial,\textsuperscript{12} and analgesic.\textsuperscript{13} Although the plant has been extensively used for its traditional value, 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 the root of \textit{G. serrata} with the purpose of contributing to the establishment of monograph.\textsuperscript{4,15}

**MATERIALS AND METHODS**

**Plant collection and authentication**

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 root 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.

**Chemicals**

All analytical grade chemicals i.e., absolute alcohol, phloroglucinol, acetic acid, chloral hydrate, \textit{H}SO\textsubscript{4}, NaOH, HNO\textsubscript{3}, FeCl\textsubscript{3}, conc. HCl and chloroform were utilized in this study, which was procured from E. Merck, Germany.

**Pharmacognostic evaluation**

**Morphological evaluation**

Organoleptic evaluation of \textit{G. serrata} root has been carried out in accordance the color, size, odor, shape, and taste as per WHO Quality Control methods of herbal medicine.\textsuperscript{16}

**Microscopic evaluation**

**Preparation of sections**

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

**Powdered microscopy**

The powdered microscopy was carried out in accordance with the procedure described in Khandelwal.\textsuperscript{17}

**Preparation of extracts and preliminary phytochemical analysis**

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

**Physicochemical analysis**

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.\textsuperscript{16}

**Fluorescence analysis**

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

**RESULTS**

**Morphological characteristics**

The morphological characteristics of \textit{G. serrata} root were described in Figure 1 and Table 1.

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

**Anatomical description**

**Root**

The transverse section of the root of \textit{G. serrata} showed the presence of Cortex was made up of thin walled parenchymatous cells with very small intercellular spaces. Cork showed the presence of periderm i.e., 2-3 layered narrow, tangentially elongated cells with dark brown granular matter. Phellogen is 1-2 layered rows of tangentially elongated thin walled cells. The endodermis showed the presence of phloem and xylem. The phloem is present in between the medullary rays. The medullary rays are parenchymatous and are uniseriate to triseriate, majorly biseriate. Radially arranged vascular bundles were present in which,
Phloem is well developed and shows the presence of phloem fibers, which are non-lignified. It also showed the presence of phloem parenchyma.

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

| Characters    | Observation       |
|---------------|-------------------|
| Colour        | Buff              |
| Odour         | Characteristic    |
| Taste         | Characteristic    |
| Texture       | Smooth            |
| Thickness     | 4-12 cm           |

The xylem region was similar to phloem region and was also surrounded by uniseriate to triseriate medullary rays. Xylem tissue consists of spiral xylem vessels, xylem fibers and xylem parenchyma (Figure 2).

**Table 2: Preliminary qualitative phytochemical analysis of *Gomphrena serrata* root**

| 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                        | +               | +                  | -                  | -                 |

**Powder microscopy**
The crude powder of root was buff in color with characteristic odor and taste. Microscopic study of the powder showed revealed different characters such as cork cells, parenchyma cells, lignified xylem vessels, and xylem fibers (Figure 3).

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

**Fluorescence analysis**
Fluorescence analysis of root powder was performed out after treating with different solvents. Fluorescence was observed at 254 and 365 nm comparing its change of color in the visible light. The observations presented in Table 4 show the variation in color.

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

| Parameters                                      | Values          |
|------------------------------------------------|-----------------|
| Moisture content (Loss on drying)              | 8.52±0.53       |
| Total ash                                      | 4.86±0.45       |
| Acid insoluble ash                             | 3.25±0.18       |
| Water soluble ash                              | 2.22±0.47       |
| Petroleum ether soluble extractive value       | 0.23±0.08       |
| Chloroform soluble extractive value            | 1.88±0.04       |
| Ethyl acetate soluble extractive value         | 3.56±0.04       |
| Methanol soluble extractive value              | 5.98±0.12       |
| Water soluble extractive value                 | 7.62±0.08       |
Indian systems of medicine utilize the 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 an 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. *G. serrata*, extensively utilized in conventional medicines has tremendous therapeutically potential due to its various biological activities. The prominent diagnostic characteristics of the root were xylem fibers, lignified xylem vessels, cork cells and parenchymatous cells. These characters can be utilized for standardization of drugs as well as used 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 the 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 that illustrate the quality and purity of the herbal medicine. Total ash consists of physiological ash, which is derived from plant tissue itself, and non physiological ash that is usually derived from atmosphere contaminations including 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. The phytochemical analysis of extracts viz., petroleum ether, chloroform, methanol, and water was analyzed and it indicated the presence of alkaloids, carbohydrates, saponins, tannins, proteins, amino acids, phytosterols, and flavonoids.

**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* root was recognized for many therapeutical properties, therefore, the current study might be beneficial to supplement the information in respect to its identification, authentication, and standardization; no such information is available for the same till date.

**AUTHOR’S CONTRIBUTION**

The manuscript was carried out, written, and approved in collaboration with all authors.

**CONFLICT OF INTEREST**

No conflict of interest is associated with this work.

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