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Research Article

Scientific Standardization of Various Extracts of *Chenopodium giganteum* D. Leaves

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ABSTRACT

In the present research the Histological examination, physiological evaluation, phytochemical screening, total phenolic content and total flavonoid content and TLC of leaf extracts of *Chenopodium giganteum* has been studied. Ethanol, aqueous, chloroform, and pet ether extracts of *Chenopodium giganteum* was prepared. Phytochemical screening shows presence of carbohydrates, proteins, amino acids, glycosides like saponin and flavonoids, tannins, phenols, alkaloids, and steroids. The physiological evaluation shows total ash 15.12% w/w and acid insoluble ash value 7.46%w/w, loss of drying was found to be 8.1% w/w, extractive value of pet ether extract was found to be 2.3% w/w, chloroform extract 4.5% w/w, ethanol extract 7.2% w/w and water 10.2% w/w. The total flavonoid content of pet ether extract was found to be 17.2227±0.0729 µg/ml, chloroform extract 23.7224±0.0878 µg/ml, ethanol extract 49.8601±0.0303 µg/ml and water extract 64.7705±0.0375 µg/ml. The total phenolic content of pet ether extract was found to be 14.477±0.0226 µg/ml, chloroform extract 17.764±0.0216 µg/ml, ethanol extract 19.518±0.0173 µg/ml and water extract 27.686±0.0233 µg/ml. TLC of pet ether extract shows constituents having Rf values 0.548, 0.274, 0.1935, and 0.080, chloroform extract shows constituents having Rf values 0.765, 0.656, 0.468, 0.234, and 0.156, ethanol extract shows constituents having Rf values 0.815 and 0.584, and water extract shows constituents having Rf values 0.704 and 0.064.

Keywords: Histological examination; physiological evaluation; phytochemical screening; Chenopodium giganteum

INTRODUCTION

India is a great source of plant and animal richness Due to its diverse geographic and agro-climatic zones. It also boasts a diverse cultural legacy in addition to a diversified biosphere. Although the Indian health care system currently includes both conventional and alternative medicine, traditional medical practices like Ayurveda, Siddha, and Unani, as well as disorganized practices like folk medicine, have been thriving. Indian-based Ayurveda and Siddha make up around 60% of the nation's overall health care system and 75% of its rural residents.[1,2] *Chenopodium giganteum*, a plant in the Amaranthaceae family, commonly referred to as tree spinach or lalbathua, is an annual, erect, many-branched shrub with an upper stem up to 5 cm diameter and a maximum height of 3 m. *Chenopodium amaranth tricolor* is another name for it.[3–6] This plant, which grows at an elevation of 4,700 meters, has been used medicinally to treat a variety of illnesses linked to nutritional deficits. The plant, which lowers the productivity of wheat, barley, mustard, and gramme crops, is a prevalent weed in waste areas and in fields throughout the summer and winter. It is also grown as a conventional leafy vegetable in India.[7–9] Nearly no *Chenopodium giganteum* is grown for sale commercially. However, the reliable and substantial yield of *Chenopodium giganteum* suggests that it may be a plant of the future.[10]

Chenopodium giganteum young shoots and leaves may be prepared and eaten like spinach. The oxalic acid and saponins present in it are greatly destroyed by cooking if cooked for two minutes at 100 °C in boiling water.[11,12] The leaves of *Chenopodium giganteum* can also be consumed raw in smaller amounts, like in a salad. Alternatively, the seeds can be crushed into flour and used with cereal flour to produce bread. The seeds can be prepared similarly to rice or quinoa. It also has decorative appeal because of its partly pink-colored leaves.[13] It is also used in assessment for accumulation of heavy metals and to

investigate the usage of Fe, Zn, Cu, Ni, Cr, and Cd for phyto extraction of heavy metals.[14] The plants belonging to the Amaranthaceae family and contains some saponins and oxalic acid, which, when present in excessive concentrations, can be harmful to human health. (e.g., Hemolysis or Kidney stone disease).[15–18]

The goal of this standardization is to guarantee the quality, safety, efficacy, and stability of final product by the careful, responsible selection and treatment of raw material. Additionally, there are no reports of comprehensive pharmacognostic research on leaves of this plant. This goal was considered when designing the current study, which aimed to evaluate the leaves scientifically. The investigation comprises physicochemical parameters, macroscopic and microscopic features, powder microscopic characteristics, TLC fingerprinting, and preliminary phytochemical screening. The data produced by this specific investigation provide pertinent pharmacognostical and physicochemical information required for accurate identification and verification of *Chenopodium giganteum* leaves.

MATERIAL AND METHODS

Collection, identification, and authentication of plant material

The leavesof *Chenopodium giganteum* (herbarium number XCH — 40416) plant was collected from the surrounding areas of Meghalaya. It was dried under shade and made into coarse powder. The plant material collected was identified and authenticated by Scientist (Dr) S. Mutheeswaran, M.Sc., M.Phil., Ph. D., Xavier Research Foundation, St Xavier's College, Tamil Nadu, India.

Morphological and macroscopic features

The fresh leaves of *Chenopodium giganteum* were examined for various macroscopic features like colour, odour and taste of leaves. Other external morphological characters like surface, base, margin, size, and shape of leaves were also studied. The microscopic examination of *Chenopodium giganteum* was done with the help of microscope. The air-dried plant material was then crushed into a coarse powder and used for further research work. The stomatal number and stomatal index of leaves of *Chenopodium giganteum* was also determined and evaluated by the methods referred from textbook authored by Kokate, Purohit and Gokhale.[19]

Physicochemical constants

The physical constants like ash value and extractive value helps in establishing the pharmacopoeial standards of the drug. The leaves of *Chenopodium giganteum* were examined for Physico-chemical constants like loss on drying, ash value and extractive value as per the methods mentioned in Pharmacopoeias.[20,21]

Preparation of extracts

Successive Solvent extraction: Leaf of *Chenopodium giganteum* was dried and milled to a coarse powder. One kg of fresh plant material was grounded and defatted using petroleum ether. About 50g of the powdered air-dried defatted plant material was extracted subsequently with chloroform, pet ether, ethanol, and water in a Soxhlet apparatus. Each time before extracting with the next solvent, the marc was air dried below 50°C. The extracts were filtered, and the solvent obtained was evaporated at room temperature and accurate weight of the extracts was taken. The extractive value (%) was calculated with reference to air dried drug.

Preliminary Phytochemical screening

Preliminary phytochemical investigations of pet ether, chloroform, ethanol and water extracts were done to reveal the presence of different secondary metabolites like Proteins & Amino acids Carbohydrates, Steroids, Phenols, Saponins, Flavonoids, Alkaloids and Tannins.[22,23]

Fluorescent analysis

Fluorescent analysis of both extracts and plant powders of *Chenopodium giganteum* leaves were carried out according to the method mentioned by Chase and Pratt et al. (1949) and Koshiet al. (1958) in day light and in UV light (254 nm and 365 nm). The plant powders and extracts were treated with different solvents and the fluorescence was observed in day light and in near and far UV light. About 10g of drug powder was taken in a petri dish and treated with different reagents and observed under different wavelengths i.e., ultraviolet and visible rays.[24]

Total Phenol Content Determination

Using gallic acid as a standard, the total phenol concentration was calculated using the Folin-Ciocalteu test. In this process, 1.5 ml of Folin-reagent Ciocalteu's (FCR) diluted 1:10 v/v and 0.5 ml of plant extracts were combined. After 5 minutes, 1.5 ml of a solution containing 7% sodium carbonate was added. With distilled water, the final volume was made up to 10 ml, and they were then left to remain at room temperature for 90 minutes. A spectrophotometer was used to test the sample's absorbance at 750 nm in comparison to the blank. The entire experiment was done three times for accuracy and data expressed as mean standard deviation in terms of phenol content (gallic acid equivalent, or GAE) per g of dry weight.

Total Flavonoid Content Determination

Using quercetin as a standard, the total flavonoid content was calculated using the aluminium chloride technique. In this process, 4 ml of water and 1 ml of plant extracts

were combined. After 5 minutes, 0.3 ml of 10% Aluminum chloride, 0.3 ml of 5% Sodium nitrite and 1ml of 1 M Sodium hydroxide was added to the reaction mixture after the mixture had been incubated at room temperature for 6 minutes the final volume was made up to 10 ml with distilled water. A spectrophotometer was used to test the sample's absorbance at 510 nm in comparison to the blank. The entire experiment was done three times for accuracy, and data were expressed as mean standard deviation in terms of flavonoid content (Quercetin equivalent, or QE) per g of dry weight.[25]

TLC of extracts

For TLC experiments, one gram of extracts was dissolved in methanol, filtered, and used. On aluminium plates that had already been coated with silica gel G, about 6μg of *Chenopodium giganteum* extract were applied. In a TLC chamber, the plate was produced using several solvent systems. Utilizing a photo documentation equipment, produced plates were seen and documented in both short and long UV.[26]

RESULTS AND DISCUSSION

Morphology of Chenopodium giganteum leaves

The morphological data of *Chenopodium giganteum* showed the colour of young leaves was pink, magenta colour and older leaves were green with smooth under surface as shown in Figure 1. It has odour and bitter characteristic taste. The shape of leaves was extremely variable as simple, deltoid, ovate to lanceolate, upper entire, rhomboid, lower toothed or irregularly lobed. The size of leaf was about 1-3 cm; petioles were of 1-2 cm in length and as long as thick blade. Its length varied from 9 to 4.5 cm broad having dentate margin. It has acute apex and upto 5 cm base.

Microscopy of C henopodium giganteum leaves

The microscopic study of *Chenopodium giganteum* leaves was done with the help of microscope. The transverse section of the leaves reveals that the *Chenopodium giganteum* leaf is dorsiventral with palisade cells pointing upward toward the top epidermal layer. Nearly half of the leaflet is covered by the lengthy palisade cells. In the upper epidermis, there were spherical, thin-walled collenchymas in place of the thick-walled parenchymatous cells that were present there. The palisade layer is broken in the midrib area underneath the top epidermis by collenchymatous cells. Near the vascular bundles, thick-walled parenchymatous cells revealed the existence of spiral and annular vessels. While the cells in the lower epidermis are spherical, those in the upper epidermis are flattened. In the midrib area, behind the collenchyma cells, the vascular bundles are organized like a bunch of grapes. The transverse section and powder microscopy (magnification 10X) of leaves of *Chenopodium giganteum* is

Fig. 1: Morphological characters of leaves of *Chenopodium giganteum*

given below in Figure 2 and Figure 3.

Determination of leaf constants

The total stomatal number and stomatal index of upper epidermis of leaves was found to be 12 and 22.2% respectively and lower epidermis was found to be 11 and 12.5% respectively.

Determination of physicochemical constants

The total ash, acid-insoluble ash and water-soluble ash value and loss on drying of leaves of *Chenopodium giganteum* were evaluated and the results are mentioned in Table 1.

Table 1: Physico-chemical constants and leaf constants of leaves of *Chenopodium giganteum*

Loss on drying	Total ash	Acid insoluble ash	Water soluble ash
8.1%	15.12%	7.46%	9.26%

Extraction of plant material

The appearance and yield of different extracts of *Chenopodium giganteum* were evaluated and the results are

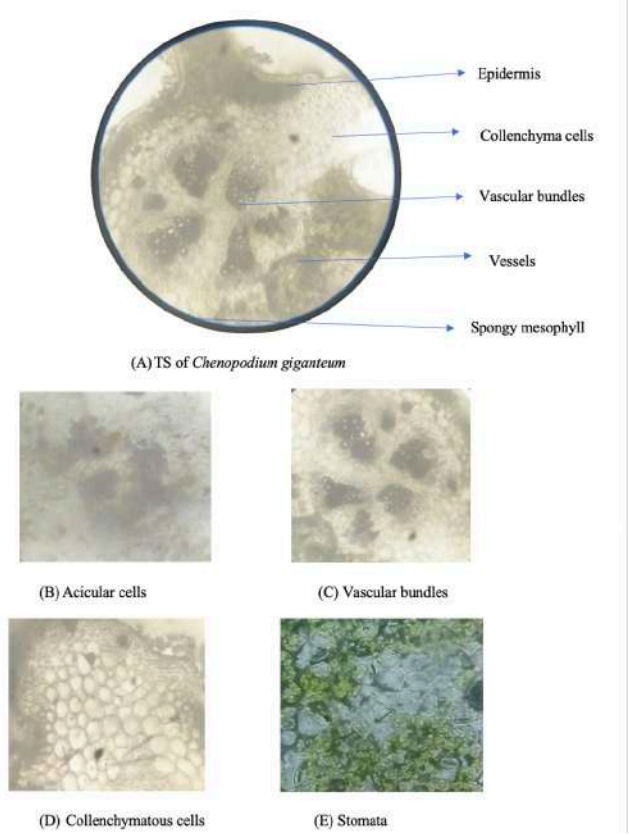

Fig. 2: A-E: Transverse section of leaves of *Chenopodium giganteum*

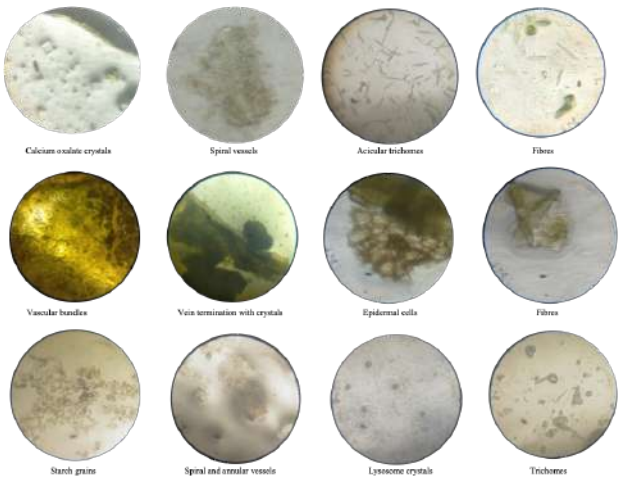

Fig. 3: Powder microscopy of leaves of *Chenopodium giganteum* (magnification 10X)

mentioned in Table 2.

Table 2: Yield of extracts obtained from successive extraction of leaves of *Chenopodium giganteum*

Plant Name	Type of Extract	Appearance	State	Yield (% w/w)
***Chenopodium giganteum* leaves**	Pet ether	Yellowish green	Semisolid	2.3% w/w
	Chloroform	Greenish black	Semisolid	4.5% w/w
	Ethanol	Dark green, black	Semisolid	7.1% w/w
	Water	Dark brown black / Semi solid Dark Brown black / Dark Dark brown, black	Semisolid	10.2% w/w

Preliminary phytochemical screening of extracts

Petroleum ether and chloroform extracts revealed the presence of steroids whereas ethanol and aqueous extracts indicated the presence of flavonoids, carbohydrates, saponins, proteins, alkaloids, phenols, steroids, and tannins respectively (Table 3).

Table 3: Preliminary phytochemical screening of leaf extracts of *Chenopodium giganteum*

Chemical tests	*Chenopodium giganteum* leaf extracts			
	Pet ether	Chloro-form	Ethanol	Water
Proteins & Amino acid	-	-	+	+
Carbohydrates	-	-	+	+
Steroids	+	+	-	-
Phenols	-	-	+	+
Saponins	-	-	+	+
Flavonoids	-	-	+	+
Alkaloids	-	-	+	+
Tannins	-	-	+	+

'-' indicates absence and '+' indicates presence

Fluorescent analysis

The selected plant is made into course powder and treated with required chemical reagents and observed under visible

and ultraviolet rays. The results are given in Table 4 and Table 5.

Table 4: Fluorescence analysis of powder of leaves of *Chenopodium giganteum*

S.No.	Treatment	Day Light	Short UV (254nm)	LONG UV (366nm)
1.	Powder	Green	Green	Green
2.	Powder + Water	Green	Green	Dark green
3.	Powder + 1NHCl	Green	Green	Dark green
4.	Powder + 1NH_2SO_4	Green	Green	Dark green
5.	Powder + 1$NHNO_3$	Green	Green	Dark-green
6.	Powder + Aceti-cacid	Green	Green	Dark green
7.	Powder + 1NNaOH	Green	Green	Dark green
8.	Powder + 1NAlc.NaOH	Green	Green	Yellowish green
9.	Powder + 1NKOH	Green	Green	Dark green
10.	Powder + 1NAlc.KOH	Green	Green	Yellowish Green
11.	Powder + Ammo-nia	Yellowish green	Green	Dark green
12.	Powder + Iodine	Yellowish brown	Dark green	Dark green
13.	Powder + $FeCl_3$	Yellowish brown	Dark green	Darkgreen
14.	Powder + Ethanol	Green	Green	Yellowish green

Table 5: Fluorescence analysis of leaf extracts of *Chenopodium giganteum*

S.No	Extracts	Day Light	UV Light	
			Short 254nm	Long 365nm
1	Pet.ether	Green	Yellowish green	Yellowish
2	Chloroform	Greenish black	Darkgreen	Reddish brown
3	Ethanol	Greenish black	Greenishblack	Reddish brown
4	Water	Brownish dark	Green	Greenish black

Total phenolic and Flavonoidal content

The total phenolic and flavonoidal content for aqueous, ethanol, chloroform, and petroleum ether extracts of *Chenopodium giganteum* were estimated and results are given in Table 6.

Table 6: Total phenolic content and total flavonoid content of leaf extracts of Chenopodium giganteum

Chenopodium giganteum plant extract	Total phenolic con-tent	Total flavonoid con-tent
Pet ether	14.477±0.0226 µg/ml	17.2227±0.0729 µg/ml
Chloroform	17.764±0.0216 µg/ml	23.7224±0.0878 µg/ml
Ethanol	19.518±0.0173 µg/ml	49.8601±0.0303 µg/ml
Water	27.686±0.0233 µg/ml	64.7705±0.0375 µg/ml

TLC studies of extracts

The extracts were undertaken for TLC profiling to assess the nature of phytochemicals present in it. A number of developing solvent systems were tried for all the extract and fractions. The solvent system, which gave the best resolution, was considered optimized, valid, and useful. The satisfactory resolution was obtained in the mobile phase mentioned in Table 7 and photo documentation is shown in Figure 4. The Rf values of different extracts was also calculated and are mentioned in Table 8. Blue and orange spots were observed which indicated the presence of phenolic compounds.[27]

Table 7: Mobile phase for TLC studies of leaf extracts of *Chenopodium giganteum*

Test extract	Solvent system	Number of Bands
Pet ether	Toluene:Chloroform (1:1)	05
Chloro-form	Toluene:Methanol (9:1)	05
Ethanol	Ethylac-etate:Methanol:Glacialacetic acid (7:2.2:0.8)	01
	Ethyl acetate: Methanol: Water (5:1:2)	02
Water	Ethylac-etate:Methanol:Glacialaceticacid (7:2.2:0.8)	01
	Ethyl acetate: Methanol: Water (5:1:2)	02

Table 8: Rf values of different extracts of *Chenopodium giganteum*

Chenopodium giganteum Leaf extracts	Color of the Spot	Rf value
	Light Blue	0.548
	Dark Blue	0.352
Pet ether	Brown	0.274
	Brown	0.1935
	Blue	0.080
	Orange	0.765
	Orange	0.656
Chloroform	Blue	0.468
	Blue	0.234
	Purple	0.156
	Orange	0.815
Ethanol	Reddish orange	0.584
	Orange	0.818
	Light blue	0.704
Water	Dark blue	0.064
	Light blue	0.731

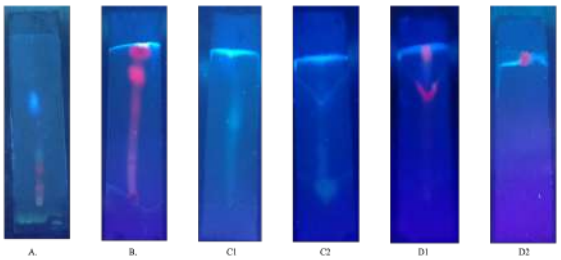

Fig. 4: TLC photo documentation of leaves of *Chenopodium giganteum*. (A) pet etherextract, (B) chloroform extract, (C1) water extract [Ethyl acetate:Methanol:Glacialaceticacid (7:2.2:0.8)], (C2) water extract [Ethyl acetate: Methanol: Water (5:1:2)], (D1) ethanol extract [Ethylacetate:Methanol:Glacialaceticacid(7:2.2:0.8)], (D2) ethanol extract [Ethyl acetate:Methanol: Water (5:1:2)]

CONCLUSION

In the current study, *Chenopodium giganteum* plant's leaves were examined for pharmacognostic characterisation, physiochemical parameter measurement, phytochemical screening, and TLC examinations of the crude extracts. The chosen plants were verified, and to prove their authenticity and purity, macroscopic examinations were carried out. To ascertain the fundamental cellular makeup of the leaf petiole, stem, kind of stomata, etc., a microscopic investigation was conducted. Physicochemical studies were carried out as per standard procedure such as ash value, acid insoluble ash values and extractive values. Fluorescence analysis was also carried out using different solvents. The important phyto-constituents were present as depicted in phytochemical screening which are well-known for their medicinal potentials. The screening of leaves indicates the presence of high phenolic content and high flavonoid content which may be due to presence of phenol, flavonoid and tannin which possess antioxidant properties. Identification of the numerous phytochemical ingredients found in the raw medicine is aided by the TLC fingerprint profile. The TLC fingerprint profile aids in locating significant phyto-constituents as well. Thus, it is concluded that our study provides the data which helps to isolate, identify, and characterize the various medicinal potential of *Chenopodium giganteum* leaves.

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