International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 19s, 2025
https://theaspd.com/index.php

Assessment Of Pharmacognostic Specification Of Xanthium Orientale Leaves & Stem

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Abstract
Xanthium orientale is widely recognized in traditional medicine for its therapeutic applications; however, the lack of standardized quality control measures poses a risk to its safety, efficacy, and global acceptability. The present study was undertaken to establish comprehensive pharmacognostic and phytochemical standards for the quality assessment and authentication of this medicinal plant. Detailed macroscopic and microscopic evaluations were conducted on the leaves and stem, including powder microscopy to document diagnostic anatomical features. Physicochemical parameters such as total ash, acid-insoluble ash, water and alcohol-soluble extractive values, and loss on drying were determined according to standard procedures. Furthermore, preliminary phytochemical screening of the aqueous and ethanolic extracts of leaves and stems revealed the presence of key secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and glycosides. The data generated from this study provide vital diagnostic and quality control parameters that can serve as a foundation for the development of a pharmacopoeial monograph for Xanthium orientale. This will ensure its safe, effective, and standardized use in herbal formulations and ethnomedicinal practices.
Keywords: *Xanthium orientale, Pharmacognostic specification, Phytochemical screening.*

INTRODUCTION

The resurgence of interest in herbal medicines globally can be attributed to their long-standing traditional use, perceived safety, affordability, and minimal side effects compared to synthetic drugs. Herbal drugs form an integral part of primary healthcare in many countries, particularly in Asia, Africa, and Latin America. However, the therapeutic efficacy and safety of herbal formulations largely depend on the quality and identity of the raw plant materials used. Therefore, the establishment of standardized pharmacognostic profiles and quality control parameters for medicinal plants is critical to ensure their authenticity, reproducibility, and global acceptability.

Xanthium orientale L., a member of the family Asteraceae, is an annual herbaceous plant traditionally used in various systems of medicine. It is native to North and South America—especially the United States, Peru, and Brazil—but has now become widely distributed across Asia, including India, Pakistan, Afghanistan, Iran, and China. Commonly known as "cocklebur" in English and "Chhota Gokhru" in Hindi, the plant thrives in temperate regions and is often found in agricultural fields, roadsides, and waste lands[1-2]. Traditionally, various parts of X. orientale have been employed for diverse therapeutic purposes. Ethnobotanical records indicate its use among the Zuni people of North America, who applied the seeds topically for wound healing and splinter removal. The seeds are also ground, mixed with cereal, and steamed to prepare food, highlighting its nutritional value. Decoctions prepared from the leaves are used to treat skin disorders, while root decoctions have been reported in folk medicine for their potential anti-cancer activity. An alcoholic tincture of the plant has been traditionally used in the management of rheumatism, and decoctions of the whole plant are employed in the treatment of dermatological ailments [3]. In Turkish folk medicine, X. orientale is used to manage central nervous system (CNS) disorders. Within the framework of Traditional Chinese Medicine (TCM), it is known as "Cang Er Zi" and is reputed for its application in treating conditions such as sinusitis, chronic nasal congestion, respiratory allergies, herpes-associated fever, and cardiovascular disorders. The widespread traditional usage of X. orientale across various cultures indicates the presence of bioactive constituents with pharmacological significance.[4-5]

Despite its extensive ethnomedicinal usage, there is a significant lack of comprehensive pharmacognostic and physicochemical studies on Xanthium orientale, especially concerning the standardization of its leaves and stem—two of the most commonly used parts. Without proper quality control parameters, there is a risk of adulteration, substitution, and loss of therapeutic efficacy in herbal preparations derived from this plant. Therefore, establishing a systematic profile through macroscopic, microscopic, physicochemical, and phytochemical evaluations is essential for its proper identification, authentication, and standardization.

The present study aims to fill this gap by investigating the detailed macroscopic and microscopic characteristics, powder microscopy, physicochemical parameters (such as total ash, acid-insoluble ash, water and alcohol-soluble extractive values, and loss on drying), and preliminary phytochemical screening of the leaves and stem of Xanthium orientale. The data generated through this study will contribute significantly to the development of a pharmacognostic monograph, thereby supporting the safe, effective, and standardized use of X. orientale in traditional and modern herbal formulations [6].
For its authentication, purity, and quality control of plant material.

MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

Fresh whole plants of Xanthium orientale were collected from the local areas surrounding Lucknow, Uttar Pradesh, India, during the post-monsoon season (November–December 2022). The collected specimens were washed thoroughly under running tap water to remove soil and other extraneous matter. The plant material was shade-dried at ambient temperature for 7–10 days. Taxonomic authentication was carried out at CSIR-NIScPR (formerly NISCAIR), New Delhi, and a voucher specimen was deposited for future reference.

2.2 Standardization and Pharmacognostic Evaluation

Standardization was performed in accordance with the guidelines outlined by the World Health Organization (WHO) for the quality control of medicinal plant materials. The evaluation included macroscopic, microscopic, powder microscopy, physicochemical, and preliminary phytochemical analyses.

2.3 Macroscopic (Organoleptic) Analysis

Organoleptic and morphological characteristics of the leaves and stems—including colour, odour, taste, shape, and size—were assessed visually and recorded following standard procedures [7].

2.4 Microscopic Analysis

2.4.1 Transverse Section of Leaf and Stem

Fresh sections of Xanthium orientale leaves (including midrib and lamina) and stems (approx. 10 cm in length) were cut into thin transverse sections using a sharp blade. These sections were mounted in glycerine-water solution and examined under a compound microscope at 40× magnification. Photomicrographs were captured to document cellular structures and tissue arrangements [8–10].

2.4.2 Quantitative Microscopy

Quantitative microscopic parameters including stomatal number, stomatal index, vein-islet number, and vein termination number were determined using standard protocols.

2.5 Powder Microscopy

Dried leaves and stems were separately powdered using a mechanical grinder and passed through a sieve (#60) to obtain coarse powder. A small amount of powder was mounted on a clean glass slide with glycerin-water, covered with a coverslip, and observed under a compound microscope for diagnostic characteristics such as trichomes, fibers, stomata, and calcium oxalate crystals [11,12].

2.6 Physicochemical Evaluation

All physicochemical parameters were assessed as per WHO guidelines [13–17].

2.6.1 Loss on Drying

Approximately 3 g of powdered leaf and stem samples were weighed separately in pre-weighed crucibles. The samples were dried in a hot air oven at 105°C until a constant weight was achieved. The percentage loss on drying was calculated with reference to air-dried samples.

2.6.2 Total Ash Value

3 g of powdered leaves and stems were taken in separate silica crucibles and incinerated at a temperature above 500°C until a carbon-free white ash was obtained. The ash was cooled in a desiccator and weighed. The total ash value (%) was calculated with reference to the air-dried drug.

2.6.3 Water-Soluble Ash

The total ash obtained was boiled with 25 ml of distilled water, filtered using ashless filter paper, and the insoluble residue was ignited to constant weight. The difference in weight between the total ash and the insoluble matter was recorded. The water-soluble ash value was expressed as a percentage.
Calculated Value: 6.65% (w/w)

2.6.4 Acid-Insoluble Ash

The total ash was boiled with 10 ml of dilute hydrochloric acid (HCl) for 5 minutes, filtered, and the insoluble matter was washed with hot water, ignited, and weighed. The acid-insoluble ash value was determined with respect to the air-dried sample.

Calculated Value: 9% (w/w)

2.7 Extraction Procedures

2.7.1 Sample Preparation

The shade-dried leaves and stems were ground separately using a mechanical grinder to obtain coarse powder, which was then stored in air-tight containers until further use [18].

2.7.2 Hot Continuous Percolation (Soxhlet Extraction)

100 g of powdered leaf and stem material were packed separately in thimbles and subjected to successive extraction using solvents of increasing polarity: petroleum ether, chloroform, ethyl acetate, and ethanol, using a Soxhlet apparatus. Each extraction was carried out for 8 hours. The respective extracts were concentrated by evaporating the solvent on a hot plate and weighed to calculate the % yield (w/w) [19–21].

2.7.3 Aqueous Extraction (Cold Maceration)

100 g of coarse powdered leaf and stem materials were macerated separately in 400 ml of distilled water in a round-bottom flask. The mixture was kept at room temperature for 72 hours with occasional shaking. After maceration, the solution was filtered and concentrated by evaporating the water. The final extract was weighed and the % yield (w/w) was calculated [22–24].

2.8 Preliminary Phytochemical Screening

The crude extracts of Xanthium orientale leaves and stems (obtained from each solvent system) were subjected to standard qualitative chemical tests for the presence of major phytoconstituents such as alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, and terpenoids, following established procedures [25-27].

The process of hot continuous extraction using a Soxhlet apparatus. Coarsely powdered plant material was placed in a thimble and successively extracted with various solvents (petroleum ether, chloroform, ethyl acetate, ethanol) for 8 hours each. Extracts were concentrated by solvent evaporation and stored for further phytochemical evaluation.

RESULTS AND DISCUSSION

3.1 Pharmacognostic Studies

3.1.1 Macroscopic Evaluation

The macroscopic evaluation of Xanthium orientale was performed to assess the organoleptic and morphological characteristics of both leaves and stems. The leaves were found to be dark green in color, bitter in taste, and characterized by a heart-shaped, three-lobed structure with serrated margins. The petiole and surfaces were hairy. Stems were cylindrical, green to reddish-brown, angular, and branched, ranging in size from 2 cm to 1 meter in height.

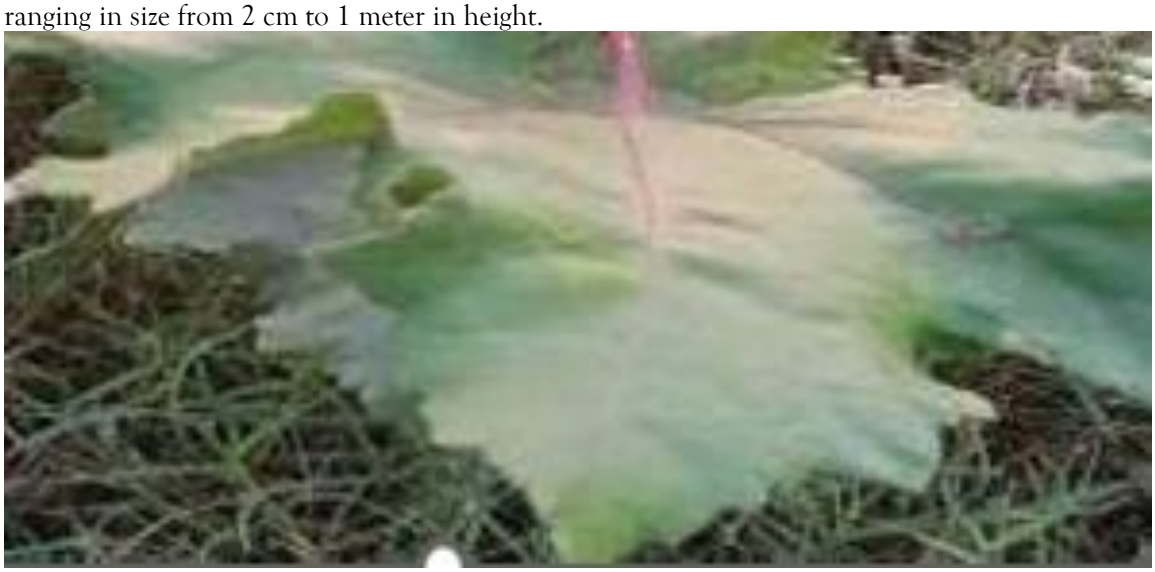

Fig.2: Leaf

Fig. 3 stem

Table 1: Macroscopic Characters of Xanthium Orientale

Sr. No.	Organoleptic Characters	Leaf	Stem
1	Colour	Dark green	Green brownish, Reddish brown
2	Odour	Characteristic	None/Slight
3	Taste	Bitter/Characteristic	Bitter
4	Shape	Simple, lobed, heart-shaped, serrate margin	Cylindrical, angular, branched
5	Size	3–8 cm (L), 2–4 cm (W)	2 cm–1 m (H), 1–2 cm (W)

3.1.2 Microscopic Evaluation

Leaf: Transverse section of the leaf revealed an upper and lower epidermis with anomocytic stomata, covered by a thick cuticle. Palisade cells were elongated and compactly arranged in three layers beneath the epidermis. The mesophyll tissue was composed of 7–10 layers of parenchymatous cells. Vascular bundles were embedded in the cortex, enclosed by a bundle sheath extending toward both epidermal layers.

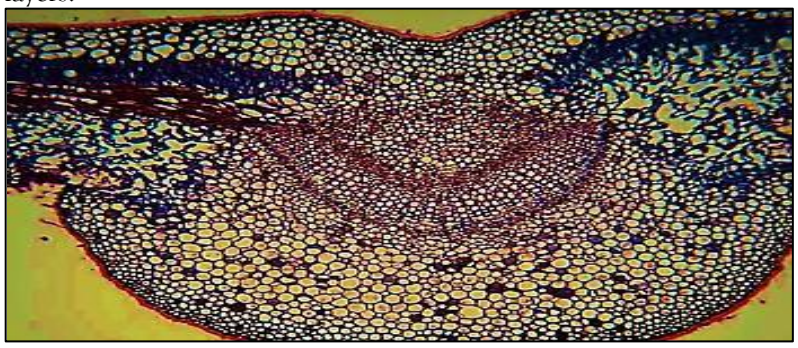

Fig.4: T.S. through midrib

Fig.5: T.S. showing epidermis

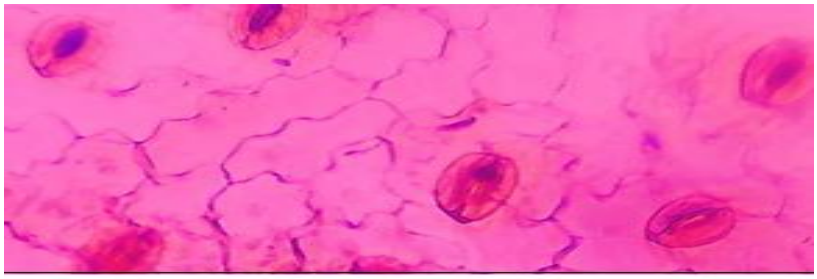

Fig.6: epidermis showing stomata

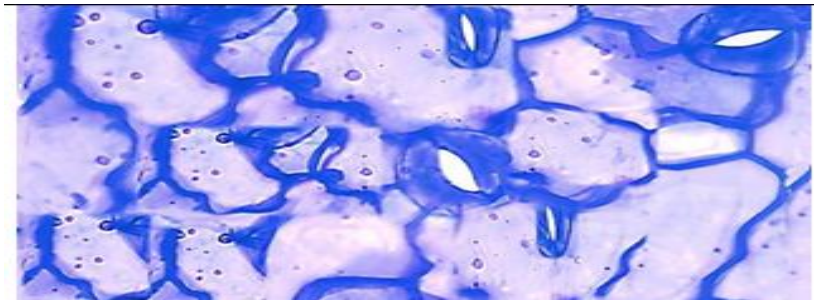

Fig.7: T.S. Showing stomata

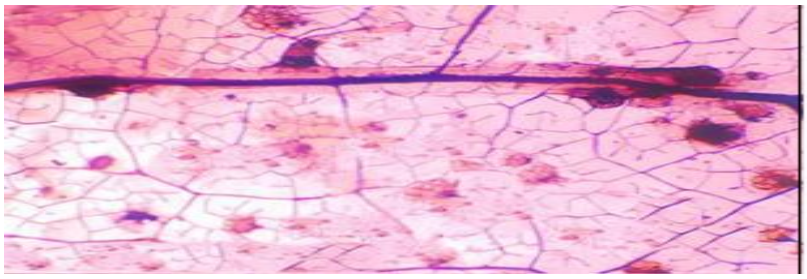

Fig.8: Leaf lamina showing veinlets, veintermination, and trichomes

Table 2: Leaf Constants of Xanthium orientale

Sr. No.	Leaf Constants	Values
1	Stomatal number (Upper Epidermis)	20.12 ± 0.289
2	Stomatal number (Lower Epidermis)	32.55 ± 0.618
3	Stomatal Index (Upper Epidermis)	21.60 ± 0.381
4	Stomatal Index (Lower Epidermis)	23.25 ± 0.023
5	Vein Islet Number	13.49 ± 0.245
6	Vein Termination Number	20.66 ± 0.761

Stem: The transverse section showed an outer epidermis, followed by a collenchymatous hypodermis. The cortex consisted of parenchymatous cells with scattered resin ducts. The pericycle was sclerenchymatous, especially near the vascular bundles. Vascular bundles were arranged in a ring, and the central pith was large and parenchymatous. A well-defined cambium ring was present, indicating secondary growth.

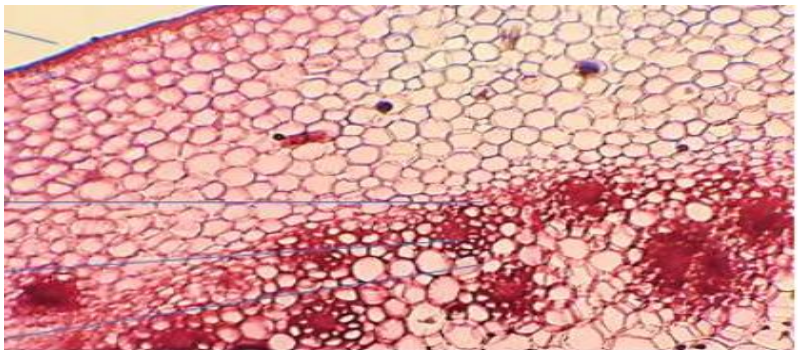

Fig.9: T.S. OF STEM (A PORTION)

3.2 Physicochemical Evaluation

The physicochemical parameters were assessed as per WHO guidelines. These parameters help determine the quality, purity, and strength of the crude drug.

Table 3: Physicochemical Evaluation of Xanthium orientale

Sr. No.	Parameter	Leaf (% w/w)	Stem (% w/w)
1	Total Ash	16.30	8.50
2	Acid-Insoluble Ash	9.00	4.85
3	Water-Soluble Ash	6.65	3.75
4	Moisture Content	12.50	8.25
5	Petroleum Ether Extractive	9.75	6.50
6	Chloroform Extractive	10.00	7.75
7	Ethyl Acetate Extractive	12.00	9.50
8	Ethanol Extractive	16.00	10.65
9	Aqueous Extractive	21.50	11.55

The high aqueous and ethanolic extractive values suggest the presence of abundant polar phytoconstituents, particularly in the leaf part of the plant.

3.3 Preliminary Phytochemical Screening

Preliminary phytochemical analysis was performed on different extracts of leaves and stems using standard qualitative methods. A wide range of bioactive compounds were detected, supporting the traditional uses of Xanthium orientale.

Table 4: Phytochemical Screening of Xanthium orientale Leaf Extracts

Constituent	Pet. Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous
Carbohydrates	+	+	–	–	–
Proteins	+	+	–	–	–
Lipids	+	–	+	+	+
Alkaloids	–	+	+	–	+
Glycosides	+	+	+	+	+
Tannins	–	–	+	+	+
Resins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Steroids	+	–	+	+	+
Flavonoids	–	+	–	–	+
Saponins	+	–	+	–	+

Table 5: Phytochemical Screening of Xanthium orientale Stem Extracts

Constituent	Pet. Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous
Carbohydrates	–	+	+	–	–
Proteins	–	–	–	–	–
Lipids	–	–	+	+	+
Alkaloids	–	+	–	+	+
Glycosides	–	+	+	+	+
Tannins	–	–	+	+	+
Resins	–	–	+	+	+
Terpenoids	–	–	+	+	–
Steroids	–	–	+	+	+
Flavonoids	–	–	+	+	+
Saponins	–	–	+	–	+

These findings highlight the abundance of key phytochemicals like glycosides, tannins, terpenoids, flavonoids, and resins, particularly in the ethyl acetate and ethanolic extracts of both parts. The differential distribution of phytoconstituents suggests their selective localization, which could influence pharmacological activity.

The comprehensive pharmacognostic, physicochemical, and phytochemical evaluation of Xanthium orientale supports its ethnomedicinal applications. The significant ash and extractive values indicate a substantial presence of organic and inorganic bioactive principles. Leaf samples generally yielded higher extractive values and phytochemical richness than stems, suggesting they may be a more potent part for therapeutic use.

The presence of secondary metabolites such as glycosides, alkaloids, terpenoids, flavonoids, and tannins may explain the traditional uses of the plant in wound healing, anti-inflammatory, and CNS disorders. The standardization parameters established in this study can serve as a reference for future research and formulation development involving Xanthium orientale.

CONCLUSION

The present study comprehensively evaluated the pharmacognostic and phytochemical characteristics of the leaf and stem of Xanthium orientale, aiming to establish standard reference parameters for its identification and potential therapeutic applications.

Macroscopic evaluation revealed distinctive morphological features such as three-lobed, serrated leaves and cylindrical, branched stems, which serve as useful diagnostic characteristics. Microscopic studies further substantiated these findings, with detailed descriptions of epidermal structures, stomatal types (anomocytic), palisade and mesophyll layers in leaves, and the presence of collenchymatous hypodermis, vascular bundles, and parenchymatous pith in the stem. Leaf constants such as stomatal number, stomatal index, vein islet number, and vein termination number offer valuable quantitative markers for authentication and quality control.

Physicochemical parameters, including ash values, moisture content, and solvent extractive values, provide essential data for assessing the purity, safety, and efficacy of plant materials. These values can be used to detect adulteration or substitution in raw drug materials and ensure consistency in herbal preparations.

Preliminary phytochemical screening revealed the presence of various bioactive constituents such as alkaloids, glycosides, tannins, resins, terpenoids, flavonoids, steroids, and saponins across different extracts of leaf and stem, indicating their potential medicinal relevance. The diversity of these phytoconstituents underscores the plant's traditional uses and supports its role in ethnomedicinal applications.

In conclusion, the macroscopic, microscopic, physicochemical, and phytochemical profiles established in this study offer critical baseline data for the standardization, authentication, and further pharmacological investigation of Xanthium orientale. These parameters not only validate traditional knowledge but also facilitate the integration of this plant material into modern phytotherapeutic practices and formulations.

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