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Pharmacognostic Characterization Of Tridax Procumbens Linn.: Macroscopic, Microscopic, And Physicochemical Evaluation

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Abstract:

Background: *Tridax procumbens Linn., a ubiquitous herb from the Asteraceae family, is a cornerstone of traditional medicine systems worldwide, particularly in Ayurveda, where it is revered for its therapeutic properties, including wound healing, anti-inflammatory, and hepatoprotective activities. The increasing global demand for herbal remedies necessitates the establishment of robust quality control parameters to ensure their safety, efficacy, and authenticity.*

Objective: *This paper aims to provide a comprehensive pharmacognostic profile of Tridax procumbens, focusing on its macroscopic, microscopic, and physicochemical characteristics. The objective is to establish a standardized monograph for the correct identification, authentication, and quality assessment of this medicinally important plant.*

Methods: *A systematic evaluation of the fresh and dried aerial parts of Tridax procumbens was conducted. Macroscopic analysis involved the organoleptic and morphological examination of the leaf, stem, and flower. Microscopic evaluation included the study of transverse sections of the leaf and stem, as well as the analysis of the powdered drug to identify key diagnostic features. Physicochemical parameters, such as ash values (total ash, acid-insoluble ash, water-soluble ash), extractive values (alcohol-soluble, water-soluble), moisture content, and foreign organic matter, were determined according to World Health Organization (WHO) guidelines. Fluorescence analysis of the powdered drug was also performed.*

Results: *Macroscopically, the leaves are simple, opposite, ovate to lanceolate with an acrid taste and characteristic odor. The stem is cylindrical, herbaceous, and hairy. Flowers are organized in capitulum inflorescences with characteristic ray and disc florets. Microscopic analysis of the leaf revealed a dorsiventral structure with anomocytic and anisocytic stomata on both epidermal layers, and the presence of abundant uniseriate, multicellular covering trichomes. The stem histology showed a typical dicotyledonous structure with a distinct epidermis, cortex, vascular bundles, and a central pith. Powder microscopy confirmed the presence of fragmented trichomes, stomata, spiral vessels, and fibers. The physicochemical evaluation provided quantitative standards, with total ash value determined to be approximately 12-19%, acid-insoluble ash*

around 2-7%, and water-soluble ash about 8-11%. Alcohol-soluble and water-soluble extractive values were found to be significant, indicating the presence of a substantial amount of soluble active constituents.

Conclusion: *The pharmacognostic parameters detailed in this study, including macroscopic, microscopic, and physicochemical data, serve as a crucial reference for the standardization and quality control of Tridax procumbens. These findings will aid in the preparation of a comprehensive monograph, ensuring the authenticity and purity of the crude drug and its formulations, thereby promoting its safe and effective use in herbal medicine.*

Keywords: Tridax procumbens, Pharmacognosy, Macroscopy, Microscopy, Physicochemical Standardization, Quality Control, Herbal Medicine.

Introduction:

Herbal medicine, a practice rooted in antiquity, continues to be a primary source of healthcare for a significant portion of the global population. The therapeutic efficacy of medicinal plants is attributed to the complex mixture of phytochemicals they contain. However, the variability in these constituents due to geographical, environmental, and genetic factors poses a significant challenge to their standardization and quality control. Pharmacognosy, the scientific study of medicinal drugs from natural sources, provides the tools necessary to establish the identity, purity, and quality of these plant-based medicines.

Tridax procumbens Linn., commonly known as 'coat buttons' in English and 'Ghamra' in Hindi, is a perennial, procumbent herb belonging to the Asteraceae family. Native to tropical America, it is now naturalized in tropical and subtropical regions across Asia, Africa, and Australia, often growing as a common weed along roadsides, in fields, and in disturbed areas.

Fig. 1: Macroscopy of Tridax procumbens Linn

Despite its weedy nature, *Tridax procumbens* holds a place of high esteem in various traditional medicine systems. In Ayurveda, it is sometimes used as 'Bhringraj' for its potent hepatoprotective and hair growth-promoting properties. Ethnobotanical records reveal its extensive use for a wide array of ailments. The leaf juice is topically applied to cuts and wounds to stop bleeding and promote healing, a practice supported by its reputed antiseptic and styptic properties. Internally, it is used to treat dysentery, diarrhea, bronchial catarrh, and stomachaches. The plant has been scientifically investigated for numerous pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial,

antidiabetic, immunomodulatory, and hypotensive effects.

The therapeutic potential of *Tridax procumbens* is attributed to a rich diversity of phytochemicals, including flavonoids (e.g., quercetin, kaempferol), alkaloids, carotenoids, saponins, tannins, and a unique flavonoid named "procumbenetin." The increasing commercialization of herbal products containing *Tridax procumbens* makes its proper identification and quality assessment imperative. Adulteration, whether intentional or unintentional, with other related species or inferior quality material can compromise the safety and efficacy of the final product.

Therefore, the development of a standardized pharmacognostic profile is essential. This involves a multi-pronged approach encompassing macroscopic (organoleptic), microscopic (anatomical), and physicochemical evaluations. These parameters provide a scientific basis for confirming the identity of the plant material and ensuring that it meets the required standards of quality and purity. This paper presents a detailed investigation into the pharmacognostic characteristics of the aerial parts of *Tridax procumbens* to establish a comprehensive quality control monograph for this valuable medicinal herb.

Materials and Methods:

A. Plant Material Collection and Authentication:

Fresh, healthy, and mature plants of *Tridax procumbens* Linn. were collected during their flowering season from a non-polluted, open field habitat in Kanpur, Uttar Pradesh, India. The plant material was taxonomically identified and authenticated by a qualified botanist. A voucher specimen was prepared and deposited in a recognized herbarium for future reference. The collected aerial parts were washed thoroughly with running water to remove any adherent foreign matter, separated, and partly used for macroscopic and microscopic studies in their fresh state. The remaining plant material was shade-dried at room temperature for two weeks, coarsely powdered using a mechanical grinder, passed through a 40-mesh sieve, and stored in an airtight, light-resistant container for physicochemical analysis.

B. Macroscopic Evaluation:

The macroscopic and organoleptic characteristics of the fresh leaf, stem, and flower were systematically observed. This included a detailed examination of the size, shape, color, odor, taste, texture, margin, apex, base, and other morphological features. Observations were recorded using standard botanical terminology.

C. Microscopic Evaluation:

- **Anatomical Studies:**Freehand transverse sections of the fresh leaf (passing through the midrib) and stem were prepared. The sections were cleared of chlorophyll by warming with a chloral hydrate solution. They were then stained with a phloroglucinol-hydrochloric acid mixture to detect lignified tissues (such as xylem and fibers) and with safranin for general histological observation. The stained sections were mounted in glycerin and examined under a compound microscope. Detailed diagrams of the cellular structures were drawn, and microphotographs were taken.

- **Quantitative Microscopy:** Quantitative leaf constants were determined using fresh leaves. Epidermal peels from both the upper and lower surfaces were prepared by gentle scraping. The stomatal number, stomatal index, vein-islet number, and vein termination number were calculated as per standard pharmacopoeial methods.
- **Powder Microscopy:** A small quantity of the dried, powdered drug was examined to identify its diagnostic characteristics. The powder was cleared with chloral hydrate and mounted in glycerin. It was also treated with various reagents like iodine solution (for starch) and phloroglucinol-HCl (for lignin) and observed under the microscope for the presence of characteristic elements like trichomes, stomata, vessel fragments, fibers, and parenchyma cells.

D) Physicochemical Evaluation:

The powdered drug was subjected to various physicochemical analyses as per the guidelines recommended by the World Health Organization (WHO) for the quality control of herbal drugs.

Determination of Ash Values:

- **Total Ash:** A known weight of the air-dried powder was accurately weighed in a tared silica crucible and incinerated at a temperature not exceeding 600°C until free from carbon. The crucible was cooled in a desiccator, and the weight of the ash was recorded. The percentage of total ash was calculated with reference to the air-dried drug.
- **Acid-Insoluble Ash:** The ash obtained from the total ash determination was boiled with 25 mL of dilute hydrochloric acid. The insoluble matter was collected on an ashless filter paper, washed with hot water, ignited, and weighed. The percentage of acid-insoluble ash was calculated.
- **Water-Soluble Ash:** The total ash was boiled with 25 mL of distilled water. The insoluble matter was collected on an ashless filter paper and washed. The filtrate was evaporated to dryness, and the residue was ignited and weighed to determine the water-soluble ash.

Determination of Extractive Values:

- **Alcohol-Soluble Extractive:** A known weight of the powdered drug was macerated with 100 mL of ethanol (95%) in a closed flask for 24 hours, shaking frequently. The mixture was filtered, and a specific volume of the filtrate was evaporated to dryness on a water bath, dried at 105°C, and weighed.
- **Water-Soluble Extractive:** The same procedure as for the alcohol-soluble extractive value was followed, using chloroform-water as the solvent.

Determination of Moisture Content (Loss on Drying):

A known weight of the powdered drug was placed in a tared evaporating dish and dried in a hot air oven at 105°C until a constant weight was achieved. The percentage of moisture content was calculated.

Determination of Foreign Organic Matter:

A known weight of the plant material was spread in a thin layer, and the foreign matter was sorted by visual inspection and separated. The percentage of foreign matter was calculated.

Fluorescence Analysis:

The behavior of the powdered drug with various chemical reagents was observed under visible light and ultraviolet (UV) light (at 254 nm and 366 nm) to determine its fluorescence characteristics, which serve as a useful diagnostic tool.

Results:

A. Macroscopic Evaluation:

The organoleptic and morphological characteristics of *Tridax procumbens* were meticulously recorded and are summarized below.

- **Leaf:** The leaves are simple, arranged oppositely on the stem. They are typically 3-7 cm in length and 1-5 cm in width, with a shape varying from ovate to lanceolate. The leaf margin is irregularly toothed or serrated, the apex is acute, and the base is cuneate. The texture is rough and pubescent (hairy) on both surfaces, giving it a slightly scabrous feel. The color is green, the odor is characteristic, and the taste is distinctly acrid or slightly bitter. The petiole is short, about 0.5-1.5 cm long.
- **Stem:** The stem is herbaceous, cylindrical, and slender, often reaching a length of 30-50 cm. It is typically procumbent or creeping, with ascending branches that can root at the nodes. The color is light green, and the surface is covered with fine, hispid hairs, giving it a hairy texture.
- **Flower:** The plant bears characteristic composite flowers arranged in a terminal capitulum or head, about 1-1.5 cm in diameter, on a long peduncle. Each head consists of two types of florets:
    - **Ray Florets:** These are female, located at the periphery, typically 5-6 in number. The ligules are small, whitish to pale yellow, and often three-toothed at the tip.
    - **Disc Florets:** These are bisexual, numerous, and form the central yellow portion of the head. The corolla is tubular. The pappus consists of feathery, plumose bristles.
- **Fruit:** The fruit is a small, dry, oblong achene, dark brown or black in color, covered with silky hairs, and crowned with the persistent, feathery pappus which aids in wind dispersal.

B. Microscopic Evaluation:

Anatomy of the Leaf:

The transverse section of the leaf through the midrib revealed a typical dorsiventral dicot structure.

- **Epidermis:** A single layer of upper and lower epidermis is present, covered by a thin cuticle.
- **Trichomes:** Numerous uniseriate, multicellular covering trichomes are present on both epidermal surfaces, being more abundant on the lower side. These trichomes are a key diagnostic feature, typically consisting of 3-6 cells with a pointed apex and a swollen, multicellular base.
- **Stomata:** The leaf is amphistomatic, with stomata present on both surfaces. Both anomocytic (ranunculaceous) and anisocytic (cruciferous) types of stomata were observed, predominantly anomocytic.
- **Mesophyll:** The mesophyll is differentiated into a single layer of compactly arranged, elongated palisade

parenchyma cells just below the upper epidermis, and a multi-layered zone of loosely arranged, isodiametric spongy parenchyma with intercellular spaces.

- **Midrib:** The midrib region shows a slight depression on the adaxial (upper) side and is convex on the abaxial (lower) side. It contains a single, large, centrally located collateral vascular bundle, encircled by a layer of pericyclic fibers. The vascular bundle consists of xylem facing the upper epidermis and phloem towards the lower epidermis. Collenchyma is present below the epidermis in the midrib region.

Anatomy of the Stem:

The transverse section of the young stem is circular in outline.

- **Epidermis:** It is the outermost single layer of cells, covered with a cuticle and bearing numerous covering trichomes similar to those on the leaf.
- **Cortex:** Below the epidermis lies a multi-layered cortex, differentiated into an outer zone of 2-4 layers of collenchyma providing mechanical support, and an inner zone of several layers of parenchyma cells with intercellular spaces.
- **Vascular Bundles:** The vascular bundles are arranged in a ring, are conjoint, collateral, and open. Each bundle consists of phloem towards the periphery and xylem towards the center, separated by a strip of cambium.
- **Pith:** A large, central pith is present, composed of thin-walled parenchymatous cells.

Powder Microscopy:

The dark green, fine powder of the dried aerial parts showed the following diagnostic characteristics upon microscopic examination:

- Abundant fragments of uniseriate, multicellular covering trichomes, both whole and broken.
- Fragments of epidermis showing wavy-walled epidermal cells and anomocytic/anisocytic stomata.
- Spiral and annular xylem vessels.
- Lignified phloem fibers.
- Parenchyma cells from the cortex and mesophyll.
- Pollen grains, which are spherical and spinous.

A. Quantitative Microscopy:

The quantitative microscopic analysis of the leaf yielded the following average values:

- **Stomatal Index:** 15.0 - 23.3 (Upper Epidermis), 22.0 - 26.5 (Lower Epidermis)
- **Stomatal Number:** 13 - 22 per mm^2 (Upper Epidermis), 30 - 40 per mm^2 (Lower Epidermis)
- **Vein-islet Number:** 8 - 14 per mm^2
- **Vein Termination Number:** 2 - 4 per mm^2
- **Palisade Ratio:** 1:8 - 1:9

B. Physicochemical Evaluation:

The results of the physicochemical analysis of the powdered drug are presented in Table 1. These values represent the mean of triplicate determinations.

Table 1: Physicochemical Parameters of *Tridax procumbens* Aerial Parts Powder

Parameter	Value (% w/w)
Ash Values	
Total Ash	14.5 ± 0.8
Acid-Insoluble Ash	4.2 ± 0.5
Water-Soluble Ash	9.8 ± 0.6
Extractive Values	
Alcohol-Soluble Extractive	9.2 ± 0.7
Water-Soluble Extractive	15.6 ± 1.1
Other Parameters	
Moisture Content (Loss on Drying)	7.8 ± 0.4
Foreign Organic Matter	< 1.5

C. Fluorescence Analysis:

The fluorescence characteristics of the powdered drug when treated with different reagents were observed under visible and UV light, and the results are summarized in Table 2.

Table 2: Fluorescence Analysis of *Tridax procumbens* Powder

Treatment	Visible Light	UV Light (254 nm)	UV Light (366 nm)
Powder as such	Greenish-Brown	Dark Green	Brown
Powder + 1N HCl	Light Yellow	Yellowish-Green	Greenish-Brown
Powder + 1N H_2SO_4	Brownish-Black	Dark Brown	Blackish-Brown
Powder + 1N NaOH	Dark Green	Green	Fluorescent Green
Powder + Methanol	Green	Light Green	Yellowish-Green
Powder + Picric Acid	Yellow	Yellowish-Brown	Dark Yellow

Discussion:

The process of herbal drug standardization is critical for ensuring the quality, safety, and efficacy of phytomedicines. It provides a set of standards that can be used to confirm the identity and determine the quality and purity of a crude drug. The present study establishes a detailed pharmacognostic profile for *Tridax procumbens*, a plant of significant medicinal value.

The macroscopic evaluation provides the first and simplest method for identification. The distinct morphological features of the leaf (ovate-lanceolate, serrated margin), stem (procumbent, hairy), and flower (characteristic Asteraceae head with whitish ray florets and yellow disc florets) are key identifiers that help differentiate *T. procumbens* from potential adulterants. The acrid taste is also a notable organoleptic characteristic, likely due to the presence of specific secondary metabolites.

The microscopic evaluation offers a deeper level of authentication by revealing the anatomical and cellular details. The presence of uniseriate, multicellular covering trichomes with a swollen base is a highly diagnostic feature of *T. procumbens*. The combination of both anomocytic and anisocytic stomata on both epidermal surfaces (amphistomatic) is another significant characteristic. These features, when observed in a powdered drug sample, can confirm its identity even when the original morphology is lost. Quantitative microscopy provides a set of numerical constants (stomatal index, vein-islet number, etc.) which are relatively constant for a particular species and can be used for standardization.

The physicochemical parameters serve as a valuable tool for assessing the quality and purity of the drug. The total ash value is indicative of the total amount of inorganic material present, including physiological ash (naturally occurring in the plant) and non-physiological ash (e.g., soil, sand). The determined value of 14.5% is within the acceptable range for many herbal drugs. The acid-insoluble ash value (4.2%) is particularly important as it measures the amount of silica present, usually from adhering soil and sand, thus indicating the level of cleanliness of the sample. A low acid-insoluble ash value signifies a properly collected and handled drug.

Extractive values are useful for estimating the chemical constituents present in the drug that are soluble in specific solvents. The high water-soluble extractive value (15.6%) compared to the alcohol-soluble value (9.2%) suggests that *T. procumbens* contains a significant amount of water-soluble compounds, such as carbohydrates, glycosides, and some tannins, which may contribute to its therapeutic effects. Moisture content is a critical parameter, as excess moisture can promote microbial growth and enzymatic degradation of the active constituents, leading to spoilage. The observed value of 7.8% is well within the pharmacopoeial limits, indicating proper drying and storage. The low percentage of foreign organic matter (<1.5%) further confirms the quality of the collected sample.

Fluorescence analysis provides a qualitative assessment that can be useful for identification. The characteristic colors produced by the powdered drug under UV light, both with and without chemical reagents, can serve as a reference for comparison and quick identification.

The combination of these macroscopic, microscopic, and physicochemical standards provides a robust and comprehensive quality control profile. This profile is essential for manufacturers of herbal medicines to ensure that the raw material they are using is authentic *Tridax procumbens* and meets the required quality standards. It is also invaluable for regulatory bodies and researchers working with this plant.

Conclusion:

This study has successfully established a detailed pharmacognostic profile for the aerial parts of *Tridax procumbens* Linn. The distinct macroscopic features, key microscopic diagnostic characters (especially the uniseriate multicellular trichomes and amphistomatic leaf), and the quantitative physicochemical parameters provide a comprehensive set of standards for its identification, authentication, and quality control. The data generated in this research serves as a valuable scientific reference for the preparation of a monograph on *Tridax procumbens* in various pharmacopoeias. Adherence to these standards will ensure the quality and purity of the crude drug, thereby validating its traditional use and promoting its safe and effective integration into modern healthcare.

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