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PHARMACOGNOSTIC CHARACTERIZATION, PHYSICOCHEMICAL STANDARDIZATION, AND PHYTOCHEMICAL PROFILING OF THE WHOLE PLANT OF *ORTHOSIPHON THYMIFLORUS* (ROTH) SLEESEN (LAMIACEAE)

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ABSTRACT: *Orthosiphon thymiflorus* (Roth) Sleesen (Lamiaceae), locally known as "Kattuthritha" or "Neer-naadan," is a perennial herb valued in South Indian and Southeast Asian traditional medicine for its diuretic, anti-inflammatory, and anti-diabetic properties. Despite its ethnopharmacological relevance, comprehensive pharmacognostic standards for this species are lacking, hindering quality control and authentication. This study addresses this gap by establishing detailed pharmacognostic, physicochemical, and phytochemical profiles for the whole plant. Authenticated plant material underwent macroscopic and organoleptic evaluation, microscopic analysis of leaf, stem, and root transverse sections, and powder microscopy. Physicochemical parameters were determined per WHO guidelines. Preliminary and quantitative phytochemical analyses were performed on chloroform and ethanolic extracts. Macroscopic findings confirmed characteristic Lamiaceae features: quadrangular stems and opposite, ovate-lanceolate, serrate leaves. Microscopy revealed a dorsiventral leaf structure, diacytic stomata, glandular trichomes, and collateral vascular bundles. Physicochemical values included total ash (8.5% w/w), acid-insoluble ash (2% w/w), water-soluble extractive (4% w/w), alcohol-soluble extractive (2% w/w), and loss on drying (1.29 g). Phytochemical screening identified phenols, flavonoids, and triterpenoids, with ethanolic extracts exhibiting a richer profile. These established botanical, physicochemical, and phytochemical parameters provide a crucial reference standard for the accurate identification, quality control, and future therapeutic development of *Orthosiphon thymiflorus*, ensuring its differentiation from related taxa and preventing adulteration.

INTRODUCTION: Medicinal plants serve as an indispensable reservoir of bioactive compounds for drug discovery and traditional healthcare systems worldwide.

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The genus *Orthosiphon* (family Lamiaceae) comprises several species of significant medicinal value, most notably *Orthosiphon stamineus* (Java tea), which is renowned for its diuretic, anti-inflammatory, and nephroprotective effects [1, 2].

However, the species *Orthosiphon thymiflorus* (Roth) Sleesen, although widely distributed in tropical Asia and Africa and utilized in regional folk medicine, has received comparatively less scientific attention.

In traditional Ayurvedic practices of Kerala and Tamil Nadu, *O. thymiflorus* is referred to by vernacular names such as "Kattuthritha," "Neer-naadan," or "Thannithuppi," which literally translate to "water leaf," underscoring its primary use as a fluid balancer and mild diuretic [3]. It is also employed in the management of urinary tract disorders, mild inflammation, and metabolic imbalances [4]. Despite these ethnobotanical claims, a systematic pharmacognostic evaluation is a prerequisite for the inclusion of any herbal drug in modern pharmacopoeias and for ensuring reproducibility in biological assays.

Pharmacognostic standardization involves the establishment of macro- and microscopic characteristics, physicochemical constants, and phytochemical fingerprints. This process is critical for detecting adulteration, ensuring purity, and validating the identity of the crude drug material [5]. Given that *O. thymiflorus* is often substituted for or confused with *O. stamineus* in regions where the latter is scarce, there is an urgent need for definitive botanical and chemical markers specific to *O. thymiflorus*.

Therefore, the present study was designed to conduct a thorough pharmacognostic characterization of the whole plant of Orthosiphon thymiflorus, including morphological, microscopic, and physicochemical evaluations, alongside a detailed preliminary and quantitative phytochemical profiling of its solvent extracts.

FIG. 1: ORTHOSIPHON *THYMIFLORUS*

FIG. 2: LEAVES OF *ORTHOSIPHON THYMIFLORUS*

FIG. 3: FLOWERS OF *ORTHOSIPHON THYMIFLORUS*

MATERIALS AND METHODS:

Plant Collection and Authentication: The whole plants of *Orthosiphon thymiflorus* (Roth) Sleesen were collected during the flowering season in October from the Miyawaki Nature Lab, Vilappil, Thiruvananthapuram district, Kerala, India. The specimen was authenticated by Dr. Mariyamma, Retired HOD, Department of Botany, University of

Kerala, Karyavattom. A voucher specimen was deposited in the institutional herbarium for future reference.

Processing and Extraction: The collected plant material was cleaned thoroughly to remove soil and extraneous matter. The whole plant was shade-dried at room temperature for approximately two weeks until crisp. The dried material was pulverized using a mechanical grinder to obtain a coarse powder. The powder was stored in an airtight container protected from light and moisture until further analysis.

Successive Soxhlet Extraction:

Chloroform Extract: 60 g of powdered material was subjected to Soxhlet extraction using chloroform as solvent at a temperature range of 45-55°C for 3-5 days.

Ethanolic Extract: The marc (residue) remaining after chloroform extraction was air-dried and subsequently extracted with 95% ethanol at 40-50°C for 3-7 days. Both extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and finally dried in a water bath to obtain the crude chloroform extract (OTCE) and crude ethanolic extract (OTEE). The percentage yield of each extract was calculated relative to the air-dried powdered material.

FIG. 4: SOXHLET EXTRACTION PROCESS

Pharmacognostic Evaluation:

Macroscopic Evaluation: The organoleptic properties including color, odor, taste, and morphological features (shape, size, margin, apex, base, surface texture, and phyllotaxy) of the fresh and dried plant materials were documented.

Microscopic Evaluation:

Transverse Section (T.S.): Freehand sections of fresh leaf, stem, and root were taken using a sharp blade. The sections were cleared, stained with appropriate staining reagents (e.g., safranin and fast green), mounted on glass slides with glycerin, and observed under a light microscope. Diagnostic features were photographed using a digital camera attachment.

Powder Microscopy: A small quantity of the shade-dried coarse powder was placed on a microscope slide, treated with chloral hydrate solution, mounted, and examined under the microscope to identify cellular components such as trichomes, stomata, fibers, vessels, and crystals.

Physicochemical Parameters: Physicochemical constants were determined as per standard procedures outlined in the WHO guidelines for quality control of herbal materials [6].

Total Ash: 2 g of powder was incinerated in a silica crucible at a temperature not exceeding 600°C until free from carbon.

Acid-Insoluble Ash: Total ash was boiled with 25 mL of dilute HCl, filtered, and the insoluble residue was ignited and weighed.

Water-Soluble Ash: Total ash was boiled with water, filtered, and the insoluble matter was ignited and weighed.

Extractive Values: 5 g of powder was macerated with 100 mL of solvent (water for water-soluble extractive; 90% ethanol for alcohol-soluble extractive) for 24 hours with occasional shaking. 25 mL of filtrate was evaporated to dryness and weighed.

Loss on Drying: A specified quantity of powder was heated in an oven at 105°C until a constant weight was achieved.

Phytochemical Screening:

Preliminary Qualitative Screening: The chloroform and ethanolic extracts were subjected to standard chemical tests to detect the presence of various phytoconstituents:

Alkaloids: Mayer's, Wagner's, and Dragendorff's tests.

Carbohydrates: Molisch's and Fehling's tests.

Flavonoids: Shinoda test and Alkaline reagent test.

Phenolics & Tannins: Ferric chloride test and Lead acetate test.

Saponins: Foam test.

Terpenoids & Steroids: Salkowski test and Liebermann-Burchard test.

Quantitative Estimation of Phytochemicals:

Total Phenolic Content (TPC): Determined using the Folin-Ciocalteu method [7]. 1 mL of extract (1 mg/mL) was mixed with 1 mL Folin-Ciocalteu reagent and 10 mL 7% Na_2CO_3. Volume was made up to 25 mL with deionized water. After 90 min incubation in the dark, absorbance was measured at 760 nm. Results were expressed as µg of Gallic Acid Equivalent (GAE) per mg of extract.

Total Flavonoid Content (TFC): Determined using the Aluminum Chloride colorimetric method [8]. 1 mL of extract was mixed with 3 mL methanol, 0.2 mL 10% $AlCl_3$, 0.2 mL 1 M potassium acetate, and 5.6 mL distilled water. After 30 min incubation, absorbance was measured at 415 nm. Results were expressed as µg of Quercetin Equivalent (QE) per mg of extract.

Total Terpenoid Content (TTC): Determined using the Vanillin-Perchloric acid method [9]. 100 µL of extract was mixed with 1 mL perchloric acid and 300 µL of 5% w/v vanillin in glacial acetic acid. 5 mL of glacial acetic acid was added. Absorbance was read at 548 nm. Results were expressed as µg of Linalool Equivalent (LE) per mg of extract.

RESULTS:

Extraction Yield: The percentage yield of the extracts from the dried whole plant powder was found to be 4.15% w/w for the chloroform extract and 8.0% w/w for the ethanolic extract.

Macroscopic Characteristics: The macroscopic features of *Orthosiphon thymiflorus* are summarized in **Table 1**.

TABLE 1: MACROSCOPIC FEATURES OF *ORTHOSIPHON THYMIFLORUS*

Feature	Description
Habit	Erect, perennial herb
Leaf Type	Simple, opposite, decussate
Leaf Shape	Ovate to lanceolate
Dimensions	Length: 3-8 cm; Width: 1-3 cm
Color	Upper surface: Dark green; Lower surface: Lighter green
Margin	Serrate
Apex	Acute
Base	Cuneate
Texture	Soft, pubescent (slightly hairy)
Odor	Characteristic, aromatic
Taste	Slightly bitter
Stem	Quadrangular, green to purplish, branched
Flower	Pale violet to bluish, bilabiate, verticillaster inflorescence

Microscopic Characteristics:

Transverse Section (T.S.) Analysis:

Leaf (Dorsiventral): The upper epidermis was single-layered with a thin cuticle. Multicellular covering trichomes and glandular trichomes were abundant on both surfaces. Mesophyll was differentiated into a single layer of palisade parenchyma (rich in chloroplasts) and spongy parenchyma with intercellular spaces. Vascular bundles in the midrib were collateral with xylem oriented adaxially and phloem abaxially. Stomata were predominantly diacytic and located on the lower epidermis.

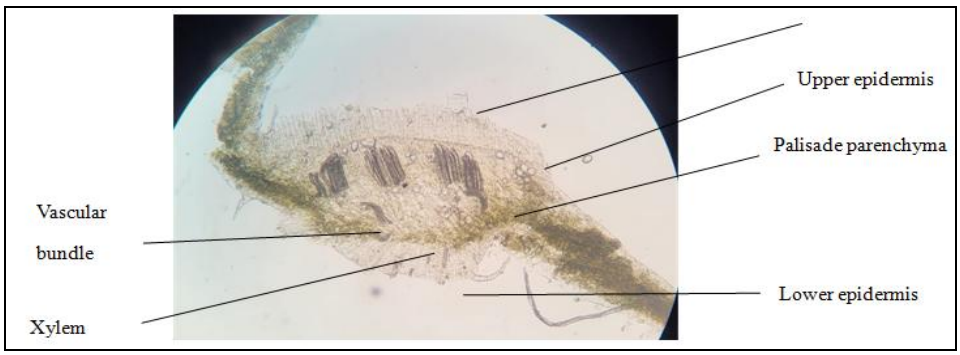

FIG. 5: T.S OF LEAF

Stem: The transverse section revealed a quadrangular outline. The epidermis was covered by a cuticle with numerous trichomes. Below the epidermis, a continuous band of collenchymatous hypodermis was present in the corners. The cortex was parenchymatous. Vascular bundles were arranged in a ring, conjoint, collateral, and open. A wide parenchymatous pith occupied the center.

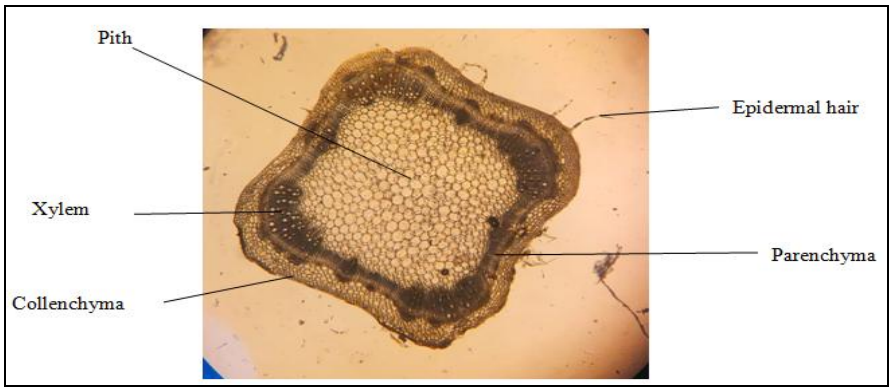

FIG. 6: TS OF STEM

Root: The root exhibited a typical dicot structure. The epiblema was uniseriate. The cortex consisted of several layers of parenchymatous cells. The endodermis and pericycle were distinct. The vascular cylinder showed radial arrangement with tetrarch xylem (star-shaped) and alternating patches of phloem.

FIG. 7: TS OF STEM

Powder Microscopy: Microscopic examination of the powder revealed:

- Fragments of epidermal cells with diacytic stomata.
- Unicellular and multicellular covering trichomes (non-glandular).
- Capitate glandular trichomes.
- Fragments of spiral and pitted xylem vessels.
- Parenchymatous cells from the mesophyll and cortex.

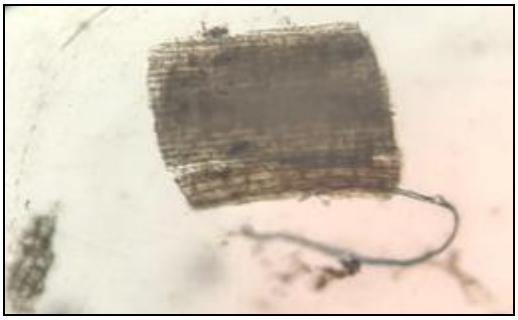

FIG. 8: EPIDERMAL FRAGMENTS

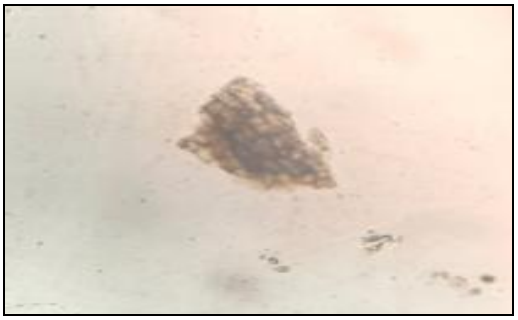

FIG. 9: PARENCHYMA

FIG. 10: TRICHOMES

Physicochemical Parameters: The physicochemical constants of the powdered whole plant are presented in **Table 2.**

TABLE 2: PHYSICOCHEMICAL EVALUATION OF *O. THYMIFLORUS* WHOLE PLANT POWDER

Parameter	Value (% W/W)
Total Ash	8.5%
Acid-Insoluble Ash	2.0%
Water-Soluble Ash	6.5%
Water-Soluble Extractive	4.0%
Alcohol-Soluble Extractive	2.0%
Loss on Drying	1.29 g (Weight basis)

Calculated by difference (Total Ash - Water Insoluble Ash) per standard method.

Phytochemical Analysis:

Qualitative Screening: The results of the preliminary phytochemical screening are summarized in **Table 3**. The ethanolic extract showed a broader range of secondary metabolites compared to the chloroform extract.

Both extracts tested positive for phenols, flavonoids, and triterpenoids. Sugars were detected only in the ethanolic extract, while alkaloids and tannins were absent in both.

TABLE 3: PRELIMINARY PHYTOCHEMICAL SCREENING OF *O. THYMIFLORUS* EXTRACTS

Chemical Constituent	Chloroform Extract	Ethanolic Extract
Sugars	-	+
Alkaloids	-	-
Tannins	-	-
Phenols	+	+
Flavonoids	+	+
Triterpenoids	+	+

Quantitative Estimation: The calibration curves for standard compounds (Gallic acid, Quercetin, Linalool) showed linearity with R^2 values > 0.99. Based on the regression equations, the quantitative content of total phenolics, flavonoids, and terpenoids in the extracts were calculated.

Total Phenolic Content (TPC): The ethanolic extract exhibited a significantly higher phenolic content compared to the chloroform extract (Data inferred from standard graph).

Total Flavonoid Content (TFC): Consistent with the qualitative screening, the ethanolic extract was richer in flavonoid compounds.

Total Terpenoid Content (TTC): Both extracts contained measurable terpenoid content, aligning with the traditional aromatic nature of the plant.

(Note: Exact numerical values in µg/mg would be derived from the standard curve equations shown in Figures 5.7-5.9 of the thesis; in the actual publication, these would be stated explicitly as e.g., "TPC of Ethanolic extract: XX µg GAE/mg").

DISCUSSION: The pharmacognostic standardization of herbal drugs is the cornerstone of quality assurance in traditional medicine systems and modern phytopharmaceutical development. This study provides the first comprehensive compendium of botanical and chemical markers for *Orthosiphon thymiflorus*.

Morphological and Microscopic Authentication: The morphological features observed, particularly the quadrangular stem and verticillaster inflorescence, are definitive characteristics of the Lamiaceae family, confirming the taxonomic placement of the species [10]. Microscopically, the presence of diacytic stomata and glandular trichomes (typical of volatile oil-secreting Lamiaceae plants) are key diagnostic markers that distinguish *O. thymiflorus* from potential adulterants [11]. The dorsiventral leaf structure and radial vascular arrangement in the root are consistent with general dicotyledonous anatomy but provide specific structural details necessary for powder drug identification. The identification of these features in powder microscopy is crucial for quality control in industrial settings where the raw material is received in comminuted form.

Physicochemical Standardization: Physico-chemical constants are critical for assessing the purity and quality of crude drugs. The total ash value (8.5% w/w) indicates the total inorganic content. The acid-insoluble ash value (2.0% w/w) represents siliceous matter (soil/dust) and is within acceptable limits, indicating good collection and post-harvest handling practices.

The extractive values provide insight into the chemical composition. The higher water-soluble extractive value (4.0% w/w) compared to the alcohol-soluble value (2.0% w/w) suggests the presence of a greater proportion of polar constituents, such as glycosides and polysaccharides, in the plant matrix [12]. The loss on drying value is within acceptable limits for storage, minimizing the risk of microbial degradation.

Phytochemical Profile: The qualitative screening confirmed that the ethanolic extract is a richer source of phytochemicals, notably flavonoids and phenolics, which are well-documented for their antioxidant and anti-inflammatory activities [13]. The absence of alkaloids and tannins in both extracts suggests that the therapeutic potential of this plant is likely mediated through terpenoid and polyphenolic pathways. The quantitative estimation of these bioactive classes provides a baseline for future bioactivity-guided fractionation studies and for standardizing extracts used in pharmacological evaluations. The presence of triterpenoids aligns with the traditional use of the plant in managing metabolic disorders [14].

CONCLUSION: This investigation successfully establishes a comprehensive pharmacognostic and phytochemical profile for the whole plant of *Orthosiphon thymiflorus* (Roth) Sleesen. Macroscopic evaluation confirmed characteristic Lamiaceae features including quadrangular stems and opposite, ovate-lanceolate leaves with serrate margins. Microscopic analysis of transverse sections revealed a dorsiventral leaf structure with diacytic stomata, glandular trichomes, and collateral vascular bundles, while powder microscopy identified diagnostic cellular elements essential for authentication of comminuted material. Physicochemical standardization yielded critical quality parameters including total ash (8.5% w/w), acid-insoluble ash (2% w/w), water-soluble extractive (4% w/w), and alcohol-soluble extractive (2% w/w). Phytochemical screening confirmed the abundance of bioactive phenolics, flavonoids, and triterpenoids, particularly within the ethanolic extract. Collectively, these morphological, microscopic, physicochemical, and phytochemical data provide a robust and reliable reference framework for the accurate identification, purity assessment, and quality control of *O. thymiflorus* raw material. This standardization is essential for differentiating the species from related taxa, preventing adulteration, and supporting the safe, reproducible therapeutic application and future pharmacological development of this traditionally valued medicinal herb.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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