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COMPARATIVE MORPHOLOGICAL AND ANATOMICAL STUDY OF *CENTELLA ASIATICA* AND *HYDROCOTYLE VERTICILLATA*

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ABSTRACT
Centella asiatica L., commonly known as Indian pennywort, is a perennial herb of the Apiaceae family renowned for its extensive pharmacological properties and traditional medicinal uses. However, its morphological similarities to *Hydrocotyle verticillata*, another creeping herb, have led to occasional misidentifications. This study presents a comparative analysis of the macro- and micro-morphological as well as anatomical characteristics of these two species to establish clear differentiation. Despite shared traits such as creeping growth patterns, palmate venation, and floral structure, significant distinctions were observed in leaf margins, stem attachment to leaves, and petiole anatomy. Employing advanced molecular and phytochemical methods alongside traditional anatomical approaches is crucial for accurate species identification, thereby supporting the safe and effective use of these plants in medicinal and scientific applications.

KEYWORDS: Apiaceae, *Centella*, *Hydrocotyle*, Anatomy, Micro and Macro morphology.

INTRODUCTION

The Apiaceae family, comprising 3,780 species across 434 genera, is a prominent group of flowering plants found worldwide, particularly in the northern temperate regions and high-altitude tropics. Species within this family share common characteristics, including an aromatic, herbaceous nature, alternate leaves with sheathing bases, hollow stems, and small flowers arranged in simple or compound umbels. The fruits or seeds contain oil ducts and do not split upon maturity.[1] The unique flavors of Apiaceae plants are due to secretory cavities filled with resin, oil, or mucilage in their fruits, stems, leaves, and roots.[2]

Centella asiatica, commonly known as Indian pennywort, mandukparni, or jalbrahmi, belongs to the Apiaceae family. It has been traditionally used for ornamental, culinary, and medicinal purposes. This herb exhibits various pharmacological properties, including antibacterial and antifungal effects. Revered for its therapeutic benefits, *Centella asiatica* has been a significant medicinal herb for centuries. It is extensively mentioned in Ayurvedic texts like the Sushruta Samhita[3&4] and has been considered a “miracle elixir of life” in China, where it is known as “getu kola,” with a medicinal history spanning over 2,000 years.[4]

Centella asiatica has recently gained attention as a promising natural antioxidant derived from plants, with potential benefits in protecting the brain's antioxidant defense system against age-related changes and enhancing memory.[5] A variety of phytochemicals, including terpenoids, triterpenoid saponins, flavonoids, coumarins, steroids, and essential oils, have been isolated from its water extract. *Centella asiatica* is also recognized for its nutritional and medicinal potential as a green leafy vegetable (GLV), particularly for Japanese consumers, due to its significant medicinal properties and nutritional value. Historically, *Hydrocotyle verticillata* was used as a minor vegetable and condiment in Pre-Columbian Mesoamerica, where it was known as “amamalacotl” or “swirl of water”.[6]

However, there have been reports of potential adulteration of *C. asiatica* with species like *Hydrocotyle verticillata* or *Merremia emarginata*, which share the local name "pegagan".[7&8] Hence, aim of the present study is to compare these plant species in terms of their micro- and macro-morphological and anatomical characteristics.

PLANT MATERIALS

Centella asiatica, commonly known as Indian pennywort or Asiatic pennywort, is a perennial herb in the Apiaceae family of flowering plants. It is found in temperate and

tropical swampy regions around the world. *Hydrocotyle verticillata*, also known as whorled pennywort, whorled marsh pennywort, or shield pennywort, is a flowering plant native to both South and North America, as well as the West Indies. Known for its creeping growth habit and distinctive whorled leaves, *Hydrocotyle verticillata* is typically found in marshy, boggy, or consistently wet areas such as wetlands, marshes, and swamps. It can even thrive in deeper water. This herbaceous perennial generally grows to a height of 5 to 20 centimeters and produces numerous creeping offshoots that can extend up to 1 meter in length.

METHODOLOGIES

Micro and Macromorphological studies

The macromorphological characteristics of *Centella asiatica* and *Hydrocotyle verticillata*, including stem, leaf, inflorescence, flower, and fruit, were carefully described and photographed using living specimens. Quantitative macromorphological measurements, such as stem length, leaf dimensions (Length and Width), calyx, corolla, stamen, bract, and seed size, were recorded from the studied samples. In addition, specific micromorphological features were examined using an ocular micrometer, including cuticle thickness, the size (Length and Width) of epidermal cells, cortex and pith width, and the size of palisade and spongy cells. These measurements provided a detailed understanding of the physical structure and characteristics of both plant species.

Anatomical studies

Young, fresh plant parts, including stems and leaves, were selected for anatomical analysis. Cross sections of the stem and leaf midrib were prepared and examined using a compound microscope. Photographs were captured using a digital camera to document the findings.

RESULTS AND DISCUSSION

Morphological characteristics of *Centella asiatica*

Centella asiatica is a creeping herb with fibrous roots that are either white or brown. Its leaves are arranged in an alternate spiral pattern, simple and fleshy, with a round shape and dentate margins that may be entire, wavy, or coarsely serrated. The leaf apex is rounded, and the base is cordate, with glabrous surfaces and palmate venation. The petiole is long, smooth on the upper surface, and hairy underneath. The thin, cylindrical stem is pink and striated, glabrous, and roots at the nodes, with stipules absent. The plant exhibits an umbel inflorescence, with pink to white, bisexual flowers arranged in lateral umbels. The flowers are pedicellate with 5 sepals and 5 oval to triangular petals, varying in color from red to purple. The androecium comprises 5 stamens alternately arranged with the petals, while the gynoecium features an inferior ovary with 2 carpels, each topped by a style and stigma. The fruit is a dull brown, oblong capsule that is laterally compressed.

Morphological characteristics of *Hydrocotyle verticillata*

Hydrocotyle verticillata is an herbaceous plant with simple, alternate, and basal leaves, each positioned singly at the base of the plant. The leaf blade has a dentate margin with teeth, and the leaves are hairless and peltate, with the stem attached to the center of the leaf rather than at the edges. The plant has a creeping stem and produces radially symmetrical flowers arranged in a spike-type inflorescence. The flowers are greenish-white and appear in elongated clusters or spikelets, with bracts present. The calyx consists of five separate sepals, and the corolla is made up of five distinct petals. The androecium contains five stamens, while the gynoecium features a single compound pistil composed of two carpels. The ovary is inferior, with two locules, each containing a pendulous, apical-axile ovule. The plant produces dry, capsule-like fruits. Comparative morphological analysis of *Centella asiatica* and *Hydrocotyle verticillata* is detailed in Table 1 and visually represented in Figures 1a–d.

Table 1: Comparison of Morphological features of *Centella asiatica* and *Hydrocotyle verticillata.*

Characters	*Centella asiatica*	*Hydrocotyle verticillata*
Habit	Creeping herb	Creeping herb
Height	1m long.	1m long.
Root	Tap root	Tap root
Stem	Glabrous, attached to the one end of the leaves, kidney-shaped, palmately compound, reticulate venation.	Glabrous, attached to the centre of leaves, umbrella like, palmately compound, reticulate venation.
Leaves	Simple, alternate, dentate margin, exstipulate.	Simple, complete, alternate, wavy margin, exstipulate.
Petiole	Long, hollow, smooth on upper surface and hairy below.	Long, solid, hairy.
Inflorescence	Umbel	Spike
Flower	Complete, hermaphrodite, bracteate, pedicellate.	Complete, hermaphrodite, bracteate, pedicellate.
Calyx	5 sepals, polysepalous	5 sepals, polysepalous.
Corolla	5 petals, polypetalous, valvate aestivation.	5 petals, polypetalous, valvate aestivation.
Androecium	5 stamens, alternate with petals, free.	5 stamens, alternate with petals, free.
Gynoecium	Inferior ovary, 2 carpels with two locules each consisting of one ovule, axile placentation.	Inferior ovary, 2 carpels with two locules each consisting of one ovule, axile placentation.

Both *Centella* and *Hydrocotyle* are creeping herbs, adapting a similar ground-covering growth pattern. Both species reach a length of up to 1 meter. Both plants have a tap root system, indicating a central primary root from which secondary roots sprout laterally. *Centella* stem is glabrous and is attached to one end of the leaves, which are kidney-shaped, palmately compound, with reticulate venation. *Hydrocotyle* stem is also glabrous but uniquely attaches to the center of the umbrella-like leaves. The leaves are also palmately compound and exhibit reticulate venation.

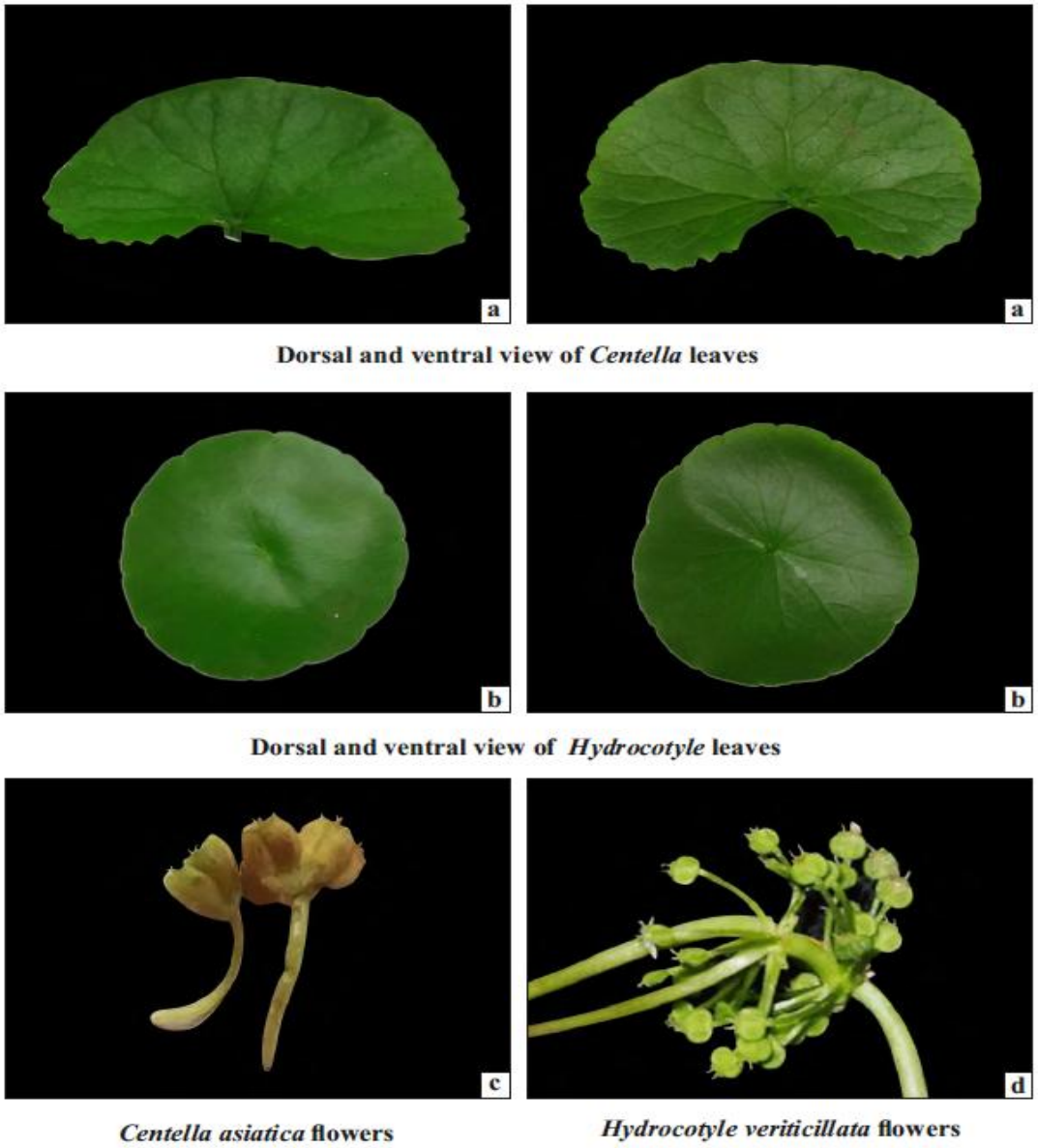

Figure 1 a-d: Morphological characteristics of *Centella asiatica* and *H. verticillata.*

Centella leaves are simple, alternate, with a dentate margin and lack stipules (estipulate). *Hydrocotyle* leaves are also simple and alternate. However, they possess a wavy margin and are also estipulate. Petiole of *Centella* is long and hollow, smooth on the upper surface, but hairy below: In contrast, *Hydrocotyle* petiole is long, solid, and covered in hair. Inflorescence in Centella adopts an umbel arrangement, *Hydrocotyle* features a spike-type inflorescence. Both species produce complete, hermaphrodite flowers that are bracteate and pedicellate. Both *Centella* and *H. verticillata* have a calyx made up of 5 separate (polysepalous) sepals. Both plants exhibit a corolla of 5 petals that are separate (polypetalous) and follow a valvate pattern of aestivation. Both species have 5 stamens that alternate with the petals, and they are free from each other. In both *Centella* and *H. verticillata*, the gynoecium features an inferior ovary comprised of 2 carpels. Each carpel contains two locules, and each locule has one ovule, following axile placentation.

Centella and *Hydrocotyle verticillata*, though similar in many aspects, can be distinguished based on the position of the stem attachment on the leaf and the leaf margin. Other features, such as the flower structure and

gynoecium components, remain consistent between the two species. Scientific research continually seeks to distinguish and differentiate between closely related plant species to ensure that plants with medicinal or economic value are correctly identified. Accurate plant identification is crucial to harness their therapeutic benefits and to avoid potential adverse effects. In 2017, Ali Alqahtani[9] and colleagues embarked on an in-depth analysis of three *Centella* species from Australia. They employed morphometric variability as a parameter to distinguish these species. The intricacy in differentiating these species underscores the need to employ a multifaceted approach. Alqahtani's team confirmed that these three species can be differentiated not only based on their physical appearance (morphometric traits) but also by diving deeper into their genetic and chemical compositions. They thereby highlighted the importance of an integrative methodology, encompassing morphometric, molecular, and phytochemical analyses, to correctly and comprehensively distinguish among plant species.

Interestingly, a similar situation exists in Thailand. *Centella asiatica* (L.) Urb., known for its myriad therapeutic benefits, has been frequently mistaken for *Hydrocotyle umbellata* L. This confusion can have consequences, especially if the therapeutic applications and chemical constituents of the two plants differ. Recognizing this, Laksanara et al.,[10] in 2023, took the initiative to clearly differentiate *C. asiatica from H. umbellata*. To achieve this distinction, the researchers applied DNA barcoding – a technique that identifies species based on a short DNA sequence from a standard and agreed-upon position in the genome. Coupled with Thin Layer Chromatography (TLC) to differentiate their phytochemical profiles and microscopic markers for precise structural differentiation, Laksanaras team provided robust tools to avoid any confusion between these two plants in the future.

Bisht et al.,[11] in 2022, expanded this narrative by emphasizing the diverse scientific techniques that can be employed to ensure plant quality, efficacy, and safety. DNA-based methods, as used by Laksanara, are cutting-edge techniques that provide accuracy at the genetic level. When combined with phytochemical analyses, such as TLC, a holistic understanding of the plant's identity and properties can be obtained. The aforementioned studies reinforce the significance of combining various scientific methods to achieve precise plant identification. As the therapeutic, cosmetic, and nutritional industries grow and evolve, ensuring the correct identification of plant species will be paramount. The synergistic use of morphometric, molecular, and phytochemical techniques offers a comprehensive approach to achieving this aim.

Micro & macro morphological and anatomical Characteristics of *Centella asiatica* and *Hydrocotyle verticillata*

Centella asiatica

The stem is circular to oval in shape. The phellem is multi-layered with squarish to rectangular cells. The phelloderm comprises several layers of parenchymatous cells, which vary from circular to polygonal, with minute intercellular spaces. The cortex resembles the phelloderm but contains slightly smaller cells. The vascular system consists of numerous collateral, strap-shaped bundles. The pith is homogeneous, consisting of 14 to 16 layers of parenchymatous cells that are circular to polygonal in shape, with small intercellular spaces.

The epidermis is a single layer of predominantly rectangular cells, with some barrel-shaped ones. Stomata are present on both surfaces and are level with the epidermal cells. The mesophyll consists of two layers of cylindrical to rectangular palisade cells and 3–5 layers of spongy parenchymatous cells with intercellular spaces. Chloroplasts are abundant, and some cells in the second palisade layer contain solitary calcium oxalate crystals (sphaero-crystals). The hypodermis comprises 5–6 layers of collenchyma on the adaxial side and 1–2 layers on the abaxial side, with cells being angular or lacunar. The vascular system features a wedge-shaped bundle in the primary veins and circular to oval bundles in the minor veins, all of which are collateral and lack pericycle.

The petiole of epidermis is single-layered, with polygonal, barrel-shaped, or oval cells that have thin walls. A few sunken stomata are present, and the outer walls of the guard cells are ledged. The ground tissue is a mix of collenchyma and parenchyma. A single layer of angular to polygonal hypodermal collenchyma is present. Large air cavities are interspersed on either side of the vascular bundles, with centrally located larger cavities. The vascular system contains 7–8 vascular bundles; larger ones are centrally placed, and smaller ones are along the margins. These bundles are oval to ovate and encased by a sclerenchymatous sheath on the abaxial side. The anatomical details are visually represented in Figures 2a, 2b and 2c.

Hydrocotyle verticillata

The stem is circular to oval in shape, with a uniseriate epidermis that lacks epidermal hairs. The hypodermis is parenchymatous, transitioning into a collenchymatous cortex. The stem contains an endodermis but lacks a pericycle. The vascular bundles are arranged in a broken ring pattern, are collateral, conjoint, and closed in type. The central region of the stem consists of a parenchymatous pith.

The epidermis is single-layered with sunken stomata. The mesophyll tissue is differentiated into two types: palisade tissue and spongy parenchyma. Each vein

contains a single centrally positioned vascular bundle within the ground tissue.

The petiole exhibits a circular outline with a multiseriate epidermis that lacks hairs. The ground tissue is composed of parenchyma, and vascular bundles are scattered throughout the petiole. The vascular system consists of xylem surrounded by phloem, and there is no endodermis or pericycle. The anatomical features are illustrated in Figures 2a (stem), 2b (leaf), and 2c (petiole).

Comparative Anatomical Analysis of *Centella asiatica* and *Hydrocotyle verticillata*

Stem: Both plants have a predominantly circular to oval-shaped stem. *Centella* has a multilayered phellem with cells ranging from squarish to rectangular, while *Hydrocotyle* has a uniseriate epidermis without epidermal hairs. *Centella's* hypodermis is parenchymatous with cells varying from circular to polygonal, leading to a phelloderm resembling the cortex but with slightly smaller cells. *Hydrocotyle's* stem also has a parenchymatous hypodermis leading to a collenchymatous cortex. Only *Centella's* structure includes a distinct phelloderm.

Centella's stem features numerous collateral, strap-shaped bundles, while Hydrocotyle's vascular bundles are arranged in a broken ring pattern and are collateral, conjoint, and closed types. *Centella's* pith is homogeneous, 14 to 16 cells in diameter, and parenchymatous. In contrast, *Hydrocotyle* simply possesses a parenchymatous pith.

Leaf: Both plants have a single-layered epidermis. *Centella's* epidermal cells are predominantly rectangular, while *Hydrocotyle's* epidermis has sunken stomata. Both species have differentiated mesophyll. In *Centella*, the mesophyll contains both palisade and spongy cells, with 2-layered adaxial palisade cells. *Hydrocotyle's* mesophyll differentiates into spongy parenchyma and palisade tissue. In *Centella*, primary veins have a wedge-shaped bundle, while minor veins possess circular to oval-shaped bundles. *Hydrocotyle's* leaf veins each possess a central vascular bundle.

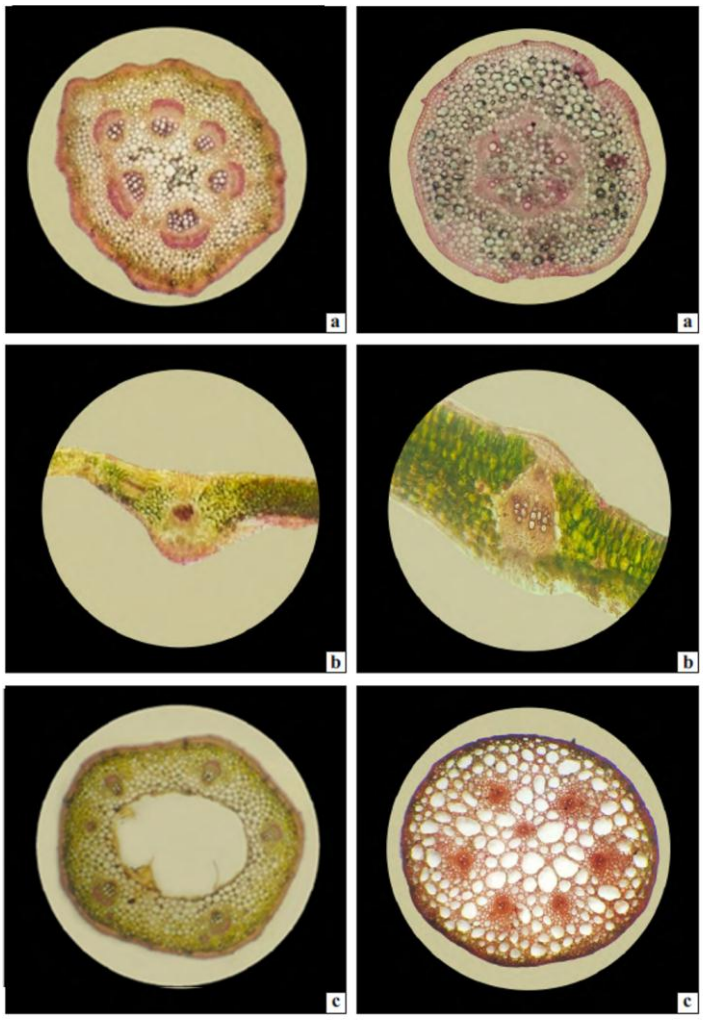

Figure 2 a. Stem anatomy of *Centella asiatica* and *Hydrocotyle verticillata,* b. Leaf anatomy of *Centella asiatica* and *Hydrocotyle verticillata,* c. Petiole anatomy of *Centella asiatica* and *Hydrocotyle verticillata,*

Petiole: *Centella's* petiole has a single-layered epidermis with polygonal to barrel-shaped adaxial cells, whereas Hydrocotyle's petiole has a multiseriate epidermis without hairs. *Centella's* petiole ground tissue is a heterogeneous mix of collenchyma and parenchyma, interspersed with large air cavities. *Hydrocotyle's* petiole has a simpler parenchymatous ground tissue. *Centella* has 7-8 vascular bundles, with larger ones centrally placed and smaller ones towards the margins. *Hydrocotyle's* petiole possesses scattered vascular bundles. While both *Centella asiatica* and *Hydrocotyle verticillata* exhibit certain similarities in their anatomical features, there are distinct differences, especially in the structure and cellular composition of their stems, leaves, and petioles. These differences can be instrumental in distinguishing between the two species when observed under anatomical study.

CONCLUSION

Centella asiatica and *Hydrocotyle verticillata* share morphological and anatomical traits indicative of their close taxonomic relationship, such as palmate venation, creeping stems, and axile placentation in flowers. However, they differ in critical aspects; *Centella asiatica* features a multilayered phellem in the stem, polygonal to barrel-shaped petiole epidermis, and larger vascular bundles in the petiole, while *Hydrocotyle verticillata* exhibits a uniseriate stem epidermis, multiseriate petiole epidermis, and scattered vascular bundles. Morphologically, *Centella asiatica* has kidney-shaped leaves with dentate margins, while *Hydrocotyle verticillata* has umbrella-like leaves with wavy margins.

The findings underline the importance of combining anatomical, morphometric, molecular, and phytochemical analyses to ensure precise species identification. Such integrative approaches are vital for harnessing the medicinal, nutritional, and economic potential of plant species while avoiding misidentification, as demonstrated by studies distinguishing closely related species in both regional and global contexts.

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