RESEARCH PAPER

Pharmacognostic, Physicochemical, and Biological Comparison of Wild and Cultivated *Centella asiatica*

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ABSTRACT

Centella asiatica is a widely used medicinal plant valued for its pharmacological potential; however, variations in ecological origin may significantly influence its quality and therapeutic efficacy. The present study aimed to comparatively evaluate wild and cultivated *C. asiatica* through pharmacognostic characterization, physicochemical standardization, phytochemical profiling, and in-vitro biological assessment. Macroscopic and microscopic analyses demonstrated distinct morphological and anatomical differences, with wild samples exhibiting higher stomatal index, palisade ratio, and vascular density. Physicochemical evaluation revealed higher ash values and extractive values in wild samples, indicating greater mineral content and bioactive constituent availability. Fluorescence analysis provided characteristic diagnostic patterns supporting identity and quality assessment. Preliminary phytochemical screening confirmed the abundance of flavonoids, phenolics, triterpenoids, and saponins, particularly in ethanol and aqueous extracts. Antioxidant activity assessed by DPPH assay showed superior radical scavenging potential of wild ethanolic extract (IC_{50} = 40.8 ± 1.4 µg/mL) compared to cultivated extract (IC_{50} = 55.2 ± 2.0 µg/mL). In-vitro anti-inflammatory activity evaluated using HRBC membrane stabilization and protein denaturation assays demonstrated higher inhibition by wild ethanolic extract (72.6 ± 2.5% and 66.8 ± 2.0%, respectively). Overall, the findings establish significant quality and bioactivity differences between wild and cultivated *C. asiatica* and emphasize the necessity of systematic standardization and source selection to ensure consistent therapeutic performance and pharmaceutical reliability.

Keywords: *Centella asiatica; pharmacognostic standardization; wild and cultivated comparison; phytochemical screening; antioxidant activity; anti-inflammatory activity.*

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1. INTRODUCTION

1.1 Botanical and Therapeutic Importance of *Centella asiatica*

Centella asiatica (L.) Urban, commonly known as Gotu kola, Mandukaparni, or Indian pennywort, is a perennial creeping herb belonging to the family Apiaceae. It is widely distributed in tropical and subtropical regions including India, Sri Lanka, China, Indonesia, Malaysia, and parts of Africa. The plant thrives in moist, marshy habitats and is characterized by kidney-shaped leaves, slender stolons, and small pinkish-white flowers arranged in umbels. Traditionally, *C. asiatica* has been extensively used in Ayurvedic, Unani, Siddha, and Traditional Chinese Medicine systems for centuries due to its diverse pharmacological properties [1,2].

Therapeutically, *C. asiatica* is recognized for its wound healing, neuroprotective, antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and antiulcer activities. It has been traditionally prescribed for enhancing memory, treating skin disorders such as eczema, psoriasis, and leprosy, improving circulation, and accelerating tissue regeneration [3,4]. Modern pharmacological investigations have validated many of these traditional claims, demonstrating its ability to stimulate collagen synthesis, angiogenesis, fibroblast proliferation, and extracellular matrix remodeling, making it particularly valuable in dermatological and regenerative medicine applications [5].

The neuroprotective and cognitive-enhancing effects of *C. asiatica* have attracted increasing scientific attention, especially in the management of neurodegenerative disorders, anxiety, depression, and cognitive decline. Experimental studies suggest that its bioactive compounds modulate neurotransmitter levels, reduce oxidative stress in neural tissues, and improve synaptic plasticity [6]. Furthermore, standardized extracts of *C. asiatica* are incorporated into various pharmaceutical formulations, nutraceuticals, cosmetic preparations, and functional foods worldwide, highlighting its commercial and therapeutic significance [7].

1.2 Phytochemical Profile and Centellosides

The pharmacological activity of *C. asiatica* is primarily attributed to its rich phytochemical composition, particularly triterpenoid saponins collectively referred to as centellosides. The major centellosides include asiaticoside, madecassoside, asiatic acid, and madecassic acid. These compounds possess potent wound healing, antioxidant, anti-inflammatory, and neuroprotective activities [8,9]. Asiaticoside and madecassoside promote collagen synthesis and angiogenesis, while asiatic acid and madecassic acid exhibit anti-inflammatory and cytoprotective effects [10].

In addition to triterpenoids, *C. asiatica* contains flavonoids (quercetin, kaempferol), phenolic acids, tannins, phytosterols, volatile oils, amino acids, carbohydrates, and minerals. These constituents contribute synergistically to its antioxidant capacity, free radical scavenging ability, and overall therapeutic potential [11]. The qualitative and quantitative composition of these phytochemicals plays a critical role in determining the efficacy, safety, and reproducibility of herbal preparations derived from the plant [12].

Recent analytical advancements such as high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), liquid chromatography–mass spectrometry (LC–MS), and gas chromatography–mass spectrometry (GC–MS) have enabled precise profiling and quantification of centellosides and other marker compounds. These techniques support quality assessment, fingerprinting, and standardization of *C. asiatica* extracts for pharmaceutical use [13].

1.3 Variability Due to Ecological and Cultivation Factors

Despite its wide therapeutic acceptance, the phytochemical composition of *C. asiatica* exhibits significant variability influenced by ecological, geographical, genetic, seasonal, and agronomic factors. Soil type, altitude, rainfall, temperature, sunlight exposure, irrigation practices, harvesting stage, and post-harvest processing significantly affect the accumulation of centellosides and secondary metabolites [14,15].

Wild and cultivated populations often differ in their phytochemical profiles due to differences in environmental stress exposure, nutrient availability, and genetic diversity. Studies have reported considerable variation in asiaticoside and madecassoside content among samples collected from different regions, which directly impacts therapeutic consistency and product quality [16]. Seasonal variations further influence metabolite biosynthesis, with certain growth phases showing higher accumulation of triterpenoids and phenolics [17].

Agricultural practices such as fertilizer application, plant density, irrigation frequency, and organic cultivation methods also modulate phytochemical yield. Improper harvesting time and inadequate drying or storage conditions can lead to degradation of active constituents, thereby reducing biological efficacy and shelf life [18]. Such variability poses challenges for reproducibility, dosage uniformity, and regulatory acceptance of herbal medicines.

1.4 Need for Standardization and Quality Control

Standardization and quality control are essential to ensure the safety, efficacy, and consistency of herbal medicinal products derived from *C. asiatica*. The presence of phytochemical variability, adulteration, substitution, microbial contamination, pesticide residues, and heavy metals necessitates stringent quality evaluation protocols [19].

Pharmacognostic parameters such as macroscopic and microscopic evaluation, physicochemical constants (ash values, extractive values, moisture content), and chromatographic fingerprinting are commonly employed to establish plant identity and purity. Quantitative estimation of marker compounds like asiaticoside and madecassoside serves as a benchmark for quality assessment [20].

Regulatory frameworks emphasize the need for validated analytical methods, batch-to-batch consistency, and traceability of raw materials. Implementing Good Agricultural and Collection Practices (GACP), Good Manufacturing Practices (GMP), and stability testing ensures product reliability and consumer safety [21]. Standardized extracts enhance clinical reproducibility, facilitate dosage accuracy, and improve global acceptance of phytopharmaceutical products [22].

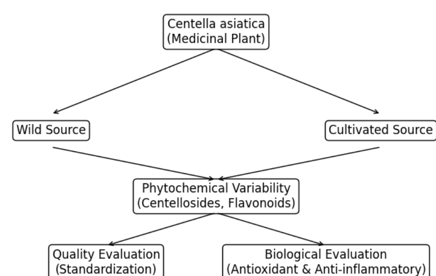

Figure 1: Graphical overview illustrating the comparative evaluation of wild and cultivated *Centella asiatica*, highlighting phytochemical

variability and its impact on quality standardization and biological activities including antioxidant and anti-inflammatory effects

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Fresh whole plants of *Centella asiatica* (L.) Urban were collected from two different sources. The wild sample was collected from naturally growing vegetation in a marshy region, whereas the cultivated sample was obtained from an organized cultivation field. Both samples were collected during the same season to minimize seasonal variation. The collected plant materials were thoroughly washed with running tap water followed by distilled water to remove adhering soil and extraneous matter. The samples were shade-dried at room temperature for 10–12 days until a constant weight was achieved.

The dried materials were coarsely powdered using a mechanical grinder and passed through a 60-mesh sieve to obtain uniform particle size. The powdered samples were stored in airtight containers protected from moisture and light until further analysis. Botanical authentication was carried out by a qualified taxonomist, and voucher specimens were deposited in the institutional herbarium for future reference.

2.2 Preparation of Wild and Cultivated Extracts

The powdered plant materials of both wild and cultivated samples were subjected to successive solvent extraction using petroleum ether, chloroform, ethanol, and distilled water. Approximately 100 g of each powdered sample was placed in a Soxhlet apparatus and extracted sequentially with each solvent in increasing order of polarity. Each extraction cycle was continued for 6–8 hours until the siphon solvent became colorless.

The extracts were filtered and concentrated under reduced pressure using a rotary evaporator. The concentrated extracts were further dried in a vacuum desiccator to obtain semisolid residues. The percentage yield of each extract was calculated with respect to the initial dry weight of the plant material. The dried extracts were stored in airtight containers at 4 °C until further experimental evaluation.

2.3 Macroscopic Evaluation

Macroscopic evaluation of the dried plant material and powdered samples was carried out to determine organoleptic and morphological characteristics. Parameters such as color, odor, taste, texture, leaf shape, surface characteristics, venation pattern, and presence of stolons were visually examined and recorded.

Organoleptic properties including appearance, consistency, and sensory characteristics were assessed using standard pharmacognostic procedures. The observations were systematically documented and compared between wild and cultivated samples to establish identity and detect any visible variations.

2.4 Microscopic Evaluation and Powder Microscopy

Microscopic evaluation was performed using freehand transverse sections of fresh leaves and stems. The sections were cleared using chloral hydrate solution and stained with safranin to enhance tissue contrast. The prepared slides were mounted in glycerin and examined under a compound microscope at different magnifications.

Quantitative microscopic parameters including stomatal number, stomatal index, palisade ratio, vein-islet number, and veinlet termination number were determined using standard microscopic methods. All measurements were performed in triplicate and mean values were calculated.

For powder microscopy, the dried powdered material was treated with specific reagents such as phloroglucinol–hydrochloric acid for lignified tissues, iodine solution for starch detection, and dilute hydrochloric acid for calcium oxalate identification. The treated powders were mounted on glass slides and observed microscopically. Diagnostic characters including vessels, fibers, trichomes, starch grains, and calcium oxalate crystals were identified and recorded.

2.5 Physicochemical Analysis

Physicochemical parameters of both wild and cultivated *Centella asiatica* samples were evaluated to establish quality control standards and detect possible variations. All determinations were performed in triplicate and expressed as mean ± standard deviation.

Ash Values

Total ash, acid-insoluble ash, and water-soluble ash were determined according to standard pharmacopoeial procedures. Approximately 2 g of accurately weighed powdered sample was incinerated in a silica crucible at 450–600 °C in a muffle furnace until carbon-free ash was obtained. The total ash content was calculated with respect to the air-dried material.

For acid-insoluble ash, the total ash was boiled with 25 mL of 2 M hydrochloric acid for 5 minutes, filtered, washed with hot distilled water, ignited, and

weighed. The residue weight represented the acid-insoluble ash content. Water-soluble ash was determined by boiling the total ash with distilled water, filtering, igniting the insoluble matter, and calculating the difference in weight.

Extractive Values

Alcohol-soluble and water-soluble extractive values were determined using the cold maceration method. Approximately 5 g of powdered sample was macerated with 100 mL of solvent (ethanol or distilled water) in a closed flask for 24 hours with intermittent shaking. The mixtures were filtered, and 25 mL of the filtrate was evaporated to dryness in a tarred dish. The residue was dried at 105 °C to constant weight, and the extractive value was calculated as percentage w/w.

Moisture Content

Moisture content was determined using the loss on drying method. About 2 g of powdered sample was weighed accurately and dried in a hot air oven at 105 °C until constant weight was obtained. The percentage moisture content was calculated based on the loss in weight relative to the initial sample weight.

2.6 Fluorescence Analysis

Fluorescence analysis of the powdered drug was performed to detect characteristic fluorescence behavior under visible light and ultraviolet light. A small quantity of powdered sample was treated separately with various reagents such as distilled water, sodium hydroxide, hydrochloric acid, nitric acid, sulfuric acid, ethanol, and iodine solution.

The treated samples were observed under visible light, short-wave UV light (254 nm), and long-wave UV light (366 nm). The observed colors and fluorescence patterns were recorded systematically for both wild and cultivated samples.

2.7 Preliminary Phytochemical Screening

Preliminary phytochemical screening of petroleum ether, chloroform, ethanol, and aqueous extracts was carried out to identify the presence of major phytoconstituent groups. Standard qualitative chemical tests were performed for alkaloids (Dragendorff's and Mayer's tests), glycosides (Keller–Killiani test), flavonoids (Shinoda test), phenolics and tannins (Ferric chloride test), saponins (froth test), steroids and triterpenoids (Liebermann–Burchard test), carbohydrates (Molisch's test), and proteins (Biuret test).

The appearance of characteristic color changes or precipitates was recorded as positive or negative for each phytochemical group.

2.8 In-Vitro Antioxidant Activity (DPPH Assay)

The free radical scavenging activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. A freshly prepared 0.1 mM DPPH solution in methanol was used for the experiment.

Various concentrations of each extract were prepared in methanol and mixed with the DPPH solution. The reaction mixtures were incubated in the dark at room temperature for a specified period to allow completion of the reaction. After incubation, the decrease in absorbance was measured spectrophotometrically at 517 nm using methanol as the blank.

The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\%\ \mathbf{DPPH\ scavenging\ activity} = \frac{(\mathbf{A_0} - \mathbf{A_s})}{\mathbf{A_0}} \times \mathbf{100}$$

where A0A_0A0 represents the absorbance of the control and AsA_sAs represents the absorbance of the sample. Ascorbic acid was used as the reference standard. The IC50_{50}50 values were determined from the concentration–response curve.

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An aliquot of 1 mL of extract solution was mixed with 1 mL of DPPH solution and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV–visible spectrophotometer. Methanol was used as blank and ascorbic acid served as the standard reference [38].

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The percentage inhibition of DPPH radicals was calculated using the formula:

$$\% \boldsymbol{Inhibition} = \boldsymbol{Acontrol}(\boldsymbol{Acontrol} - \boldsymbol{Asample}) \times \mathbf{100}$$

The IC_{50} value (concentration required to inhibit 50% of free radicals) was calculated from the plotted inhibition curve.

2.9 In-Vitro Anti-Inflammatory Activity

Protein Denaturation Method

The anti-inflammatory activity was assessed by inhibition of protein denaturation using bovine serum albumin (BSA). Reaction mixtures consisting of 1 mL of extract solution and 1 mL of 1% BSA solution were prepared. The pH was adjusted to 6.3 using dilute hydrochloric acid.

The mixtures were incubated at 37 °C for 20 minutes followed by heating at 57 °C for 3 minutes. After cooling, the absorbance was measured at 660 nm. Diclofenac sodium was used as the standard drug. Percentage inhibition of protein denaturation was calculated [39].

HRBC Membrane Stabilization Method

Fresh human blood was collected from a healthy volunteer and mixed with Alsever's solution. The blood was centrifuged at 3000 rpm for 10 minutes and washed three times with isotonic saline. A 10% v/v HRBC suspension was prepared using normal saline.

Reaction mixtures containing extract solution, phosphate buffer, hypotonic saline, and HRBC suspension were incubated at 37 °C for 30 minutes and centrifuged. The absorbance of the supernatant was measured at 560 nm. Diclofenac sodium served as the reference standard. The percentage membrane stabilization was calculated [40].

3. RESULTS AND DISCUSSION

Macroscopic and organoleptic evaluation revealed clear morphological and sensory differences between wild and cultivated *Centella asiatica* samples. As summarized in **Table 1**, the wild sample exhibited deeper green coloration, stronger characteristic odor, coarse fibrous texture, and prominent venation, whereas the cultivated sample showed lighter coloration, smoother texture, and milder sensory characteristics. These variations reflect differential growth environments and physiological maturity, which influence cellular structure and metabolite accumulation.

Microscopic analysis further confirmed quantitative anatomical variation between the samples. The wild sample demonstrated higher stomatal index, stomatal number, palisade ratio, vein-islet number, and veinlet termination number compared to the cultivated sample (**Table 2**). Increased stomatal density and palisade ratio indicate enhanced photosynthetic

efficiency and metabolic activity, supporting higher biosynthesis of secondary metabolites in wild populations. The greater abundance of calcium oxalate crystals in wild samples further supports higher mineral deposition and metabolic turnover.

Physicochemical evaluation demonstrated consistent differences in mineral content, extractable constituents, and moisture levels between the samples, as illustrated in **Figure 2**. Higher total ash, acid-insoluble ash, and water-soluble ash values in the wild sample indicate increased inorganic and mineral composition. Elevated alcohol-soluble and water-soluble extractive values suggest greater availability of bioactive phytoconstituents, particularly polar compounds. In contrast, slightly higher moisture content in cultivated samples may influence storage stability and susceptibility to microbial degradation.

Fluorescence analysis provided additional diagnostic support for sample differentiation and quality evaluation. Distinct fluorescence responses were observed under ultraviolet light following treatment with various reagents (**Table 3**). The wild sample generally exhibited stronger fluorescence intensity, indicating higher concentration of fluorescent phytoconstituents such as flavonoids and phenolic compounds. These fluorescence patterns serve as rapid qualitative markers for authentication and quality screening.

Preliminary phytochemical screening demonstrated the presence of diverse secondary metabolites across all extracts, with solvent polarity influencing phytochemical distribution (**Table 4**). Ethanol and aqueous extracts showed strong presence of flavonoids, phenolics, tannins, glycosides, and saponins, whereas petroleum ether extracts primarily contained lipophilic constituents such as steroids and triterpenoids. The abundance of phenolics and flavonoids explains the strong antioxidant and anti-inflammatory activities observed in polar extracts.

Antioxidant evaluation using the DPPH assay demonstrated concentration-dependent free radical scavenging activity across all extracts (**Table 5**). Ethanolic extracts exhibited the lowest IC_{50} values, indicating superior antioxidant efficacy, followed by aqueous extracts. The wild extracts consistently showed lower IC_{50} values compared to cultivated extracts, confirming higher antioxidant potential. This enhanced activity can be attributed to greater phenolic and flavonoid content observed during phytochemical screening.

Anti-inflammatory activity assessed through HRBC membrane stabilization and protein denaturation assays further validated the biological superiority of wild extracts. As depicted in **Figure 3** and **Figure 4**, ethanolic extracts exhibited maximum inhibition in both models, followed by aqueous extracts. Wild extracts consistently demonstrated higher percentage inhibition compared to cultivated samples. Petroleum ether extracts showed comparatively minimal activity, confirming that polar phytoconstituents contribute predominantly to anti-inflammatory efficacy.

Collectively, the integration of pharmacognostic evaluation (**Tables 1 and 2**), physicochemical profiling (**Figure 2**), fluorescence behavior (**Table 3**), phytochemical composition (**Table 4**), antioxidant performance (**Table 5**), and anti-inflammatory activity (**Figures 3 and 4**) establishes a strong correlation between ecological origin, phytochemical richness, and therapeutic potential. These findings emphasize the importance of source selection and standardization strategies for ensuring consistent quality and pharmacological reliability of *Centella asiatica*–based formulations.

Table 1: Macroscopic and Organoleptic Characteristics of *Centella asiatica*

Parameter	Wild Sample	Cultivated Sample
Color	Deep green	Light green
Odor	Strong characteristic	Mild characteristic
Taste	Bitter–astringent	Mildly bitter
Texture	Coarse and fibrous	Smooth and soft
Leaf appearance	Thick, well developed	Thin, moderately developed
Venation	Prominent	Less prominent

Table 2: Quantitative Microscopic Parameters

Parameter	Wild Sample	Cultivated Sample
Stomatal Index	14.92 ± 0.58	12.41 ± 0.46
Stomatal Number (/mm²)	318 ± 17	276 ± 14
Palisade Ratio	6.28 ± 0.31	5.14 ± 0.27
Vein Islet Number (/mm²)	25 ± 2	21 ± 1
Veinlet Termination Number (/mm²)	11 ± 1	9 ± 1
Calcium Oxalate	Abundant	Moderate

Crystals		

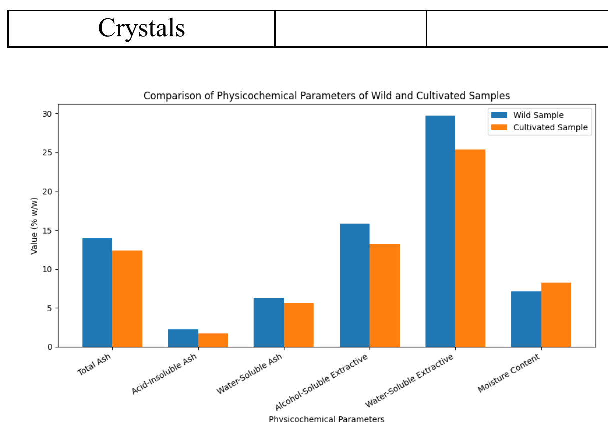

Figure 2: Comparison of Physicochemical Parameters of Wild and Cultivated Samples

Table 3: Fluorescence Behavior of Powdered Drug

Treatment	Visible Light	UV 254 nm	UV 366 nm
Powder as such	Green	Dull green	Fluorescent green
+ NaOH	Brownish green	Bright green	Yellowish fluorescence
+ HCl	Light brown	Pale green	Weak fluorescence
+ H_2SO_4	Dark brown	Quenched	Dark fluorescence
+ Iodine	Reddish brown	Dark	No fluorescence

Table 4: Preliminary Phytochemical Screening of Extracts

Phytoconstituent	Petroleum Ether	Chloroform	Ethanol	Aqueous
Alkaloids	–	+	+	+
Flavonoids	–	+	+++	++
Phenolics / Tannins	–	+	+++	+++
Saponins	–	–	++	+++
Glycosides	–	+	++	++
Steroids / Triterpenoids	+	++	++	+
Carbohydrates	–	–	+	++

(+: present, ++: moderate, +++: abundant, –: absent)

Table 5: Antioxidant Activity (DPPH IC_{50} Values)

Extract	Wild (µg/mL)	Cultivated (µg/mL)
Petroleum Ether	112.6 ± 4.8	136.2 ± 5.6
Chloroform	78.4 ± 3.1	92.7 ± 3.9
Ethanol	40.8 ± 1.4	55.2 ± 2.0
Aqueous	46.9 ± 1.8	61.8 ± 2.4
Ascorbic Acid	21.6 ± 0.9	—

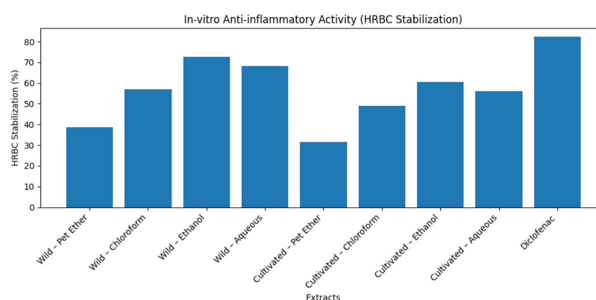

Figure 3: In-vitro Anti-inflammatory Activity – HRBC Membrane Stabilization (%)

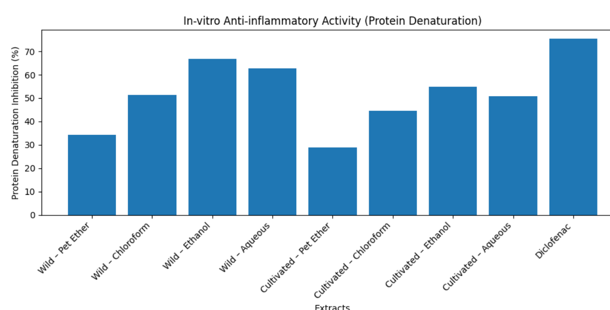

Figure 4: In-vitro Anti-inflammatory Activity – Protein Denaturation Inhibition (%)

4. CONCLUSION

The present investigation established clear comparative differences between wild and cultivated *Centella asiatica* with respect to botanical characteristics, physicochemical quality, phytochemical composition, and biological activities. Macroscopic and microscopic evaluations confirmed the authenticity of both samples, while quantitative microscopy demonstrated higher stomatal index, palisade ratio, and vascular density in wild samples, suggesting enhanced physiological maturity and metabolite accumulation.

Physicochemical analysis revealed higher ash values and extractive values in wild samples, indicating greater mineral content and higher availability of bioactive constituents. Slightly higher moisture content in cultivated samples reflected increased hygroscopicity, which may influence storage stability. Fluorescence analysis provided characteristic diagnostic profiles useful for rapid identification and quality control.

Preliminary phytochemical screening confirmed the abundance of flavonoids, phenolics, triterpenoids, and saponins, particularly in ethanol and aqueous extracts. The biological evaluation demonstrated superior antioxidant and anti-inflammatory activities

in wild samples, with ethanolic extracts exhibiting maximum efficacy among all tested extracts.

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