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ARTICLE

Taxonomic and Anatomical Characterization of Flowering Medicinal Plants Restricted to Riverbank Habitats

Atheer Hashim Abdulmajeed

Department of Horticulture and Landscape Engineering, College of Agriculture, University of Anbar, Ramadi, Anbar, Iraq

ABSTRACT

Mentha is among the most important flowering medicinal plants; they are also a perennial, aromatic plant. The *Mentha* genus belongs to the Lamiaceae family. The *Mentha* genus comprises approximately 30 species, 14 hybrids, and numerous varieties. *Mentha* species are characterized by high essential oil concentrations, which are widely used in the pharmaceutical industry. Mint species are of medicinal and nutritional relevance, but studies dealing with their identification and categorization are few, especially in Iraq. Therefore, the present study was carried out to identify and categorize the flowering medicinal *Mentha* species growing along the riverbanks in Iraq, using morphological, anatomical, and biochemical analysis. The three *Mentha species* studied were *Mentha spicata*, *Mentha piperita*, and *Mentha longifolia*. Morphological and anatomical studies were carried out on leaves, flowers, and stems of *M. spicata*, *M. piperita*, and *M. longifolia*, and the results revealed obvious changes in most features between the species. The results also revealed that *M. spicata* and *M. piperita* were highly comparable for some morphological leaf characters, but *M. piperita* and *M. longifolia* were highly similar for some anatomical leaf characters. The biochemical study results showed that *M. piperita* had the greatest levels of DPPH radical scavenging activity (60.17%), total phenolic content (TPC: 46.11 mg/g), total flavonoid content (TFC: 28.12 mg/g), and essential oil content (EOs: 0.57%), followed by *M. spicata* and *M. longifolia*. The study revealed that morphological, anatomical, and biochemical analyses are effective and complementary ways to identify and classify species of the same genus. Although these methods have been successful in identifying and classifying the three mint species, there is a need to extend the study to a larger number of species and ensure their correct identification. Therefore, this study suggests future research for better identification and characterization of a broader range of *Mentha species* by adding additional methodologies, e.g., molecular characterization.

Keywords: *Flowering; Medicinal Plant; Mentha Species; Morphology; Taxonomy, Biochemical.*

Corresponding Author: Atheer Hashim Abdulmajeed
E-mail: ag.atheer.hashim@uoanbar.edu.iq
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INTRODUCTION

The Medicinal plants are becoming increasingly important due to their effectiveness in treating different illnesses and the related health advantages (Dolatkhahi *et al*., 2014; Porwal *et al*., 2025). These plants have been important in traditional medicine and the world economy for a long time due to their low cost and easy availability (Porwal *et al*., 2025). Furthermore, pharmaceutical research and development can utilize bioactive chemicals from medicinal plants, including flowering plants (Jaberian *et al*., 2013). Medicinal plants have been used for therapeutic purposes in various places, including developed countries and the Middle East, including Iraq (Ahmad *et al*., 2017; Porwal *et al*., 2025). The World Health Organization studies verify the extensive use of medicinal plants in the food business and clinical practice in most affluent nations (Ahmad *et al*., 2017).

Reports and research have shown that about 80% or more of the world's population depend on traditional medicine for treatment of common ailments and health maintenance, especially therapies involving medicinal plants, particularly those containing essential oils (EOs) (Parvaiz *et al*., 2014; Porwal *et al*., 2025). The importance of medicinal plants and the need for their thorough study are underscored by the fact that most countries worldwide, including Iraq, continue to value and utilize traditional medicine to address a wide range of health issues (Porwal *et al*., 2025). *Mentha* is among the most important flowering medicinal plants; they are also a perennial, aromatic plant (Abd El Maksoud and Azer, 2013; Naseem *et al*., 2025). The *Mentha* genus belongs to the Lamiaceae family, also known as the mint family or traditionally as Labiatae, which comprises 280 genera and 7,000 species distributed worldwide (Baimakhanova *et al*., 2025; El Boukhari and Fatimi, 2025; Marie *et al*., 2026; Alarkwazi *et al*., 2026). This family is divided into two subfamilies: Lamioideae and Nepetoideae. The Nepetoideae subfamily includes the tribe Mentheae, one of the largest tribes, comprising 65 genera and over 2,000 species; the genus Mentha belongs to this tribe, as do many other genera used in folk medicine (Chaker *et al*., 2011; Abd El Maksoud and Azer, 2013; Naseem *et al*., 2025). *Mentha* spp. are also known for their high content of essential oils, which have wide applications in the pharmaceutical industry (Moon *et al*., 2009; Jabeen *et al*., 2025; Marie *et al*., 2026). The *Mentha* genus comprises approximately 30 species, 14 hybrids, and numerous varieties (El-Kashoury *et al*., 2013; Salehi *et al*., 2018; Mohammed *et al*., 2024). This genus is distributed globally, with a wide ecological range resulting from its capacity for significant genetic diversity driven by frequent interspecific hybridization (Tyler *et al*., 2026; Marie *et al*., 2026). This diversity highlights the importance of identifying and characterizing new species both morphologically and genetically—to ensure their conservation and survival, given their significance in traditional medicine (Póvoa *et al*., 2023; Olofsson *et al*., 2024). The *Mentha* genus includes several species, such as *M. aquatica*, *M. arvensis*, *M. pulegium*, *M. spicata*, *M. piperita*, and *M. longifolia* (Shinwari *et al*., 2011; Octavia *et al*., 2023). Among the most widely distributed mint species in Iraq are *M. spicata*, *M. piperita*, and *M. longifolia*. These species have significant medical and economic importance because their leaves and flowers have therapeutic and aromatic properties (Al-Bayati, 2009; Porwal *et al*., 2025; Marie *et al*., 2026). The biological activity of these species is linked to their content of bioactive compounds, including EOs (such as menthol, isomenthone, menthone, menthofuran, linalyl acetate, linalool, and limonene), flavonoids, terpenes, and phenolic compounds (Salehi *et al*., 2018; Salama *et al*., 2019; El Boukhari *et al*., 2025; Naseem *et al*., 2025; Shirokova *et al*., 2026). All these compounds are primarily used in the pharmaceutical, cosmetic, and food industries (Naseem *et al*., 2025). Furthermore, *M. spicata, M. piperita*, and *M. longifolia* are distinguished by their high concentration of volatile oils and bioactive compounds (Yousefian *et al*., 2023; El Boukhari *et al*., 2025; Alarkwazi, 2026). This has led to their widespread use in treating various ailments, most notably digestive disorders, dental issues, and skin diseases, as well as their use for antioxidant, anti-inflammatory, anti-tumor, antimicrobial, and antiviral purposes (Salama *et al*., 2019; El Boukhari *et al*., 2025; Naseem *et al*., 2025). Furthermore, M. C, *M. piperita*, and *M. longifolia* possess high concentrations of bioactive compounds and volatile oils, owing to the presence of peltate and capitate glandular trichomes on their leaf surfaces (Hanafy *et al*., 2019). These glandular trichomes secrete volatile oils and bioactive compounds, while also protecting the plant from external factors and ensuring its survival (Choi and Kim, 2013; Hanafy *et al*., 2019). The characteristics of glandular trichomes vary in leaves across plant genera and species; consequently, these leaf traits can be used to identify and classify various and closely such as Mentha species (Choi and Kim, 2013; Hanafy *et al*., 2019). The climatic variety, ecological variation, and river systems in Iraq have led to plenty of therapeutic flowering plants of different genera and species (Porwal *et al*., 2025). In the river plains, species *M. spicata*, *M. piperita*, and *M. longifolia* are abundant, and especially *M. spicata* and *M. longifolia* are capable of naturally cross-pollinating and creating many unique hybrids (Naseem *et al*., 2025; Porwal *et al*., 2025). Therefore, the determination and identification of *Mentha* plants at the genus and species levels based on physical parameters such as leaf

and flower characteristics were crucial. The identification and demarcation of medicinal plant species such as Mentha still pose a major issue and need more investigations and taxonomic competence (Mohammed *et al*., 2024; Naseem *et al*., 2025). To our knowledge, there is minimal research on the identification of Mentha species in Iraq. Correct identification and separation are vital to avoid the extinction of these species. The climate and habitat in Iraq are favorable for the growth of *Mentha* species of medicinal and economic interest, but there is a considerable gap in their identification, categorization, conservation, and maintenance of genetic variety. Some *Mentha* species create hybrids that are critical in traditional medicine; therefore, it is vital to identify these species based on their physical traits. The present work is an attempt to identify and categorize three species of the genus *Mentha* based on morphological and anatomical features and biochemical evaluations. It tries to provide a complete account of these morphological and microscopic characters based on variations of leaf, flower, and stem characters.

MATERIALS AND METHODS

Study Area

The investigation was conducted from June to August at six sites on the banks of the Euphrates River in Al-Anbar Governorate, Iraq. These places experience hot and dry summers and warm and wet winters. During the winter season, the vegetation cover is dense. The geographical coordinates (latitude and longitude) of these sites are shown in Table 1, and the geographical distribution of the locations of the study and sampling regions are shown in Figure 1. The location is classed as having moderate rainfall, with annual precipitation between 108 and 148 mm; summer temperatures are between 30 °C and 39 °C, while winter temperatures are between 6°C and 10 °C.

Table (1) Geographical coordinates (latitude and longitude) of six regions (study site).

Sr. No.	Site	Latitude (N)	Longitude (E)
1	Al-Qaim	34° 15′ 18″	40° 08′ 31″
2	Ana	33° 55′ 33″	42° 08′ 34″
3	Haditha	33° 45′ 25″	43° 06′ 15″
4	Heet	33° 28′ 21″	42° 40′ 10″
5	Ramadi	33° 26′ 34″	44° 16′ 40″
6	Fallujah	33° 22′ 38″	44° 46′ 12″

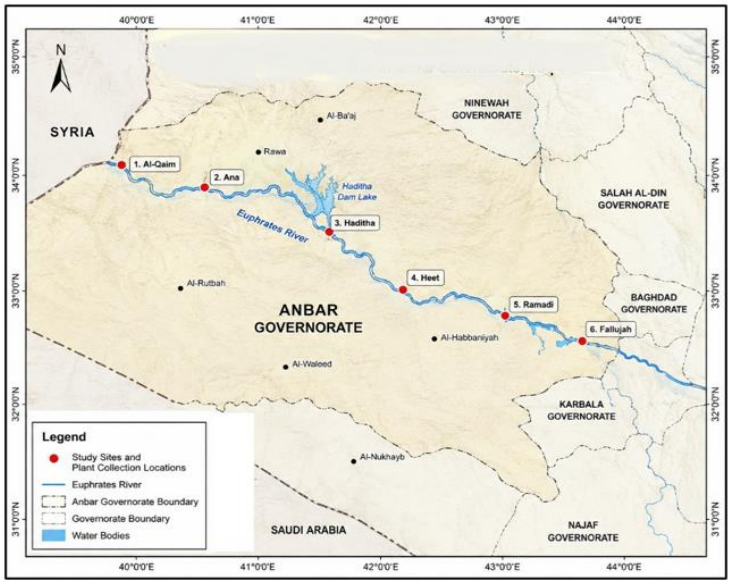


Figure (1) Map of the geographic distribution of the sampling area and sites from six regions on the banks of the Euphrates River comprising Ramadi, Al-Qaim, Fallujah, Ana, Heet and Haditha, Anbar Governorate, Iraq

Plant Materials Collection

Plant samples of the genus *Mentha* were collected during field trips to six locations in Anbar Governorate, Iraq, during the flowering period (June to August 2025). Three separate plants of each taxon were collected from each location where the taxon was found, depending on their availability in the natural habitat along the Euphrates River. The plant name, collection site, and elevation above sea level were recorded for each sample. The collected plants were used to examine the morphological features of the leaves and flowers. One mature leaf from the middle part of the stem of a representative plant of each taxon was selected for morphological and anatomical examination. Identification was made according to the Flora of Iraq (Ghazanfar *et al*., 2026) and other botanical references, including Plants of the World Online (POWO). The collected specimens were not deposited in a herbarium, and no voucher numbers were assigned. The scientific names of the studied *Mentha* taxa were verified according to Plants of the World Online (POWO, 2026).

Table (2) The selected *Mentha* taxa and their collection locations in Anbar Governorate, Iraq.

Sr. No.	Scientific name	Local name	Common name	Family	Distribution in Iraq	Number of plants per site
1	*Mentha spicata L.*	Spearmint	Spear mint	Lamiaceae	Haditha, Heet, Ramadi, and Fallujah	3
2	*Mentha piperita L.*	Peppermint	Pepper mint	Lamiaceae	Al-Qaim, Ana, and Haditha	3
3	*Mentha longifolia L.*	horsemint	White mint	Lamiaceae	Haditha, Heet, and Ramadi	3

Morphological Characterization

One representative plant of each of the three *Mentha* taxa was selected for morphological examination. One mature leaf from the middle part of the stem was used to examine leaf characteristics, while flower and stem features were observed during the flowering season. Photographs of the plants and their parts were taken using a Sony digital camera (model ZV-E10, 12 MP). The observed morphological characteristics were compared with descriptions in the Flora of Iraq and other taxonomic references. The ranges of leaf length and width presented in Table 3 were obtained from these references and were not based on direct measurements of the collected specimens. The examination was descriptive and focused on the diagnostic characteristics of each taxon rather than variations within the species.

Anatomical Characteristic Investigation

Fresh mature leaves from the three *Mentha* taxa were used for anatomical examination. Thin sections were prepared manually using a sharp blade and stained with 5% safranin solution for 5–10 minutes. The prepared sections were placed on microscope slides and examined using an Optika B-125 light microscope with 10X and 40X objective lenses. One representative slide was examined for each taxon. The anatomical features of the leaves were photographed and recorded, including the internal leaf tissues and epidermal characteristics. The thickness of the sections was not measured because the sections were prepared manually.

Biochemical Parameters Investigation

Measurement of Total Phenolic Content

Total phenolic content (TPC) of leaf samples of three *Mentha* species was estimated using the colorimetric Folin-Ciocalteu method as described by Singleton *et al*. (1999). For the manufacture of the leaf extracts, 150 mg of finely powdered leaves were added to 2 ml of 75% ethanol in a glass flask, and the liquid was stirred until homogeneity. The

mixture was filtered with Whatman No. 1 filter paper. Then 2 ml of the extract was added to 2 ml of Folin-Ciocalteu reagent (10% concentration, diluted with distilled water). To this mixture anhydrous sodium carbonate (Na_2CO_3) solution (7.5% concentration) was added to a volume of 1 ml, and the required final volume was obtained by adding distilled water (DW). The mixture was left in the dark at room temperature for 30 minutes to allow the blue color to develop. Finally, the absorbance of the blue-colored plant extract was measured at a wavelength of 765 nm using a UV-Vis spectrophotometer (Unico EW-83059-10). Gallic acid was used as the reference standard, and the total phenolic content was expressed in mg g^{-1} using the calibration approach reported in previous studies.

Measurement of Total Flavonoid Content

Total flavonoid content (TFC) of the sample extracts was determined using the Aluminum Chloride ($AlCl_3$) colorimetric method, in accordance with the procedure described by Marinova et al. (2005). 1 mL of the extract was added to 4 mL of DW in a 10 mL test tube. Then, 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution was added to the mixture, and the mixture was allowed to stand for 10 minutes. Subsequently, 0.3 mL of 10% $AlCl_3$ was added and left for one minute, followed by the addition of 2 mL of 1 M sodium hydroxide (NaOH) solution. DW was added to reach a final volume of 10 mL. The mixture was stirred until homogeneous and left in the dark at 27 °C for 30 minutes. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer. Catechin was used as the reference standard, and a calibration curve was prepared using different concentrations of catechin. Total flavonoid content was calculated from the calibration curve and expressed as milligrams of catechin equivalents per gram of plant material (mg CE g^{-1}).

Measurement of DPPH Scavenging Activity

The DPPH radical scavenging activity of extracts from three *Mentha* taxa was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, following the method described by Brand-Williams *et al*. (1995). A 0.1 mM DPPH solution was prepared in methanol and stored in the dark. The plant extracts were tested at a concentration of 1 mg/mL. One milliliter of each plant extract was added to a test tube containing 2 mL of methanolic DPPH solution. The mixture was mixed well and incubated at 37 °C for 30 minutes. The methanolic DPPH solution was used as the control (A0), while the mixture containing the plant extract and DPPH was used as the sample (As). A sample blank containing the plant extract without DPPH was also prepared by replacing the DPPH solution with methanol to correct for the background absorbance of the extract (Ab). The determination of the absorbance of the control, samples, and sample blanks was done at 515 nm by using a UV-Vis spectrophotometer. The percentage of inhibition, indicating DPPH radical scavenging activity, was calculated using the following equation (Alhakmani *et al*., 2013): DPPH scavenging activity (%) = $[(A0 - (As - Ab)) / A0] \times 100$

Where A0 represents the absorbance of the control, as represents the absorbance of the sample containing DPPH, and Ab represents the absorbance of the sample blank without DPPH. The assay was performed once for each taxon, and the results were expressed as percentage inhibition.

Determination of Extracted Essential Oils

Essential oil was obtained from three *Mentha* samples by hydrodistillation according to Başer *et al*. (1999). The *Mentha* species under research were collected separately in polyethylene bags throughout the flowering time with the particular data for each species and were mature and intact. After cleaning, the leaves were dried in shade at room temperature. They were weighed, and the weights (w) were recorded. The weighed dried leaf samples were placed in a Clevenger-type distillation apparatus and subjected to hydrodistillation using distilled water for 4 h. The extracted EOs

were collected in the apparatus's collection vessel, and water was removed using anhydrous sodium sulfate. The extracted oils were stored in dark glass vials of specific volumes (v). Finally, the essential oil yield was calculated based on the volume of extracted oil and the dry weight of plant material using the following equation:

Essential oil yield (mL/100 g) = (V/W) × 100

Where V represents the volume of extracted essential oil (mL), and W represents the dry weight of plant material (g). The extraction was performed once for each taxon, and the results were expressed as preliminary measurements.

RESULTS AND DISCUSSION

The present investigation was carried out for the identification and characterization of three blooming medicinal plant species of the genus *Mentha*, such as *M. spicata*, *M.* × *piperita*, and *M. longifolia*, as shown in Figure 2. The morphological characters of leaves, flowers, and stems were researched for the distinction of the taxa under consideration. Anatomical characteristics of leaves were also explored. Biochemical assays were carried out to provide further information on their chemical properties. Table 3 presents morphological and anatomical features of the three studied species. Therefore, the plants studied are often known as spearmint, peppermint, and horsemint (white mint). Of these taxa, *M.* × *piperita* is a recognized hybrid.

Morphological and Anatomical Characteristics Analysis

Morphological Characteristics Analysis

The results reported in Table 3 show that *M. spicata* and *M.* × *piperita* grow in an erect habit along riverbanks and in locations with moist soil, with simple and sparse stem branching. Conversely, *M. longifolia* exhibited an upright and densely branching habit in riverbank habitats and semi-mountainous areas. All three taxa are perennial herbs. Differences in internode length and appearance were also detected among the plants examined. These morphological features are in agreement with earlier studies on the identification and categorization of *Mentha* taxa (Yousefian *et al*., 2023; Naseem *et al*., 2025; Tyler *et al*., 2026).

The morphometric analysis demonstrated variation in various leaf traits among the three taxa tested. The leaf aroma of *M. spicata* was sweet, mild, and pleasant, whereas in both *M.* × *piperita* and *M. longifolia* it was powerful, pungent, and aromatic. The leaves had diverse morphologies: oblong-ovate to lanceolate leaves in *M. spicata*, ovate to oblong-ovate leaves in *M.* × *piperita,* and oblong-ovate to oblong-lanceolate leaves in *M. longifolia* (Figure 2, Table 3). All three taxa have simple leaves with sharp apices and opposing leaf configurations. The leaf bases of *M. spicata* were cordate to rounded, those of *M.* × *piperita* were cordate to sub-rounded, and those of *M. longifolia* were cordate. M. spicata had serrate margins with separated teeth, M. × piperita had serrate margins with closely spaced teeth, and M. longifolia had irregularly serrated margins.

M. spicata had dark green leaves, *M.* × *piperita* had light green leaves, and *M. longifolia* had green to grayish green leaves. The leaf venation was sunken reticulate in *M. spicata* and *M.* × *piperita* and non-sunken reticulate in *M. longifolia*. The lower leaf surface was glabrous to rugose in *M. spicata* and glabrous to weakly rugose in *M.* × *piperita, but it was* pubescent in *M. longifolia*. The upper surface of the leaf was usually glabrous in both *M. spicata* and *M.* × *piperita*, but *M. longifolia* had a velvety-woolly surface. In addition to the differences in leaf shape, petiole features also varied, with *M. spicata* having different petioles, *M.* × *piperita* having very short petioles, and *M. longifolia* having sessile to subsessile leaves (Table 3). Descriptive comparison was based on the approximate leaf dimensions

given in Table 3, irrespective of these values as evidence of quantitative variance within each taxon. Such morphological traits are often employed for identification and taxonomic descriptions of plants (Abd El Maksoud and Azer, 2013; Naseem *et al*., 2025; Ya'ni *et al*., 2018).

In terms of flower morphological traits, the shape of inflorescence, color of flowers, structure of calyx, and characteristics of corolla and floral bracts were different. The inflorescence of *M. spicata* was a long, narrow spike; of *M.* × *piperita*, an elongated spike; and of *M. longifolia*, a thin spike. *M. spicata* had purple flowers, and *M.* × *piperita* had light purple flowers. *M. longifolia's* flower color varied from white to mauve. The calyx in M. spicata and *M.* × *piperita* was tubular to campanulate and purple, but *M. longifolia* had a narrowly campanulate green calyx. The calyx surface was glabrous in *M. spicata* and glabrous with secretory glands in *M.* × *piperita*, but it was densely hairy in M. longifolia. The corolla was tubular in both *M. spicata* and *M.* × *piperita*, whereas in *M. longifolia* it was short and tubular. Corolla color ranged from white to pale pink in *M. spicata*, light purple in *M.* × *piperita*, and purple in *M. longifolia*. The stamens were equal and glabrous in *M. spicata* and *M.* × *piperita* and subequal and glabrous in *M. longifolia*. In *M. spicata* and *M.* × *piperita* floral bracts were oblong-lanceolate to linear and glabrous, whereas linear and pubescent bracts were documented in *M. longifolia* (Table 3).

Moreover, different stem characters were observed in the studied *Mentha* taxa. The stem color was dark red in *M. spicata,* purple-red in *M.* × *piperita,* and pale to light green in *M. longifolia.* The stems of *M. spicata* and *M.* × *piperita* were glabrous, but *M. longifolia* had pubescent stems. Differences were also documented in the approximate length and thickness of the stems, as shown in Table 3. These features, together with leaf and floral characters, gave descriptive information to distinguish the taxa studied. A few morphological similarities were detected between *M. spicata* and *M.* × *piperita*, notably in terms of leaf arrangement, surface features, and branching pattern.

The morphological features recorded in the present study were compared with prior studies on distinct *Mentha* species. Abd El Maksoud and Azer (2013) studied the morphological characters of *M. longifolia,* such as leaf shape, edge, apex, base, color, venation, and petiole characters. Abbaszadeh *et al*. (2009) reported similar features in their study on morphological variation across species of *Mentha*. In addition, Abdalla *et al*. (2022) evaluated a number of morphological features of *M. spicata* leaves, while Kieltyka-Dadasiewicz *et al*. (2017) found differences in *M.* × *piperita*, such as serrated leaf margins, leaf color, leaf base, and leaf shape. Such studies provide the comparative description of different features noticed in studied plants.

Erum *et al*. (2012) studied the quantitative and qualitative morphological traits of 17 *Mentha* taxa, such as *M. spicata*, *M. piperita,* and *M. longifolia*. Their study was based on quantitative variables such as leaf length, width, area, and stem dimensions and qualitative features such as leaf arrangement, color, scent, margin, blade form, and floral traits. The traits examined in that study resemble several of those recorded in the present investigation. However, the present study was mostly based on descriptive morphological findings, and no statistical comparisons were made to test significant differences among the taxa investigated.

The investigated plants exhibited several morphological traits, such as simple green leaves, opposite leaf arrangement, acute leaf apices, serrate leaf margins, terminal spike-like inflorescences, and tubular corollas. Salama *et al*. (2019) also reported similar morphological traits in their study on *M. spicata, M. piperita, M. longifolia, and M. sativa*. They mentioned certain common traits such as the arrangement of leaves, the margins of leaves, the forms of leaves, and the differences in leaf bases. Other studies also observed morphological variation among the *Mentha* taxa in plant height, stem color, leaf size, internode length, petiole features, scent of leaves, flower color, and inflorescence structure (Hoque *et al*., 2014; Abasi *et al*., 2019; Devi *et al*., 2022).

A morphological study of *M. spicata*, *M. × piperita,* and *M. longifolia* revealed similarities and differences in various leaf, blossom, and stem characters. These observations contain descriptive information that can be compared with known taxonomic descriptions of the genus *Mentha*. The extensive morphological analysis, however, was based on representative plant material, and the results do not represent the complete range of diversity within each taxon. Further material and molecular analyzes may shed more light on the taxonomic status of these species.



Figure (2) Morphological features of the three categorized and identified flowering medicinal plant species: *Mentha spicata*, *Mentha piperita*, and *Mentha longifolia*.

Table (3) Morphological and anatomical properties of three flowering medicinal plant species.

Morphological Characters		Flowering Medicinal Plants (Mentha Species)		
		M. spicata	***M. piperita***	***M. longifolia***
Habitat		Riverbanks and areas with moist soil	Riverbanks and areas with moist soil	Riverbanks and submontane
Growth Habit		Erect	Erect	Erect
Duration		Herbaceous perennial	Herbaceous perennial	Herbaceous perennial
Internodes		Cylindrical, short to medium length	Quadrangular, short to medium	Rectangular, medium to long
Stem Branching		Branched	Simple and sparsely branched	Strongly branched
Leaf morphology	Leaves Odor	Sweet mild refreshing	Aromatic strong pungent	Pleasantly Strong pungent
	Leaf shape	Oblong-ovate to lanceolate	Ovate to oblong-ovate	Oblong-ovate to oblong-lanceolate
	Leaf blade shape	Orbicular	lanceolate	lanceolate
	Leaf type	Simple	Simple	Simple
	Leaf apex shape	Acute	Acute	Acute
	Leaf base shape	Cordate to round	Round to sub-cordate	Cordate
	Leaf margin shape	Regularly serrate (spaced)	Regularly serrate (closely)	Irregularly serrated
	Leaf arrangement	Opposite	Opposite	Opposite
	Leaf persistence	Evergreen / Regenerating	Evergreen / Regenerating	Evergreen /Semi-deciduous
	Leaf venation	Sunken reticulate	Sunken reticulate	Non-sunken reticulate
	Leaf color	Dark Green	Light green	Green to grayish-green
	Lower leaf texture	Glabrous to rugose	Glabrous to weakly rugose	Pubescent (downy)
	Upper leaf texture	Glabrous (smooth)	Glabrous (smooth)	Velvety-woolly
	Leaf length (cm)	2-6	3-8	4-10
	Leaf width (cm)	1-3	1.5-4	2-4
	Leaf area (cm^2)	2-18	4.5-32	8-40
	Leaf petiole	Petiolate	Very shortly petiolate	Sessile or sub-sessile
Flower morphology	Inflorescence shape	Long narrow spike	Elongated spike	Slender spike
	Flower type	Spike terminal	Spike terminal	Spike terminal
	Flower color	Purplish	Light purple	White to mauve
	Calyx shape	Tubular/campanulate	Tubular/campanulate	Narrowly campanulate
	Calyx surface	Glabrous	Glabrous secretory glands	Densely hairy
	Calyx color	Purple	Purple	Green
	Corolla shape	Tubular	Tubular	Tubular short cylindrical
	Corolla surface	Glabrous	Glabrous	Puberulous
	Corolla color	White to pale pink	light purple	Purple
	Stamens	Equal glabrous	Equal glabrous	Subequal glabrous
	Floral bracts	Lanceolate-oblong to linear, glabrous	Lanceolate-oblong to linear, glabrous	Linear pubescent
Stem morphology	Stem color	Dark red	Purple-red	Whitish to light green
	Stem thickness (mm)	1-3	1-4	1.5-6
	Stem Hair	Glabrous	Glabrous	Pubescent
	Stem length (cm)	20-90	20-80	25-100
Upper epidermal morphology	Stomatal type	Anisotricytic	Diacytic	Diacytic
	Stomatal level	Superficial	Superficial	Superficial
	Trichomes type	Glandular and non-glandular (1–2 celled)	Glandular and non-glandular multilocular	Glandular and non-glandula multilocular
	Sculpture pattern	Smooth	Smooth to finely striated	Granulate rugose
	Epidermal cell wall	Irregularly polygonal cells	Irregularly sinuous cells	Irregularly polygonal cells
Lower epidermal morphology	Stomatal type	Diacytic	Diacytic	Diacytic
	Stomatal level	Semi-raised	Raised	Semi-raised
	Trichomes type	Glandular and non-glandular (unicellular)	Glandular and non-glandular multilocular	Glandular and non-glandula multilocular
	Sculpture pattern	Rugose	Smooth to striated	Rugose
	Epidermal cell wall	Irregularly sinuate cells	Irregularly sinuous cells	Irregularly oval cells

Anatomical Characteristics Analysis

The present work aimed to analyze the anatomical structure of the leaves of three *Mentha* taxa to describe their anatomical features and investigate their utility in plant identification and classification. Leaf anatomical features are important for the discrimination of closely related plant taxa, although they can be affected by varied environmental conditions (Moon *et al*., 2009).

The results revealed several anatomical characteristics of the upper and lower epidermal layers of the examined *Mentha* taxa, as presented in Table 3 and Figures 3, 4, and 5. Anatomical examination showed that the upper epidermis of all three taxa consisted of a single layer of cells. Stomata were observed in the upper epidermis of *M. spicata*, although their exact type could not be conclusively determined from the available microscopic images, while diacytic stomata were recorded in *M. × piperita* and *M. longifolia*. The stomatal level was described as shallow in the examined samples of all three taxa. Both *M. × piperita* and *M. longifolia* showed glandular and non-glandular multicellular trichomes, whereas *M. spicata* exhibited glandular and non-glandular trichomes consisting of one or two cells. Differences were also observed in the surface ornamentation of the upper epidermis, which appeared smooth in *M. spicata*, smooth to finely striate in *M. × piperita*, and granular to rugose in *M. longifolia*. The epidermal cells of *M. spicata* and *M. longifolia* showed irregular polygonal outlines, while those of *M. × piperita* appeared irregular with sinuous walls. Examination of the lower epidermis showed that it consisted of a single cell layer in all three taxa, with diacytic stomata recorded in the examined samples. The stomata were described as semi-erect in *M. spicata* and *M. longifolia*, while erect stomata were recorded in *M. × piperita*. The lower epidermis of *M. × piperita* and *M. longifolia* contained glandular and non-glandular multicellular trichomes, whereas *M. spicata* showed glandular and non-glandular unicellular trichomes. Differences in surface ornamentation were also recorded, with a rugose pattern observed in *M. spicata* and *M. longifolia*, while *M. × piperita* exhibited a smooth to striated surface. The epidermal cells of *M. spicata* and *M. × piperita* showed irregular sinuate outlines, whereas those of *M. longifolia* were irregular and oval-shaped. The microscopic examination included transverse sections of the leaf tissues and surface views of the epidermis, as illustrated in Figures 3, 4, and 5, where panel B represents a surface view showing the stomata and surrounding epidermal cells. These observations are comparable to previous studies that described the importance of leaf anatomical characteristics in the identification and differentiation of *Mentha* taxa (Chaker *et al*., 2011; Abdalla *et al*., 2022; Marat, 2022).

Anatomical examination of medicinal plant leaves provides useful descriptive information, particularly when the examined taxa share several external morphological characteristics. Previous investigations have also discussed the application of anatomical features in medicinal plant identification and quality assessment (Solanki *et al*., 2025; Garas *et al*., 2026). In the present study, the anatomical observations provided additional descriptive characters for comparing the three examined *Mentha* taxa. However, the examination was based on one representative slide for each taxon, and the recorded features do not represent the full range of anatomical variation within each taxon. Therefore, these observations are considered supporting morphological evidence rather than independent confirmation of taxonomic identity.

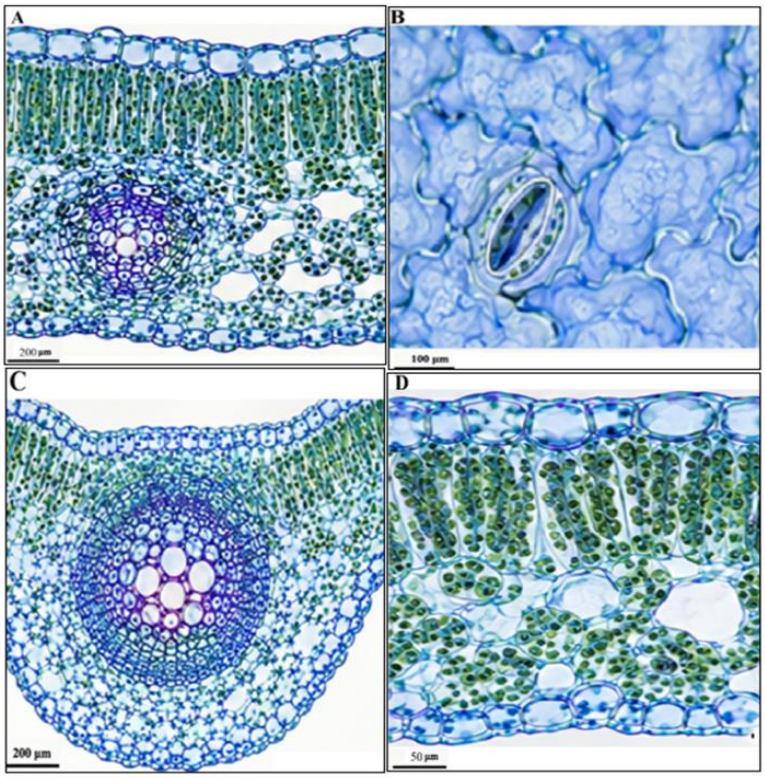


Figure (3) Microscopic characteristics and anatomical structure of the leaf of *Mentha spicata*. A: Cross-section showing the upper and lower epidermal layers and leaf vascular bundles; B: Cross-section of stomatal complexes in the epidermis; C: Cross-section of parenchyma tissue surrounding the vascular region; and D: Cross-section of palisade and spongy parenchyma in the leaf mesophyll. Bars=50µm,100µm, and 200µm.

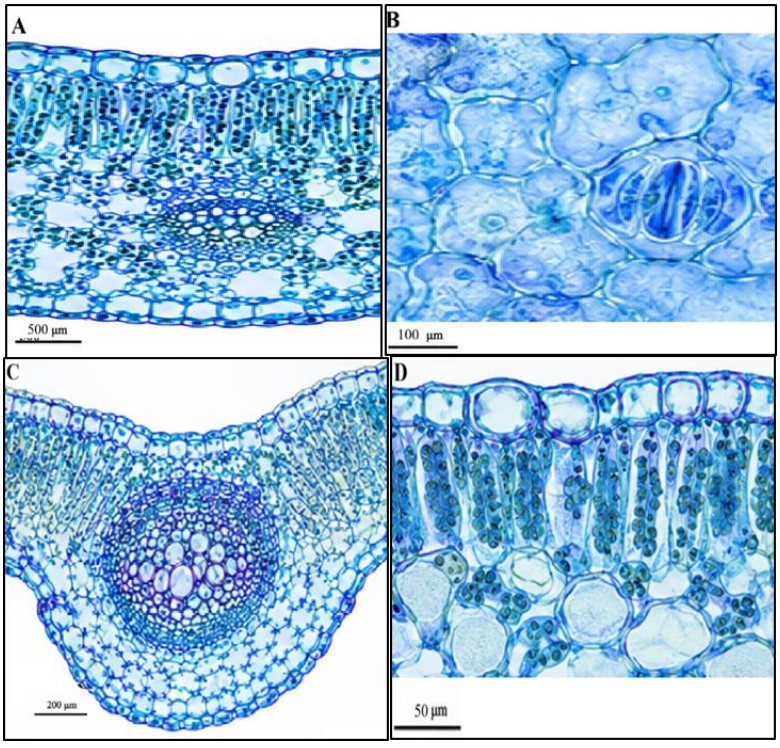


Figure (4) Microscopic characteristics and anatomical structure of the leaf of *Mentha piperita*. A: Cross-section showing the upper and lower epidermal layers and leaf vascular bundles; B: Cross-section of stomatal complexes in the epidermis; C: Cross-section of parenchyma tissue surrounding the vascular region; and D: Cross-section of palisade and spongy parenchyma in the leaf mesophyll. Bars=50µm,100µm,200µm, and 500µm.

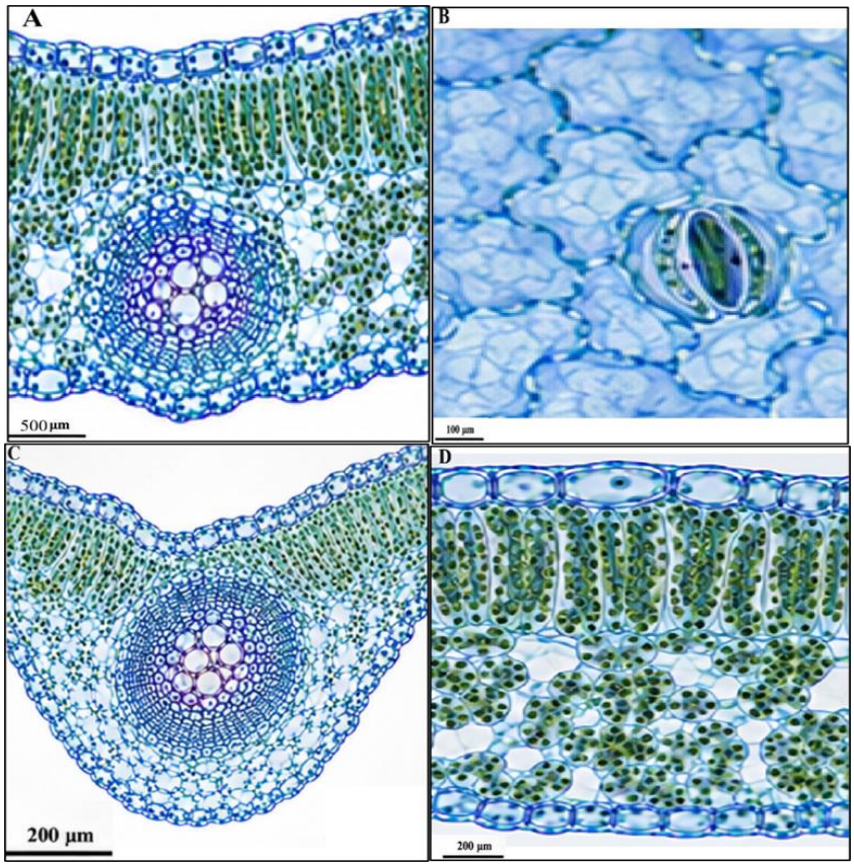


Figure (5) Microscopic characteristics and anatomical structure of the leaf of *Mentha longifolia*. A: Cross-section showing the upper and lower epidermal layers and leaf vascular bundles; B: Cross-section of stomatal complexes in the epidermis; C: Cross-section of parenchyma tissue surrounding the vascular region; and D: Cross-section of palisade and spongy parenchyma in the leaf mesophyll. Bars=100µm,200µm, and 500µm.

Biochemical Parameters Analysis

Table 4 presents the results of the biochemical analysis of three *Mentha* taxa, including DPPH radical scavenging activity, total phenolic content (TPC), total flavonoid content (TFC), and essential oil yield. The results showed differences in DPPH scavenging activity among the examined taxa. *M.* × *piperita* recorded the highest DPPH inhibition at 60.17%, followed by *M. spicata* at 50.63%, while *M. longifolia* showed the lowest inhibition at 43.15%. Similarly, differences were observed in total phenolic content (TPC), with *M.* × *piperita* recording the highest value of 46.11 mg g^{-1}, followed by *M. spicata* at 38.14 mg g^{-1} and *M. longifolia* at 33.85 mg g^{-1} (Table 4). Regarding total flavonoid content (TFC), *M.* × *piperita* also recorded the highest value at 28.12 mg g^{-1}, whereas *M. spicata* and *M. longifolia* showed values of 22.30 mg g^{-1} and 20.73 mg g^{-1}, respectively. Differences were also recorded in essential oil yield among the three taxa. *M.* × *piperita* exhibited the highest oil yield at 0.57%, followed by *M. spicata* at 0.46%, while *M. longifolia* showed the lowest yield at 0.41%, as presented in Table 4. These biochemical measurements were obtained from a single analysis for each taxon and are presented as preliminary descriptive results without statistical comparisons.

Biochemical constituents of *Mentha* species, including phenolic compounds, flavonoids, and essential oils, have been widely investigated because of their association with various biological activities, particularly antioxidant properties (Hanafy *et al*., 2019; Naseem *et al*., 2024). Essential oils are also important components of aromatic medicinal plants and have been studied for their potential antioxidant and antimicrobial activities and their applications

in the pharmaceutical and food industries (Sfaxi *et al*., 2025; Shirokova *et al*., 2026). In the present study, the biochemical measurements provided additional information about the chemical characteristics of the examined *Mentha* taxa. However, these measurements were considered complementary to the morphological and anatomical observations rather than independent evidence for confirming their taxonomic identification.

The results of the present study were compared with previous investigations on the biochemical properties of *Mentha* species. Naseem *et al*. (2025) reported TPC, TFC, and essential oil values of 22.95 mg g^{-1}, 11.72 mg g^{-1}, and 0.37%, respectively, for *M. spicata*. These values differed from those recorded in the present study, although both investigations examined similar biochemical parameters. Benabdallah *et al*. (2016) also investigated DPPH radical scavenging activity, total phenolic content, and total flavonoid content in *M.* × *piperita*, providing comparative information on its biochemical characteristics. Similarly, Nickavar *et al*. (2008) evaluated DPPH radical scavenging activity and phenolic content in five *Mentha* species, including *M. longifolia*, *M. spicata*, and *M.* × *piperita*, and reported differences in their antioxidant activity and phenolic content. Brown *et al*. (2019) examined TPC and DPPH activity in *M. longifolia*, providing additional information for comparison with the present findings. Regarding essential oil yield, Yousef *et al*. (2015) reported values ranging from 0.10% to 0.46% in mint species, including *M.* × *piperita* and *M. spicata*. These previous investigations provide comparative data on the biochemical properties of *Mentha* taxa, although differences in extraction procedures, experimental conditions, and analytical methods should be considered when comparing the reported values. The biochemical results of the present study describe the characteristics of the examined samples and do not represent the full variation within each taxon.

Table (4) Biochemical characteristics of three flowering medicinal plant species.

Flowering medicinal plant species	Biochemical Parameters			
	DPPH inhibition (%)	Total Phenols (mg g^{-1})	Total Flavonoids (mg g^{-1})	Oil percentage (%)
Mentha spicata L.	50.63	38.14	22.30	0.46
Mentha piperita L.	60.17	46.11	28.12	0.57
Mentha longifolia L.	43.15	33.85	20.73	0.41

CONCLUSIONS

The current study concludes that the morphological, anatomical, and biochemical characteristics of *Mentha* species are important for their identification and differentiation, particularly as these plants are widely recognized for their medicinal, nutritional, and industrial applications. The examined characteristics showed several differences among the three studied taxa*, M. spicata*, *M.* × *piperita*, and *M. longifolia*, particularly in the morphological features of leaves, flowers, and stems, as well as in the anatomical structure of the leaves. Biochemical analyses also revealed differences in the recorded values among the three taxa. Moreover, the morphological and anatomical observations provided useful descriptive characteristics for distinguishing the examined taxa, while the biochemical measurements offered additional information about their chemical properties rather than independent confirmation of their taxonomic identity. Although the examined plants showed differences in several characteristics, the findings were based on representative anatomical samples and single biochemical measurements for each taxon. Therefore, the results are limited to the examined plant material and do not represent the full variation within each taxon. This study highlights the importance of further investigations involving additional plant samples and more advanced identification methods, including molecular characterization, to provide a more comprehensive understanding of the taxonomic characteristics of *Mentha* taxa.

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