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MORPHOCHEMICAL AND ANATOMICAL STUDY OF *COLEUS SCUTELLARIOIDES* (L.) BENTH. (LAMIACEAE) IN IRAQ

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SUMMARY

The presented research comprised an anatomical study of the species *Coleus scutellarioides* (L.) Benth. in Iraq. The vertical section of the leaf blade, which passed through the midrib, appeared in a semi-circular form, and the upper surface of the epidermis showed the eglandular trichomes covering the surface of the leaf. The stomata that seemed to occur on both surfaces of the leaf, called amphistomatic, were of a paracytic stomatal type. The needle-type crystals also emerged on the surface of the epidermis. The vein course was of the brochidodromous type. The pollen grains were zonocolpate, hexacolpate, prolate to subprolate, and exine ornamentation, as recognized with three types. Moreover, the study determined the phenolic and flavonoid compounds, and the phenol and flavonoid concentrations were 1.993 ± 0.005 and 191.682 ± 2.273 , respectively.

Keywords: *Coleus scutellarioides* (L.) Benth., leaf veins, stomata, pollen grains, phenol, flavonoid

Key findings: The *C. scutellarioides* is notably palatable and has medicinal values based on its biochemical and pharmacological formatting.

INTRODUCTION

The species *Coleus scutellarioides*, commonly known as coleus and carpet plant, a flowering plant widely grown for the highly decorative

variegated leaves, belongs to the family Lamiaceae. Family Lamiaceae is native to Southeast Asia and Australia, which includes 200 genera of Angiospermae (mint family), with 3500 species spread worldwide. About

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103 wild species exist in the Iraqi flora, with only five species being cultivated (Al-Kateb, 1988). This plant grows well in partly shaded, moist, rich, loose soil but can tolerate full shade.

In this family, most of the plant species are aromatic herbs that contain volatile oils with a fragrant smell, such as mint, lavender, and rosemary. Additionally, most species are desirable in food preparations for their special flavors, such as thyme, basil, and mint. Some plants are also ingredients in the preparation of different medicines (Nasrallah, 2007; 2014). Moreover, they have ornamental plants, such as *Coleus* sp., characterized by its colorful leaves, as well as *Salvia* sp., a plant distinct for its beautiful red flowers. *Coleus scutellarioides* is applicable to treat unspecified medicinal disorders as a medicine, and it also has environmental and social uses aside from food.

The most valuable characteristic of the said family plants is the flowers made with bilateral symmetry and the ovary with four lobes; each one has a single ovule, the basal style ends in two branches, the stamens are four, and the leaves are opposite (Al-Mashhadani *et al.*, 2008). Environmental factors and geographical variations are crucial to influencing the said family plants and require attention. These environmental factors influence the phenotypic characteristics of the individual species of plants; thus, several ways to know the diagnosis for the classification of different plants are essential (Nasrallah, 2007).

Coleus has attractive foliage and succulent stems and comes in many colors, providing year-round interest. It could serve as a tender annual outdoors in a bed, a border, or a hanging container. If grown as a houseplant, it requires bright light. The family Labiatae is outstanding for medicinal plants; hence, the following study includes the pharmaceutical assessment of the species *Coleus scutellarioides* (Nasrallah, 2014) and anatomical classification through pollen grains and chemical compounds.

MATERIALS AND METHODS

Sample collection

The latest study relied on the soft leaf samples of the *Coleus scutellarioides*, collected during February-April 2021 from the orchards of Al-Adhamiya (longitude 44.360° East and latitude 33.369° N), Iraq. For diagnosis, the study used the classification keys of the family Lamiaceae in the Flora e Funga of Brazil (Antar *et al.*, 2024), as well as from internet sources. The plant's soft leaves bore cuts within 2–3 cm from the center for the section to pass through the midrib and to the edge of the leaf (Al-Shami *et al.*, 2024). After fixation with F.F.A. solution for 24 h at room temperature (Johansen, 1940), the leaf pieces received a 70% alcohol washing to remove traces of the fixative solution before being kept in the same alcohol concentration in the refrigerator/freezer until used.

Epidermis preparation

The leaf epidermis of the *C. scutellarioides* incurred scraping in the center between the apex and the base. The scraping process proceeded using a sharp dissecting lancet. After the process, the samples washed with distilled water underwent placement in a petri dish in a solution of sodium hypochlorite (artificial bleach) at a concentration of 5% for 5 min. Afterward, washing the samples with distilled water followed before transferring to another petri dish containing KOH at a concentration of 10% for 5-10 min. Then, the replacement of the solution with ethyl alcohol had a concentration of 70% for 10-15 min. Next, the samples' transfer to a petri dish contained safranin stain at a concentration of 1% for 30-45 min. Subsequently, the sections' washing with distilled water occurred, followed by passing through a descending series of ethyl alcohol (70%, 95%, and 100%) successively. At each concentration, placing

the samples remained for 10 min. The sections then underwent transfer to a tube containing xylene for 10 min before being placed on a slide with a drop of water mixed with a drop of xylene. Afterward, placing a slide cover made it ready for microscopic examination. The examination took place with a KRÜSS compound microscope, with the imaging made with an AmScope (Model MU 1000) microscope-mounted camera (Al-Khazraji and Aziz, 1989; Al-Hadeethi *et al.*, 2020).

Vertical sections elaboration

The sections' manual preparation followed the methodology of Hutchinson (1954) with some modifications (Al-Janabi *et al.*, 2024): the leaves bore cutting in the center into tiny pieces and slices; the small parts' soaking in a hypochlorite AL-sodium (artificial bleach-0.5%) for 5 min removed the chlorophyll; the parts received staining with safranin (5%) for one hour before washing the sections with 70% ethyl alcohol to remove the excess dye; then, the parts moved to 90% ethyl alcohol for 5 min before being transferred to 95% alcohol and absolute alcohol, respectively, for 2 min for each concentration, and then moved to a mixture of absolute alcohol and xylene of 1:1 for 2 min; subsequently, the parts obtained pure xylene and remained for 2 min; and finally, the examination continued with a KRÜSS compound microscope, carrying out photography with an AmScope, Model MU 1000 camera mounted on the microscope.

Pollen grains study

The fresh stamens of the *C. scutellarioides*, as collected from the field, followed the procedure of Alanbari *et al.* (2023). Pollen grains' fixing with Carnoy's solution consisted of three volumes of ethyl alcohol 100% and one volume of glacial acetic acid for 18–24 h before washing with 70% ethyl alcohol, preserving the specimens with alcohol in the same concentration. Dry pollen grains, after being placed on a slide, received a drop of safranin and glycerin dye, remaining on it for 10–20 min

and then covering the slide to be ready for examination. The slides' scrutiny proceeded under an Olympus light microscope with an oil immersion lens. Ten pollen grains sustained their assessment for each type of the genus under study. This comprised measuring the diameter of the equatorial axis and the polar axis, the diameter of the germination hole, and the wall thickness of each pollen grain using an ocular lens.

Venation pattern and leaf architecture

Placing the leaves in 0.5% sodium hypochlorite for 15 minutes removed the chlorophyll before fixing them on the slide for photography using a microscope. For the preparation of leaf venation, the study employed the methodology of Al-Hadeethi *et al.* (2019), with the terminology defined by Metcalfe and Chalk (1950).

Phenolic content determination

From the earlier prepared specimen mixture, adding 2.0 ml of Na₂CO₃ (75%) to the mixture after 5 min continued into brooding at 50 °C for 10 min with irregular stirring. Afterward, the sample sustained refrigeration before calculating the absorbance using a Shimadzu UV-1800 spectrophotometer at 765 nm against a blank without extract. The data outcomes' definition was mg/g of gallic acid equals mg g⁻¹ (mg GAE/g) of parched extract, following the formula of Lee *et al.* (2015).

Total flavonoid determination

Using methanol alcohol with a concentration of 50% prepared 50 ml of 100% concentrated methanol alcohol. Adding 45 ml of purified water helped it reach the required concentration. A sodium nitrate solution with a concentration of 5% utilized 5 g of sodium nitrate powder, with 95 ml of distilled water added to it to reach the required concentration. A sodium hydroxide solution with a concentration of 10% comprised 10 g of

sodium hydroxide dissolved in 90 ml of purified water to reach the required concentration. Aluminum chloride solution with a concentration of 10% had 10 g of aluminum chloride dissolved in 90 ml of water to reach the required concentration.

For the assay procedure of total flavonoid, following the technique of Sakanaka *et al.* (2005) progressed: the prepared solutions at the required concentrations had the total content of flavonoid compound determined chemically in the aqueous extract of the plant based on rutin (flavonoid standard) using the aluminum chloride colorimetric method, as explained earlier. The 3.2 mg of the German extract of the chasteberry plant incurred dissolving in 5 ml of 50% methanol alcohol before adding 1 ml of the 5% sodium nitrate solution. After 6 min, the addition of 1 ml of 10% aluminum chloride solution to the mixture followed, then the mixture stood for 5 min before adding 10 ml of the 10% sodium hydroxide solution. The volume of the mixture reached completion with 50 ml of distilled water, followed by mixing well. After leaving the mixture for 15 minutes, measuring the absorbance used a spectrophotometer at a wavelength of 450 nm to determine the absorbance of flavonoids in the chasteberry plant. The same method continued to six different concentrations of rutin (2.5, 5, 10, 20, 40, and 80 µg/ml), wherein the standard curve preparation employed the curve-fitting equation for the standard curve.

RESULTS AND DISCUSSION

The results detailed that the stomata appeared on both surfaces of the leaf, with this type of stomata called amphistomatic in *C. scutellarioides*. The stomatal type was paracytic, which surrounds stomata with two subsidiary cells, and this was the distinctive feature, appearing on both surfaces of the leaves in the same type (Figure 1). The results were greatly analogous to the findings of Al-Ani and Saleh (1979), who also studied the shapes of epidermal cells and the stomata types. The ordinary epidermal cell walls appeared semi-straight on both surfaces, and the oil droplets also spread into epidermal cells (Figure 1). These results follow Davis (1975) in the Flora of Turkey and the East Aegean Islands.

The vertical section of the leaf blade and midrib of the species *C. scutellarioides* showed the main tissues making up the leaf were the epidermis, mesophyll tissue, and vascular tissue. The epidermis, as characterized by the upper and lower epidermis, comprised a simple single layer of quadrilateral cells stacked together. The epidermis layer displays interspersing with the stomatal complexes and eglandular trichomes, and the prismatic crystals were diffuse in the mesophyll (Figure 2). These results were consistent with past findings based on the comparative anatomical study of Lamiaceae species, including *C. scutellarioides*, where the trichomes and crystals appear in the mesophyll and epidermis (Venkateshappa and Sreenath, 2013).

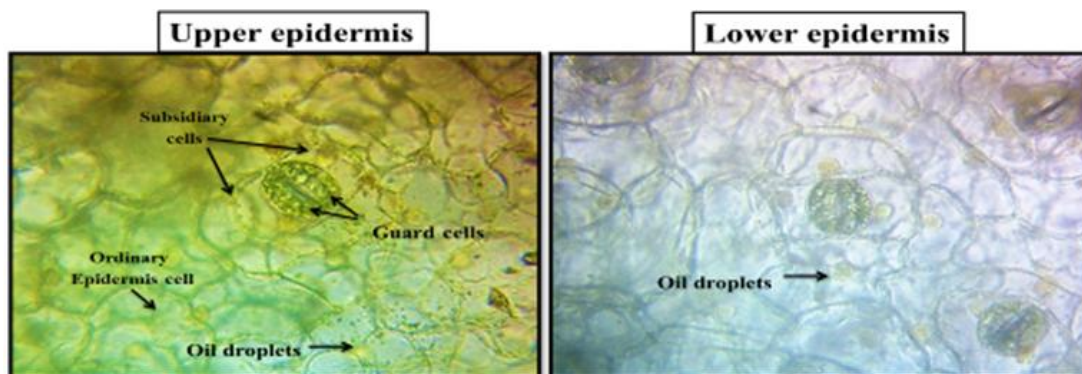


Figure 1. The stomata shape and type in the leaf epidermis of *Coleus scutellarioides*.

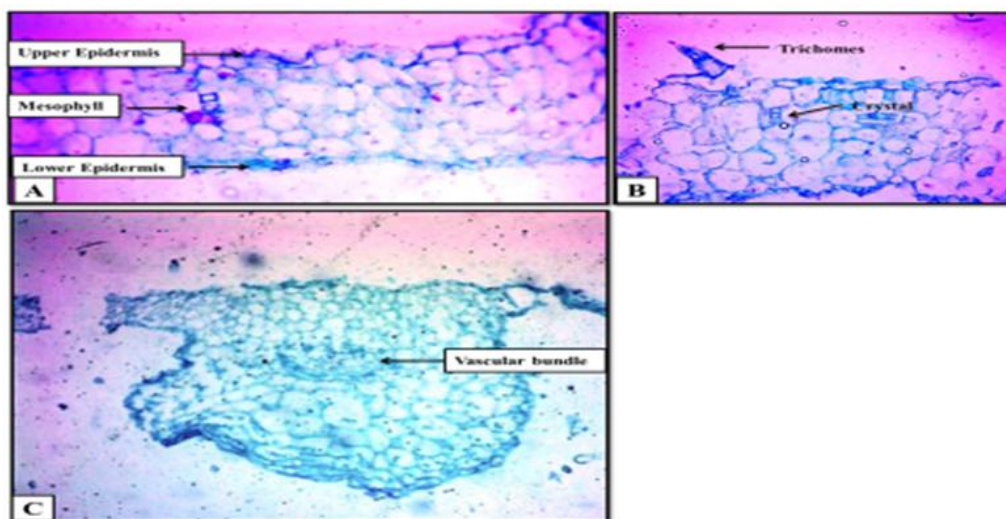


Figure 2. Vertical section of leaf appears the blade and midrib of *Coleus scutellarioides*.

The lower surface epidermis appeared to be thicker than the upper surface. The thickness of the upper epidermis layer emerged to be 33 μ , which was larger than its lower surface at 28 μ . The epidermis cells covered the outside of the thin, smooth cuticle layer. The essential tissue appearing was the larger part of the leaf blade, consisting of the mesophyll tissue and the tissue surrounding the large veins. The mesophyll tissue consists of spongy tissue only, making these results agree with the findings of Hullatti and Bhattacharjee (2011).

This type of tissue is distinctly unifacial and is the common form. The spongy tissue consists of 3–6 rows of parenchyma cells that fill the area below the midrib, as its thickness reaches approximately 220 μ (Figure 2). Al-Ani and Saleh (1979) reported the parenchyma cells are an important necessity because they carry out functions such as storage, transport, and photosynthesis. Moreover, they serve as support when filled with cellular juice in soft plant organs, viz., leaves and young stems, despite the thinness of their walls.

The veins appearing in the leaf blade of the species *C. scutellarioides* were of the brochidodromous type. The secondary veins do not end at the edge of the leaf, and each

secondary vein connects with the higher vein. These further have connections to each other by a series of prominent arches, which also share the lateral loop formed by the junction of veins (Figure 3). Similarly, Paton *et al.* (2019) reported the nomenclatural variations in the *Coleus* and *Plectranthus* of the family Lamiaceae.

The pollen grains were zonocolpate, hexacolpate, and prolate to subprolate. Three types of exine ornamentation were distinct (Figure 4). Özaltan and Koçyiğit's (2022) findings revealed recognizing the pollen grains as zonocolpate, hexacolpate, and prolate types of exine ornamentation.

The phenolic and flavonoid compounds gained successful determination, and the concentration of phenol reached 1.993 ± 0.005 , and flavonoid reached 191.682 ± 2.273 (Table 1). Ekor (2014) and Paton *et al.* (2018) also pointed out the abundance of phenols and flavonoids in the species *C. scutellarioides*, which makes it an effective medicinal plant in treating some diseases. They also recommended further testing of the said species on several laboratory animals to determine its medical and therapeutic effectiveness.

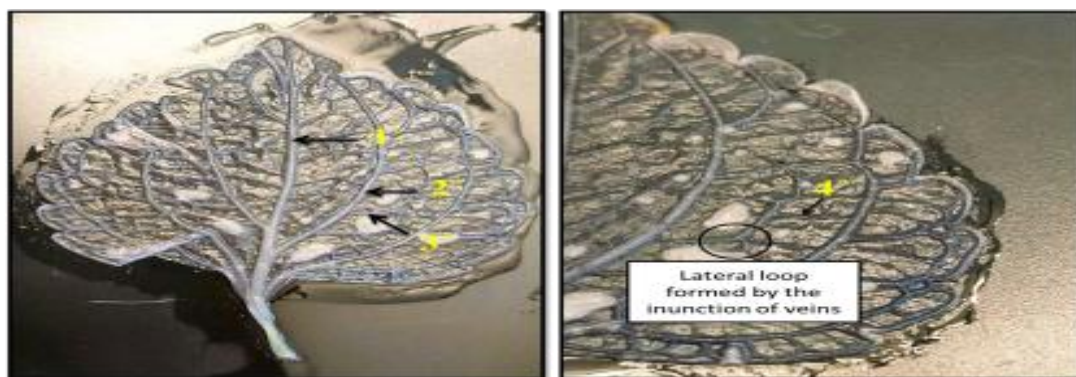


Figure 3. Venation of leaf appears. 1: the first veins, 2: the secondary veins, 3: the third veins, 4: the fourth veins and the lateral loop formed by the inunction of veins in the *Coleus scutellarioides*.

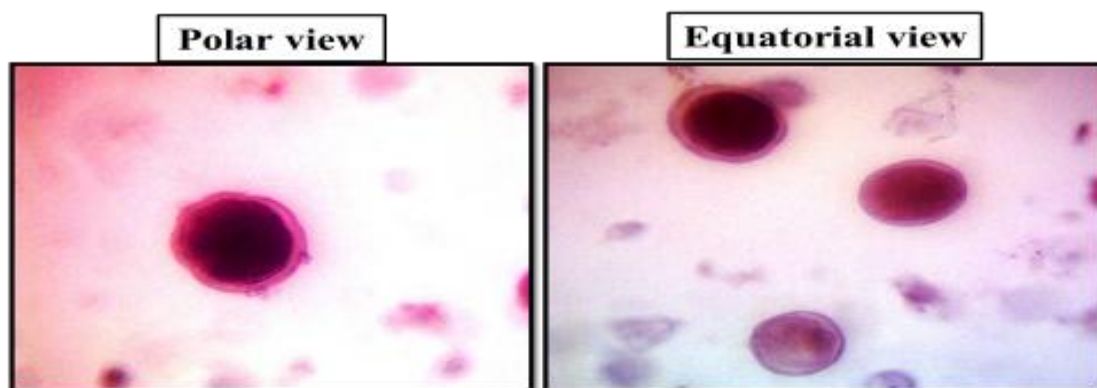


Figure 4. Pollen grains in the polar and equatorial views of the *Coleus scutellarioides*.

Table 1. The concentration of chemical compounds in the *Coleus scutellarioides*.

No.	Name	Conc. $\pm$ SD
1	Phenol	1.993 $\pm$ 0.005
2	Flavonoid	191.682 $\pm$ 2.273

CONCLUSIONS

In *Coleus scutellarioides*, the study indicated the anatomy of the leaf parts and pollen grains, in addition to the determination of phenolic content and total flavonoid. In herbal medicines, the leaves appeared broadly effective. The promising results based on anatomical features and chemical compounds will substantially promote its use in herbal medicines.

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