Anatomical study of Combretum indicum (L.) DeFilipps cultivated in Iraq

¹Muazaz A. AL-Hadeethi^{*}, ²Ali T. AL-Taie and ³Jwad K. Ali

¹College of Edu, for Pure Sciences-Ibn Al- Haitham, University of Baghdad, Iraq, ²Continuous Learning Center, Mustansiriyah University, Iraq. ³College of Agriculture, University of AL-Qasim Green, Iraq. Corresponding author: lubni_a75@yahoo.com.

Abstract

The current study included the anatomical characteristic Keywords: Anatomical study, Combretum indicum, of epidermal cells, indumentum and cross sections of Combretaceae family. roots, stems, leaves and petioles of Combretum indicum from Combretaceae family, which collected from the herbal garden at AL-Rashidia city north of Baghdad. The anatomical study showed that these characteristics are important in assessing morphological characteristics which can be used to diagnostic, isolation characteristics and study of this species.

Anatomical characteristics of plant parts (root, stem and leaf) were studied, and the most important research findings that the cross-section of the root is circular and in the secondary growth stage and the vascular bundles are bicollateral consist from the axial system and radial system of xylem and phloem, so in the xylem area appear clearly the early (spring) and late (summer) wood. on the other hand, the cross-section of stem is circular and in the secondary growth stage, consists from uniseriate epidermis covered by cuticle and many trichomes diffused in the service of epidermis after it has many layers of the cortex and the vascular bundles are bicollateral consist of axial system and radial system of xylem and phloem like as the root. As for the leaf epidermis appear stomata paracytic in shape and the cross-section of leaf contains upper and lower epidermis uniseriate and in the mesophyll showed numerous druses crystals diffuse in the cells. The midrib region of leaf in cross-section appeared crescent shape mainly composed of upper and lower epidermis, collenchyma layers contain many druses crystal, central vascular bundle, furthermore the petiole cross sections was recorded as oval in shape and the open collateral vascular bundles appeared in central of petiole crescent in shaped.

INTRODUCTION

Combretum indicum, also known as the Rangoon or Chinese honeysuckle creeper from the Combretaceae

family, is a tree, sharp or vine with clusters of red flower and the species is found in Asia and tropical Africa also it is found in many regions of the world as a cultivated or

wild and have many other names depending on growing in this countries, like as *Quiscual* spread out in Spain, *Niyog-niyogan* spread out in Filipina, *Madhumalti* spread out in India, *Akar Dani* spread out in Malay and *Madhuvi lota* spread out in Bengal [1].

The species is used as an herbal medicine in many country because have many chemical compounds and essential oils so the therapists used the root, seed and fruit extracts to treat the anthelmintic and for relieving diarrhea, so used the extract of root to treat rheumatism and used the fruit boiling for gargling and treatment the combat nephritis also the leaves of plants can be used to remove the pain caused by fever [2].

[3] refer to the *Combretum indicum* and description as a variable in flower shape, color and size and have been distinguished in tropical Asia. In Iraq, the species inter as a cultivated plant from India and China spread out of in many gardens and roads, also not have any study or description of the plant in Iraq so the aim of this study refer to the important parts of this species by anatomical way to appear the characters parts of root, stem, leaf and all the appendix of this parts.

METHODS AND MATERIALS

1. Collection and authentication of plant material

Combretum indicum plant was collected from the herbal garden at AL- Rashidiya city north of Baghdad (Latitude of Rashidiya: 33, 5528 (3333'10.080"N), Longitude of Rashidiya: 44, 3725 (4422'21.000"E)) at April 2017. The plant material was identified and authenticated by Flora of China [4] (Fig. 1).

2. Preservation the samples

The fresh samples of the root, stem and leaf are kept in formalin acetic acid (FAA) which was prepared according to [5] for 24-48 hours then preserved in 70% alcohol until the date of experiments.

3. Study of leaf epidermis

Peeling the epidermis of the leaf was done by mechanical scraping using the razor blade, followed by washing with distilled water and putting in 10% KOH, then passed through alcohol series for 10-15 minute, then stained in 1% Safranin for 30-45 minute, excess stain was washed off with distilled water, dehydrated by Alcohol series (70, 95, and 100%) and cleared by pure xylene at 10 minutes, finally, the epidermal samples were put on slides and mounted by cover slides with Dextrin Plasticizer Xylene [6].

The epidermis using stomatal index [7] as follows: Stomatal index =

(number of stomata)/(number of stomata

+ number of ordinary epidermal cells) × 100

4. Study the sectioning samples

For doing sectioning parts of root, stem, leaf and petioles of leaf, the fresh material of it was fixed in formalin acetic acid alcohol solution (FAA) at 48 hours and changed the solution after this time and put in the (70%) ethanol, then sectioned on a rotary microtome and stained with safranin and fast green stain and then mounted with Dextrin Plasticizer Xylene, the prestaining and staining procedure was performed according to [8].

RESULT AND DISSECTION

1. Study of leaves

In the surface view of leaf the epidermis consist from the stoma complex, the shape of stomata was paracytic type

that's mean has two subsidiary cells on the side of guard cells, the guard cells have kidney shape, the anticlinal walls of epidermal cells were winding (Fig. 2) and the measurements of epidermal cells and stomata are appearing in table 1, the results accepted with [9].

The cross section of leaf consist from the dermal tissue system this system represented the upper and lower uniseriate epidermis. From the current study it was clear that the leaf have upper epidermis covered by cuticle with the thickness ranging from 3.1μ m, also the thickness of upper epidermis was ranged from 10.5μ m, while thickness of lower epidermis ranged from 5.5μ m. The ground tissue system was represented by mesophyll which differentiated into palisade parenchyma and spongy parenchyma. The thickness of palisade layers was 30.1μ m and the thickness of spongy layers was 32.7μ m. The mesophyll of the species showed numerous druses crystals diffuse in the cells (Table 2 and Fig. 3)

The cross section of midrib region in the species under study revealed as crescent shape showed upper and lower epidermis after it located the cortex, which mainly composed of collenchyma located under on the both upper and lower epidermis followed by ordinary parenchyma cells contain many druses crystal, the vascular bundle central, open, collateral and crescent shape ranged to 88.5 μ m include xylem with variable size of vessels ranged by 6.1-6.7 μ m and phloem (Table 4 and Fig. 4). The results of this study agree with [9, 10 and 11].

2. Cross sections of root

Measurements of anatomical characters of the root are shown in the table 3. The shape outline of the root is circular and in the secondary growth stage, the cross section of it consists from uniseriate epidermis after it has located a thin layer of cortex, the cortex consists from parenchyma tissue and between as appears ordinary schizogenous intercellular space.

Vascular bundles are bicollateral consist from the axial system and radial system of xylem and phloem because the stem in the secondary growth stage and in the xylem area clear the early (spring) and late (summer) wood, pith located in the center of stem contain from isodiametric parenchyma cells. (Figures 5). The results of study agree with [11 and 12].

3. Cross section of stem

Measurements of anatomical characters of stems are shown in the table 4, the shape of outline stem is circular and in the secondary growth stage, the cross section of stem consists from uniseriate epidermis covered by cuticle and many trichomes diffused in the service of epidermis after it has many layers of the cortex.

Vascular bundles are bicollateral consist of axial system and radial system of xylem and phloem because the stem in the secondary growth stage, pith located in the center of stem contain from parenchyma cells of isodiametric cells. (Figures 6). The results of study agree with [11 and 12].

4. Cross section of petiole

The shape of petiole cross sections oval, the epidermis was uniseriate covered with cuticle, the average thickness of it 1.7 μ m and the average thickness of petiole epidermis reached to 5.3 μ m. After the epidermis represented the cortex consist from the collenchyma layer below the epidermis and the mass area of this cortex was occupied by ordinary parenchyma cells separated from other by ordinary schizogenous

intercellular space, the average thickness of cortex ranged from 109.1 µm. The vascular tissue system appeared in central of petiole open collateral in type and crescent in shaped, the average thickness of it reached to 59.7 μ m (Table 5 and fig. 7). The results of this study agree with [11, 13 and 14].

Table 1. Characters of epidermis and stomata in Combretum indicum (in	n micrometer).
---	----------------

Species	Epidermal cells				Stomata					
	Ada	Adaxial		Abaxial		Adaxial		Aba	axial	Stomatal index
	Length	Width	Length	Width	Length	Width	index	Length	Width	
Combretum indicum L.	10.5-30 (22.5)	12-15 (13.5)	22-33 (25.5)	11-15 (13.75)	21-25 (22.5)	11-17 (15.5)	12.75	15-20 (19.5)	15-18.5 (17.5)	14.5

*Between brackets = mean of 10 replicates

Table 2. Leaves anatomical characters in Combretum indicum (in micrometer).

Species		_	Blade	Midrib				
	Cuticle Upper thickness Epidermis thickness		Lower	Palisade layer	Spongy layer	Epidermis	Vascular	Vessel
			epidermis	thickness	thickness	thickness	bundle thickness	diameter
	(µm)	thickness	thickness				CHICKIC33	
Combretum indicum L.	2.3-3.4 (3.1)	10.5-11.3 (10.5)	5.4-6 (5.5)	28.3-32.5 (30.1)	29.8-35.7 (32.7)	4.5-5.1 (4.8)	86.4-90.5 (88.5)	6.1-6.7 (6.5)

*Between brackets = mean of 10 replicates.

Table 3. Root anatomical characters in *Combretum indicum* (in micrometer).

Species	shape	Epidermis thickness (µm)	Pith diameter	Root diameter			
Combretum indicum L.	circular	11.5-18.5 (15.5)	130-170 (155.5)	800.5-1120 (959.5)			
*Between brackets - mean of 10 replicates							

Between brackets = mean of 10 replicates

Table 4. Stem anatomical characters in Combretum indicum (in micrometer).

	Species	shape	Cuticle thickness (µm)	Epidermis thickness (μm)	Cortex diameter	Stem diameter			
	Combretum indicum L.	circular	2.5-3.5 (3.5)	9.5-15.5 (10.4)	140-200 (143)	500.5-820 (709.5)			
*Between brackets = mean of 10 replicates									

Between brackets = mean of 10 replicates

Table 5. Petiole anatomical characters in Combretum indicum (in micrometer).

Species	shape	Cuticle thickness (µm)	Epidermis thickness (µm)	Pith diameter	Vascular bundle thickness
Combretum indicum L.	circular	1.5-1.9 (1.7)	5.1-5.5 (5.3)	97.3-121.5 (109.1)	55.9-63.7 (59.7)

*Between brackets = mean of 10 replicates.



Figure 1. The shape of the species Combretum indicum.

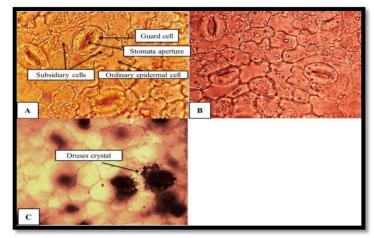


Figure 2. Stomata and ordinary cells in epidermis, A. Adaxial epidermis, B. Abaxial epidermis, C. Druses crystals in *Combretum indicum*.

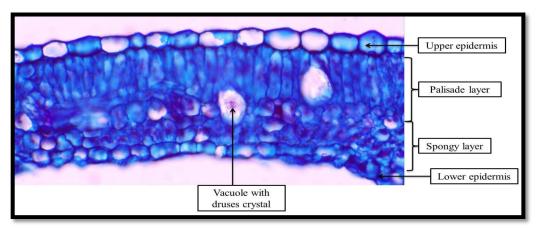


Figure 3. The cross section of blade of leaf in *Combretum indicum*.

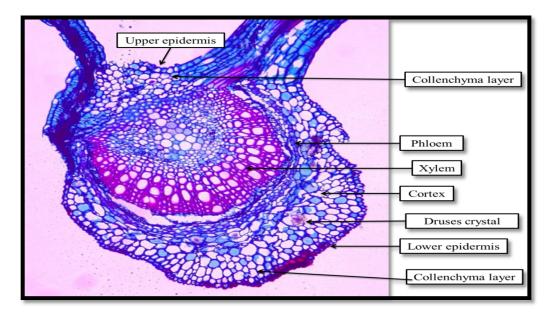


Figure 4. The cross section of midrib of leaf in *Combretum indicum*.

Figure 5. The cross section of root in *Combretum indicum*.

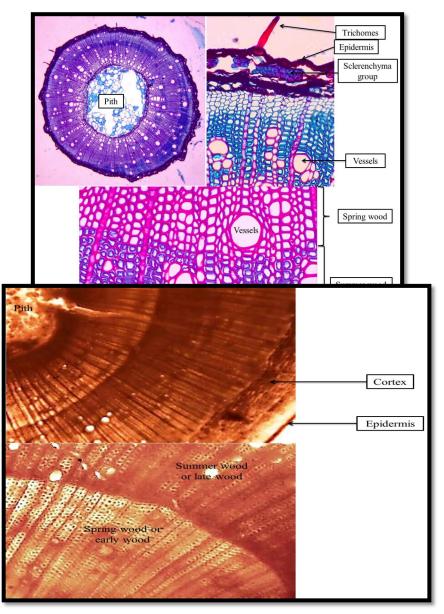


Figure 6. The cross section of stem in *Combretum indicum*.

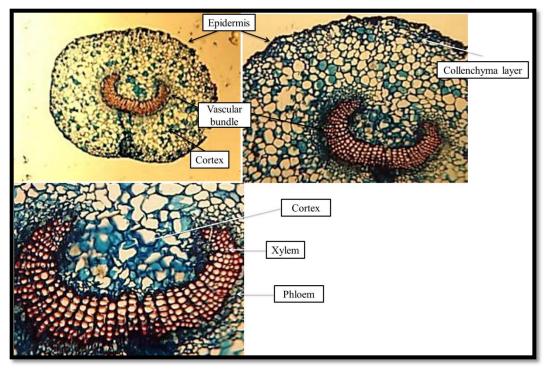


Figure 7. The cross section of petiole of leaf in *Combretum indicum*.

REFERENCES

- 1. Munir, M.; Lu, L. and Mcgonigl, P. (1995). Excitotoxic cell death and delayed rescue in human neurons derived from NT2 cells. Journal of Neuroscience, 15 (12): 7847–7860.
- 2. Murphy, T. H.; Schnaar, R. L.; Coyle, J. T. and Sastre, A. (1988). Glutamate cytotoxicity in a neuronal cell line is blocked by membrane depolarization. Brain Research, 460 (1):155–160.
- 3. Burkill, H.M., 1985. The useful plants of West Tropical Africa. 2nd Edition. Volume 1, Families A–D. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 960 pp.
- 4. Jie C. and Turland N. J., 1984. COMBRETACEAE. Flora of china. ed., Fl. Reipubl. Popularis Sin. 53(1): 1–28.
- 5. Johanson AD, 1940. Plant Microtechnique .1st ed. Mc. Graw-Hill Book Company, New York and London, 523 PP.
- 6. Foster WD, 1977. Freehand sectioning of bryophytes. Bull. Brit. Bryol. Soc. 29: 21.
- 7. Steel RGD, and Torrie JH. 1981. Principles and Procedures of Statistic. Mcgraw. Hill Book Co., Inc. N. Y. pp. 485.
- 8. Thammathaworn, A. (1996). Handbook by paraffin method. Department of biology, Faculty of science, Khon Kaen Universality, Thailand.
- 9. Exell, A. W. (1931). The genera of Combretaceae. J. Bot. (Lond.), 69: 113-128.
- Eames, A. J. (1929). The role of floral anatomy in the determination of angiosperm phylogeny. Proc. Int. Cong. Plant Sci. Ithaca, N. Y. I, 423-427.
- 11. Esau K, 2006. Esau's Plant anatomy: meristems, cells, and tissues of the plant body, their structure, function, and development. Ray F. Evert. (3rd Ed.). Hoboken, New Jersey. Canada, 607PP.
- Aguilar, N.O., 1999. Quisqualis L. In: de Padua, L.S., Bunyapraphatsara, N. & Lemmens, R.H.M.J. (Editors). Plant Resources of SouthEast Asia No 12(1). Medicinal and poisonous plants 1. Backhuys Publishers, Leiden,

Netherlands. pp. 421–424.

- 13. Eames, A. J. (1931). The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. Am. J. Bot., 18: 147-188.
- Eames, A. J. and Mac-Daniels, L. H. (1951). An Introduction to Plant Anatomy. McGraw. Hill Book Co., N.Y.