



Stem histochemistry analysis of three medicinal species from Iraq

Marwan Noori Mohammed and Issra Kareem Nassrullah*

Dep. of Biology, College of Education For Pure Science (Ibn AL-Haitham), University of Baghdad, Iraq.

*Corresponding author: israanassrullah@yahoo.com

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Abstract

The chemical constituents act as protection and defense the plant body, but a lot of these compounds are extracted and used for medical purposes, active chemical compounds are used for treatment diseases, so it necessary to study the chemical contents in the secretory structure of the plants. The objective of the study was to determine the secretory structures and its chemicals in the stem of *Anethum graveolens* L., *Catharanthus roseus* (L) G.Don and *Ruta graveolens* L. The samples were collected from different region in Baghdad before the flowering period and used fresh for anatomical and histochemical analysis, test conducted for detection total lipids, terpenes, acidic and neutral Lipids, phenolic compounds, lignin, tannins, pectin, starch, alkaloids and proteins. The secretory structures found mainly as idioblast among the species and especially as secretory canals in *Anethum graveolens*, lactiferous canals in *Catharanthus roseus* and as secretory cavities in *Ruta graveolens*.

Keywords: Stem, Histochemistry , *Anethum graveolens* , *Catharanthus roseus*, *Ruta graveolens*.

Introduction

Medicinal plants possess therapeutic properties or beneficial pharmacological effects on the body (Majeed and Khairallah, 2020). *Anethum graveolens* commonly known as dill, is a member of the family Apiaceae (Umbelliferae), in Iraq, this family include about 60 genera and 143 species (Al-Mousawi, 1987). The gas chromatography of seeds oil shows the presence of two major compounds which are D-carvone and D-limonine (Al-Bazaz *et al.*, 2020), the oil contain 43-63 % of carvone this oil used for flavoring and pharmaceuticals (Everitt and Lockwood, 1995). Dill oil has a therapeutic value and great activity against many bacteria (Nawrooz, 2022; Mahdi, 2016), Studies concerning the insecticidal activity have shown toxicity to the rice weevil *Sitophilus oryzae* (Su, 1985), cowpea beetle *Callosobruchus maculatus* (Essa and Daoud, 2005), corn aphid *Rhopalosiphum maidis* (Kadhim and Kadhim, 2017).

Apocynaceae represent by four species in Iraq, others are culutured as *Catharanthus roseus* (Al-Mousawi, 1987), which is an important medicinal plant of the family Apocynaceae that used for diabetes, blood pressure, asthma, constipation, and cancer (Sain and Sharma, 2013), also have antibacterial effect (Al-Hayanni and El-Shora, 2020), Studies showed insecticidal activity against corn

aphid *Rhopalosiphum maidis* (Kadhim and Kadhim, 2017).

Rutaceae members include permanent shrubs and trees that have glandular dotted and aromatic, in Iraq represented by one genus *Haplophyllum* and other genus are cultured as *Ruta* (Al-Mousawi, 1987), which have antibacterial effect (Al-Bazaz, 2011), insecticidal effect against workers of termites *Microcerotermis gabriles* (Thamer, 2008), bugs *Monosteria uncostata* (Al-Obada, 2011) and *Culex pipines molestus* (Mekhlif and AbdulRazzak, 2019).

The anatomical study of the secretory structures is important to ensure the correct identification of the species (Fahn, 1988). The secondary metabolism compounds that product by the plant contribute in protection and defense in addition to attract the pollinators (Evert, 2006).

Materials and Methods

The species *Anethum graveolens*, *Catharanthus roseus* and *Ruta graveolens* were collected from different region in Baghdad before the flowering period and used fresh, for anatomical study the cross section take from the middle of the internode by free hand sectioning, then stained with 2 % safranin and 1% fast green (Nassrullah, 2007). For the histochemical study also used fresh samples and free hand sectioning but with noticed that when used razor blade for lipid analysis, we must to wash it with tap water to remove the oil from the blade

margin. The reagents used are Sudan IV for total lipids, NADI reagent for terpenes, Nile blue for acid and neutral lipids, Ferric chloride for total phenolic compounds (Demarco, 2017), Toluidine blue O for tannins (Yeung *et al.*, 2015), Floroglucinol for lignins, Ruhtenium red for pectins and mucilages, Lugol's

reagent for starch, Wagner's reagent for alkaloids and Comassie blue for proteins (Demarco, 2017), the color of the reaction between reagent and chemical compounds illustrated in table (1), the slides photographed by light microscope coupled with digital camera.

Table 1. Color of the reaction between reagent and chemical compounds.

Reagent	Chemical compound	Reaction color
Sudan IV	total lipids	red or red-orange
NADI	Essential oils (monoterpenes and sesquiterpenes)	blue
	resins (diterpenes, triterpenes, tetraterpenes and derivatives)	red
	Mixtures of essential oils and resins.	violet to purple
Nile blue	acidic lipids	blue
	neutral lipids	pink
Ferric chloride	total phenolic	usually black sometimes brown.
Toluidine blue O	tannins	greenish blue or bright blue
Floroglucinol	lignins	pink to red
Ruhtenium red	pectins and mucilages	magenta or red
Lugol	starch	dark blue to black
Wagner	alkaloids	red or red-brown
Coomassie blue	proteins	blue

Results and Discussion

The anatomical characters of the stems illustrated in table (2 and 3), the secretory structure on the epidermis occurred as Trichome in *Catharanthus roseus* only, other structures founded internally as secretory canal, lactiferous canal, secretory cavity and as idioblast. In the idioblast cells the vacuoles occupied by chemicals compounds, these cells found in the parenchyma tissue. Some chemicals found in the phloem.

Anethum graveolens: The stem appears with ribs associated with collenchyma patches which found under the epidermis and alternate with chlorenchyma (Fig.1, A), chlorenchyma appear like palisade tissue, the pith region is hollow, the vascular cylinder composed of isolated vascular bundles, the secretory canals placed between the collenchyma patches and the vascular bundles (Fig.1, B).

In the histochemical tests, the lipids restricted to the cuticle as essential oil (Fig.1, D), phenolic compound found in some epidermal cells (Fig.1, E), lignin found in the cell wall of the vessels (Fig.1, F), tannins observed in the epithelial cells of the secretory canals (Fig.1, G) and some epidermal cells (Fig.1, H),

pectins observed densely in the cell wall of collenchyma tissue (Fig.1, I), starch found in the parenchyma (Fig.1, J), alkaloids found in the cortex near the vascular bundles (Fig.1, K) and the proteins distribute densely in the phloem (Fig.1, L).

Catharanthus roseus: The siphonostelic with intraxylary phloem (Fig.2, A), the intraxylary phloem found as isolated bundles in the margin of the pith (Fig.2, B), the parenchyma of the cortex include lactiferous canals near the epidermis and embedded in the chlorenchyma, hairs found glandular, unicellular with average length 138 μm and 12 μm diameter.

In the histochemical tests, the lipids found in the lactiferous canals and the droplets that dispersed from it (Fig.2, C), the lactiferous canals have mixtures of essential oils and resins (Fig.2, D), some droplets appear have neutral lipids (Fig.2, H) other acidic lipids (Fig.2, I), also some trichome appear it have acidic lipids (Fig.2, F) and some have neutral lipid (Fig.2, G). Lignin found in the xylem region (Fig.2, J), tannins is absent, pectins found in the cell wall of the collenchymas, starch absent, alkaloids observed in the intraxylary phloem (Fig.2, K) and proteins in the most of the phloem (Fig.2, L).

Ruta graveolens: The siphonostelic was with narrow parenchyma rays, the vascular bundles have a bond of sclerenchyma strands represent a cup of fibers (Fig.3, A), the secretory cavities placed in the cortex under the epidermis (Fig.3, B)

In the histochemical tests, the lipids accumulate in the secretory cavities as essential oil (Fig.3, D) and as acidic lipids (Fig.3, E) , phenolic compounds observed in the cells that surround the secretory cavities (Fig.3, F), lignin found in the xylem region (Fig.3, G) and in the stone cells (Fig.3, H), tannins absent, pectins observed in the xylem parenchyma near the pith (Fig.3, I), starch found in the parenchyma of cortex and pith (Fig.3, J), alkaloids observed as idioblast in the pith (Fig.3, K) and proteins found in the phloem and xylem parenchyma near the cortex (Fig.3, L)

Most of the chemical contents are found in the idioblasts, idioblasts are cells that different by having distinct shape, size or chemicals from other cells of a tissue, the idioblasts accumulate or secrete a compounds like oil, resin, mucilage and tannin (Fahn, 1988), these different cells occurred in a large distribution in the stems of the medicinal species that studied.

The stem of *A.graveolens* appears with ribs and palisade-like chlorenchyma and this agree with Metcalf' and Chalk (1950) conclusion that the stems of Umbelliferae family have ribs of collenchyma or sclerenchyma and sometimes with palisade chlorenchyma between the ribs. The secretory canals found with tannin epithelial cell and this did not match with Metcalf opinion of that the secretory canal may contain oils, resin or mucilage in Umbelliferae stems. The occurrence of alkaloids in

the stem of *Anethum* agree with Muhammad and Al-Khesraji (2021) they indicated the presence of alkaloids in the shoot. Dill tablet are used as lipid lowering due to the presence of tannins which possess potential therapeutic influence as anti-hyperlipidemia agents (Yaseen and Jasim, 2020)

Lactiferous canals always present in Apocynaceae stem and the pericycle with intraxylary phloem except the genus *Pachypodium* Metcalf and Chalk (1950). The medicinal important of *C. roseus* related to accumulation of Alkaloides (Sain and Sharma, 2013), the therapeutic agent for cancer may be related to the alkaloids vincalkebostine which possess anticancer properties. (Al-Rawi and Chakravarty, 1988; Mohammed, 2012)

Hairs not observed in *R.graveolens* and the secretory cavities found with essential oils contents, placed in the parenchymatous tissue and that agree with Metcalf and Chalk (1950), the therapeutic effect is due of presence alkaloids (Petit-paly *et al.*, 1989), stem has the presence of a bundle cap fibers (bundle sheath) which consists of sclerenchyma fibers and this observe in many plant(Al-Joboury and Aliwy, 2022).

The investment in starch and proteins reserves at the stem may be associated with the fact that the species is annual, with the need for fast metabolism in a short period of time. (Nurit-Silva and Agra, 2011).The medicinal properties of the species associated with the distribution of secondary compound in the different organs, histochemical technique are fast and cheap methods that can be used to identify bioactive classes of compounds in the tissues (Demarco, 2017).

Table 2. Anatomical characters of the stems measured with micrometer

species	Cuticle		Epidermis		Collenchyma			Chlorenchyma		Parenchyma		Pith dim.
	Th.	Th.	No.	Th.	No.	Type	Th.	No.	Th.	No.		
<i>A. graveolens</i>	3.98	17.33	1	97.06	8-10	annular	64.13	3-4	83	3-4	1960	
<i>C. roseus</i>	4.06	17.16	1	32.90	1-2	tangential	26.82	2-3	212	7-9	728	
<i>R. graveolens</i>	5.37	15.60	1	-	-	-	47.30	2-4	150	8-11	1548	

Table 3. Vascular bundle characters measured with micrometer

species	Bundle			Fiber Th.	Phloem Th.	Cambium Th.	Xylem Th.
	Length	Width	No.				
<i>A. graveolens</i>	234.33	34.58	24-26	-	39.86	32.06	138.66
<i>C. roseus</i>	-	351	1	31.5	47.66	29.46	252.48
<i>R. graveolens</i>	307.30	234	14-18	36.7	60.60	46.15	243.75

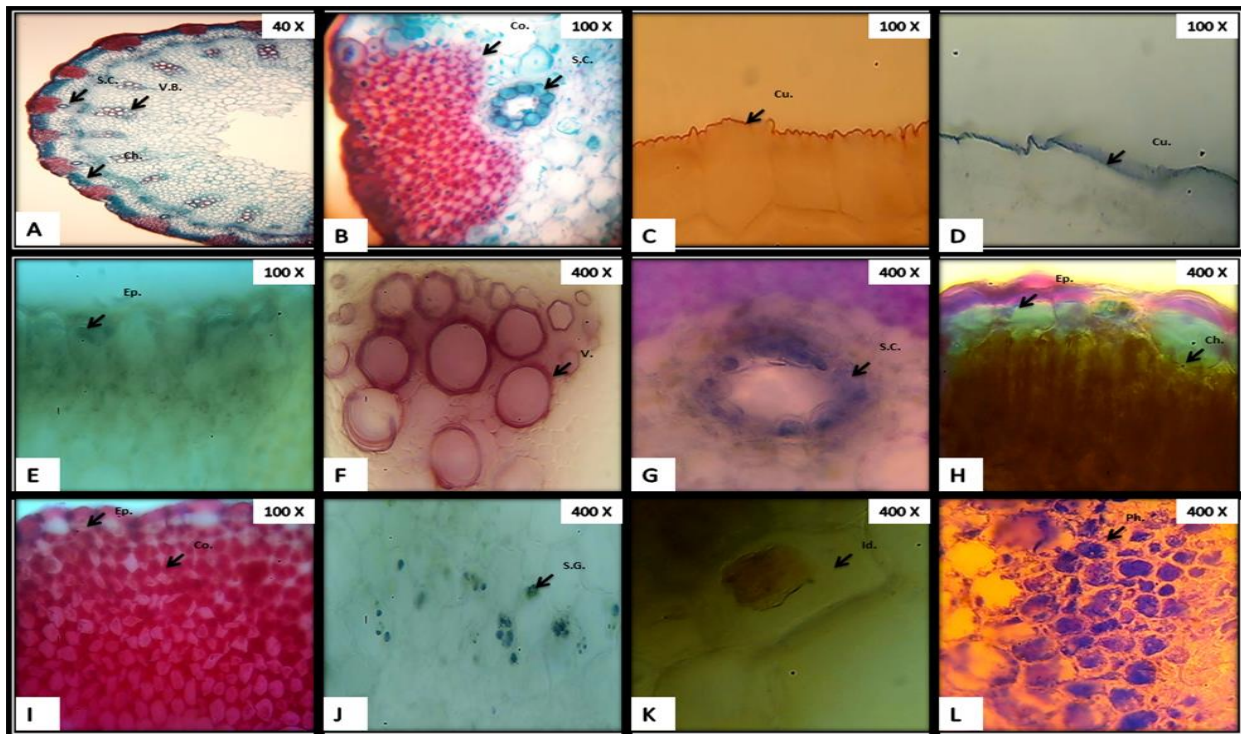


Figure 1: Transverse sections of *Anethum graveolens* stem, (A, B) anatomical characters, (C-L) histochemical test: C sudan IV, D nadi, E ferric chloride, F floroglucinol, G, H toluidine, I ruthenium, J Lugol, K wagner, L comassie. Abbreviations: S.C. Secretory canal, V.B. vascular bundle, Ch. Chlorenchyma, Co. Collenchyma, Cu. Cuticle, Ep. Epidermis, V. Vessels, S.G. Starch grain, Id. Idioblast, Ph. Phloem.

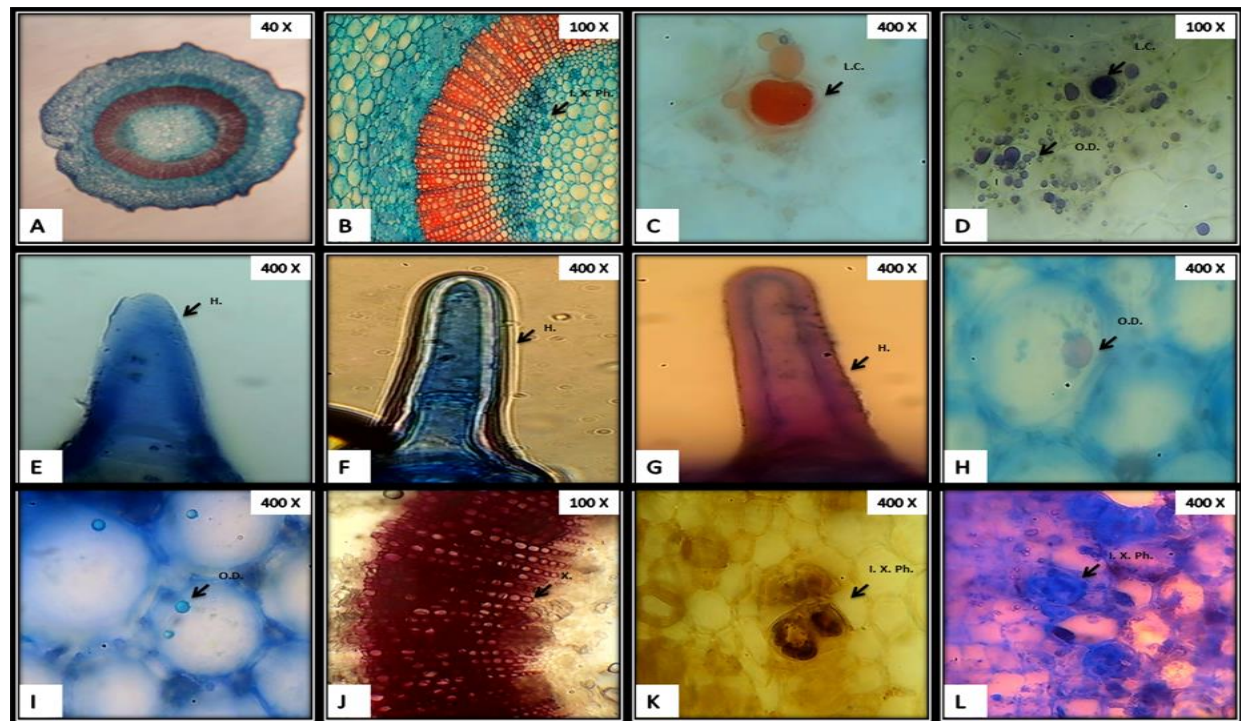


Figure 2: Transverse sections of *Catharanthus roseus* stem, (A, B) anatomical characters, (C-L) histochemical test: C sudan IV, D nadi, (E-I) Nile blue, J floroglucinol, K wagner, L comassie. Abbreviations: I.X.Ph. Intraxylary phloem, L.C. Lactiferous canal, O.D. Oil droplet, H. Hair, X. Xylem.

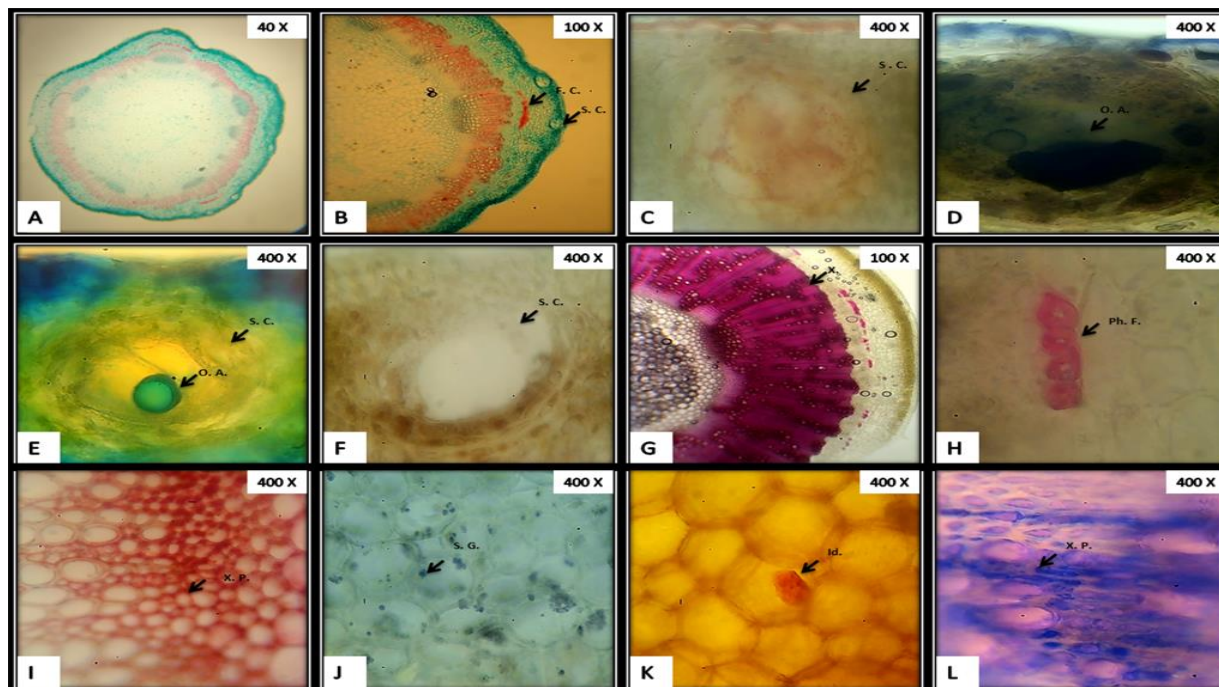


Figure 3: Transverse sections of *Ruta graveolens* stem, (A, B) anatomical characters, (C-L) histochemical test: C sudan IV, D nadi, E Nile blue, F ferric chloride, G, H floroglucinol, I ruthenium, J Lugol, K wagner, L comassie. Abbreviations: F.C. Fibre cup, S.C. Secretory cavity, O.A. Oil accumulation, X. Xylem, Ph.F. Phloem fibre, X.P. Xylem parenchyma, S.G. Sarch grain, Id. Idioblast.

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