

## MACRO- AND MICROMORPHOLOGICAL STUDY OF *SALVIA MERJAMIE* FORSSK GROWING IN SAUDI ARABIA

Ebtesam S. Al-Sheddi<sup>1</sup>, Fawkeya A. Abbas<sup>2\*</sup> and Adnan J. Al-Rehaily<sup>1</sup>

<sup>1</sup> Pharmacognosy Department, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.

<sup>2\*</sup> Pharmacognosy Department, College of Pharmacy, Zagazig University, P.O. Box 44519 Zagazig, Egypt.

### ABSTRACT:

The macro- and micromorphological characters of the leaves, stems and roots of *Salvia merjamie* Forssk growing in Saudi Arabia are described to find out the diagnostic features of these organs by which the plant can be identified in both entire and powdered forms. Moreover, microscopical numerical values of leaves and certain Pharmacopoeial constants such as crude fibre, total ash, water soluble ash, acid-insoluble ash and moisture content were determined according to the British Pharmacopoeia to authenticate the plant species.

### INTRODUCTION

*Salvia merjamie* Forssk belongs to the family Lamiaceae, which has 252 genera and some 6700 species<sup>(1)</sup>. The genus *Salvia* belongs to the Nepetoideae, which is the largest sub-family and it comprises about 900 species of shrubs, subshrubs, perennial or annual herbs<sup>(2)</sup>. The genus is distributed in the tropics, temperate regions and endemics to Turkey<sup>(3)</sup>. *Salvia* species contain monoterpenes with antiseptic characteristics<sup>(4)</sup>. In recent studies on this plant species, it has been observed that the compounds decrease DNA synthesis in the cell. This feature is important in the diagnosis and treatment of cancer<sup>(5)</sup>. *Salvia* species are grown in parks and gardens as ornamental plants<sup>(6)</sup>. Essential oil, which gives off a fragrance, is a characteristic feature of many species of *Salvia*<sup>(7)</sup>. For this reason, it is widely used in perfumery and as a sweetener in the food industry<sup>(8)</sup>. *Salvia* species are used as traditional medicines all around the world possessing antibacterial<sup>(6,7)</sup>, antioxidant, antidiabetic<sup>(9)</sup>, antitumor<sup>(9)</sup>, and antituberculous<sup>(10)</sup>. In Saudi Arabia, nine species are reported, which are *S. aegyptiaca* L., *S. deserti* Decne, *S. lanigera* Poir, *S. merjamie* Forssk, *S. palaestina* Benth., *S. schimperii* Benth., *S. spinosa* L., *S. tingitana* Edl. and *S. verbenca* L.<sup>(2,11)</sup>. Phytochemical screening of *Salvia merjamie* Forssk revealed the presence of carbohydrates, flavonoides, steroids and/or triterpenes. Nothing, however, could be traced dealing with macromorphology and micromorphology of *Salvia merjamie* Forssk growing in Saudi Arabia. This encouraged the authors to carry out the present study, with the aim of finding out the diagnostic features by which the plant could be identified.

In the present work, the macro and micromorphology of the leaves, stems and roots of *Salvia merjamie* Forssk were studied to show the diagnostic characters of each organ to ease the identification of the plant in both entire and powdered form. The study also includes determination of numerical values of leaves and certain Pharmacopoeial

constants such as crude fibre, total ash, water soluble ash, acid-insoluble ash and moisture content to authenticate the plant species<sup>(12)</sup>.

### EXPERIMENTAL

#### Plant Material

The plant materials used for this work was collected during the flowering period in March 2006 from Al-Souda, Southeast Abha City, which is located in Southern region of Saudi Arabia. The plant was kindly identified by Dr. M. Atiqur Rahman in the Taxonomy division of the Department of Pharmacognosy, College of Pharmacy, King Saud University, and a voucher specimens were deposited in its herbarium. Fresh materials and samples kept in 70% glycerol were used.

#### I. Macromorphology

*Salvia merjamie* Forssk (Fig. 1) is aromatic multi-stemmed pilose, erect, annual herb, reaches up to 40 cm in height. The flowers (March - Jun) are variable in colour either blue, purple, dark red or pink and white. Verticillasters are 5 to 8-flowered arranged in racemose inflorescence. Bract are 14-17x15-20 mm, broadly ovate, lower surface lanate and upper surface green coloured. Calyx is 10 to 15-nerved, tubular-campanulate, bilabiate, upper lip usually shortly 3-dentate, rarely entire, lower lip with almost lanceolate teeth. Floral leaves are smaller than calyx. Corolla is purple to white, 12 mm long bilabiate with falcate upper lip. The corolla tube is scarcely exerted. Stamens are also exerted and the anthers are black 2.775 x 2 mm ovoid keeled on inner face and mucilaginous on wetting<sup>(1,11)</sup>.

The root (Fig.1 and A<sub>1</sub>): is a dark brown tap root, bearing several short tapering lateral roots and measures 13.5 to 21 cm in length and 0.6 to 1.6 cm in diameter at the widest part with pale-brown hard bark. The fresh root is flexible, while the dry breaks with short fracture.

The stem (Fig.1 and A<sub>2</sub>): is more or less quadrangular, up to 40 cm in length and 0.2 to 0.3 mm in diameter. It is green in colour, sympodially

\* Corresponding author

branched; densely short hairy or spreading long hairy, densely glandular inflorescence axils. The stem is flexible, but when dry, it breaks with a fibrous fracture.

The leaves (Fig. 1 and A<sub>1</sub>): The leaves are simple, cauline, mostly rosetted and exstipulate with crenate margin. They are lanceolate and measure 5 to 7.5 cm in length and 1.5 to 2 cm in breadth. The leaves have grayish green surfaces covered with glandular and eglandular hairs and show obtuse or acute apices, symmetric bases and papery texture. They have reticulate venation with prominent midrib and main veins on the lower surface only with the area between veinlets strongly depressed. The petiole is winged and measures 1.5 to 5 cm length and 0.1 to 0.2 cm width. The leaves have an agreeable odour and slight characteristic taste.

## II. Micromorphology

### The root

The transverse section in the root (Fig. 2) is circular in outline showing an outer brown cork followed by a wide parenchymatous phelloderm containing scattered groups of stone cells surrounding a central cylinder of vascular tissue. The vascular tissue comprises a narrow phloem containing sclerenchymatous cells and a wide xylem with a central tetrarch primary xylem.

The cork (Fig. 2 and Fig. 3) is wide, consists of about 7 to 10 rows of polygonal thin-walled, tangentially elongated cells arranged in radial rows. The cells have slightly lignified walls, with brownish contents acquiring dark violet colour with alkali. They measure 23 to 92  $\mu$  in length, 14 to 51  $\mu$  in breadth and 38 to 50  $\mu$  in height.

The phelloderm (Fig. 2) is wide, formed of 12 to 20 rows of thin-walled, more or less tangentially elongated parenchymatous cells containing starch granules and scattered small or large groups of sclereids (3 to 26) measuring 52 to 162  $\mu$  in length and 19 to 34  $\mu$  in diameter. These vary in structure from isodiametric to elongated, having wide to very narrow lumen, thin or thick lignified walls, with simple pits, and stained green with 66% H<sub>2</sub>SO<sub>4</sub>.

The vascular tissue (Fig. 2) is formed of an outer cellulose phloem containing sclereids similar to that present in the phelloderm and a wide lignified central xylem; the cambium is almost distinguishable. The xylem is formed mainly of fibres (Fig. 2 and Fig. 3) having almost thin lignified walls with slit-like pits, narrow or wide lumen and acute or blunt apices. They measure 70 to 180  $\mu$  in length and 15 to 18  $\mu$  in diameter. Vessels (Fig. 2 and Fig. 3) are isolated or in radial rows of 2-4 up to 8 or in small groups, they are mostly pitted with bordered pits, or occasionally spiral, annular, pitted and measure 18 to 59  $\mu$  in diameter. Tracheids and tracheidal vessels (Fig. 3) are few showing moderately thick lignified walls with numerous oval bordered pits and measure 160 to 170  $\mu$

in length and 15 to 17  $\mu$  in diameter. Wood parenchyma are diffuse, formed of moderately thick-walled pitted or lignified polygonal axially elongated cells. The medullary ray (Fig. 2) are 2 to 5 cells wide being cellulose in the phloem and lignified in the xylem and containing starch granules.

### Powdered root

The powdered root (Fig. 3) is brownish-yellow in colour. It is characterized microscopically by the following features;

1. Fragments of brown cork showing polygonal cells with thin, slightly lignified walls.
2. Fragments of isodiametric or elongated sclereids with wide or very narrow lumen, thin or thick lignified walls and showing simple pits.
3. Fragments of lignified spiral, annular and pitted vessels.
4. Numerous fragments of lignified wood fibres having moderately thick walls, wide lumen and acute apices.
5. Fragments of lignified polygonal and moderately thick-walled pitted wood parenchyma, tracheids and tracheidal vessels.
6. Abundant, small simple polyhedral starch granules with centric hilum.

### The stem

The transverse section in stem (Fig. 4) is more or less quadrangular in outline. It shows an outer epidermis surrounding a relatively narrow parenchymatous cortex with outer 1 to 3 rows of collenchyma and lined by differentiated endodermis enclosing a ring of 12 to 41 separated vascular bundles each with an arch of non-lignified pericyclic fibres surrounding wide non-lignified pith.

The epidermal cells (Fig. 5) are polygonal axially elongated with straight anticlinal walls and covered with thick striated cuticle. They measure 81 to 120  $\mu$  in length, 23 to 47  $\mu$  in breadth and 16 to 45  $\mu$  in height.

Stomata (Fig. 5) are few of the anisocytic type each is surrounded by 3 to 4 cells and measures 30 to 43  $\mu$  in length and 17 to 35  $\mu$  in breadth.

Trichomes (Fig. 5) are very numerous. They are of glandular and eglandular types. The glandular hairs have a short uni- or bi-cellular stalks and large uni- or bi-cellular secretory heads, the cuticle was thin. They measure 30 to 55  $\mu$  in length. The eglandular hairs are abundant and larger. They are uniseriate multicellular (1 to 6) with thick walls, acute apices, warty cuticle and measure 69 to 471  $\mu$  in length.

The cortex (Fig. 4) is parenchymatous with subepidermal layer of collenchyma. The collenchyma consists of 1 to 3 rows of small thick-walled cellulose cells present particular at the corner of stem. The parenchyma is formed of 5 to 7 rows of large thin-walled cells with narrow intercellular spaces.

The endodermis is well differentiated and having no casparian strip.

The pericycle (Fig. 4) is formed of 10 to 12 rows of thick-walled cellulosic fibres present as an arch between the cortex and the vascular bundle region and present particularly at the corner of the stem. The fibres (Fig. 5) have thick walls with acute to blunt apices. They measure 100 to 225  $\mu$  in length and 12 to 15  $\mu$  in diameter.

The vascular tissue consists of an outer cellulosic phloem and a wide lignified xylem; the cambium is almost indistinguishable. The xylem is formed of fibres (Fig. 4 and Fig. 5) having moderately thick - lignified walls, narrow or wide lumen and acute apices. They measure 210 to 306  $\mu$  in length and 11 to 33  $\mu$  in diameter. Vessels (Fig. 4 and Fig. 5) are lignified, diffused either isolated or in radial rows being spiral, annular and pitted and measure 11 to 25  $\mu$  in diameter. Wood parenchyma (Fig. 5) is diffused and formed of moderately thick-walled pitted and lignified polygonal axially elongated cells. Medullary rays (Fig. 4 and Fig. 5) are 2 to 3 cells wide being cellulosic in the phloem and lignified in the xylem.

The pith (Fig. 4) is formed of moderately thick cellulosic parenchyma with narrow intercellular spaces.

#### Powdered Stem

The powdered stem (Fig. 5) is green in colour and characterized microscopically by:

1. Fragments of epidermal cells; polygonal, axially elongated with straight anticlinal walls, covered with thick-striated cuticle and showing few anomocytic stomata.
2. Numerous glandular hairs with short uni- or bi-cellular stalk and large uni- or bi-cellular secretory heads.
3. Numerous eglandular hairs with uniseriate multicellular (1 to 6) with thin walls, wide lumen, acute apices and warty cuticle.
4. Fragment of lignified spiral, annular and pitted vessels.
5. Fragments of lignified wood parenchyma.
6. Fragments of non-lignified pericyclic fibres.
7. Fragments of lignified wood fibres.
8. Calcium oxalate and starch are absent.

#### The leaf

The transverse section of the leaf (Fig. 6A and Fig. 6C) shows dorsiventral structure with three rows of upper palisade being interrupted in the midrib region. The midrib projects on the both sides and shows a single crescent - shaped vascular bundles, the pericycle is collenchymatous

The upper epidermal cells of the leaf (Fig. 6A and Fig. 7A) are formed of a tabular, polygonal nearly isodiametric cells having beaded anticlinal walls and covered with thick striated cuticle. They measure 28 to

95  $\mu$  in length, 11 to 46  $\mu$  in breadth and 16 to 37  $\mu$  in height. The lower epidermis of the leaf (Fig. 7A) consists of tabular, polygonal, isodiametric cells with wavy anticlinal walls and covered with thick striated cuticle. They measure 45 to 126  $\mu$  in length, 11 to 52  $\mu$  in breadth and 15 to 34  $\mu$  in height.

Stomata (Fig. 7A) are present on both surfaces. They are more frequent on the lower epidermis. They are sunken, they are of diacytic and occasionally of the anomocytic type. They measure 32 to 39  $\mu$  in length and 18 to 26  $\mu$  in width. Both epidermis show glandular and eglandular trichomes similar to those of the stem. The second type of trichomes is labiate. The hair consists of a short stalk and large globular head. The stalk is short unicellular measuring 20 to 30  $\mu$  in length and 10 to 25  $\mu$  in width. Head is multicellular composed of 8 radiating cells. It measures 50 to 102  $\mu$  in diameter and covered with thin cuticle.

The upper and lower neural epidermal cells (Fig. 7B) are polygonal axially elongated with beaded anticlinal walls, granular surface and covered with smooth cuticle. They measure 55 to 141  $\mu$  in length and 10 to 44  $\mu$  in breadth.

The mesophyll (Fig. 6A and Fig. 6C) is dorsiventral with an upper palisade formed of three rows of radially elongated columnar cells with straight anticlinal walls and measure 32 to 43  $\mu$  in length and 6 to 15  $\mu$  in breadth. It is interrupted by collenchyma in the midrib region. The spongy tissue (Fig. 6C) is formed of 2 to 3 rows of thin-walled more or less rounded parenchymatous cells with wide intercellular spaces.

The cortex of the leaf (Fig. 6A and Fig. 6B) is parenchymatous with upper and lower subepidermal collenchyma abutting the upper and lower epidermises and formed of 2 to 3 rows of thick-walled cellulosic cells.

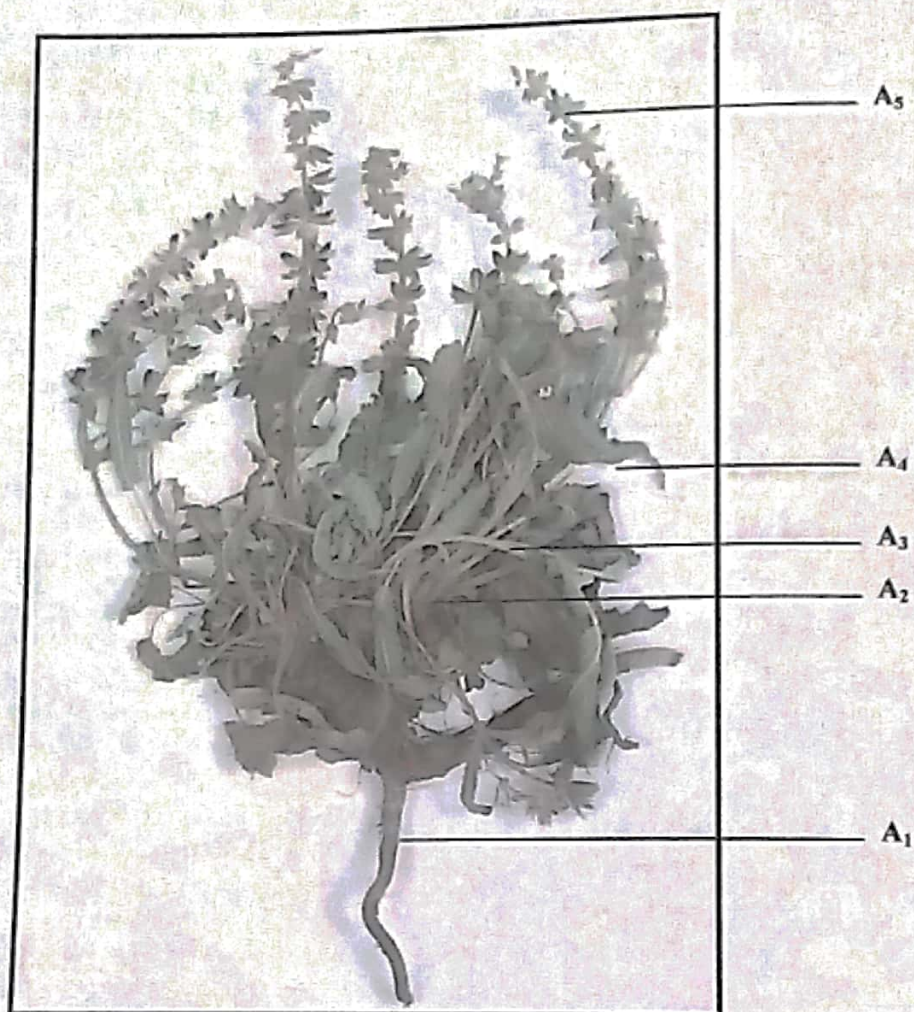
The pericycle (Fig. 6A and Fig. 6B) consists of two arcs of collenchymatous cells above and below the vascular bundle.

The vascular bundle (Fig. 6A and Fig. 6B) consists of cellulosic phloem of thin-walled elements and xylem formed of lignified spiral and annular vessels, 10 to 20  $\mu$  in diameter and thin-walled cellulosic parenchyma.

#### Powdered Leaf

The powdered leaves (Fig. 7) are dark green in colour and having characteristic odour. The powder is characterized microscopically by following features:

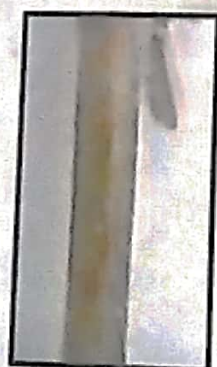
1. Numerous fragments of the upper and lower epidermises showing polygonal cells with beaded anticlinal walls, covered with thick striated cuticle and showing sunken stomata of diacytic or anomocytic types on both surfaces.
2. Numerous fragments of upper and lower neural epidermises showing polygonal axially elongated



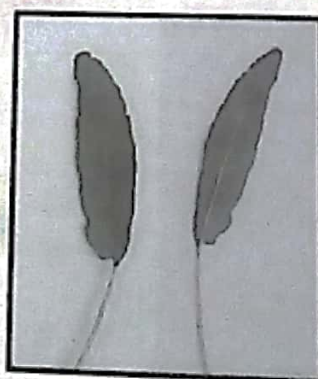
**Figure (1):** Photograph of *Salvia merjamie* Forssk.



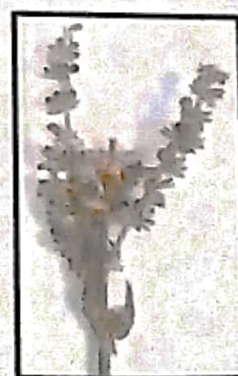
**A<sub>1</sub>**



**A<sub>2</sub>**



**A<sub>3</sub> and A<sub>4</sub>**



**A<sub>5</sub>**

- A<sub>1</sub>: The root.
- A<sub>2</sub>: The stem.
- A<sub>3</sub>: The petiole.
- A<sub>4</sub>: The leaf.
- A<sub>5</sub>: The inflorescence.

3. cells with beaded anticlinal walls, granular surface and covered with smooth cuticle.
  4. Numerous capitate and labiate hairs.
  5. Numerous glandular trichomes with uniseriate, multicellular (3 to 6) with thick walls, acute apices and warty cuticle. The basal cell may be enlarged.
  6. Fragments of lignified spiral vessels.
- The microscopical numerical values of the leaf were listed in (Table 1).

#### The petiole

A transverse section in the petiole (Fig. 8A and Fig. 8B) is almost planoconvex in outline. The epidermal cells (Figs. 8A, 9A and 9B) are hexagonal, measuring 25 to 93  $\mu$  in length, 16 to 47  $\mu$  in width and 13 to 31  $\mu$  in height in abaxial surface and 23 to 85  $\mu$  in length and 5 to 38  $\mu$  in width in adaxial surface. There are a lot of glandular and eglandular hairs and anomocytic stomata similar to those of the stem and leaf (Fig. 9). The cortex is formed of narrow collenchyma (2 to 4) layers under epidermis followed by a central wide parenchyma (5 to 7) layers. There are three large vascular bundles on the median region with additional four small lateral ones. The vascular bundles are similar in structure to that of leaf except that the xylem is more developed than that of the leaf.

The isolated element of petiole (Fig. 9) is characterized microscopically by the following features:

1. Numerous fragments of polygonal epidermal cells with straight or beaded anticlinal walls, covered with smooth or thick striated cuticle and showing stomata of anomocytic type.
2. Fragment of glandular and eglandular hairs similar to those of the stem and leaf.
3. Fragment of lignified spiral vessels.

Table (1): Microscopical numerical values of *Salvia merjamie* Forssk leaves.

The Numerical Value	Number
Stomatal index of upper epidermis	11-12
Stomatal index of lower epidermis	16-18
Palisade ratio	6-7
Vein-islet number	3-4
Vein termination number	6-8

#### III. Determination of certain Pharmacopoeial constants:

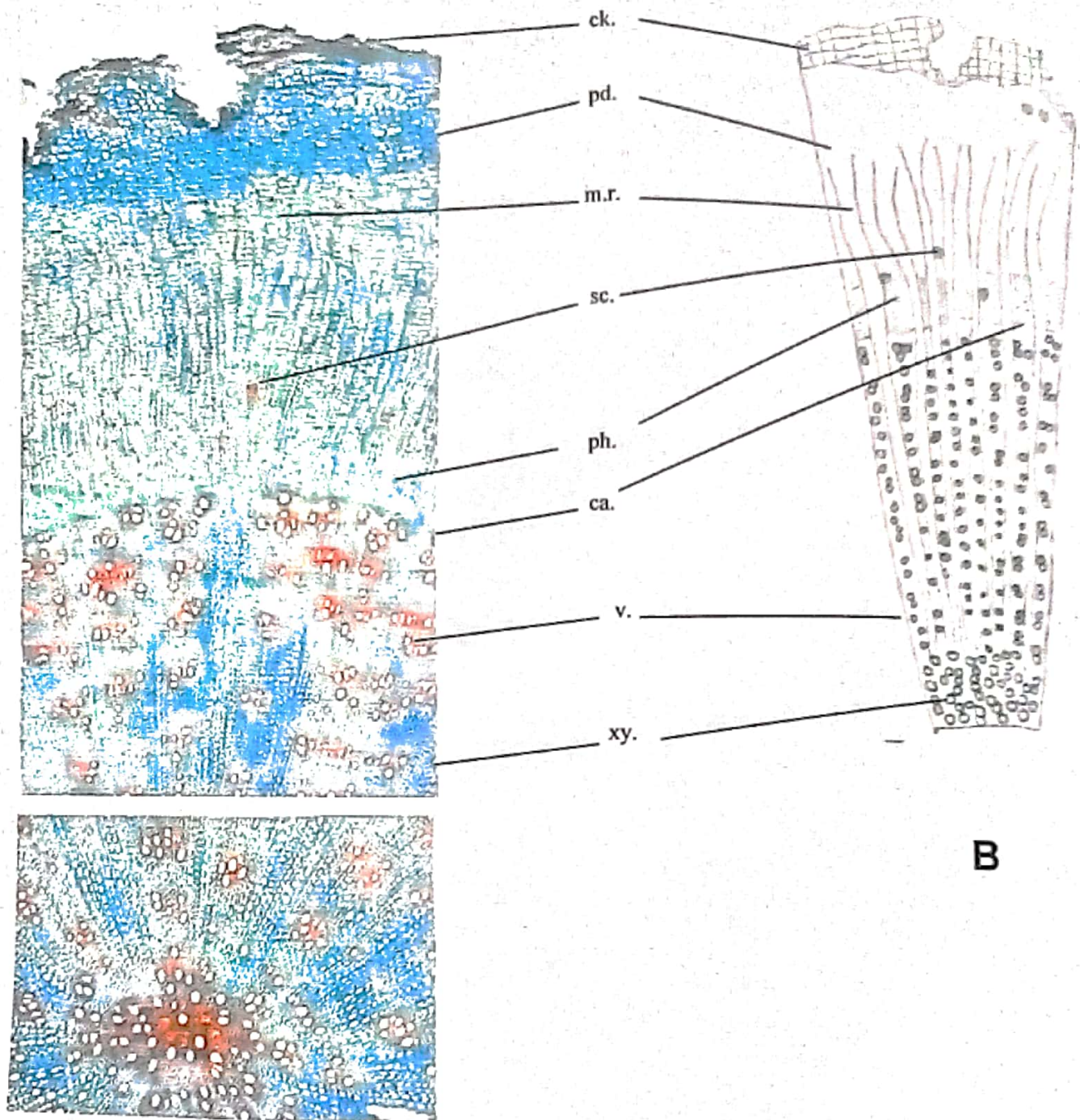
The determination of crude fibre, total ash, water soluble ash, acid-insoluble ash and moisture content of the plant was carried out according to British Pharmacopoeia <sup>(12)</sup> and the results were tabulated in (Table 2).

Table (2): Certain Pharmacopoeial constants of *Salvia merjamie* Forssk.

The Pharmacopoeial Constant	Value
Crude fibre	21.86%
Total ash	15.81%
Water soluble ash	12.5%
Acid-insoluble ash	10.02%
Moisture content	6-8%

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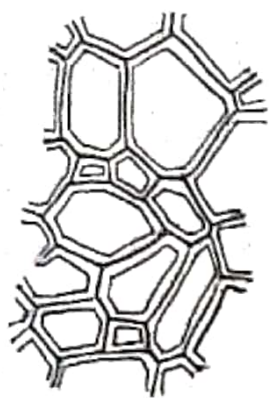
**A****B**

**Figure (2):** The root of *Salvia merjamie* Forssk.

A- Detailed transverse section of the root. (x 25)

B- Diagrammatic transverse section of the root. (x 10)

ca., cambium; ck., cork; m.r., medullary ray; pd., pelloderm; ph., phloem; Sc., sclereids; v, vessels; xy., xylem.



ck..



s.



sc.



tr.v.



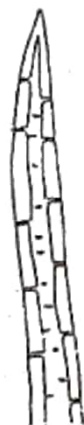
tr.



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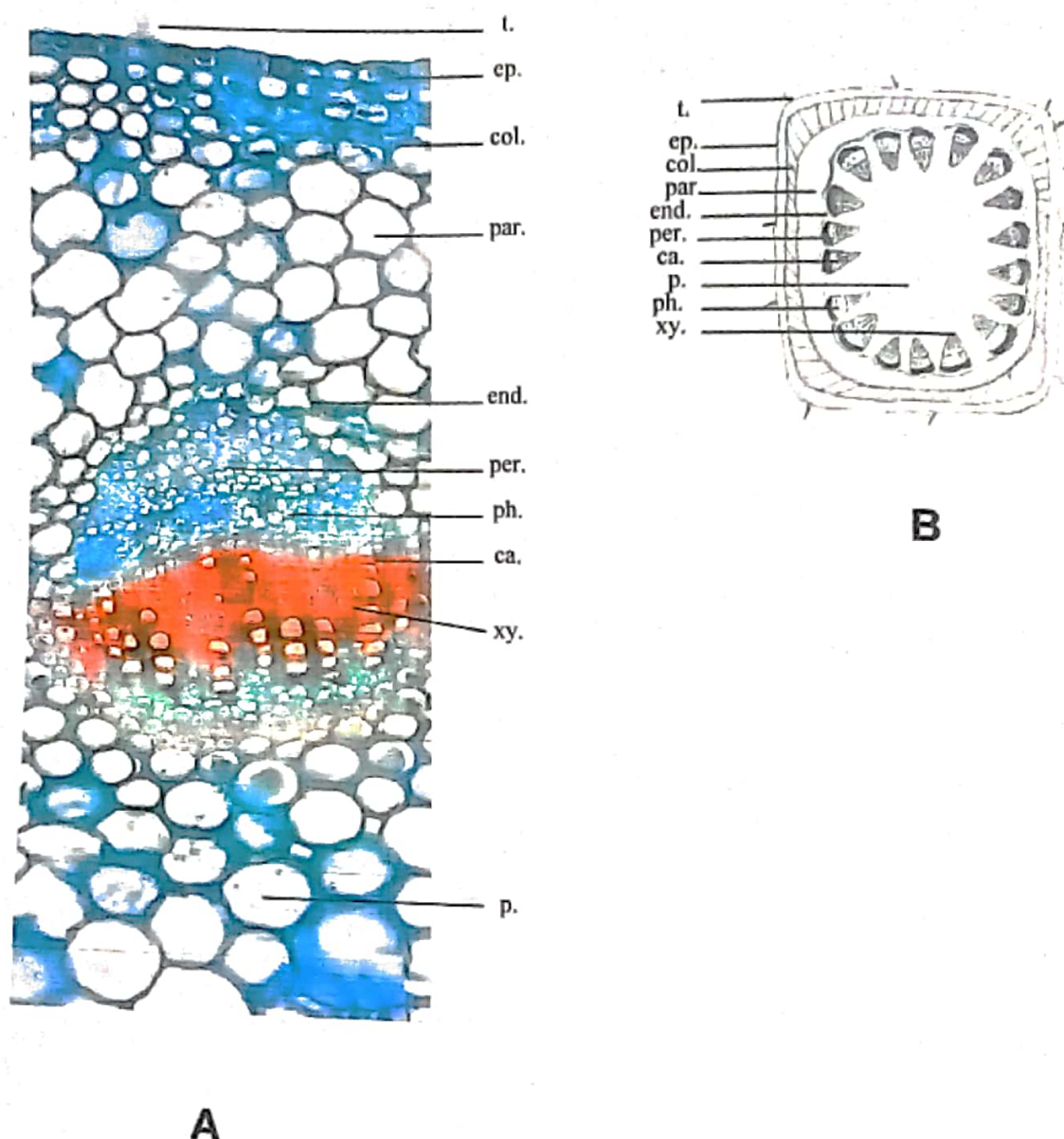
w.f



w.p.

Figure (3): The isolated elements from the root. (x 209)

ck., cork; m.r., medullary ray; s., starch; sc., sclereids; tr., tracheids; tr.v., tracheidal vessel; v, vessels; w.f., wood fibre; w.p., wood parenchyma.



**Figure (4):** The stem of *Salvia merjamie* Forssk.

A- Detailed transverse section of the stem. (x 177)

B- Diagrammatic transverse section of the stem. (x 27)

ca., cambium; col., collenchyma; end., endodermis; ep., epidermis; p., pith; par., parenchyma; per., pericycle; ph., phloem; t., trichome; xy., xylem.



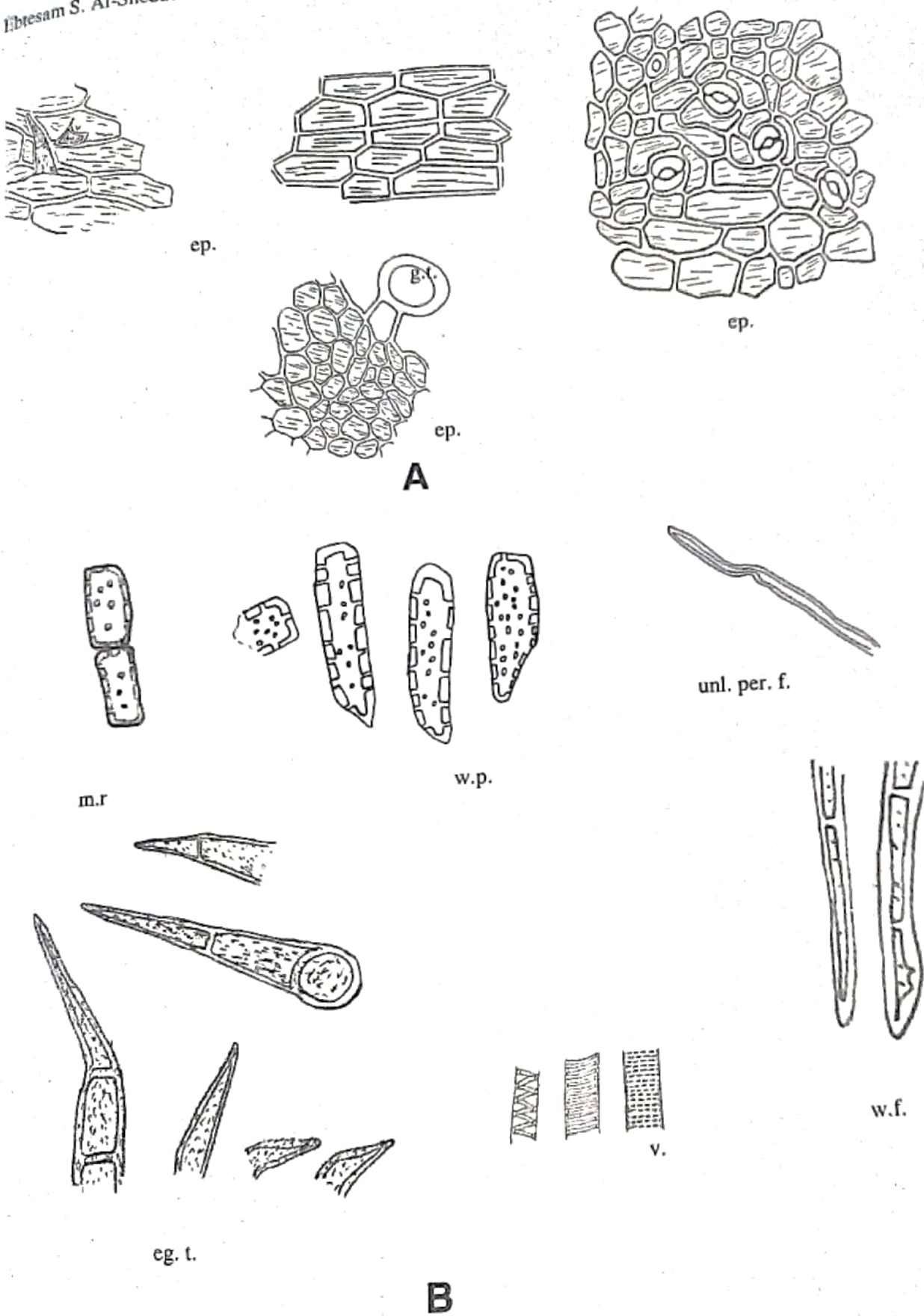
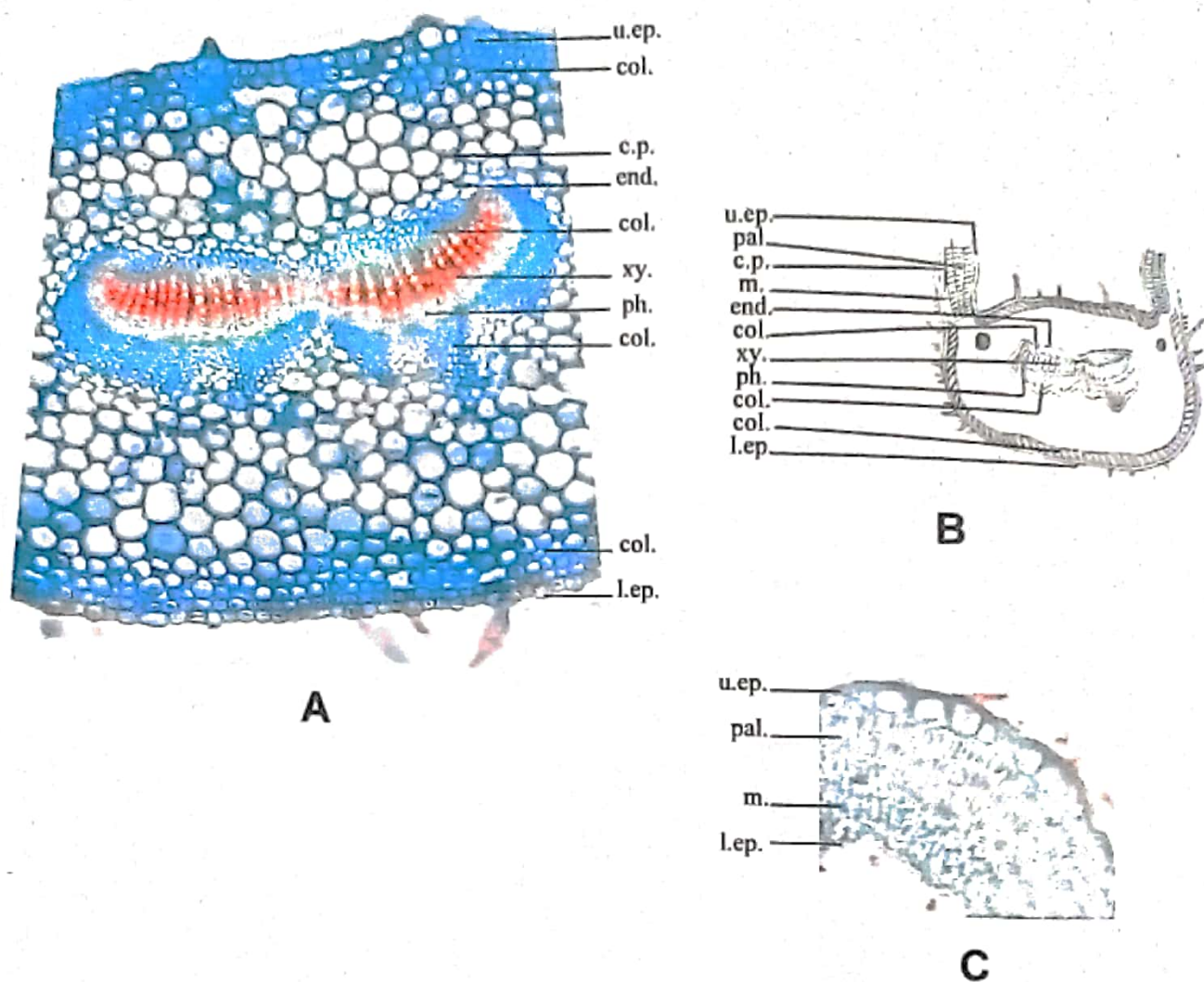


Figure (5): The characteristic elements of the stem.

A- Epidermal cells of the stem. (x 127)  
 B- Isolated elements from the stem. (x 197)  
 eg.t., eglandular trichome; ep., epidermis; g.t., glandular trichome; m.r., medullary ray; unl.per.f., unilignified pericyclic fibre; v., vessels; w.f., wood fibre; w.p., wood parenchyma; xy., xylem.



**Figure (6):** The leaf of *Salvia merjamie* Forssk.  
 A- Detailed transverse section of the leaf. (x 80)  
 B- Diagrammatic transverse section of leaf. (x 23)  
 C- Detailed transverse section of the lamina. (x 93)  
 c.p., cortical parenchyma; col., collenchyma; eg.t., eglandular trichome; end., endodermis; g.t., glandular trichome;  
 l.ep., lower epidermis; m., mesophyll; pal., palisade; par., parenchyma; per., pericycle; ph., phloem; u.ep., upper  
 epidermis; xy., xylem.

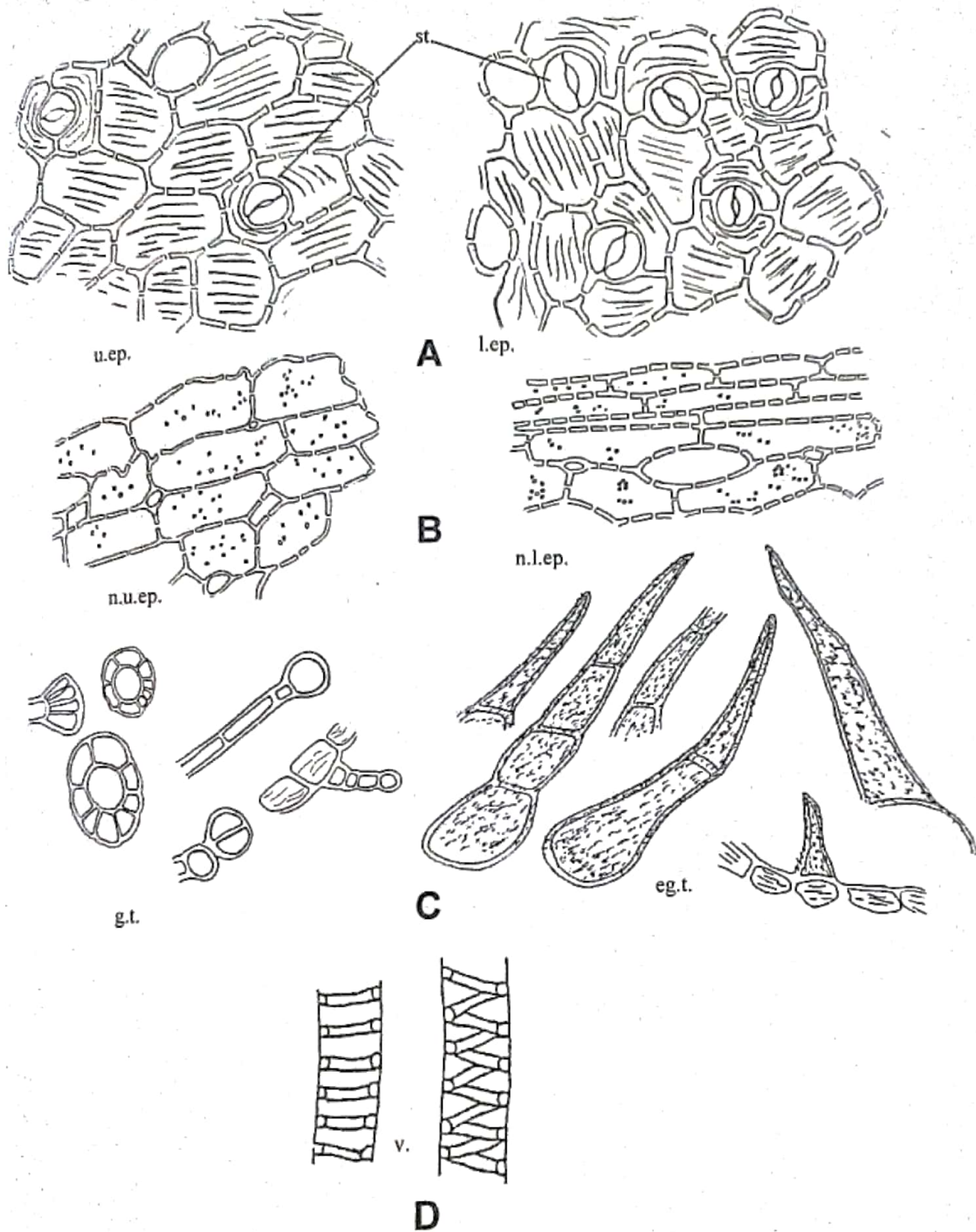
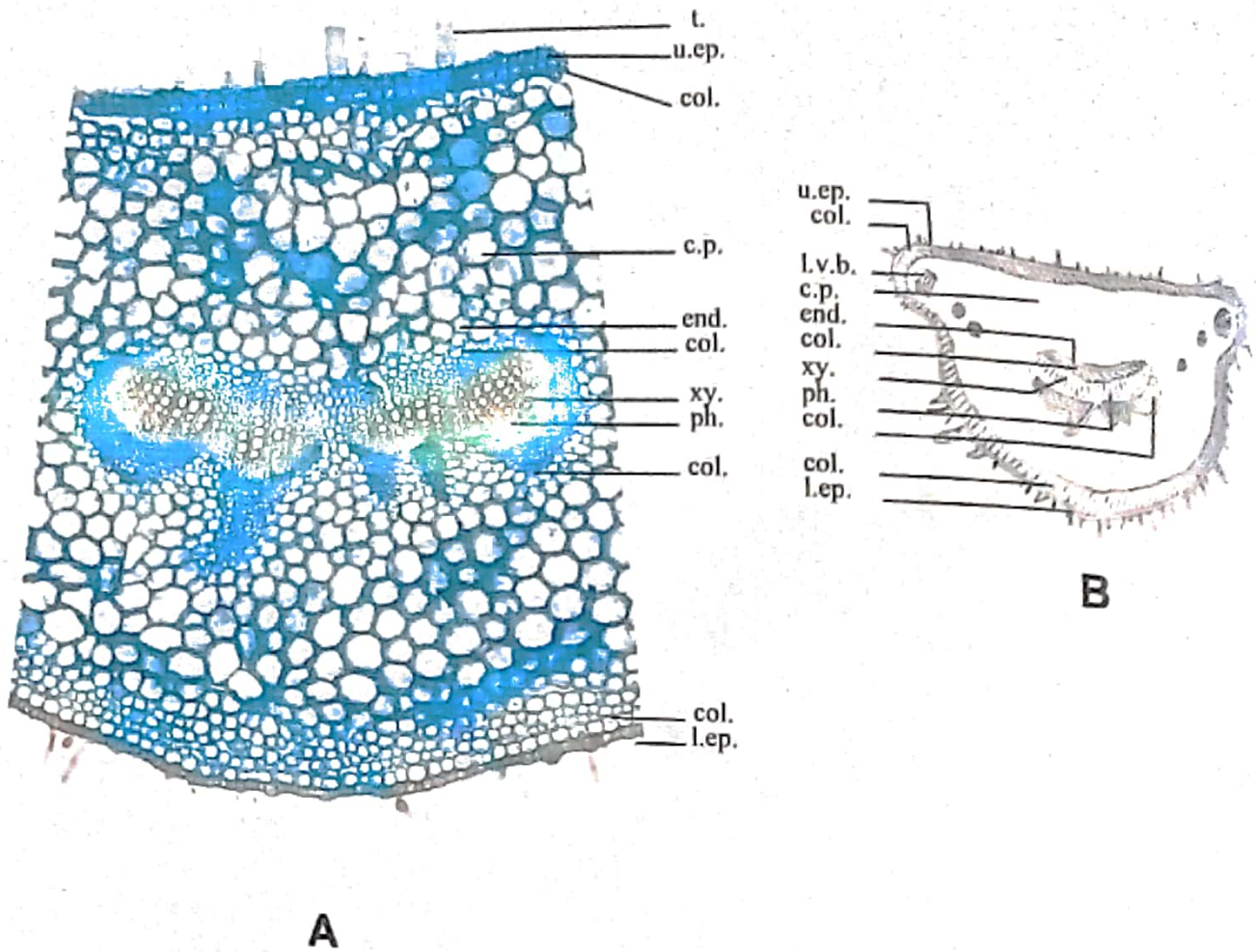


Figure (7): The isolated elements of the leaf.

- A- Epidermis of the leaf. (x 222)
  - B- Neural epidermis of the leaf. (x 200)
  - C- Glandular and eglandular trichomes. (x 176)
  - D- Vessels of the leaf. (x 500)
- eg.t., eglandular trichome; g.t., glandular trichome; l.ep., lower epidermis; n.l.ep., neural lower epidermis; n.u.ep., neural upper epidermis; st., stomata; u.ep., upper epidermis.



**Figure (8):** The petiole of *Salvia merjamie* Forssk.

A- Detailed transverse section of the petiole. (x 75)  
 B- Diagrammatic transverse section of the petiole. (x 19)  
 c.p., cortical parenchyma; col., collenchyma; eg.t., glandular trichome; end., endodermis; g.t., glandular trichome;  
 l.ep., lower epidermis; l.v.b., lateral vascular bundle; par., parenchyma; per., pericycle; ph., phloem; u.ep., upper  
 epidermis; xy., xylem.

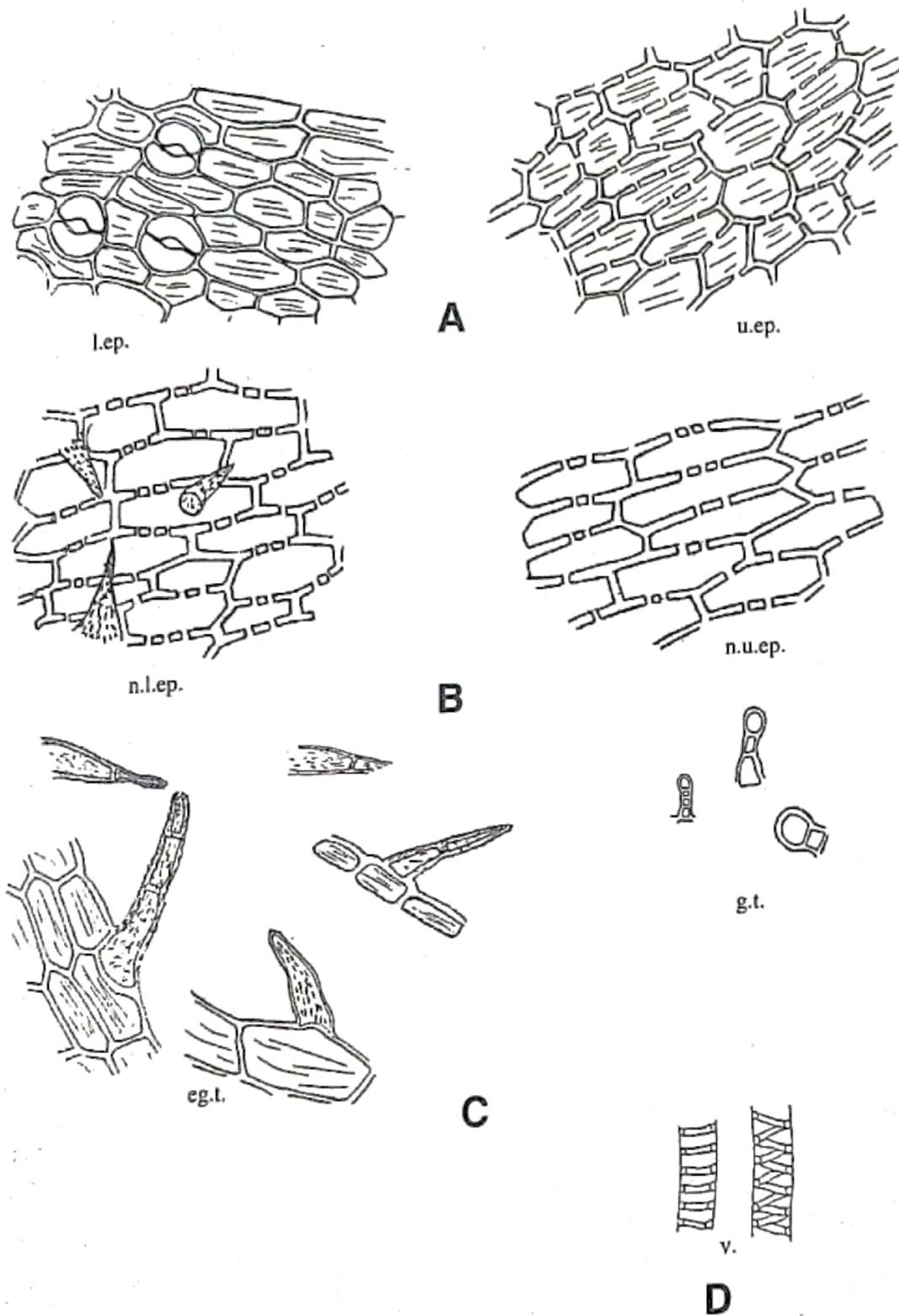


Figure (9): The isolated elements of the petiole.

- A- Epidermis of the petiole. (x 196)
  - B- Neural epidermis of the petiole. (x 156)
  - C- Glandular and eglandular trichomes. (x 173)
  - D- Vessels of the petiole. (x 500)
- eg.t., eglandular trichome; g.t., glandular trichome; l.ep., lower epidermis; n.l.ep., neural lower epidermis; n.u.ep., neural upper epidermis; st., stomata; u.ep., upper epidermis.

## دراسة الصفات العيانية والمجهريّة لجذور وسيقان وأوراق نبات الضرو الذي ينمو في المملكة العربيّة السعوديّة

ابتسام سعد الشدي - فوقيّة عبدالله عباس - عذنان جزلان الرحيلي

قسم العقاقير - كلية الصيدلة - جامعة الملك سعود - الرياض ١١٤٥١ - ص. ب. ٢٤٥٧ - المملكة العربيّة السعوديّة

قسم العقاقير - كلية الصيدلة - جامعة الزقازيق - الزقازيق ص. ب. ٤٤٥١٩ - مصر

تم في هذا البحث دراسة الصفات العيانية والمجهريّة لجذور وسيقان وأوراق نبات الضرو لتحديد الصفات العيانية والمجهريّة التي يمكن الاعتماد عليها في التعرف على النبات في حالة وجوده على صورة كاملة أو على هيئة مسحوق. كما تم حساب بعض القيم العددية الميكروسكوبية لأوراق النبات وتعيين بعض الثوابت الدستورية مثل تعيين محتوى الرطوبة والرماد الكلي، الرماد الذائب في الماء والرماد الغير ذائب في الحمض وكذلك الألياف الخام لتوثيق النبات.