An-Najah National University

Faculty of Graduate Studies

Systematic study of the genus *Salvia* L. (Labiatae) in West Bank/Palestine

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Dedication

To my parents, to my fiancee Hanan

and

to my brothers and sisters.

Acknowledgments

I cannot find words to express my gratitude to my supervisor Dr. Ghadeer Omar for her continuous assistance, supplement with literature, constructive criticism, and great support during my work.

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الاقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل العنوان:

Systematic study of the genus *Salvia* L. (Labiatae) in West Bank/Palestine

أقر بأن ما اشتملت عليه هذه الرسالة انما هو نتاج جهدي الخاص، باستثناء ما تمت الاشاره اليه حيثما ورد، وان هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أيّ درجة أو لقب علميّ لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced. Is the researcher`s own work and has not been submitted from anywhere else, for any other degree or qualification.

Student`s name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

W	West, western
E	East, eastern
C	Central
N	North, northern
S	South, southern
S.	***Salvia***
L.	Carl Linnaeus
Boiss.	Pierre Edmond Boissier
Poir.	Jean Louis Marie Poiret
Mill.	Philip Miller
Fl	Flowering
E	The equatorial axis
P	The polar axis
PT	The Palestinian Territory
D.P.X	Distrene, Plasticiser, Xylene
SEM	Scanning Electron Microscope
Met	mountain
ANNU	An-Najah National University
DoBS	Department of Biological Sciences
RMP	Revolutions Per Minute

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Abstract

The status of the genus ***Salvia*** in West Bank/Palestine was investigated in a comprehensive biosystematic study taking into consideration various biosystematic evidences. This work was based on studying plant specimens, which were collected from their wild natural habitats as well as herbarium specimens deposited at ANNU herbarium, DoBS, ANNU.

The morphological taxonomic characters of ***Salvia*** species have been studied. Moreover, synopsis of taxa, key for species, complete description, literature citation and geographical distribution were provided through this work. It was found that morphological characters should be associated with other evidences to achieve the exact identification of ***Salvia*** in West Bank /Palestine.

Pollen morphological characteristic of ***Salvia*** species have been investigated by both light and scanning electron microscope. All information considering symmetry, polarity, shape, size, apertures and surface sculpturing were detected. All studied taxa of ***Salvia*** were found to have pollen grains varied from suboblate to oblate- spheroidal; size ranging from 27/30 µm to 45/55 µm (P/E); radial symmetry with hexazonocolpate aperture; isopolar or rarely heteropolar; sculpturing semitectate reticulate to reticulate-perforate with variable degree in reticulation and perforation.

Similarities and variations of pollen characters cannot be used alone to separate taxa at the species level.

Leaf anatomy has been investigated using transverse section of fresh leaves, which were studied and characterized in details using light microscopy. Differences in leaves anatomical characters have been recorded. Results showed that the studied species have bifacial leaves. They varied in mesophyll layers ranging from the largest; 200-230 µm thick of ***S. verbenaca*** to the smallest; 90-112.5 µm thick of ***S. viridis***. Also, it was found that the mesophyll of all studied taxa composed of both palisade chlorenchyma and spongy chlorenchyma. In addition to that midrib vascular bundle size ranging from the largest; 122-644×161-763 µm thick of ***S. heirosoloymitana*** to the smallest; 74-61×51-58 µm thick of ***S. viridis***. Moreover, the examined species have either glandular hairs of various types or eglandular hairs or both. Data obtained were found to be significant and contributed with other biosystematics evidences for the delimitation and characterization of ***Salvia*** species, recorded in West Bank / Palestine.

In addition to that, petiole anatomy has been examined using transverse section of fresh petioles. Data gained showed that the anatomical characters of petiole can be used as a biosystematics tool to distinguish one species from another. Especially, the central vascular bundle which can be divided or not. Results showed that the central vascular bundle of the species ***S. viridis***, ***S. verbenaca*** and ***S. hierosolymitana*** are divided into lobes while, the others species under study is not divided.

Chapter One

Introduction

1.1 General Background

Palestine is located in South-West Asia and it is at the heart of the Middle East, between the Mediterranean Sea and the Jordan River and Dead Sea. Therefore, we can consider Palestine as a meeting point between Europe, Asia and Africa. The Palestinian Territories (PT) is sectioned into two special land bodies, the major of these two areas are the West Bank (covering 5,700 km^2), and the Gaza section (covering only 365 km^2). The West Bank runs along the mountain area and down to the Jordan Valley and Dead Sea. While, the Gaza Strip runs over the Mediterranean Sea on the border of the Sinai and Negev deserts (The Palestinian Institute for Arid Land and Environmental Studies, 1996).

The climate of Palestine remains moderate in nature and it is traditionally qualified as 'Mediterranean', which is recognized by winter rain and summer aridity. However, there is a major diversity in this climate, which is modified locally by latitude and altitude, (Mimi et al., 2009). This is particularly obvious in the West Bank climatic areas ranging from highly arid to humid climate according to the De Martonne aridity index classification for arid areas (Land Research Centre, 2007). Yearly, the rainfall in the West Bank is higher in the north (up to 700mm around Jenin) and lowest in the Dead Sea zone of the south (80-100mm). Beside this latitudinal difference, there is an orographic one. which is that, the western downhill receives 500-600 mm, while the eastern downhill welcomes 150-450 mm (Ministry of Agriculture, 2008).

The Mediterranean region conserves its biological importance due to the high level of plant endemism and the appearance of many relict species (Alhamad, 2006). Palestine is a unique area of biodiversity that hosts a large set of plants of almost 2500 species appear in the West Bank and Gaza, performing a high degree of biological diversity (Zohary, 1966). This number of species was updated to 2076 species appear in the West Bank and Gaza according to a survey done by a specialized ARIJ (Applied Research Institute – Jerusalem) team in the year 2006 (Ghattas, 2008). As part of the rich crescent, it has been specified as a remarkable centre of genetic diversity for the life nutritious crops of wheat, barley, vines, olives, onions, and pulses, which all originated within the geographical land of Palestine (Ghattas, 2008).

It is worth adding that Palestine is identified by its magnificent variable ecosystems that facing various floral associations. Palestine location also supplies biological diversity, through which climatic zones, desert, steppe, Mediterranean wood land, and even oases, connect one another in this consolidated geographical area. Therefore, in spite of the small size of Palestine, the Palestinian Territory (PT) involves almost three percent of the global biodiversity. It contains a high intensity and a large number of species which are only found in limited regions such as Caper, Palestinian sea blite, Marjoram, Iris, Fluellen and others, showing a high plant diversity for such a small area (Ghattas, 2008).

1.2. Lamiaceae (Labiatae) Family

Lamiaceae (Labiatae) is the sixth largest family of the flowering plants which is also called the mint family (Harley et al., 2004). Lamiaceae is composed of more than 250 genera and 7000 species (Thorne, 1992). Lamiaceae family is known for the fortune of species with curative properties, which are frequently used in cooked dishes, and are known as a significant preventive factor of many diseases (Chalchat and Özcan, 2008; Hussain et al., 2008 and Baser et al., 2009). Essential oils and extracts of Lamiaceae species are known to have anti-inflammatory and antimicrobial activities (Burt, 2004; Skocibusic et al., 2006 and Bozin et al., 2006).

1.3. *Salvia* Genus

The genus ***Salvia*** name "***Salvia***" came from "***Salveo***" which means "to save or to recover" in Latin (Hamlyn, 1969). ***Salvia*** genus is the largest genus of the family Lamiaceae which represents around 1000 species displaying a notable diversity in growth forms, secondary compounds, floral morphology and pollination biology. The genus ***Salvia*** has spread widely in three districts of the world which are Central and South America (500 species), Western Asia (200 species) and Eastern Asia (100 species) (Walker and Sytsma, 2007).

This genus is important as its cultivated for medicinal, condiment uses and decorative objectives. Therefore, it presents pharmacological and economic significance, particularly for small farmers (Kalemba and Kunicka, 2003; Maksimovic et al., 2007 and Taarit et al., 2009).

In addition to that, the seeds of ***Salvia*** species predominantly make mucilage on wetting (Hedge, 1982). This mucilage product used for

lacquerware and often mixed with fruit juices to make mucilage products tasteful drinks (Estilai et al., 1990). Where in the Eastern countries, the traditionally used for the treatment of eye diseases (Aktas et al., 2009).

However, the genus ***Salvia*** was not lately reviewed in West Bank. Therefore, a reversion of the genus ***Salvia*** in West Bank/Palestine is needed and was conducted through this study.

1.4. Synopsis of Taxa (Danin, 2006).

Kingdom: Plantae

Superdivision: Spermatophyta

Division: Angiospermae

Class: Dicotyledoneae

Family: Labiatae (Lamiaceae)

Genus: ***Salvia***

Species: 1. ***S. fruticosa*** Mill.

2. ***S. dominica*** L.

3. ***S. hierosolymitana*** Boiss.

4. ***S. verbenaca*** L.

5. ***S. lanigera*** Poir.

6. ***S. viridis*** L.

7. ***S. judaica*** Boiss.

1.5. Palynology

Palynology (from the Greek words, to sprinkle, fine meal; cognate with pollen flour or dust), it is the study of pollen grain and spore (Walker and Doyle, 1975). Palynology is unique in that one can take out a large amount of information from a little material in short time (Walker and Doyle, 1975). The constant features and the sculpturing of exine make pollen grains a highly recognizable object by which parent genera or even species may be recognized (Moore, 1978).

There is a board relation between applied palynology and plant taxonomy caused by the fact that palyological aspects are effective as any other biosystematic supplementary evidence.

Application of pollen grains morphology in plant taxonomy is the best evidence in the flowering plant, especially in the angiosperms. The major variety of pollen morphotypes take place among the angiospermous plant. Advances in microscopy techniques, revealed that the information obtained from the scanning electron microscope is the most considerable and reliable in that field (Moore, 1978).

Scanning electron microscopes provide an image of unequaled depth of field, which is ideal for comparative studies of pollen

surface, providing a taxonomical discrimination tool among different taxa (Walker and Doyle, 1975).

The pollen features of the family labiates have been reported to have considerable taxonomic importance (Erdtman, 1945).

As a result the aim of present work was to fund out the morphological characteristics of the pollen grains of the genus ***Salvia*** studied species to be added as further biosystematic evidence for the delimitation of ***Salvia*** species in the study area.

1.6. Leaf Anatomy

Anatomical characters are vastly used as a biosystematic proof to construct phylogenetic relationships between different levels of taxa. Various authors have talked about the use of anatomical data in classification and phylogeny (Omar, 1994).

The advantage of anatomy in individual identification is backward proportional to the plasticity of the characters used. Some characters are more fixed than others, upon which systematic anatomists relied. Those fixed characteristics may are related to the genome rather than to the habitat. Those one which change or modify due to specific environmental condition, cannot be used as taxonomic indicators (Omar, 2006).

The plant parts that can be used for anatomical study are several, as stems, leaves, roots, corolla, and others. These features may be significant to

distinguish between the species. The plant parts that to be chosen depends on both the aim of study and the nature of plant under the study. Mostly leaves generally show a wide range of forms (Radford et al., 1974).

Anatomical characters of leaves, which to be used as biosystematics evidences are several. Mainly considering various leaf layers of tissue and these are: epidermis, mesophyll structure, distribution of stomata on upper and lower epidermis, vascular system and other associated structures.

The epidermal cells show variations from species to another, especially variations in size, shape and arrangement. Measurements of epidermal cells have been made to try to distinguish between studied taxa (Al-Eisawi, 1977).

Mesophyll features are another important anatomical aspects, by which on can differentiate between distinct taxa. Its characters consider the tissue type of which is composed either to be palisade chlorenchyma, or spongy chlorenchyma or both. Moreover, the thickness of palisade and spongy layers and mesophyll region have been measured to discriminate between species (Al-Eisawi, 1977). However, within a group of related plants there may be close resemblances of arrangement. These arrangements cannot be altered by environmental condition, because they are rigidly controlled by genome (Jones and Luchsinger, 1979).

In addition, screening of vascular system may introduce a taxonomic indication within a higher level of taxa, rather than the species level. The

number of bundles may vary in the leaves of plants of any one species found growing under arrange of condition. Also the structure of vascular bundles in leaf anatomy of ***Salvia*** species can be used as a very useful key for distinguishing the species (Esau, 1977). Therefore, families can be distinguished by having particular vascular system characters, in the majority. Studying the vascular system can be achieved considering the components, position, vascular bundle size, and others (Esau, 1977).

Furthermore, the leaf thickness (from upper epidermis to lower epidermis passing through midrib vein) have been measured to discriminate one species from another. Other anatomical characters of leaves can be used as storage materials, trichomes. Not necessarily all anatomical characters are to be used in all condition. Their use depends on their variation and presence (Al-Eisawi, 1977).

In spite of, there is general information about the anatomical structure of Lamiaceae (Metcalf and Chalk, 1972), most of ***Salvia*** species have not been investigated in details considering their anatomical characters except for a few species (Shirsat et al., 2012).

Therefore, the present study was undertaken with a view to give a detailed account of the anatomical characters in seven species of ***Salvia*** growing in West Bank /Palestine.

1.7. Petiole Anatomy

The anatomical characters of petiole are used as a biosystematic evidence to distinguish one species from another. The arrangement of vascular bundles in petiole of Lamiaceae (Labiatae) is important in their taxonomy (Metclafe and Chalk, 1972), which was taken into account in the current study.

Other anatomical characters of petiole, which to be used as a taxonomic evidence, are the petiole thickness, the central vascular bundle, which could be divided or not and the number of small bundles at each end of the petiole.

Therefore, the goal of present study was to fund out the anatomical characters of petiole of the genus ***Salvia*** studied species to be added as further biosystematic evidence for distinguish between ***Salvia*** species.

1.8. Aim of this Study

The goal of this study is to assess classification alternatives, revealing further evidences for either within species at the intraspecific level or between species. Therefore, it is expect that the results are going to be useful for the differentiation between the ***Salvia*** species based on different taxonomic tools.

Traditional biosystematic evidences are looking forward to be obtained from morphological, palynological, leaf anatomy and petiole anatomy examination. It is desirable, that a wide range of information from diverse sources will be obtained and utilized to perform the best sort and the closest to natural classification. Accordingly, the following approaches are going to be made to accomplish this target:

- Morphological studies of taxa under study.
- Preparation of a key for the species under study to help in the identification of the species.
- Determination of the geographical distribution throughout the country as will presented by a map for each species under study.
- Pollen grains morphology, based on external features of the grains as revealed under the light microscope and scanning electron microscope.
- Comparative anatomical studies of transverse section of leaves and petioles of taxa under study.

1.9. Literature Review

1.9.1 Morphological Studies

Seventy-five of ***Salvia*** species were recorded by Boisser in Turkey in 1875, based on morphological characteristics (Kahraman et al., 2009). Furthermore, the ***Salvia*** genus has been the object of a number of studies

on its species morphological characteristics in Turkey (Hedge, 1982). After that, Baran and Özdemir (2006), studied the morphological characteristics of ***Salvia napifolia***, in Turkey, using different morphological parameters. For example, stems, leaves, petioles and flower morphology of the studied species have been examined. Later on, ***Salvia belpharoclaena*** which is an endemic species in Turkey, was studied and described morphologically based on samples collected from different localities in Turkey (Özkan and Say, 2007).

Moreover, the genus ***Salvia*** was also studied in Egypt based on the morphological features. Ten species were recorded in Egypt (Tackholm, 1974). Later on, 19 species of ***Salvia*** were recorded in Jordan (Al-Eisawi, 1982). In addition to that, in the field guide to wild flowers of Jordan and neighboring countries, 10 species of ***Salvia*** were described, photographed and their geographical distribution in Jordan and neighboring regions were cited (Al-Esawi, 1998).

Then the genus ***Salvia*** was studied systematically in Palestine depending on the morphological characteristics. In Flora Palestina, 21 species of the genus ***Salvia*** were recorded (Naomi,1978). This number was updated to 23 species in 2006 (Danin, 2006).

Furthermore, morphological characteristics such as features of stem, leaves, calyx, corolla, and nutlets were used to discriminate between the two species, ***Salvia glutinosa*** and ***Salvia staminea***. It was found that such

features are significant to distinguish the two species (Kahraman et al., 2009).

After that, morphological studies were carried out on ***Salvia glutinosa*** specially on the morphology of glandular hairs which are distributed on the aerial organs of the species (Coisin and Gostin, 2011). A year later, the ***Salvia plebeian*** species has been subjected to morphological studies, based on samples that were collected from different ranges in India (Shirsat et al., 2012).

1.9.2. Anatomical Studies

The genus ***Salvia*** has been the subject of a number of studies on its anatomical characters (Metcalfe and Chalk, 1965). Later on, Oran (1997), described the nutlet anatomy of 19 Jordanian ***Salvia*** species. She found significant differences between the studied species such as the coat thickness and mucilage production. Moreover, the ***Salvia napifolia*** species has been studied based on the anatomical characters. It was found out that dense collenchymatous cells which are located at the corners of the stem, is a distinguishing anatomical characteristic of the family Lamiaceae (Baran and Özdemir, 2006).

Anatomical studies were carried out on fresh samples or samples kept in alcohol of ***Salvia belpharoclaena***, which were collected from different areas in Turkey. The anatomical features in this study were root, stem, leaf, and petiole (Özkan and Soy, 2007). In addition to that, the anatomical characteristics of ***Salvia glutinosa*** and ***Salvia staminea*** were studied, in

order to distinguish the two species and the sections to which they belong (Kahraman et al., 2009). In another study, Koyuncu et al. (2009), conducted a detailed account of the anatomical characters of ***Salvia verticillata*** which was collected from Turkey. After that, anatomical studies were carried out on ***Salvia glutinosa*** especially on the anatomy of glandular hairs which are distributed on the aerial organs of the species. They found out that upper and lower epidermis of ***Salvia glutinosa*** leaves are covered with densely glandular trichomes (Coisin and Gostin, 2011).

Furthermore, the anatomical characteristics of ***Salvia plebeia*** species were studied based on the anatomy of stem and surface features like hairs and trichomes (Shirsat et al., 2012).

1.9.3. Palynological Studies

Earlier, studies were carried out on the palynological characteristics of ***Salvia*** species (Erdtman, 1945). Later on, the palynological characteristics such as pollen size and exine ornamentation were used to distinguish between the two species ***Salvia glutinosa*** and ***Salvia staminea***. It was concluded that such features are important in differentiating between these two species (Kahraman et al., 2009). Moreover, the palynological characteristics of ***Salvia verticillata*** were studied and found that the pollen grains were suboblate-subprolate and stephanocolpate, while, exine was seen to be tectate-granulate (Koyuncu et al., 2009). In another study, the palynological characteristics of ***Salvia glutinosa***, were studied. It was indicated that the pollen micro morphology is an important taxonomic index for correct determining of the species (Coisin and Gostin, 2011).

Chapter Two

Materials and Methods

2.1. Morphology

The material which was used to study the morphology of the genus ***Salvia*** was based mainly on 80 specimen which were freshly collected from West Bank/Palestine, in-addition to herbarium specimens deposited at the herbarium, Department of Biological Sciences, Faculty of Science, An-Najah National University. Coloured photographs, field observations, as well as some ecological notes were made.

When collecting plant material of ***Salvia***, field notes on the following characters of taxonomic importance were made on colour of perianth segments, leaves colour, life form, general distribution and frequency in the site from which each species was collected. Measurements were taken for different parts of each studied specimen. These measurements were made on at least 10-15 specimens on average for each studied species provided that enough material is available. The collected material of plant specimens were pressed until drying, then poisoned chemically using a mixture of mercuric chloride and ammonium chloride (150 g of mercuric chloride, HgCl, and ammonium chloride, NH_4Cl, were dissolved in as little water as possible. After that 10 L of 96% ethanol were added to the previous mixture). The poisoned plant specimens were identified, based on Flora Palestina (Zohary, 1966). Then labeled, mounted and finally deposited at the herbarium, Department of Biological Sciences, Faculty of Science, An-Najah National University providing each specimen with a specific voucher number can be referred to.

2.2. Pollen Grains Morphology

2.2.1. Light Microscopy

Pollen grains morphology was studied by light microscopy:

1. Acetolysed pollen grains were used in the study which are obtained as follows:

    i. Mature anthers of each species were taken and soaked in distilled water.

    ii. Anthers were smeared to allow the pollen grains to suspend in the water.

    iii. Water with the pollen grains were sieved through a suitable mesh sieves allowing the pollen grains to pass to a centrifuge tube.

    iv. Centrifugation was made for 3 minutes at 3000 rpm.

    v. Supernatant was decanted and pollen grains pellets were resuspended in 10 ml acetic acid.

    vi. Again centrifugation was carried out for 3 minutes at 3000 rpm.

    vii. Supernatant was decanted, then 10 ml of acetolysis mixture (acetic anhydride: sulfuric acid 9:1) will be added.

    viii. Incubation for 5-10 minutes in water bath at 70°C was carried out.

    ix. Centrifugation for 3 minutes at 3000 rpm, was carried out.

    x. The acetolysis mixture was decanted in a sink filled with water.

xi. This was followed by washing twice with distilled water, each time and then followed by centrifugation for 3 minutes at 3000 rpm and decanting.

xii. A pellet of pollen grains was obtained.

xiii. A glycerine-jelly pieces and a drop of safranine stain were added.

xiv. A glycerine-jelly piece swapped with the pollen grains and then transferred to clean slide. A cover slip was placed on the piece of the glycerine-jelly.

xv. The slide then was placed on a hot plate to allow the melting and spreading of the glycerine-jelly under the cover slip. Slides were also be allowed to cool for solidification of glycerine-jelly.

2. Slides were examined under the light microscopy.

3. The size of pollen grains was measured by calculating the average of polar and equatorial axis (P/E) of at least 50 pollen grains of each taxon at 1000 x magnification.

4. The aperture, the overall shape, and the outline were also observed.

5. Microscopic photographs of the pollen grains of each taxon under study were obtained.

2.2.2. Scanning Electron Microscopy (at The University of Jordan& Al-Yarmouk University)

1. A mature anther was taken and opened.

2. The pollen grains were scattered on clean stub.

3. The stubs were then coated by platinum by (Suptter Coater Emitech K 550 X Platinum target).

4. Examination under scanning electron microscope (Inspect F50 Schottky FEG High Vacuum < 6e-4pa), at different magnifications.

5. SEM photographs were taken to show the shape, aperturing and sculpturing of pollen grains.

2.3. Anatomy of the Leaf and Petioles

The method which was used in the histological work is following that used by Oran (1991), with any modification could be required based on the plant nature.

i. Fixation.

1. Small leaf or petiole segments were taken. Including the midrib and margins.

2. Fixed in formalin-acetic alcohol (F.A.A.) 1:1:10 cc. (70% alcohol), for 72 hours at room temperature.

ii. Washing and dehydration.

1. The segments, were removed from fixative.

2. The segments, were washed several times with distilled water.

3. Washed with two changes of 50 % alcohol (ethanol).

4. Dehydration with a series of different concentration of alcohol as follows was carried out:

a. 50% alcohol 3 hours

b. 60% alcohol 3 hours

c. 70% alcohol over night

d. 90% alcohol 2 hours

e. 95% alcohol 2 hours

f. 100% 30 minutes

iii. In-filtration and pre-staining.

1. Xylene	10 minutes except ***S. viridis*** 4minutes
2. Xylene	10 minutes except ***S. viridis*** 4minutes
3. Xylene: Alcohol (1:1)	10 minutes except ***S. viridis*** 4minutes
4. Xylene: Alcohol (1:1)	10 minutes except ***S. viridis*** 4minutes
5. Xylene: melted wax (1:1)	30 minutes except ***S. viridis*** 10minutes

5. Removal of all xylene: melted wax.
6. Small chips of paraffin wax (60 °C melting point) were added.
7. Infiltration with wax was carried out by placing the vials in the oven for 1-1.30 hours at 60-65 °C, except ***S. viridis*** 35 minutes at 60-65 °C.

iv. Embedding.

1. Metal moulds were used.

2. New molten wax was poured in the moulds.

3. Leaf or petiole segment was placed inside the moulds.

4. The leaf or petiole segment was oriented with the help of two hot needle.

5. The moulds were placed over ice bath and left wax to get solid.

6. Then placed in a cold water bath to harden the wax containing the leaf or petiole material.

v. Mountaining of ribbons and sectioning by rotary microtome.

1. Wax block were trimmed to appropriate size and then fixed to a block holder.

2. Blocks holder was oriented vertically and horizontally at the microtome to get right angled sections.

3. Wax blocks were sectioned at a thickness of 6 µm.

4. The ribbons were received then spread on the surface of hot water bath at 40°C.

5. The ribbons were picked up on a clean glass slide.

6. The slides were kept overnight to dry for the following steps.

vi. Removal of wax and staining.

For removing the wax and staining, slides were passed through the following chemicals:

A. Wax removal:	**Time**
1. Xylene	10-15 minutes
2. Xylene:absolute alcohol 1:1	10-15 minutes
B. Dehydration :	
3. absolute alcohol	1minute
4. 95% alcohol	1 minute
5. 90% alcohol	1 minute
6. 80% alcohol	1 minute
7. 70% alcohol	1 minute
8. 60% alcohol	1 minute
9. 50% alcohol	1 minute
C. Staining:	
10. safranin stain	Over night
11. 50% alcohol	10-20 seconds
12. 60% alcohol	10-20 seconds
13. 70% alcohol	10-20 seconds
14. 80% alcohol	10-20 seconds
15. 90% alcohol	10-20 seconds
16. 95% alcohol	10-20 seconds
17. absolute alcohol	10-20 seconds
18. Fast green	1-2 minutes
D. Clearing:	
20. Pure clove oil	10-15 seconds
21. Xylene	10-15 seconds
E. Mounting:	
22. Mountend in D.P.X and left to dray on a hot plate at 30°C.	

Then the stained slides were examined under the light microscope (trinocular microscope with ccd camera) and photographed to show anatomical differences between the different studied taxa.

Chapter Three

Results and discussion

3.1. Morphological Characterization of the Genus *Salvia* and Studied Species

3.1.1. Description of the Genus *Salvia*

The genus ***Salvia*** can be described as perennial or annual herbs, chamaephyets or shrubs, leaves entire, dentate, crenate or variously divided; flora leaves mostly differing from cauline leaves. Verticillasters 2 -to many-flowerd, mostly in spike or raceme- like inflorescence; bracteoles mostly small. Calyx campanulate, ovoid or tubular, more or less distinctly 2-labiate; upper lip shortly 3-dentate, rarely entire or with middle tooth obsolete; lower lip more deeply 2-dentate. Corolla 2-labiates; tube naked inside or hairy-ringed or with a fringed scale; upper lip concave, straight or falcate; lower lip spreading, 3- lobed, with middle lobe the largest. The 2 anterior stamens fertile; filament usually short; connective elongated, usually articulated with the filament; the posterior arm of the connective bearing a fertile theca; the anterior arm more or less sterile, often expanded and flattened distally; the 2 posterior stamens either reduced to small staminodes or lacking. Style- branches either equal or the posterior longer and flattened. Nutlets ovoid, smooth, sometimes nearly triquetrous, often mucilaginous on wetting.

3.1.2. Key to Studied *Salvia* Species

1. Basal leaves present

2. Annual herb. Corolla purplish-pink, rarely white, 10-14 mm length. Calyx 6-8 mm length.

……..……..….***S. viridis***

2. Perennial herb. Corolla wine-red or purplish- pink or blue-lavender, 10-12 mm or 23-28 mm length. Calyx 10-14mm or 5-9 mm length.

3. Leaves dentate. Corolla wine-red or purplish- pink, 23-28 mm length. Calyx glandular hairy, 10-14mm length.

……..…...……. ***S. hierosolymitana***

3. Leaves crenate or lobed. Corolla blue to lavender, 10-12 mm length. Calyx hispid with glandular hairy, 5-9mm length.

……..… ***S. verbenaca***

1. Basal leaves absent

4. Plant tomentose

5. Shrub. Leaves margin tripartite, ovate-oblong to lanceolate. Corolla purplish-pink, rarely white. Calyx texture viscid with glandular and partly eglandular hairs.

……..……..……***S. fruticosa***

5. Perennial herb. Leaves margin pinnatisect, oblong to triangular. Corolla deep violate. Calyx texture covered with long white hairs.

…….…….…..…..…..……***S. lanigera***

4. Plant not tomentose

6. Chamaephyte, strong smell. Corolla cream, verticillasters 4-6 number. Calyx campanulate, texture dense long white hairs.

……..…… ***S. dominica***

6. Perennial herb. Corolla violet, verticillasters 6-12 number. Calyx tubular, texture scabrous.

…….……..... ***S. judaica***

3.1.3. Description of *Salvia* Species

3.1.3.1. *Salvia fruticosa* Mill., Gard. Dict. ed. 8, no. 5 (1768); Hedge, op. cit. 23 (1974). *S. triloba* L. fil., Suppl. 88 (1781); Bossi., Fl. 4:595; Post, Fl 2:349. *S. libanotica* Bossi. & Gaill. in Boiss., Digan. ser. 2, 4:16 (1859) (Zohary, 1966). **(Plate 1, A, B).**

Shrub, appressed-wooly-tomentose, canescent. Leaves 2-8.5 cm in length while, it 1-3 cm in width, rugulose, crenate, tripartite, ovate-oblong to lanceolate, truncate or nearly cordate at base, greyish-white on lower face, greenish on the upper; petiole length 1-3cm with dense long hairs, leaves beneath the inflorescences with 1-2(-4) small, ovate or elliptic segment at base; flora leaves shorter than verticillasters, ovate, acuminate, sessile, later deciduous. Inflorescences paniculate or racemose, viscid; verticillasters 4-6- to many-flowered, mostly remote, bracteoles minute , membranous, deciduous; flowers pedicellate. Calyx campanulate, 7-8 mm, scarcely accrescent, viscid with glandular and partly eglandular hairs and with sessile gland; teeth triangular, acute, nearly equal. Corolla about 13-21mm, purplish-pink, rarely White (forma *albiflora*). Fl. March-June.

Habitat: Common and abundant in plant association of garigue and maquis, often stand-forming. Coastal Galilee, Coast of Carmel; Upper and Lower Galilee, Mt. Carmel, Mt. Gilboa, Samaria, Judean Mts (Zohary, 1966).

Area: Mainly E. Mediterranean (including S. Italy).

Distribution:

Kafr Thulth /Qalqilia and Kifl Haris/Salfeet. **(Plate 5).**

3.1.3.2. *Salvia dominica* L., Sp. Pl. 25 (1753). *S. graveolens* Vahl, Enum. Pl. 1:273 (1804); Boiss., Fl. 4:615; post, Fl. 2:356 (Zohary, 1966). **(Plate 1, C, D)**.

Strong-smelling chamaephyte, many-stemmed, grey, with dense spreading white hairs. Stems erect or ascending, simple or often forming narrow panicles. Leaves appressed-canescent, strongly rugose, 1.4-5.5 cm in length while, it 0.5-3.6 cm in width, triangular-ovate to oblong, generally obtuse, truncate or cordate at base, crenate and often undulate-lobed; leaves have a short petioles 0.5- 1.4 cm with long hairs, upper leaves sessile; flora leaves cordate-ovate, acuminate, glabrous on upper face, shorter than calyx. Verticillasters 4-6 flowered, in long and rather dense racemes. Calyx campanulate, 7-10mm, covered with dense long white hairs and sessile glands; lips about as long as tube, widely spreading; teeth of upper lip very short; teeth of lower lip lanceolate. Corolla about 14-21 mm, cream-coloured, with a yellow lower lip; upper lip falcate, long; tube abruptly dilated above, with a fringed scale at base of throat. Fl. February-May.

Habitat: Batha on Senonian and Eocenian chalky hills. Abundant, Upper and Lower Galilee, Mt. Carmel, Esdraleon Plain, Mt. Gilboa, Samaria, Shefela, Judean Mts., Judean Desert, N. and C. Negev; Upper Jordan

Valley, Beit Shean Valley, Lower Jordan Valley. Dead Sea area; Golan, Gilead, Ammon, Moav, Edom (Zohary, 1966).

Area: E. Mediterranean.

Distribution:

Al-Nsarya/ Nablus, Al- Auja /Jericho, Al-Badhan / Nablus, Tammun /Jenin and Jeet / Qalqilia. **(Plate 5).**

3.1.3.3. ***Salvia hierosolymitana*** Boiss., Diagn. ser. 1, 12:61 (1853); Boiss Fl. 4:627; Post, Fl. 2:358 (Zohary, 1966). **(Plate 2, A, B).**

Perennial herb, branched from base. Stems erect, leafy, ending in a long glandular-hairy and viscid panicle, often with sparse eglandular hair; lower part of stem nearly glabrous, mostly scabrous at angles. Leaves 5.5-17 cm in length while, it 3.5-12 in width, thin, green, sparsely pubescent, ovate to ovate-oblong, obtuse, irregularly dentate and incised-lobed; cauline leaves 1-2 pairs, smaller, sessile; flora leaves glandular-hairy, triangular-ovate, acuminate-subulate, much shorter than calyx. Petioles length 1.5-15cm on both upper and basal leaves with short hairs, basal leaves showing long-petiolate, 10-15 cm. Verticillaster 4-6-flowerd, remote; pedicels hirsute. Calyx glandular-hairy, 10-14 mm; teeth of upper lip minute, aristate; teeth of lower lip aristate-spinulose. Corolla wine-red or purplish-pink 23-28mm; upper lip falcate, compressed. Fl. March -June.

Habitat: Rocky places in batha and maquis. Upper Galille (rare), Samaria, Judean Mts., Judean Desert; Gilead, Moav (Zohary, 1966).

Area: E. Mediterranean (Palestine, Lebanon , Syria).

Distribution:

Nablus and Kafr Thulth /Qalqilia. **(Plate 5)**

3.1.3.4. ***Salvia verbenaca*** L., Sp. Pl. 25(1753); Boiss., Fl. 4:629; Post, Fl. 2:359 (Zohary, 1966). **(Plate 2, C, D).**

Perennial herb, branched from base, short-pubescent below, villose and often glandular- hairy above. Stems erect or ascending, simple or sparingly branched. Leaves 4-6.8 cm in length while, it 1.5-2.6 cm in width, green and nearly glabrous, rugose, ovate to oblong in outline, rounded or cordate at base, crenate or more often variously lobed or incised; petiole length of basal leaves 1.7-6.9 cm with short hairs; upper leaves sessile, clasping, acute or cuspidate; floral leaves orbicular-cordate, acuminate, later reflexed. Verticillasters 4-6 flowered, more or less remote or close together. Calyx ovate-campanulate, hispid or in addition glandular-hairy, 5-9mm, often coloured; upper lip somewhat shorter, semi-orbicular, minutely 3-dentate; teeth of lower lip lanceolate-subulate; sinus between the lips ciliate. Corolla blue to lavender, 10-12mm; upper lip nearly falcate. Fl. November-May.

Habitat: Roadsides, fallow fileds and batha. Coastal Galilee, Acco Plain, Coast of Carmel, Sharon Plain, Philistean Plain, Upper Galilee, Mt. Carmel, Samaria, Shefela, Judean Mts., Judean Desert, C. Negev; Upper Jordan Valley, Gilead, Ammon, Moav (Zohary, 1966).

Area: Mediterranean, with extensions into the W. Irano-Turanian and Euro-Siberian.

Distribution:

Nablus and Salfeet. **(Plate 5).**

3.1.3.5. ***Salvia langira*** Poir. in Lam., Encycl. Meth. Bot. Suppl. 5:49 (1817); Hedge, op. cit. 99 (1974); Lacaita, Jour. Bot (London) 65:320 (1927); Post, Fl. 2:359. *S. controversa* auct. non Ten., Fl. Neap. Syll. (1830); Boiss., Fl. 4:630 (Zohary, 1966). **(Plate 3, A, B).**

Perennial herb, with a pungent smell, canescent, densely tomentose and with long spreading hairs. Stem numerous, simple or branched from near base. Leaves 0.5-4 cm in length while, it 0.5-7 in width, oblong to triangular in outline, pinnatisect; segment linear, set at right angles to rachis, obtuse, crenulate, revolute-margined, bullate-rugose; leaves with short petioles 0.5-1 cm with long hairs, flora leaves ovate-orbicular, acuminate. Verticillaster 6-8-flowered. Calyx 7-8 mm, covered with long white hairs upper lip minutely 3-dentate. Corolla 15-20 mm, deep violate; tube long-exserted; upper lip nearly falcate. Fl. February- May.

Habitat: Loess, sandy chalky soils in steppes and desert. Judean desert, N., C. and S. Negev; Dead Sea area; Ammon, Moav, Edom (Zohary, 1966).

Area of species: S. Mediterranean and Saharo-Arabian.

Distribution:

Wadi Al-Qilt /Jericho. **(Plate 5).**

3.1.3.6. *Salvia viridis*: L., Sp. Pl. 24 (1753); Boiss., 4:630; Post, Fl. 2:360. *S horminum* L. var. *viridis* (L.) Caruel in parl., Fl. Ital. 6:246 (1884) (Zohary, 1966). **(Plate 3, C, D).**

Annual, with white hairs and sessile glands, sometimes scabrous or glabrescent. Stem erect, simple or branched from base or above. Leaves 1.2-4.2 cm in length while, it 0.5-2 cm in width, ovate to oblong, obtuse, crenate, crenate or rounded to cordate at base; petioles length of both basal and upper leaves 0.3-4 cm with short hairs; flora leaves bract-like, sessile, ovate, broad, acute, about as long as calyx or longer; terminal leaves sterile, violet, membranous, elliptic to obovate, or with spikes devoid of tuft of coloured sterile floral leaves, Verticillasters 4-6 flowered, generally remote. Calyx 6-8 mm, corolla 10-14 mm, purplish-pink, rarely white (forma *albiflora*). Fl. February-May.

Habitat: Fields and batha. Acco Plain, Sharon Plain, Philistean Plain; Upper and Lower Galilee, Mt. Carmel, Esdralelon Plain, Judean Mts., N. Negev; Dan Valley, Upper and Lower Jordan Valley, Arava Valley; Golan, Moav (Zohary, 1966).

Area: Mediterranean, extending into the W. Irano-Toranian.

Distribution:

Al-Nsarya / Nablus**. (Plate 5).**

3.1.3.7. ***Salvia judaica*** Boiss., Diagn. ser. 1, 12:61 (1853); Boiss., Fl. 4:635; Post, Fl. 2:361 (Zohary, 1966). **(Plate 4, A, B).**

Perennial herb, sparsely setulose to aculeolate-scabrous. Stem erect, ending in panicle; flowering stems purple. Leaves 1.6-6.5 cm in length while, it 1-2.5cm in width, rugose-bullate, repand-crenate or dentate-lobed; cauline leaves lyrate-pinnatipartite or pinnatisect, with ovate obtuse segments; petioles length 1-6.5 cm with long hairs, uppermost leaves sessile, cordate-ovate to oblong acute; flora leaves membranous, acuminate, much shorter than verticillasters.; Verticillasters 6-12 flowered, remote, in long raceme-like inflorescences; pedicles shorter than calyx. Calyx tubular, 7-8 mm, purple, scabrous; upper lip with 2 lateral triangular spinulose teeth, middle tooth obsolete; teeth of lower lip slender, subulate; fruiting calyces nodding. Corolla violet, 10-15mm. Fl. April-June.

Habitat: Mediterranean batha and fallow fileds. Rather common. Sharon Plain; Upper and Lower Galilee, Mt. Carmel, Mt. Gilboa, Esdraelon Plain, Samaria, Shefela, Judean Mts.; Upper Jordan Valley; Gilead, Ammon (Zohary, 1966).

Area: E. Mediterranean (Palestine, Lebanon, Syria).

Distribution:

Beit-Leed /Tulkarm, Al-Yamwn /Jenin, Taluza /Nablus and Ramallah. **(Plate 5).**

Table 1: Morphological Characteristics of *Salvia* Studied Taxa

Taxon	Leaves				Flower				Petiole	
	Leaf length (cm)	**Leaf width (cm)**	**Leaf margin**	**Shape**	**Calyx length& texture (mm)**	**Corolla length (mm)**	**Corolla colour**	**No . of verticillasters**	**Petiole length (cm)**	**Petiole texture**
S. fruticosa	2-8.5	1-3	crenate tripartite	ovate-oblong to lanceolate	7-8 viscid with glandular & partly eglandular hairs	13-21	purplish-pink, rarely white	4-6 to many-flowered	1-3	dense, long hairs
S. dominica	1.4-5.5	0.5-3.6	crenate	triangular-ovate to oblong	7-10 covered with dense long white hairs	14-21	cream-coloured	4-6	0.5-1.4	long hairs
S. hierosolymitana	5.5-17	3.5-12	dentate	ovate to triangular - ovate	10-14 glandular-hairy	23-28	Wine-red or Purplish-pink	4-6	1.5-15	short hairs
S. verbenaca	4-6.8	1.5-2.6	crenate & mostly lobed	ovate to oblong	5-9 hispid &glandular-hairy	10-12	blue to lavender	4-6	1.7-6.9	short hairs
S. lanigera	0.5-4	0.5-0.7	pinnatisect	oblong to triangular	7-8 covered with long white hairs	15-20	deep violate	6-8	0.5-1	long hairs
S. viridis	1.2-4.2	0.5-2	crenate	ovate to oblong	6-8	10-14	purplish-pink, rarely white	4-6	0.3-4	short hairs
S. judaica	1.6-6.5	1-2.5	crenate	ovate to oblong	7-8 scabrous	10-15	violate	6-12	1-6.5	short hairs

plate 1: Coloured photographs showing the plant as taken in thier natural habitat.

A: ***S. fruticosa*** general view .

B: ***S. fruticosa*** showing the flowring parts.

C: ***S. dominica*** general view.

D: ***S. dominica*** showing the flowring parts.

plate 2: Coloured photographs showing the plant as taken in thier natural habitat.

A: ***S. hierosolymitana*** general view .

B: ***S. hierosolymitana*** showing the flowring parts.

C: ***S. verbenaca*** general view.

D: ***S. verbenaca*** showing the flowring parts.

plate 3: Coloured photographs showing the plant as taken in thier natural habitats.

A: ***S. lanigera*** general view.

B: ***S. lanigera*** showing the flowring parts.

C: ***S. viridis*** general view.

D: ***S. viridis*** showing the flowring parts .

Plate 4: Coloured photographs showing the plant as taken in thier natural habitats.

A: ***S. judaica*** general view.

B: ***S. judaica*** showing the flowring parts.

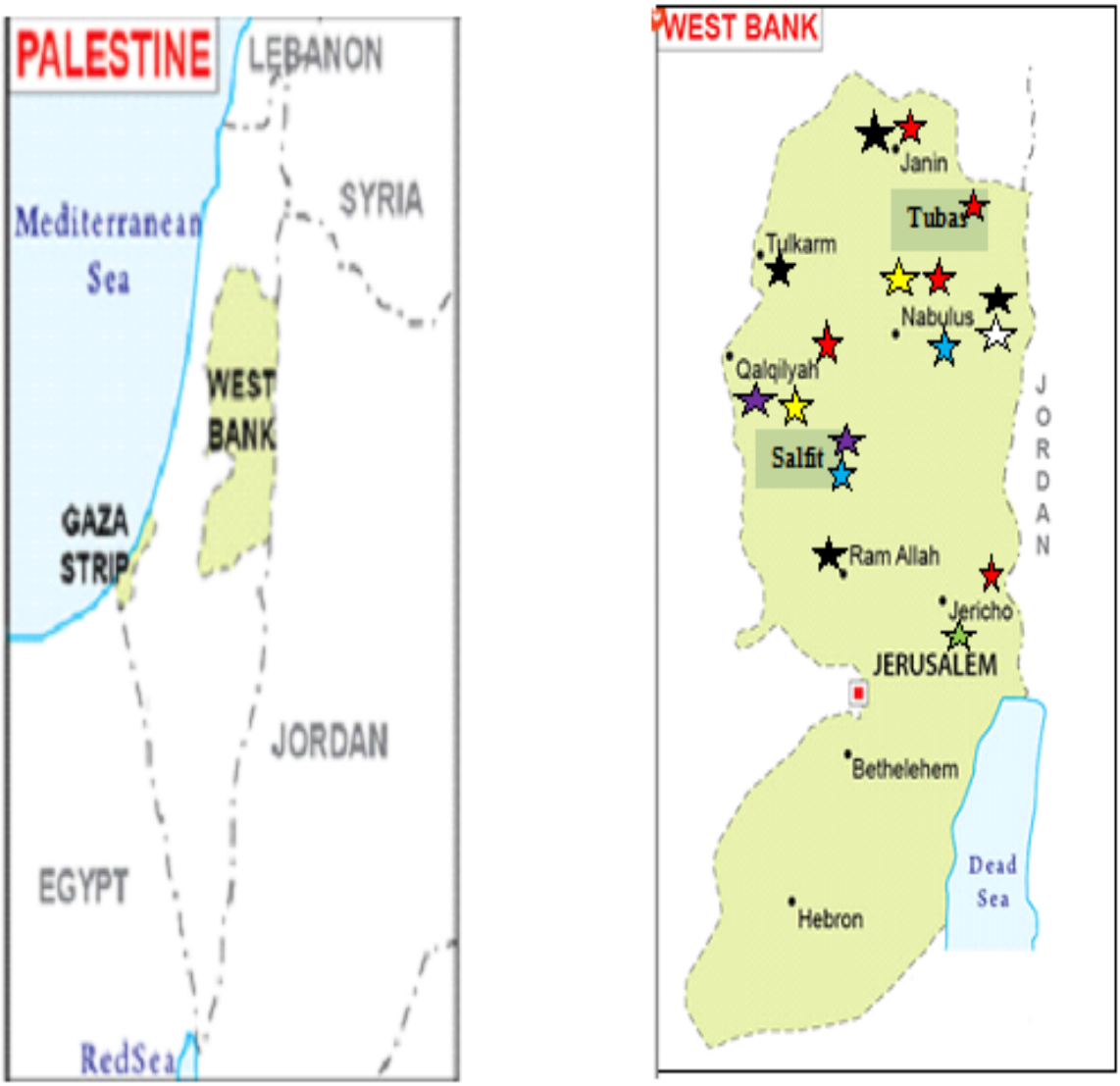

Plate 5: Map of Palestine showing the distribution of *Salvia* taxa.

Showing the distribution of *S. fruticosa*

Showing the distribution of *S.dominica*

Showing the distribution of *S. hierosolymitana*

Showing the distribution of *S. verbenca*

Showing the distribution of *S. lanigera*

Showing the distribution of *S. viridis*

Showing the distribution of *S. judaica*

3.1.4. Discussion and Conclusions

The main morphological characters of ***Salvia*** species such as leaf shape, margin, calyx, corolla and petiole length are taxonomically important features to identify these species.

The constancy of the morphological characters upon which, the plant classification depends on, increases their taxonomic value. Mostly, there is no clear discrimination between some plant species regarding a specific character. Instead, the consideration of overall characters is more helpful in the classification of studied taxa.

Leaves margin vary between ***Salvia*** species. The leaf margin of ***S. hierosolymitana*** is dentate while, it is pinnatisect in ***S. lanigera*** but appears as crenate in all other ***Salvia*** spices under study.

Leaves shape also vary between ***Salvia*** species. ***Salvia fruticosa*** has ovate-oblong to lanceolate leaves, ***S. dominica*** has triangular-ovate to oblong, ***S. hierosolymitana*** has ovate-triangular ovate, ***S.verbenaca*** has ovate to oblong, ***S. lanigera*** has oblong to triangular, ***S. viridis*** has ovate to oblong and ***S. judaica*** has triangular ovate ones.

Leaves length range from the largest; 5.5- 17 cm in ***S. hierosolymitana*** to the smallest; 0.5-4 in ***S. lanigera***. While, leaves width range from the largest; 3.5-12 cm in ***S. hierosolymitana*** to the smallest; 0.5-0.7 cm of ***S. lanigera***.

The calyx length and texture important taxonomical tools to distinguish between ***Salvia*** species. The calyx length and texture for studied ***Salvia*** species are as follow:

The only ***Salvia*** species with a scabrous calyx is ***S. judaica***. But ***S. fruticosa***, ***S. verbenaca*** and ***S. hierosolymitana*** have calyx with glandular hairs, with being partly eglandular in ***S. fruticosa***. However, both ***S. dominica*** and ***S. lanigera*** have calyx with long white hairs.

While, considering the calyx length, it was observed that ***S. hierosolymitana*** has the largest one with 10-14 mm length. Other studied species calyx length was ranging from 5mm to 10 mm as maximum.

Morphological differences regarding petioles length were recorded between studied taxa. The variation ranged from the largest; 1.5-15 cm of ***S. hierosolymitana*** to the smallest; 0.5-1 cm of ***S. lanigera***. This indicates that petiole length can be used to distinguish between some species under study.

Corolla length and colour introduce taxonomical characters by which we can differentiate among ***Salvia*** species. These characters as follow:

Cream-coloured corolla is recorded in ***S. dominica***. Purplish-pink to rarely white corolla was observed in both ***S. fruticosa*** and ***S. viridis***. This colour turns to be more wine red in ***S. hierosolymitana***. Violet corolla is recorded in ***S. judaica*** and being deeper in colour in ***S. lanigera.*** However, the blue

to lavender corolla is observed in ***S. verbenaca.*** While, the largest corolla was recorded in ***S. hierosolymitana*** and the smallest one in ***S. verbenaca***.

As a conclusion, there is an overlapping between different species in measurements of parts of the plants under study. In spite of that, some morphological characters of ***Salvia*** species can be useful in identifying between species such as corolla colour and length. While, the consideration of overall characters may be more precise to discriminate among studied taxa.

3.2. Palynology Results

3.2.1. Results

Pollen grains of seven ***Salvia*** species have been isolated from mature anthers of either herbarium or fresh specimens. Their pollen grains were studied using scanning electron microscope through which, general shape, symmetry, aperture type and sculpturing type. Pollen grains were also examined under the light compound microscope mainly for the measurements of their size and investigation of possible variation in shape and aperture. Pollen grains characters obtained results are summarized in (Table 2).

Studied pollen grains of the species of the genus ***Salvia*** occurring in West Bank/Palestine showed the following characterization:

3.2.1.1. *Salvia fruticoza* Mill. (Plate 6, A) & (Plate 8, A, B).

Pollen radially symmetrical, non-angular, convex, obtuse, rectangular, isopolar, suboblate, hexazonocolpate. Size P/E= 27-38/35-47 µm, sculpturing reticulate-perforate.

3.2.1.2. *Salvia domonica* L. (Plate 6, B) & (Plate 8, C, D).

Pollen radially symmetrical, non-angular, convex, obtuse, elliptical-circular, isopolar, oblate-spheroidal, hexazonocolpate. Size P/E= 35-42/38-54 µm, sculpturing reticulate-perforate.

3.2.1.3. *Salvia hierosolymitana* Boiss. (Plate 6, C) & (Plate 9, A, B).

Pollen radially symmetrical, non-angular, convex, obtuse, elliptical, heteropolar, suboblate, hexazonocolpate. Size P/E= 34-45/41-55 µm, sculpturing reticulate-perforate.

3.2.1.4. *Salvia verbenca* L. (Plate 6, D) & (Plate 9, C, D).

Pollen radially symmetrical, non-angular, convex, obtuse, rectangular, isopolar, oblate-spheroidal, hexazonocolpate. Size P/E= 32-42/38-49 µm, sculpturing semitectate reticulate.

3.2.1.5. *Salvia lanigera* Poir. (Plate 7, A) & (Plate 10, A, B).

Pollen radially symmetrical, non-angular, convex, rectangular, isopolar, suboblate, hexazonocolpate. Size P/E= 36-43/41-50 µm, sculpturing reticulate-perforate.

3.2.1.*6. Salvia viridis* L. (Plate 7, B) & (Plate 10, C, D).

Pollen radially symmetrical, non-angular , convex, obtuse, rectangular-elliptical, isopolar, oblate-spheroidal, hexazonocolpate. Size P/E= 31-37/39-50 µm, sculpturing reticulate-perforate.

3.2.1.7. *Salvia judaica* Boiss. (Plate 7, C) & (Plate 11, A, B).

Pollen radially symmetrical, non-angular, convex, rectangular, isopolar, oblate-spheroidal, hexazonocolpate. Size P/E= 27-34/30-40 µm, sculpturing reticulate-perforate.

Table 2 :Pollen Grains Morphology of the studied *Salvia* Species

Taxon	Symmetry	Polarity	Aperture	Shape	Size(P/E) (µm)	Sculpture
S. fruticosa	radially	isopolar	hexazonocolpate	suboblate	27-38/35-47	reticulate-perforate
S. dominica	radially	isopolar	hexazonocolpate	oblate-spheroidal	35-42/38-54	reticulate-perforate
S. hierosolymitana	radially	heteropolar	hexazonocolpate	Suboblate	34-45/41-55	reticulate-perforate
S. verbenaca	radially	isopolar	hexazonocolpate	oblate-spheroidal	32-42/38-49	semitectate reticulate.
S. lanigera	radially	isopolar	hexazonocolpate	suboblate	36-43/41-50	reticulate-perforate
S. viridis	radially	isopolar	hexazonocolpate	oblate-spherodeal	31-37/39-50	reticulate-perforate
S. judaica	radially	isopolar	hexazonocolpate	oblate-spheroidal	27-34/30-40	reticulate-perforate

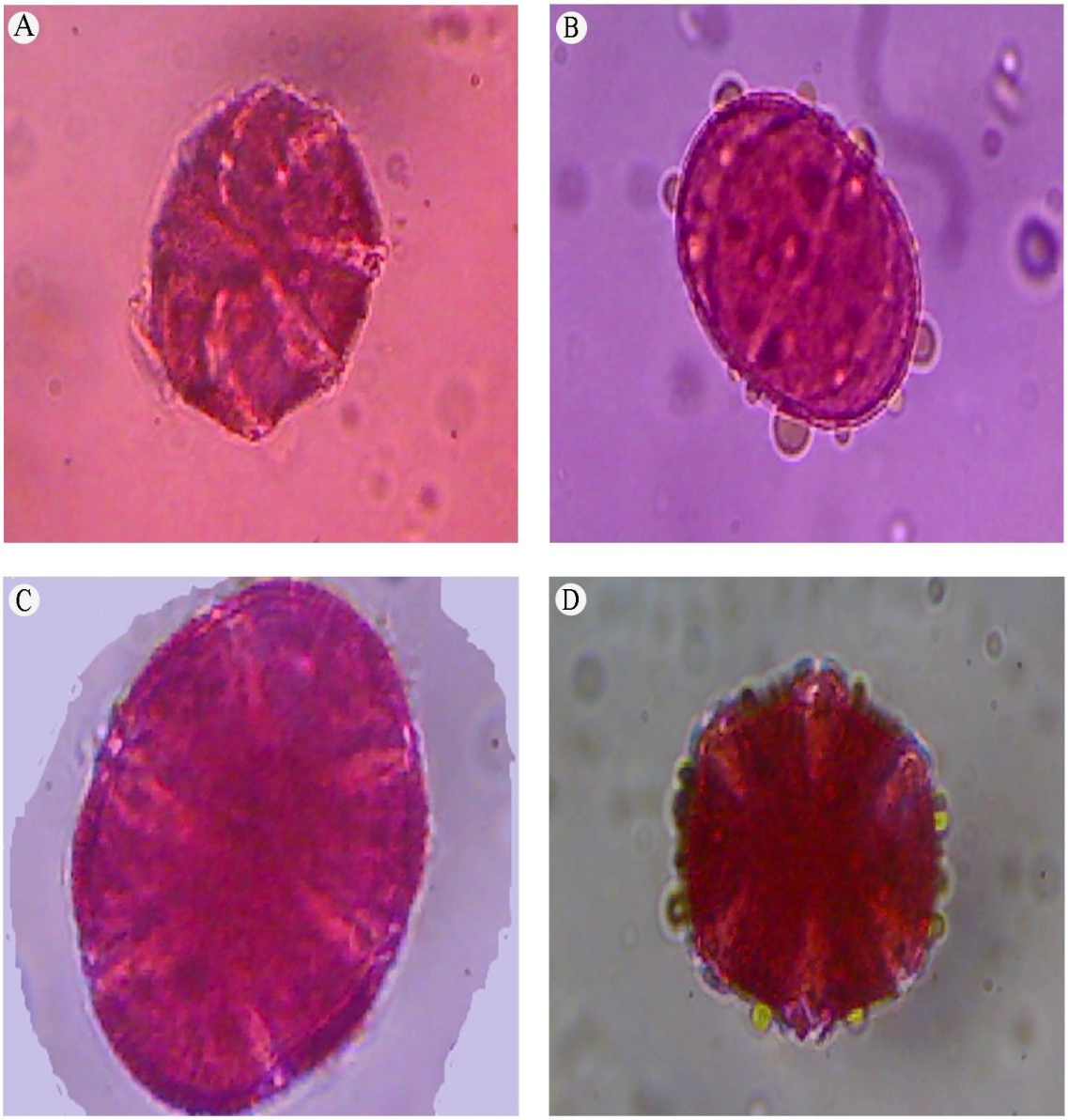

Plate 6: Light micrographs of pollen grains of *Salvia* species showing out line shape, and aperture.

A. ***S. fruticosa*** showing polar view and hexazonocolpate apertures, (1000x).

B. ***S. dominica*** showing equatorial view and hexazonocolpate apertures, (1000x).

C. ***S. hierosolymitana*** showing polar view and hexazonocolpate apertures, (1000x).

D. ***S. verbenaca*** showing equatorial view and hexazonocolpate apertures, (1000x).

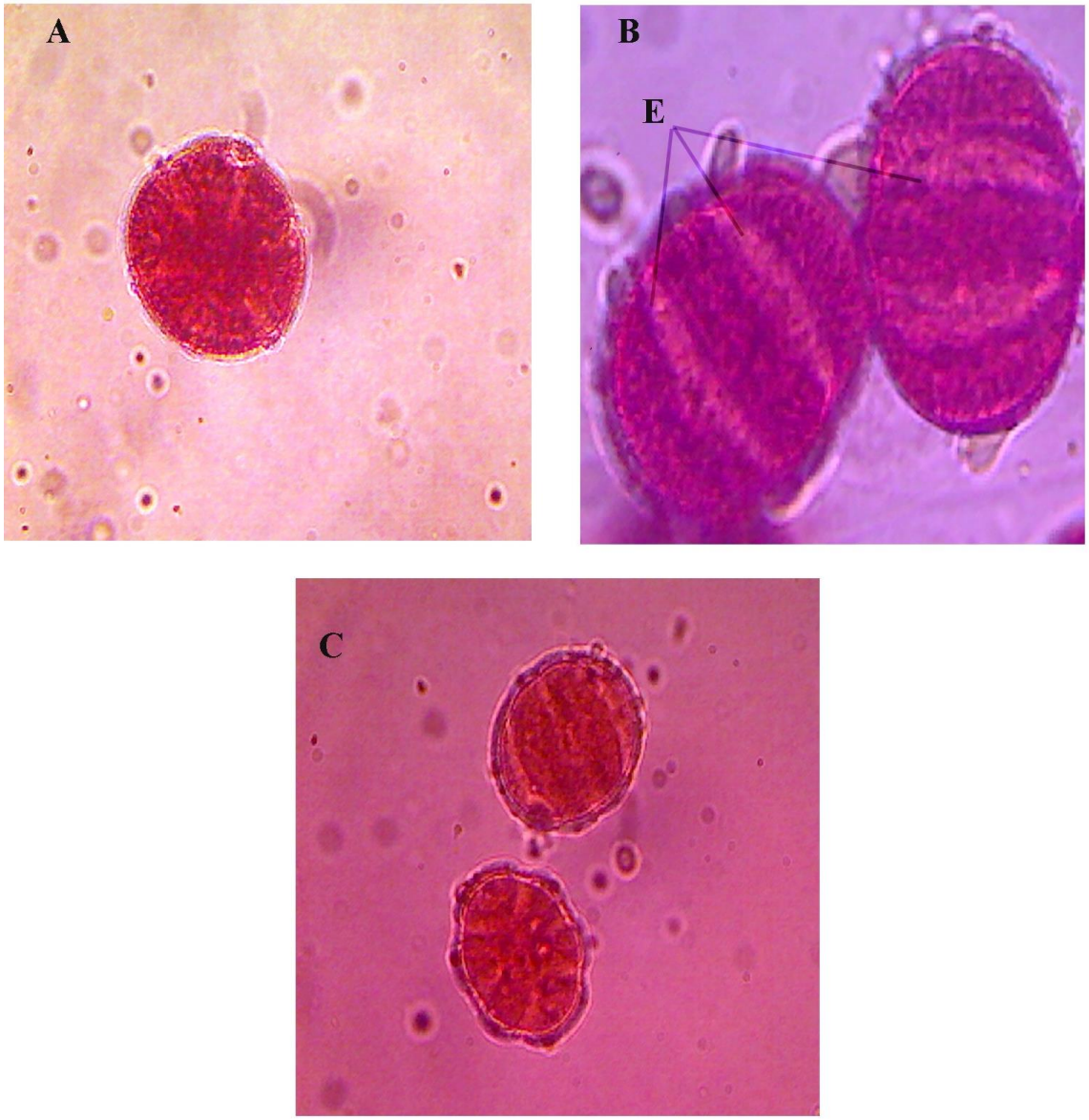

Plate 7: Light micrographs of pollen grains of *Salvia* species showing out line shape, and aperture pattern.

A. ***S. lanigera*** showing polar view and hexazonocolpate aperture, (1000x).

B. ***S. viridis*** showing equatorial view and hexazonocolpate aperture, (1000x).

C. ***S. judaica*** showing polar and equatorial view and hexazonocolpate aperture, (1000x).

E. Aperture pattern hexazonocolpate.

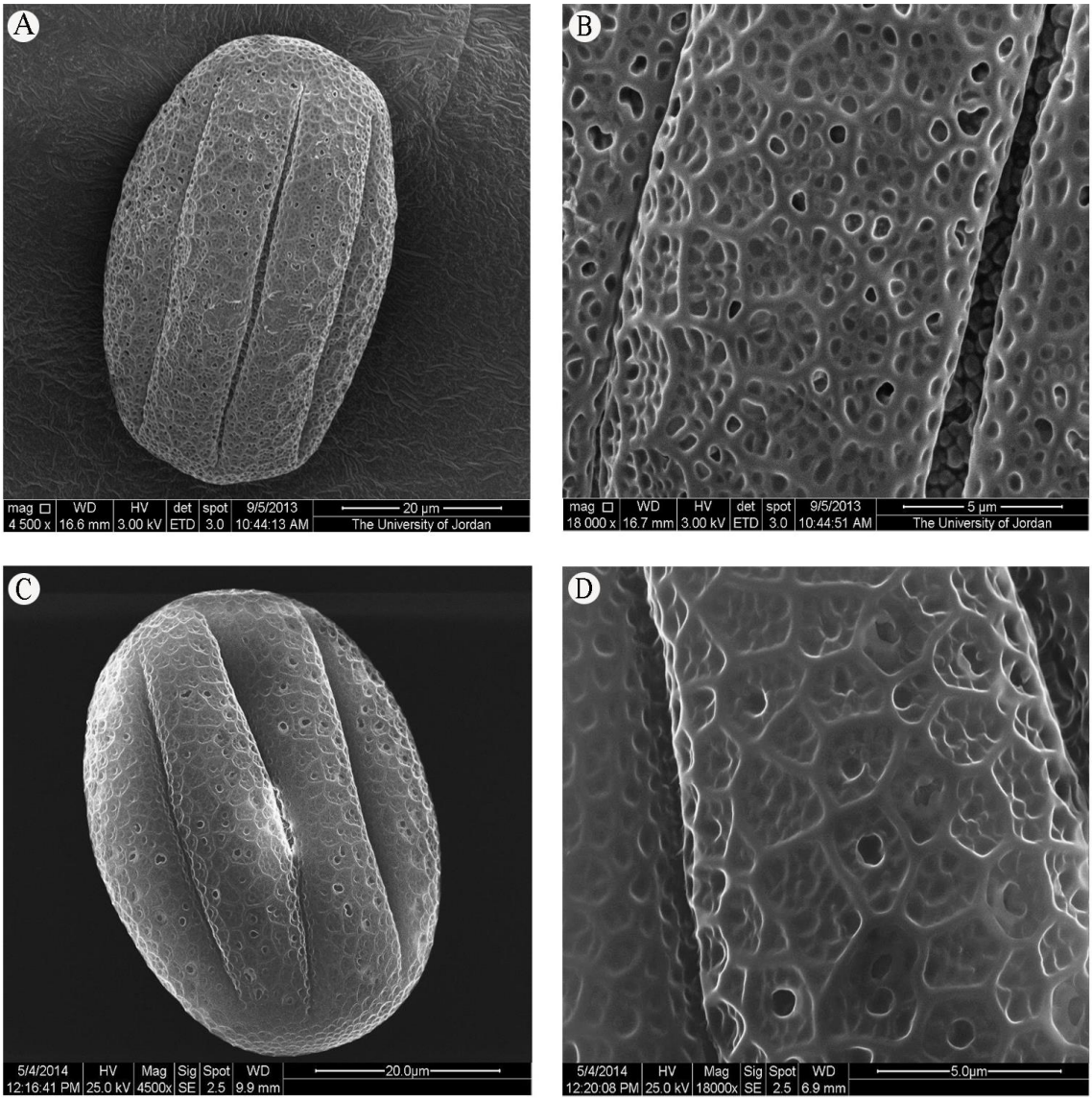

Plate 8: Scanning electron micrographs of pollen grains of *Salvia* species showing out line shape, polarity, and surface sculpturing pattern.

A: ***S. fruticosa*** showing suboblate shape general equtaorial view and isopolarity, (4500x).

B: ***S. fruticosa*** showing reticulate-perforate sculpturing, (18000x).

C: ***S. dominica*** showing oblate-spheroidal shape general equtaorial view, and isopolarity, (4500x).

D: ***S. dominica*** showing reticulate-perforate sculapturing, (18000x).

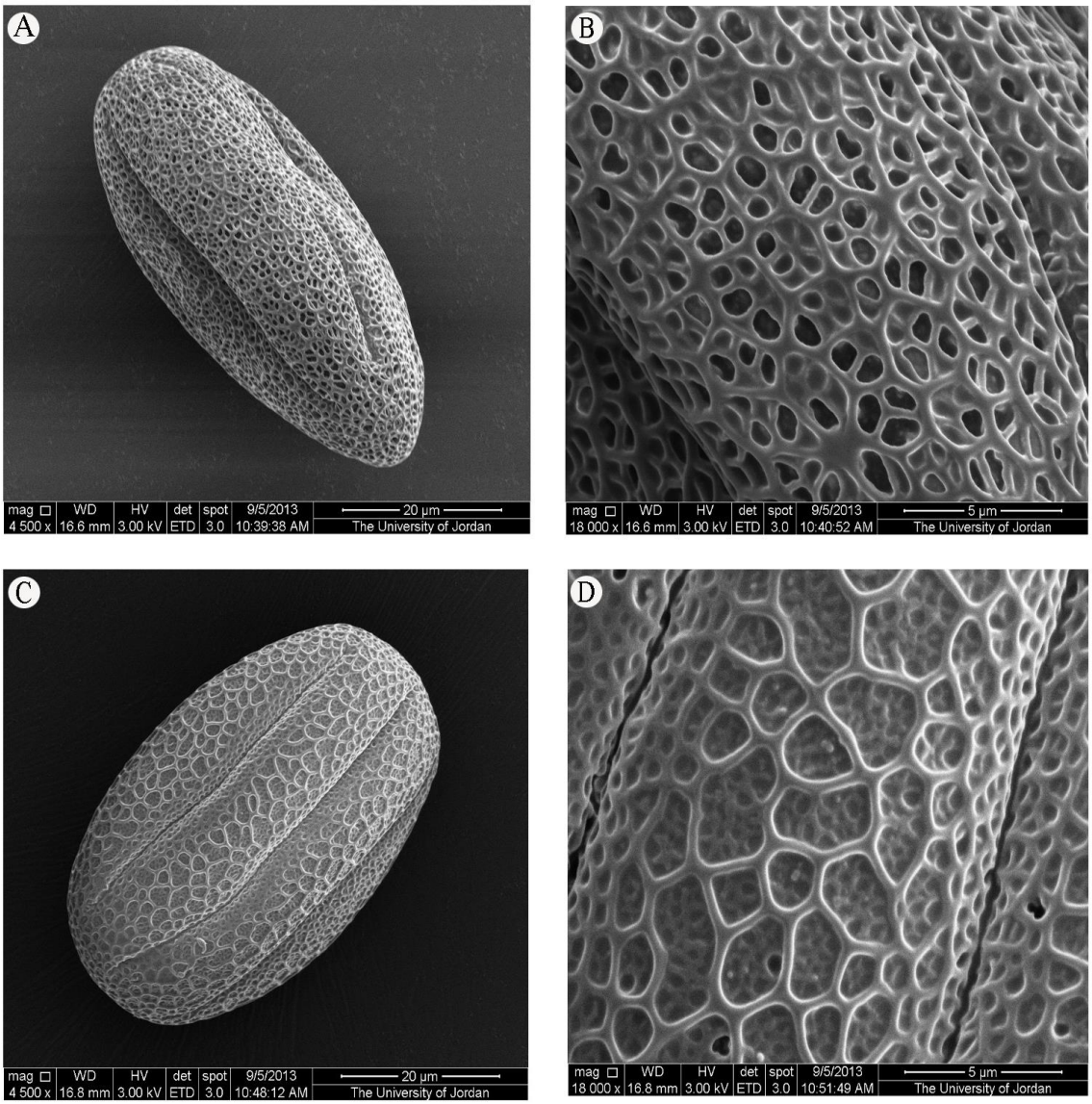

Plate 9: Scanning electron micrgraphs of pollen grains of *Salvia* species showing out line shape, polarity and surface sculpturing pattern.

A: ***S. hierosolymitana*** showing suboblate shape general equatorial view and heteropolarity, (4500x).

B: ***S. hierosolymitana*** showing reticulate-perforate sculpturing. (18000x).

C: ***S. verbenaca*** showing oblate-spherodeal shape general equatorial view and isopolarity, (4500x).

D: ***S. verbenaca*** showing semitectate reticulate sculpturing, (18000x).

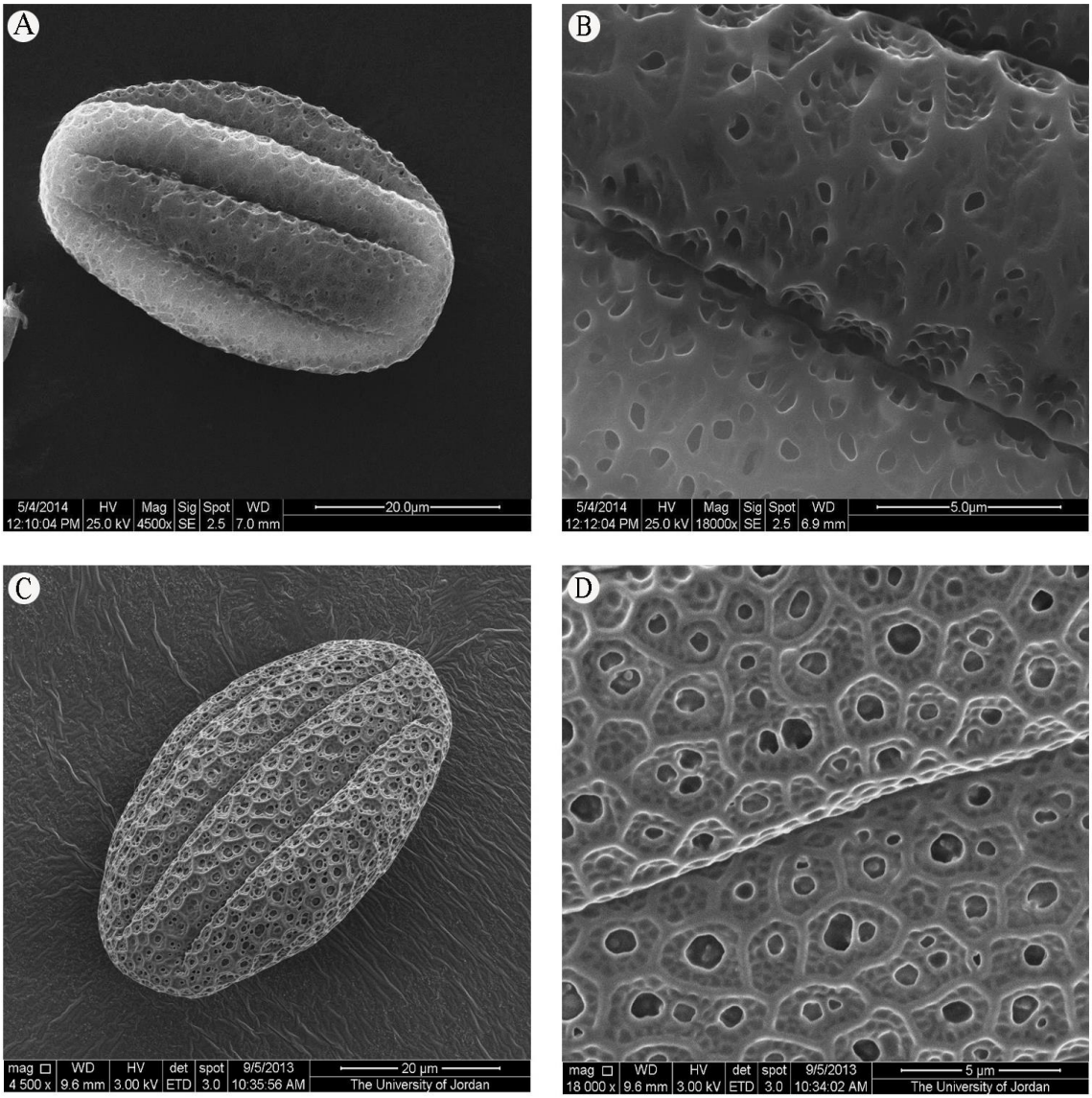

Plate 10: Scanning electron micrgraphs of pollen grains of *Salvia* species showing out line shape, polarity and surface sculpturing pattern.

A: ***S. lanigera*** showing suboblate shape general equatorial view and isopolarity, (4500x).

B: ***S. lanigera*** showing reticulate-perforate sculpturing, (18000x).

C: ***S. viridis*** showing oblate-spherodeal shape general equatorial view and isopolarity, (4500x).

D: ***S. viridis*** showing reticulate-perforate sculpturing, (18000x).

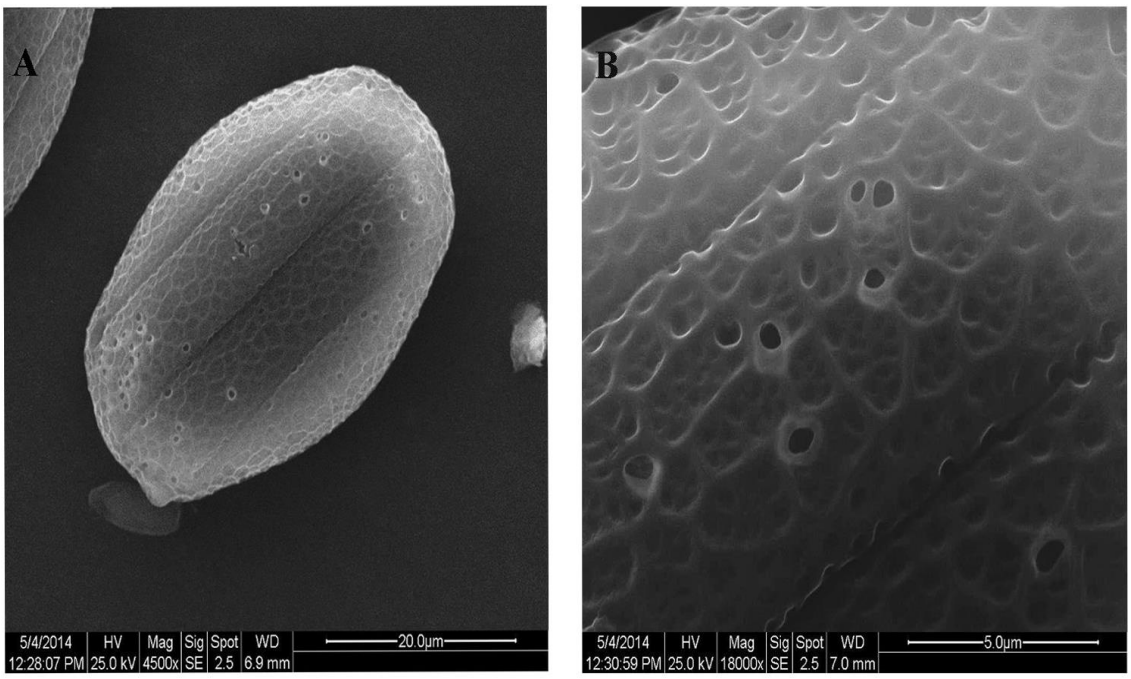

Plate 11: Scanning electron micrographs of pollen grains of *Salvia* species showing out line shape, polarity and surface sculpturing pattern.

A: ***S. judaica*** showing suboblate shape general equtaorial view and isopolarity, (4500x).

B: ***S. judaica*** showing reticulate-perforate sculpturing. (18000x).

3.2.2. Discussion and Conclusion

The examination of pollen grains morphology of ***Salvia*** species have been achieved by both light and scanning electron microscopy. Data obtained from light microscopy study was related to the symmetry, apertures, shape and size. While, the scanning electron microscopy was based on the shape, poles, apertures and sculpturing pattern.

Obtained data (Table 2), showed some differences between all studied taxa. ***Salvia*** species in general have isopolar pollen grains, except ***S. hierosolymitana*** which has heteropolar pollen grains, that one pole is wider than the other. However, all studied taxa have radial symmetry.

The pollen grains size is variable among the ***Salvia*** studied species. It ranges from 27/30 to 45/55 µm (P/E), with some variations among the examined species.

The shape of pollen grains varies between studied taxa, from suboblate for ***S. fruticoza***, ***S. hierosolymitana*** and ***S. lanigera*** to oblate-spheroidal shape, for ***S. domonica, S. verbenaca***, ***S. viridis*** and ***S. judaica***, considering the ratio between the polar axis (P) and the equatorial axis (E). They can also be described as elliptical to rectangular or rectangular-elliptical according to Moore and Weeb aspects (Moore and Weeb, 1978). In spite of that, the shape of pollen grain cannot be used to a great deal in separating taxa, because it can vary considerably within one grain type or even within one species. This fact agrees with the pollen grains of most

plant (Moore and Webb, 1978). The apertures of all studied taxa are hexazonocolpate.

Most ***Salvia*** species have reticulate-perforate sculpturing, except ***S. verbenaca*** wich has semitectate reticulate sculpturing. The degree and pattern of reticulation and tectation of the pollen grains surface of the studied taxa varied. Variations in this aspect, could not be reflected to numbers or description, but could be observed by looking to their sculpture as shown by the electron micrographs for each ***Salvia*** species

As a conclusion, the combination of information from palyonological studies and other biosystematic evidences, may allow the opportunity to make use of the palynological data in the delimitation and classification of ***Salvia*** species.

3.3. Leaf Anatomy Results

3.3.1. Results

Plant samples were freshly collected from natural population. Some of the samples were used for anatomical observation and some of them were dried as herbarium sample. Then leaves specimens were fixed and treated as mentioned in materials and methods chapter previously.

Transverse sections of 6 µm thickness for several specimens of each taxon under study were obtained. Different light microscopic photographs were

taken for several cross sections for the studied taxa. The most ideal ones have been selected, examined and photographed.

The slides of the cross leaf section were studied and interpreted and described for each species in details. The description and measurements of the leaves cross section parts are summarized in (Table 3) following the detailed description.

3.3.1.1. *Salvia fruticosa* Mill. **(Plate 12).**

Leaf is bifacial in cross section. Leaf thickness is 461-737 µm (from upper epidermis to lower epidermis passing through midrib vein). Cuticle thickness is 3-7.5 µm. Epidermis is 3-24×10-47 (l.×w.) µm. Mesophyll is 64-172 µm thick, made of 3-4 layers of palisade chlorenchyma; 42-90µm thick and spongy chlorenchyma; 21-111 µm thick.

Midrib area is made of a central vascular bundle; 194-271× 187-367 (l.×w.) µm. All secondary vascular bundles; 40-138×22-150 (l.×w.) µm, are surrounded by a sheath of layers of 2-3 irregular parenchyma cells. Adaxial epidermal cells are larger than abaxial epidermal cells. Especially, eipedermal cells at the region of median vein are larger than others. Stomata present on the lower and upper epidermis of leaf. The leaf in cross section has capitate glandular hairs.

3.3.1.2. *Salvia dominica* L. **(Plate 13).**

Leaf is bifacial in cross section. Leaf thickness is 372-426 µm (from upper epidermis to lower epidermis passing through midrib vein). Cuticle

thickness is 1.3-6.5µm. Epidermis is 7-31×6-36 (l.×w.) µm. Mesophyll is 73-122 µm thick, made of 3-4 layers of palisade chlorenchyma; 39-62 µm thick and spongy chlorenchyma; 36-61 µm thick.

Midrib area is made of a central vascular bundle; 125-445×80-326 (l.×w.) µm. All secondary vascular bundles; 34-145×24-113 (l.×w.) µm are surrounded by a sheath of layers of 1-2 irregular parenchyma cells. Adaxial epidermal cells are larger than abaxial epidermal. Stomata present on the lower and upper epidermis of leaf. The leaf in cross section has capitate glandular hairs.

3.3.1.3. *Salvia hierosolymitana* Boiss. **(Plate 14).**

Leaf is bifacial in cross section. Leaf thickness is 403-632 µm (from upper epidermis to lower epidermis passing through midrib vein). Cuticle thickness is 1.4-6 µm. Epidermis is 6-23×12-45 (l.×w.) µm. Mesophyll is 93-128 µm thick, made of 3-5 layers of palisade chlorenchyma; 65-72.5µm thick and spongy chlorenchyma; 27.5-35 µm thick.

Midrib area is made of a central vascular bundle; 122-644×161-763 (l.×w.) µm. All secondary vascular bundles; 31-171×290-229 (l.×w.) µm. Adaxial epidermal cells are larger than abaxial epidermal cells. Stomata present on lower and upper epidermis of leaf. The leaf in cross section has capitate glandular hairs.

3.3.1.4. *Salvia verbenaca* L. **(Plate 15).**

Leaf is bifacial in cross section. Leaf thickness is 309-555 µm (from upper epidermis to lower epidermis passing through midrib vein). Cuticle

thickness is 2-8.5 µm. Epidermis is 14-33×15-46 (l.×w.) µm. Mesophyll is 200-230 µm thick, made of 3-5 layers of palisade chlorenchyma; 120-130 µm thick and spongy chlorenchyma; 77.5-110 µm thick.

Midrib area is made of a central vascular bundle; 110-293×194-293 (l.×w.) µm. All secondary vascular bundles; 40-110×24-71 (l.×w.) µm, are surrounded by a sheath of layers of 2-3 irregular parenchyma cells. Adaxial epidermal cells are larger than abaxial epidermal cells. Especially, eipedermal cells at the region of median vein are larger than others. Stomata present on the lower and upper epidermis of leaf. The leaf in cross section has peltate glandular hairs.

3.3.1.5. *Salvia lanigera* Poir. **(Plate 16).**

Leaf is bifacial in cross section. Leaf thickness is 288 µm (from upper epidermis to lower epidermis passing through midrib vein). Cuticle thickness is 2.6-8.6µm. Epidermis is10-27×11-40 (l.×w.) µm. Mesophyll is 123-175 µm thick, made of 3-5 layers of palisade chlorenchyma; 54-112µm thick and spongy chlorenchyma; 55-97 µm thick.

Midrib area is made of a central vascular bundle; 100×262 (l.×w.) µm. All secondary vascular bundles; 41-72×26-56 (l.×w.) µm are surrounded by a sheath of layers of 1-2 irregular parenchyma cells. Adaxial epidermal cells are larger than abaxial epidermal cells. Stomata present on the lower and upper epidermis of leaf. The leaf in cross section has eglandular hairs.

3.3.1.6. *Salvia viridis* L. **(Plate 17).**

Leaf is bifacial in cross section. Leaf thickness is 124-180 µm (from upper epidermis to lower epidermis passing through midrib vein). Cuticle thickness is 2.2-7.5 µm. Epidermis is 9-28×12-42 (l.×w.) µm. Mesophyll is 90-112.5 µm thick, is made of 3-4 layers of palisade chlorenchyma; 57.5-65µm thick and spongy chlorenchyma; 47.5-60 µm thick.

Midrib area is made of a central vascular bundle; 74-61× 51-58 (l.×w.) µm. All secondary vascular bundles; 25-40×22-28 (l.×w.) µm are surrounded by a sheath of layers of 1-2 irregular parenchyma cells. Adaxial epidermal cells are larger than abaxial epidermal cells. Stomata present on the lower and upper epidermis of leaf. The leaf in cross section has eglandular hairs.

3.3.1.7. *Salvia judaica* Boiss. **(Plate 18).**

Leaf is bifacial in cross section. Leaf thickness is 295-388 µm (from upper epidermis to lower epidermis passing through midrib vein). Cuticle thickness is 1.7-7.5µm. Epidermis is 7-28×6-43 (l.×w.) µm. Mesophyll thickness is 83-143 µm thick, made of 3-4 layers of palisade chlorenchyma; 57-89 µm thick and spongy chlorenchyma; 30-65 µm thick.

Midrib area is made of a central vascular bundle; 119-162×155-278 (l.×w.) µm. All secondary vascular bundles; 45-113×28-113 (l.×w.) µm are surrounded by a sheath of layers of 1-2 irregular parenchyma cells. Adaxial epidermal cells are larger than abaxial epidermal cells. Stomata present on the lower and upper epidermis of leaf**.** The leaf in cross section has eglandular hairs.

Table 3: Leaf Anatomical Characteristic of *Salvia* Studied Taxa

Taxon	Leaf thickness (µm)	Cuticle thickness (µm)	Epidermis length (µm)	Epidermis width (µm)	Mesophyll layer (µm)	Palisade thickness (µm)	Spongy thickness (µm)	Midrib vascular bundle (length × width) (µm)	Small vascular bundle (length × width) (µm)
S. fruticosa	461-737	3-7.5	3-24	10-47	64-172	42-90	21-111	194-271× 187-367	40-138×22-150
S. dominica	372-426	1.3-6.5	7-31	6-36	73-122	39-62	36-61	125-445×80-326	34-145×24-113
S. hierosolymitana	403-632	1.4-6	6-23	12-45	93-128	65-72.5	27.5-35	122-644×161-763	31-171×290-229
S. verbenaca	309-555	2-8.5	14-33	15-46	200-230	120-130	77.5-110	110-293×194-293	40-110×24-71
S. lanigera	288	2.6-8.6	10-27	11-40	123-175	54-112	55-97	100×262	41-72×26-56
S. viridis	124-180	2.2-7.5	9-28	12-42	90-112.5	57.5-65	47.5-60	74-61× 51-58	25-40×22-28
S. judaica	295-388	1.7-7.5	7-28	6-43	83-143	57-89	30-65	119-162×155-278	45-113×28-113

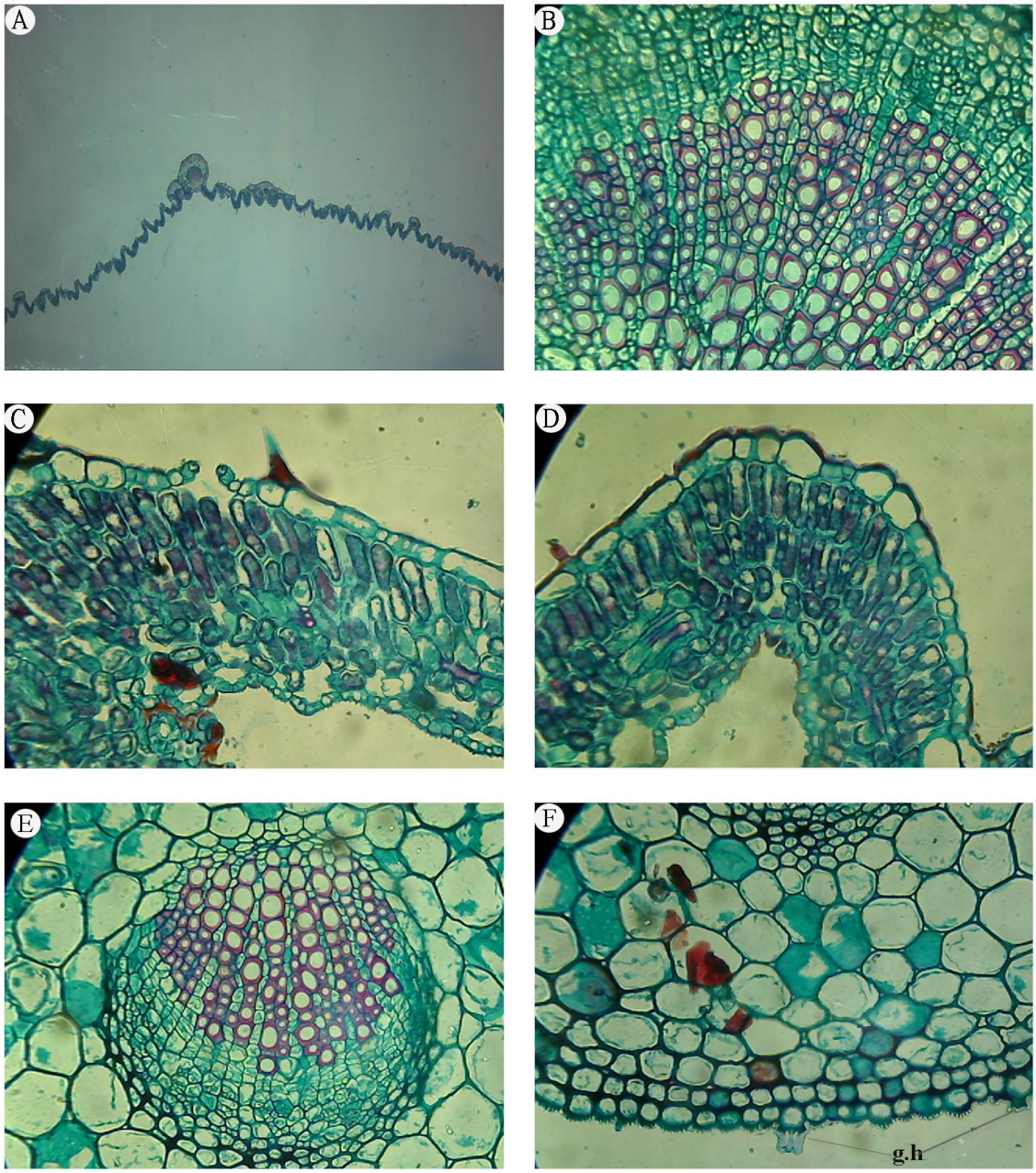

Plate 12:Light microscopic photographs of leaf transverse section of *S. fruticosa* showing the general out line, cuticle, epidermis, mesophyll, stomata and vascular bundles.

A. Leaf out line, (8x).

B. Midrib area showing arc-shaped central vascular bundle, (400x).

C. Stomata, on both lower and upper epidermis, (400x).

D. Leaf layers showing cuticle, epidermis, mesophyll including palisade and spongy cells, (400x).

E. A vascular bundle srounded by a sheath of 2-3 layers of irregular shaped parenchyma, (400x).

F. Leaf section showing capitate glandular hairs (g.h), (400x).

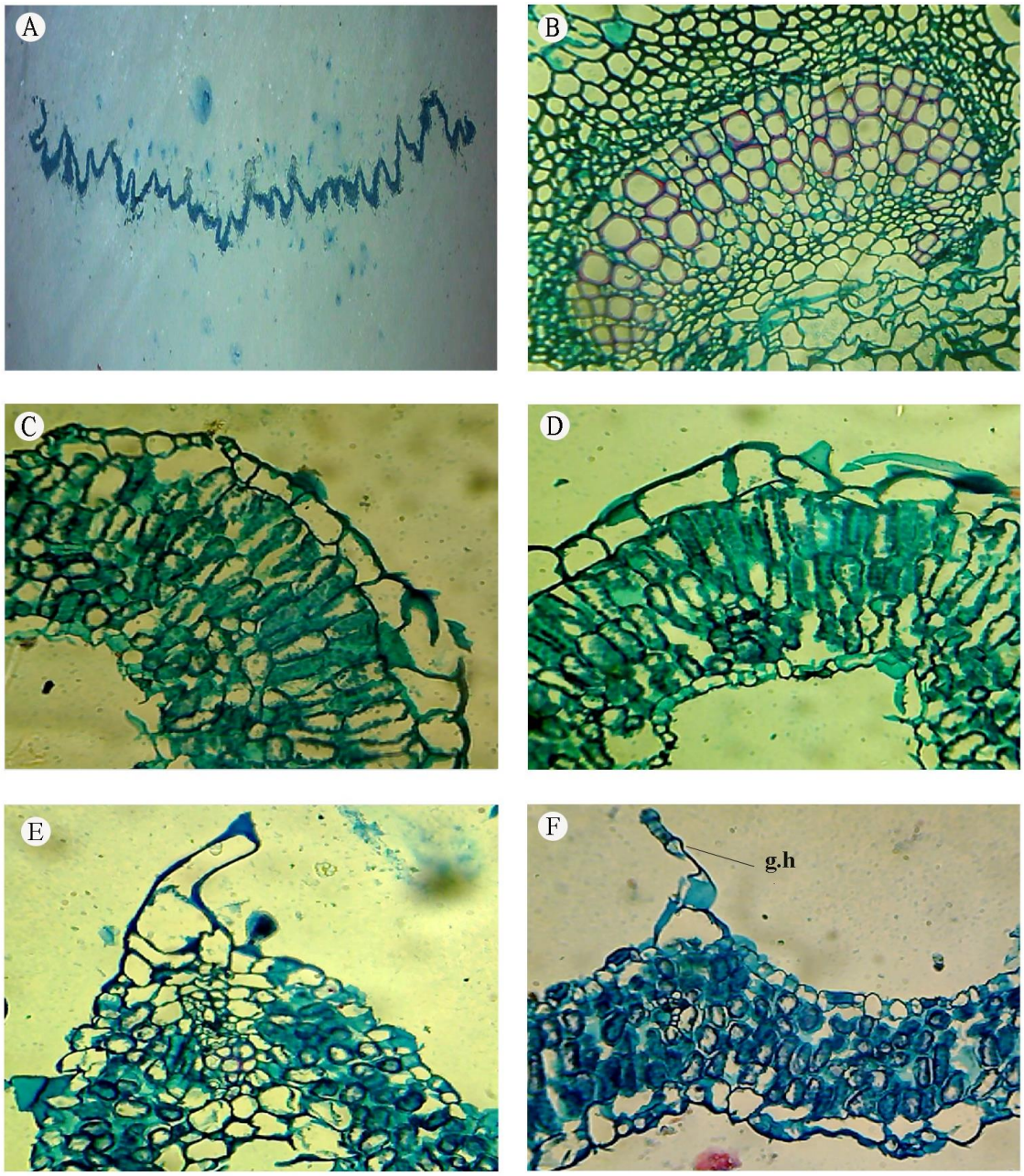

Plate 13: Light microscopic photographs of leaf transverse section of *S. dominica* showing the general out line, margins, cuticle, epidermis, mesophyll, stomata and vascular bundles.

A. Leaf out line, (10x).

B. Midrib area showing the arc- shaped central vascular bundle, (400x).

C. Stomata, on the lower and upper epidermis, (400x).

D. Leaf layers showing cuticle, epidermis, mesophyll including palisade and spongy cells, (400x).

E. A vascular bundle srounded by a sheath of 1-2 layers of irregular shaped parenchyma, (400x).

F. Leaf layers showing capitate glandular hair (g.h), (400x).

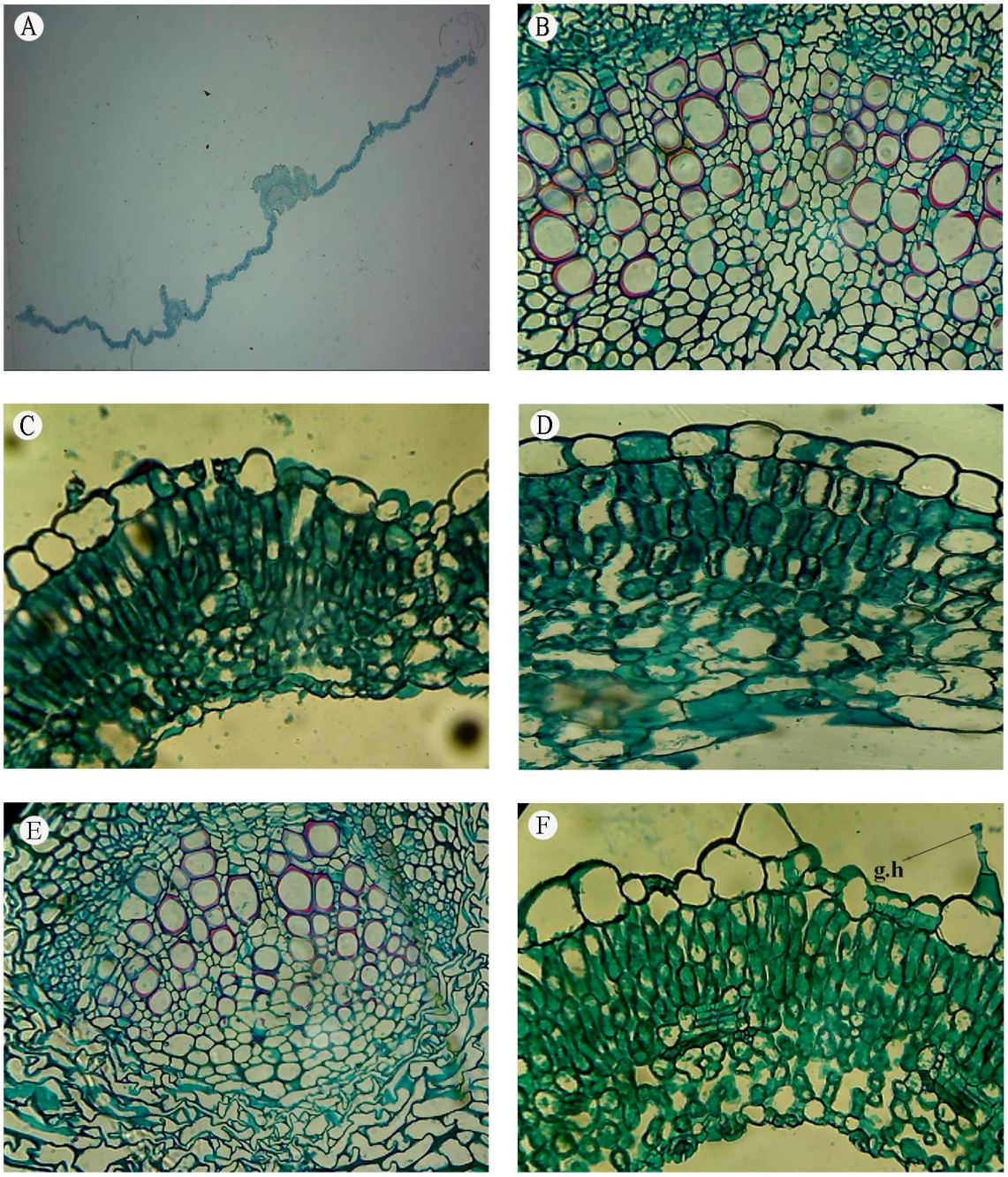

Plate 14: Light microscopic photographs of leaf transverse section of *S .hierosolymitana* showing the general out line, cuticle, epidermis, mesophyll, stomata and vascular bundles.

A. Leaf out line, (10x).
B. Midrib area showing arc-shaped central vascular bundle, (400x).
C. Stomata, on the lower and upper epidermis, (400x).
D. Leaf layers showing cuticle, epidermis mesophyll including palisade and spongy cells, (400x).
E. A small vascular bundle, (400x).
F. Leaf layers showing capitate glandular hair (g.h), (400x).

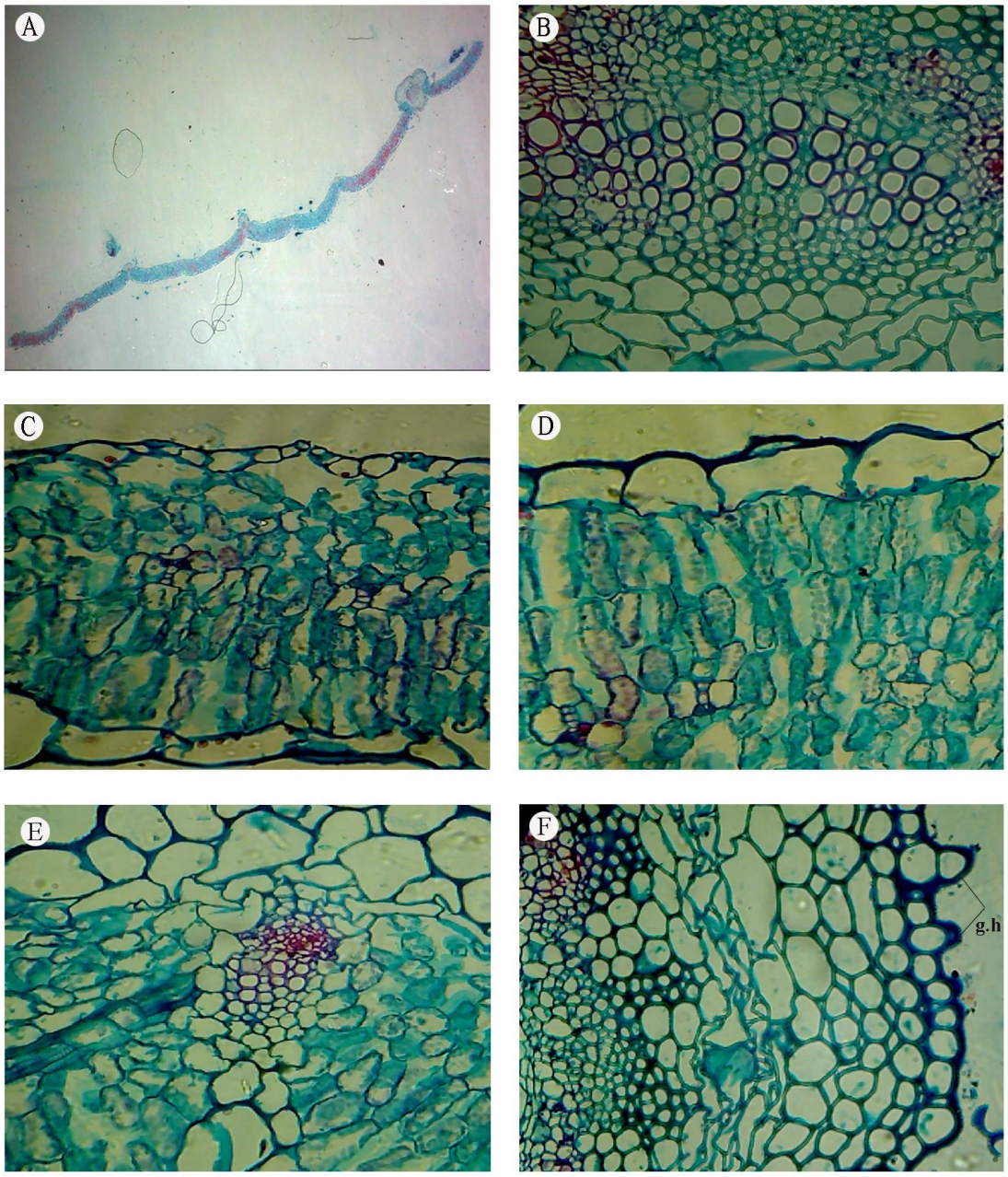

Plate 15 : Light microscopic photographs of leaf transverse section of *S. verbenaca* showing the general out line, cuticle, epidermis, mesophyll, stomata and vascular bundles.

A. Leaf out line, (10x).

B. Midrib area showing arc-shaped central vascular bundle, (400x).

C. Stomata, on the lower epidermis, (400x).

D. Leaf layers showing cuticle, epidermis ,mesophyll including palisad and spongy cells, (400x).

E. A vascular bundle surounded by a sheath of 2-3 layers of irregular shaped parenchyma, (400x).

F. Leaf section showing peltate glandular hair (g.h), (400x).

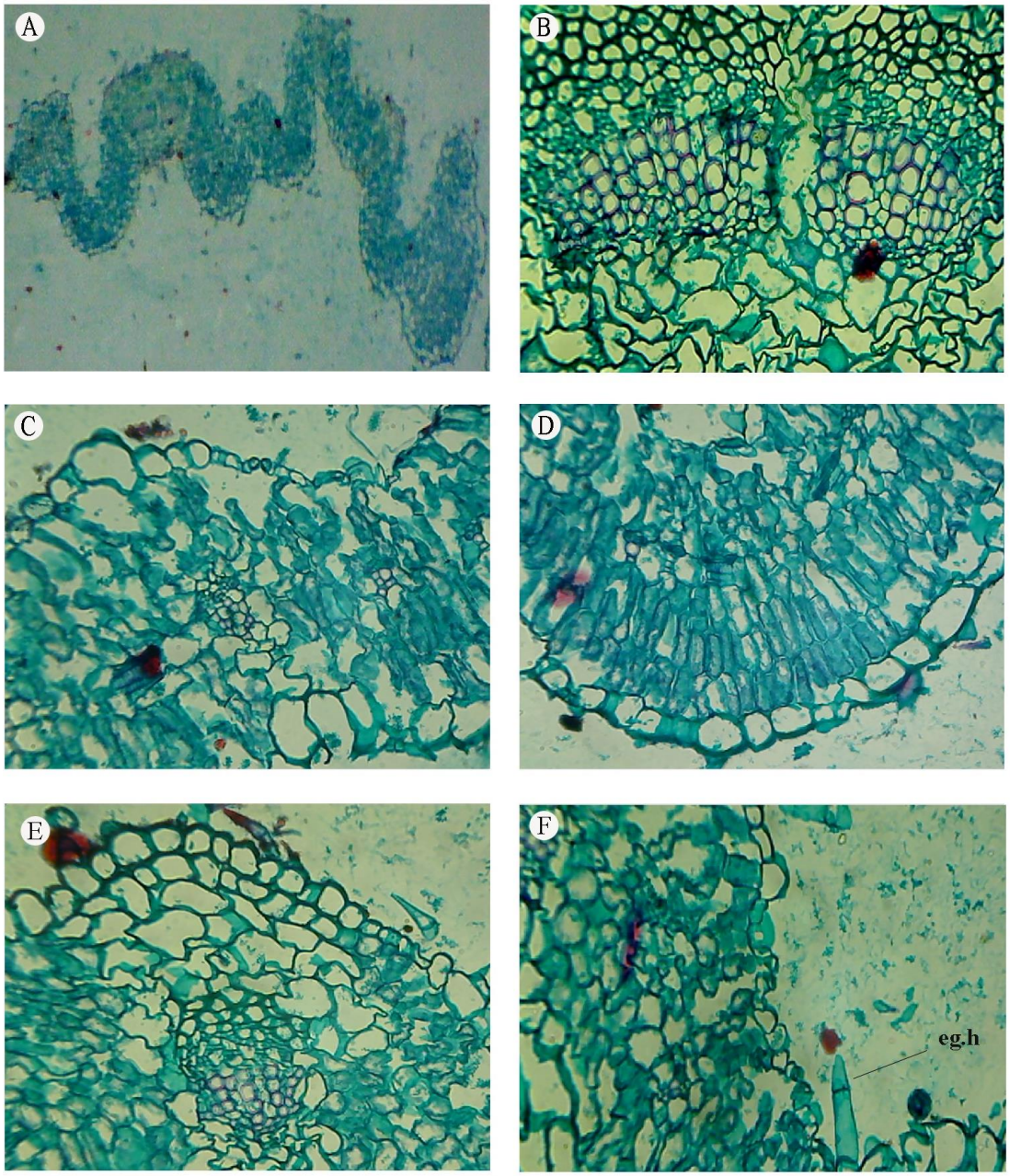

Plate 16: Light microscopic photographs of leaf transverse section of *S. lanigera* showing the general out line, margins, cuticle, epidermis, mesophyll, stomata and vascular bundles.

A. Leaf out line, (30x).

B. Midrib area showing the central vascular bundle, (400x).

C. Stomata, on the lower epidermis, (400x).

D. Leaf layers showing cuticle, epidermis, mesophyll including palisade and spongy cells, (400x).

E. A vascular bundle srounded by a sheath of 1-2 layers of irregular shaped parenchyma, (400x).

F. Leaf layers showing eglandular hair (eg.h), (400x).

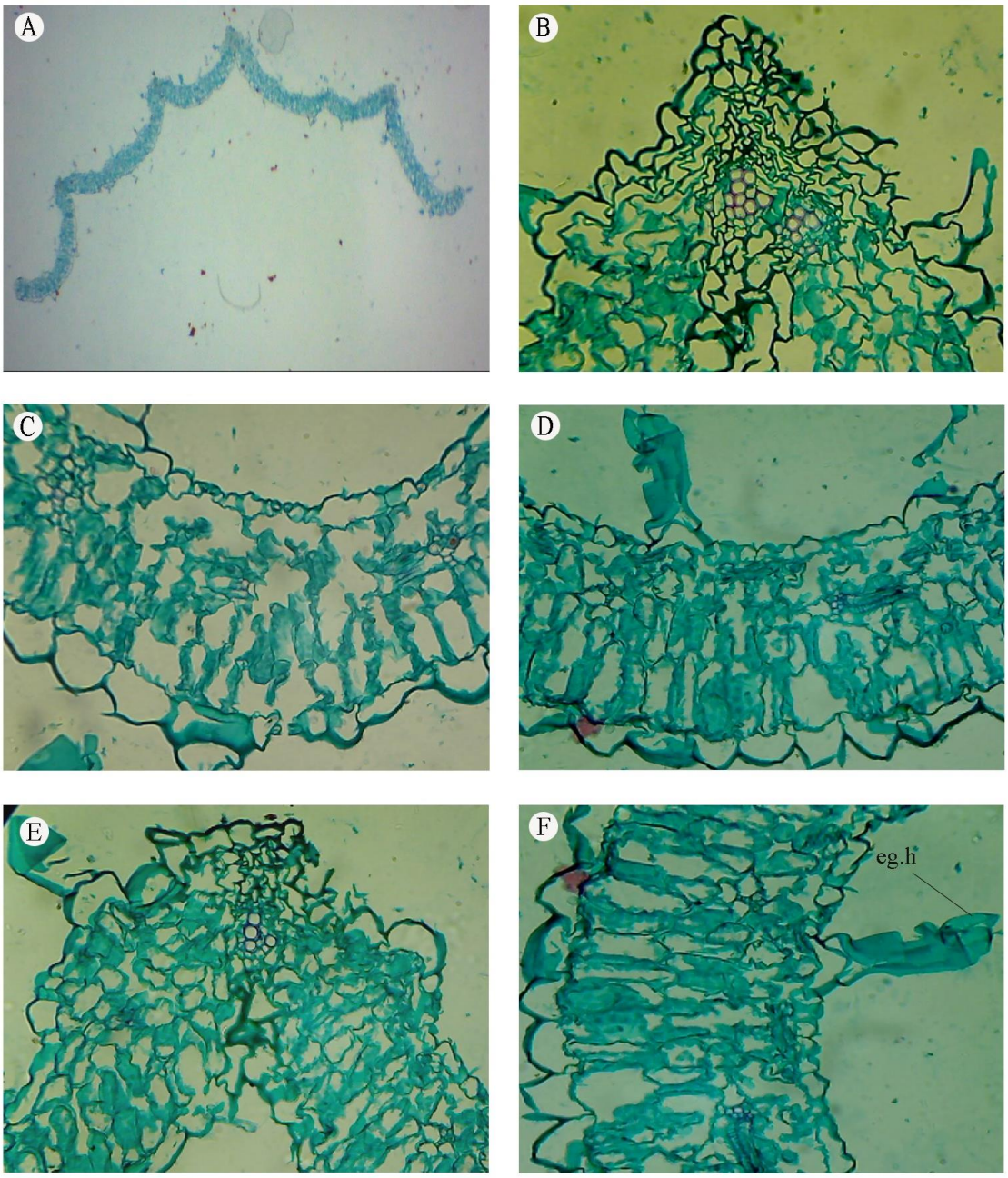

Plate 17: Light microscopic photographs of leaf transverse section of *S. viridis* showing the general out line, margins, cuticle, epidermis, mesophyll, stomata and vascular bundles.

A. Leaf out line, (30x).

B. Midrib area showing central vascular bundle, (400x).

C. Stomata, present on the lower and upper epidermis of leaf, (400x).

D. Leaf layers showing cuticle, epidermis, mesophyll including palisade and spongy cells, (400x).

E. A vascular bundle surounded by a sheath of 1-2 layers of irregular shaped parenchyma, (400x).

F. Leaf layers showing eglandular hair (eg.h), (400x).

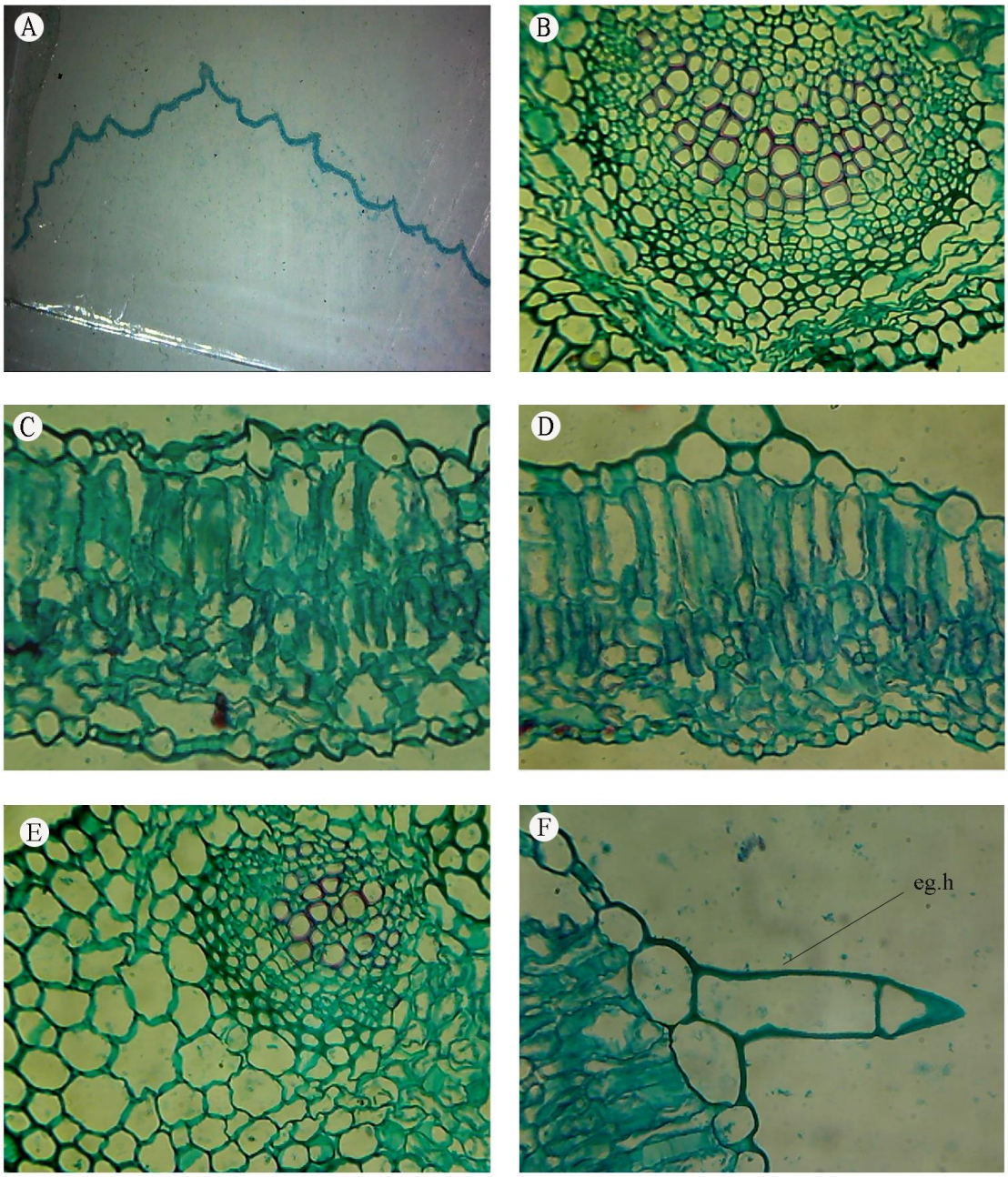

Plate 18: Light microscopic photographs of leaf transverse section of *S. judaica* showing the general out line, cuticle, epidermis, mesophyll, stomata and vascular bundles.

A. Leaf out line, (8x).

B. Midrib area showing arc-shaped central vascular bundle, (400x).

C. Stomata, present on the lower and upper epidermis of leaf, (400x).

D. Leaf layers showing cuticle, epidermis, mesophyll including palisade and spongy cells, (400x).

E. A vascular bundle srounded by a sheath of 1-2 layers of irregular shaped parenchyma, (400x).

F. Leaf layers showing: eglandular hair (eg.h), (400x).

3.3.2. Discussion and Conclusion

The leaf anatomy of seven species of ***Salvia*** recorded in West Bank/Palestine have been investigated of transverse sections of fresh leaves. The leaf characters, which were examined are the following:

3.3.2.1. Out line

It was found that the out line shape is bifacial in cross section in all studied taxa. Therefore, the outline shape cannot be used as discrimination factor among studied taxa. The sections of leaves vary in their thickness at midrib area from the upper to the lower epidermis. This variation ranges from the largest; 461-737µm thick of ***S. fruticosa*** to the smallest; 124-180 µm thick of ***S. viridis***. Therefore, this variation can be used to distinguish between studied species to a certain extent.

3.3.2.2. Cuticle

The cuticle was noted in all species with range varies from 1 -8.6 µm thick. All species have a cuticle on both upper and lower epidermis. This character cannot be used to distinguish between studied species. This is due to that it highly depends on environmental condition under which they grow, which may even result in variation within one species.

3.3.2.3. Epidermis

The epidermis length varies among species from 3 µm to 33 µm with great overlapping. This overlapping is due to the variation of length within one

species and within even the same section also. While, the epidermis width varies from 6 µm to 47 µm and showed the same overlapping.

Therefore epidermis length and width cannot be used as identification tool. The lower epidermis is always smaller than upper epidermis. Similarities in the epidermal cells shape are recorded between same species, while differences are observed between other. In general they are of irregular shape for both upper and lower epidermis.

3.3.2.4. Mesophyll

The mesophyll layers characteristics were studied from two points of view, the tissue components and measurements of mesophyll components. It was found that the mesophyll of all studied taxa composed of both palisade chlorenchyma and spongy chlorenchyma. The mesophyll thickness ranges from the largest; 200-230 µm thick of ***S. verbenaca*** to the smallest; 90-112.5 µm thick of ***S. viridis***. The palisade thickness varies from the largest; 120-130 µm thick of ***S. verbenaca*** to the smallest; 39-62µm thick of ***S. dominica***. Also the thickness of spongy layers varies from the largest; 21-111 µm thick of ***S. fruticosa*** to the smallest; 27.5-35 µm thick of ***S. hierosolymitana***.

3.3.2.5. Midrib

The size of the central vascular bundles varies among studied taxa, ranging from the largest; 122-644×161-763 µm thick of ***S. hierosoloymitana*** to the smallest; 74-61×51-58 µm thick of ***S. viridis***.

Hence in conclusion, leaf anatomical characters in over all can perform a good biosystematics tool to distinguish studied taxa of ***Salvia***. That in spite of the overlapping in some of anatomical features among examined species.

3.4. Petiole Anatomy Results

3.4.1. Results

Measurements of size of central vascular bundles and small vascular bundles at the end of petiole have been made to try to distinguish between different species. The results of the studied anatomical characters of petioles are summarized in (Table 4).

Anatomical petiole studies of ***Salvia*** species were not investigated before. Therefore in the present study, a comparative anatomy study of ***Salvia*** species under study has been under taken. Transverse sections of 6 µm thick of petioles have been prepared, examined and photographed.

3.4.1.1. *Salvia fruticosa* Mill. **(Plate 19).**

Petiole thickness is 684 µm (from adaxial epidermis to abaxial epidermis passing through central vascular bundle). The central vascular bundles are 194×377 (l.×w.) µm, large undivided. All small vascular bundles are 84×88 (l.×w.) µm at the end of petiole. The number of these small vascular bundles are 2 at the end of petiole. Petiole section surface has capitate glandular hairs.

3.4.1.2. *Salvia dominica* L. **(Plate 20).**

Petiole thickness is 484 µm (from adaxial epidermis to abaxial epidermis passing through central vascular bundle). The central vascular bundles are 194×573 (l.×w.) µm, large undivided. All small vascular bundles are 89×100 (l.×w.) µm at the end of petiole. The number of these small vascular bundles are 2 at the end of petiole. Petiole section surface has eglandular and capitate glandular hairs.

3.4.1.3. *Salvia hierosolymitana* Boiss. **(Plate 21).**

Petiole thickness is 1261 µm (from adaxial epidermis to abaxial epidermis passing through central vascular bundle). The central vascular bundles are 166-430×138-405 (l.×w.) µm, large divided into 5 lobes. All small vascular bundles are 225×170 (l.×w.) µm at the end of petiole. The number of these small vascular bundles are 5 at the end of petiole. Petiole section surface has capitate glandular hairs.

3.4.1.4. *Salvia verbenaca* L. **(Plate 22).**

Petiole thickness is 588 µm (from adaxial epidermis to abaxial epidermis passing through central vascular bundle). The central vascular bundles are 157-200×129-180 (l.×w.) µm, large divided into 3 lobes. All small vascular bundles at the end of petiole are 157×161 (l.×w.) µm. The number of these small vascular bundles are 2 at the end of petiole. Petiole section surface has peltate glandular hairs.

3.4.1.5. *Salvia lanigera* Poir. **(Plate 23).**

Petiole thickness is 431 µm (from adaxial epidermis to abaxial epidermis passing through central vascular bundle). The central vascular bundles are 94×290 (l.×w.) µm, large undivided. All small vascular bundles are 38×32 (l.×w.) µm at the end of petiole. The number of these small vascular bundles are 2 at the end of petiole. Petiole section surface has capitate glandular hairs.

3.4.1.6. *Salvia viridis* L. **(Plate 24).**

Petiole thickness is 206 µm (from adaxial epidermis to abaxial epidermis passing through central vascular bundle). The central vascular bundles are 47-55×54-87 (l.×w.) µm, large divided into 3 lobes. All small vascular bundles are 33×21(l.×w.) µm at the end of petiole. The number of these small vascular bundles are 4 at the end of petiole. Petiole section surface has eglandular hairs.

3.4.1.7. *Salvia judaica* Boiss. **(Plate 25).**

Petiole thickness is 677 µm (from adaxial epidermis to abaxial epidermis passing through central vascular bundle). The central vascular bundles are 178 ×712 (l.×w.) µm, large undivided. All small vascular bundles are 118×128 (l.×w.) µm at the end of petiole. The number of these small vascular bundles are 4 at the end of petiole. Petiole section surface has capitate glandular hairs.

Table 4: Petiole Anatomical Characteristic of *Salvia* Studied Taxa.

Taxon	Petiole thickness (µm)	Central vascular bundle (length × width) (µm)	small vascular bundle (length × width) (µm)	Large vascular bundle No. of lobes	small vascular bundle No.
S. fruticosa	684	194×377	84×88	-	2
S. dominica	484	194×573	89×100	-	2
S. hierosolymitana	1261	166-430×138-405	225×170	5	5
S. verbenaca	588	157-200×129-180	157×161	3	2
S. lanigera	431	94×290	38×32	-	2
S. viridis	206	47-55×54-87	33×21	3	4
S. judaica	677	178×712	118×128	-	4

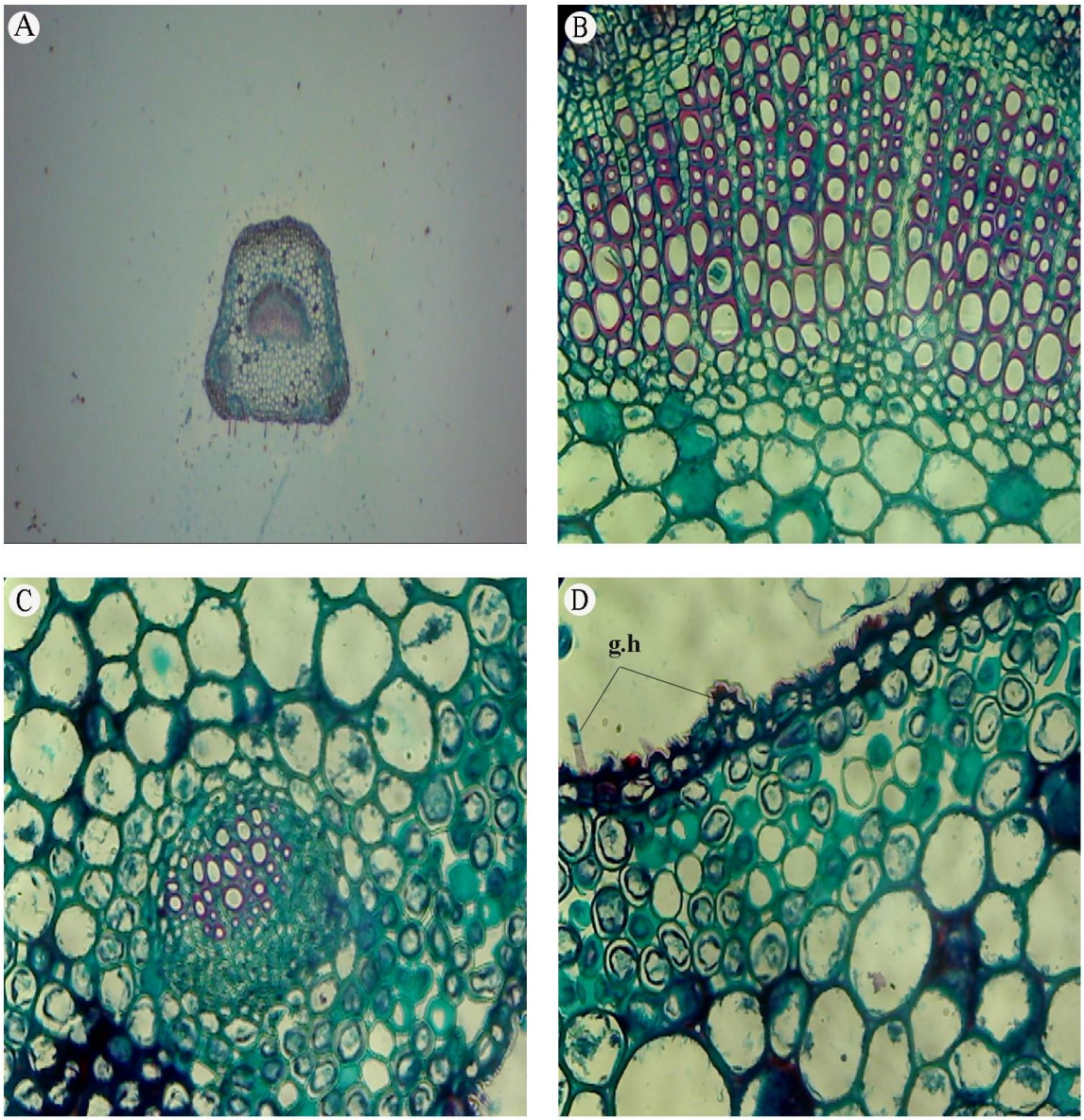

Plate 19: Light microscopic photographs of petiole section of *S. fruticosa* showing the general out line, epidermis, parenchyma cells, large and small vascular bundles.

A. Petiole out line, (30x).

B. Petiole section showing large single central vascular bundles, (400x).

C. Petiole section showing small vascular bundles at the end of petiole, (400x).

D. Petiole section showing capitate glandular hair (g.h), (400x).

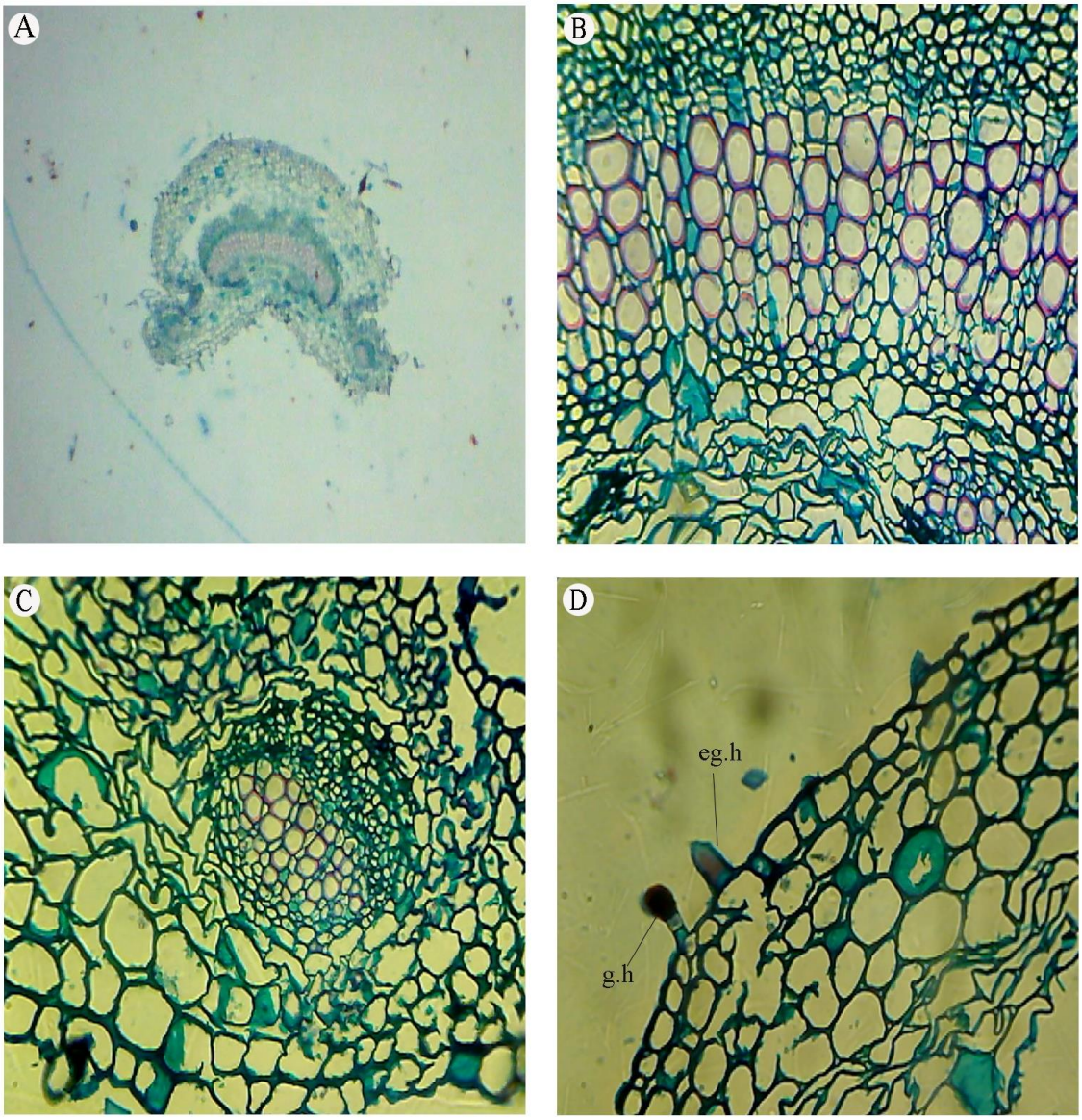

Plate 20: Light microscopic photographs of petiole section of *S. dominica* showing the general out line, epidermis, parenchyma cells, large and small vascular bundles.

A. Petiole out line, (30x).

B. Petiole section showing the large single vascular bundles, (400x).

C. Petiole section showing small vascular bundles at the end of petiole, (400x).

D. Petiole section showing eglandular (eg.h) and capitate glandular hairs (g.h), (400x).

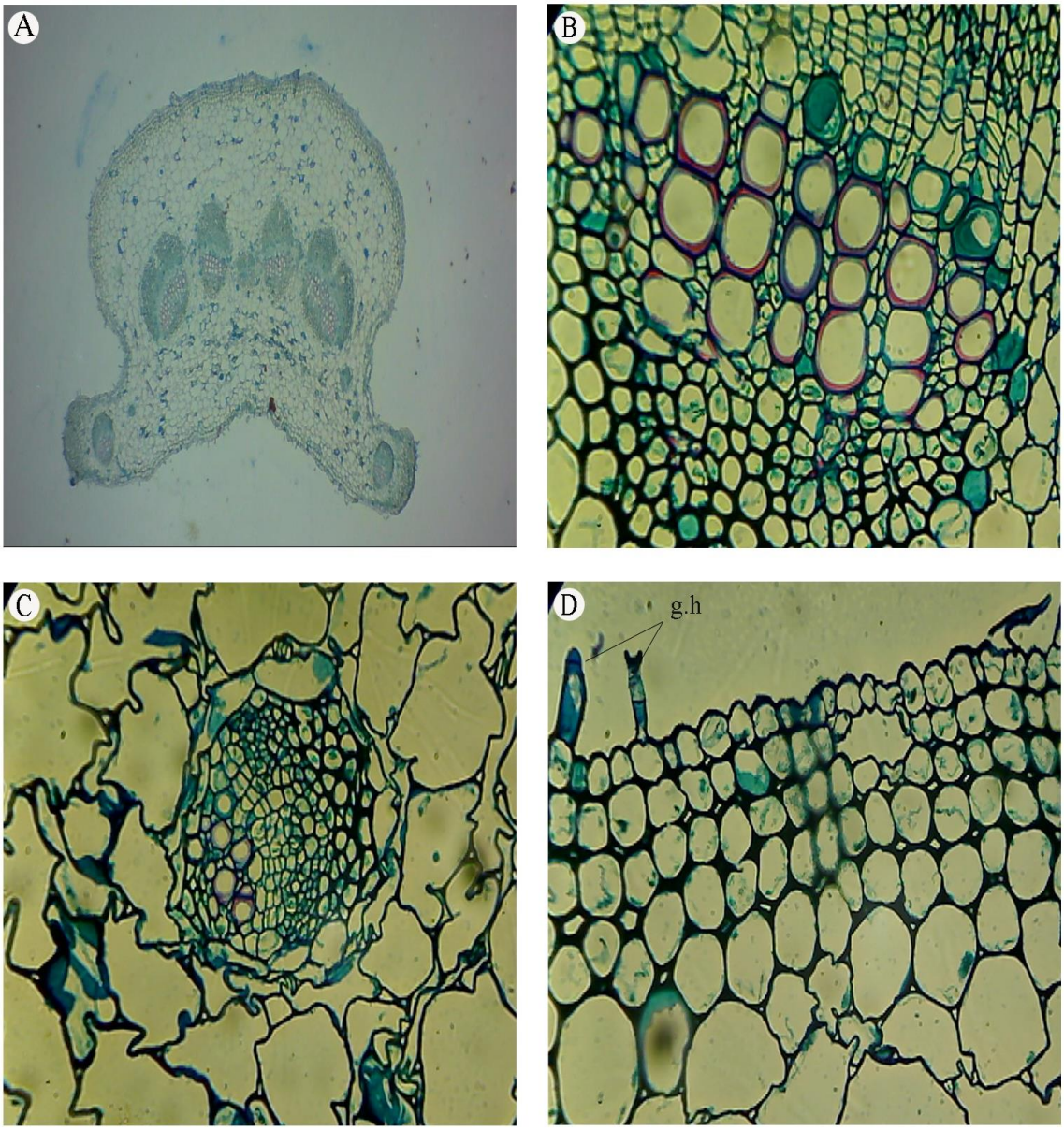

Plate 21: Light microscopic photographs of petiole section of *S. hierosolymitana* showing the general out line, epidermis, parenchyma cells, large and small vascular bundles.

A. Petiole out line, (25x).

B. Petiole section showing one lobe of central vascular bundles, (400x).

C. Petiole section showing small vascular bundles at the end of petiole, (400x).

D. Petiole section showing capitate glandular hairs (g.h), (400x).

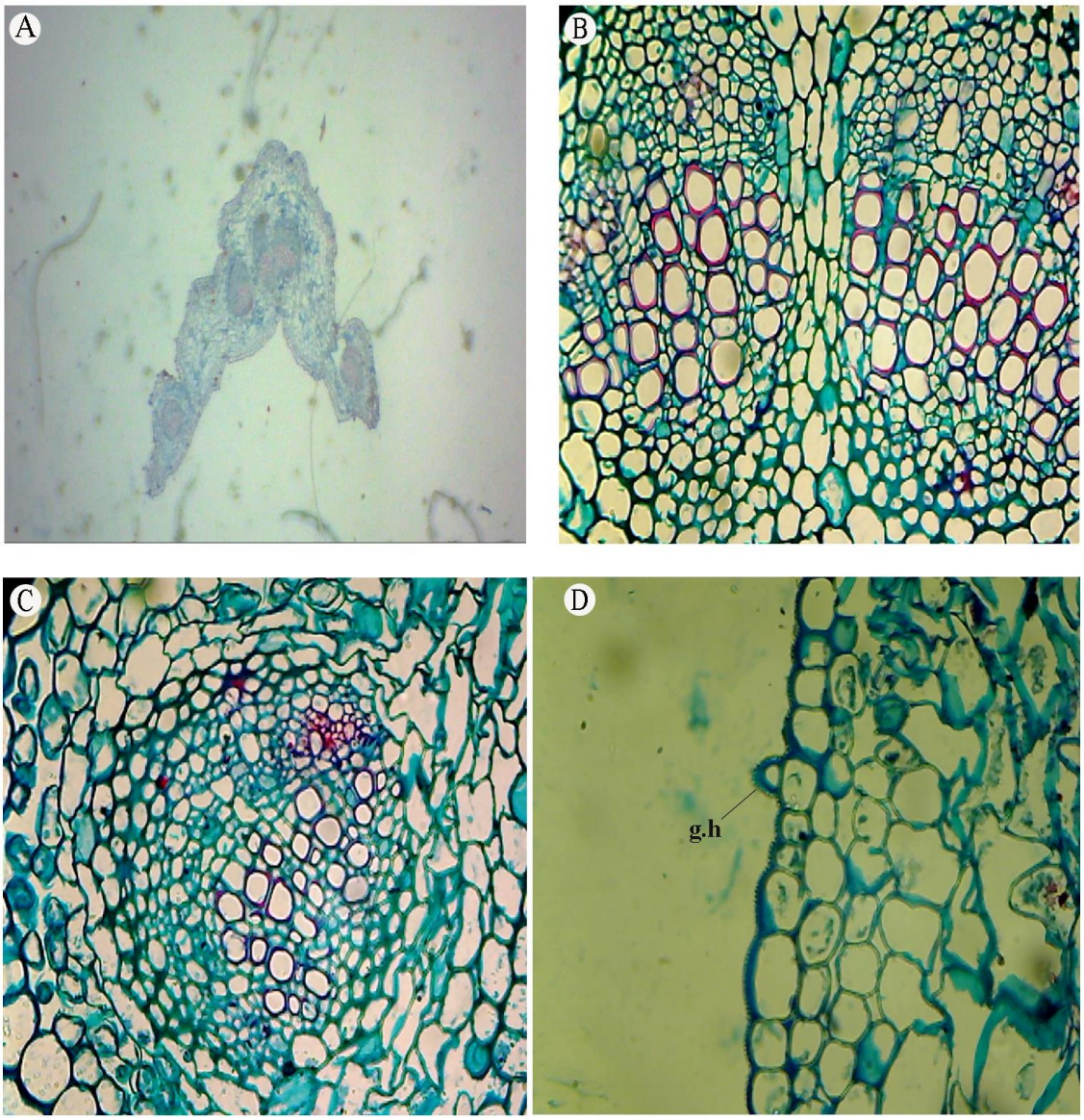

Plate 22: Light microscopic photographs of petiole section of *S. verbenaca* showing the general out line, epidermis, parenchyma cells, large and small vascular bundles.

A. Petiole out line, (30x).

B. Petiole section showing two lobes of central vascular bundles, (400x).

C. Petiole section showing small vascular bundles at the end of petiole, (400x).

D. Petiole section showing peltate glandular hair (g.h), (400x).

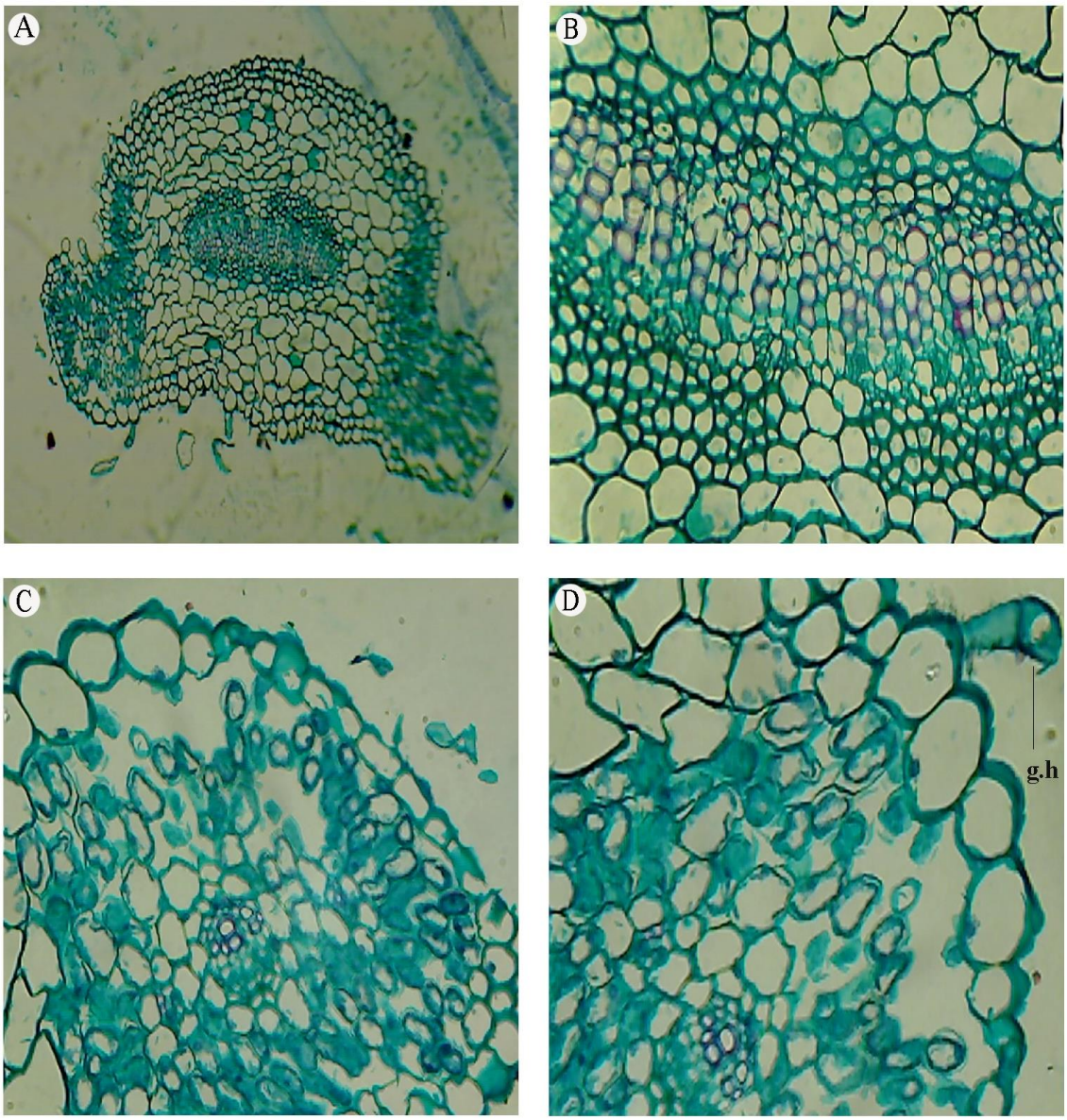

Plate 23: Light microscopic photographs of petiole section of *S. lanigera* showing the general out line, epidermis, parenchyma cells, large and small vascular bundles.

A. Petiole out line, (100x).

B. Petiole section showing the large single vascular bundles, (400x).

C. Petiole section showing small vascular bundles at the end of petiole, (400x).

D. Petiole section showing capitate glandular hair (g.h), (400x).

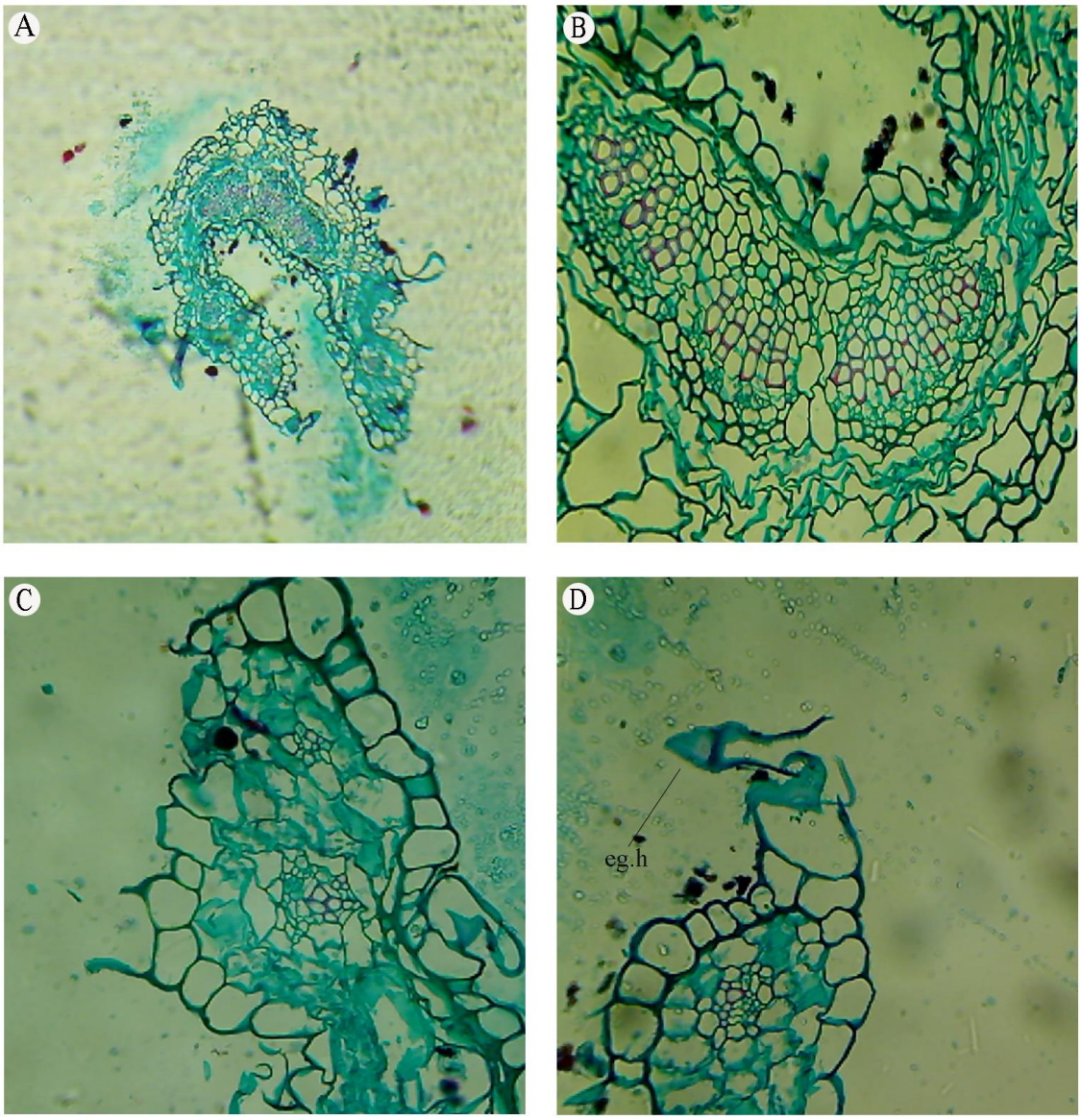

Plate 24: Light microscopic photographs of petiole section of *S .viridis* showing the general out line, epidermis, parenchyma cells, large and small vascular bundles.

A. Petiole out line, (100x).

B. Petiole section showing central vascular bundles divided into three lobes, (400x).

C. Petiole section showing small vascular bundles at the end of petiole, (400x).

D. Petiole section showing eglandular hair (eg.h), (400x).

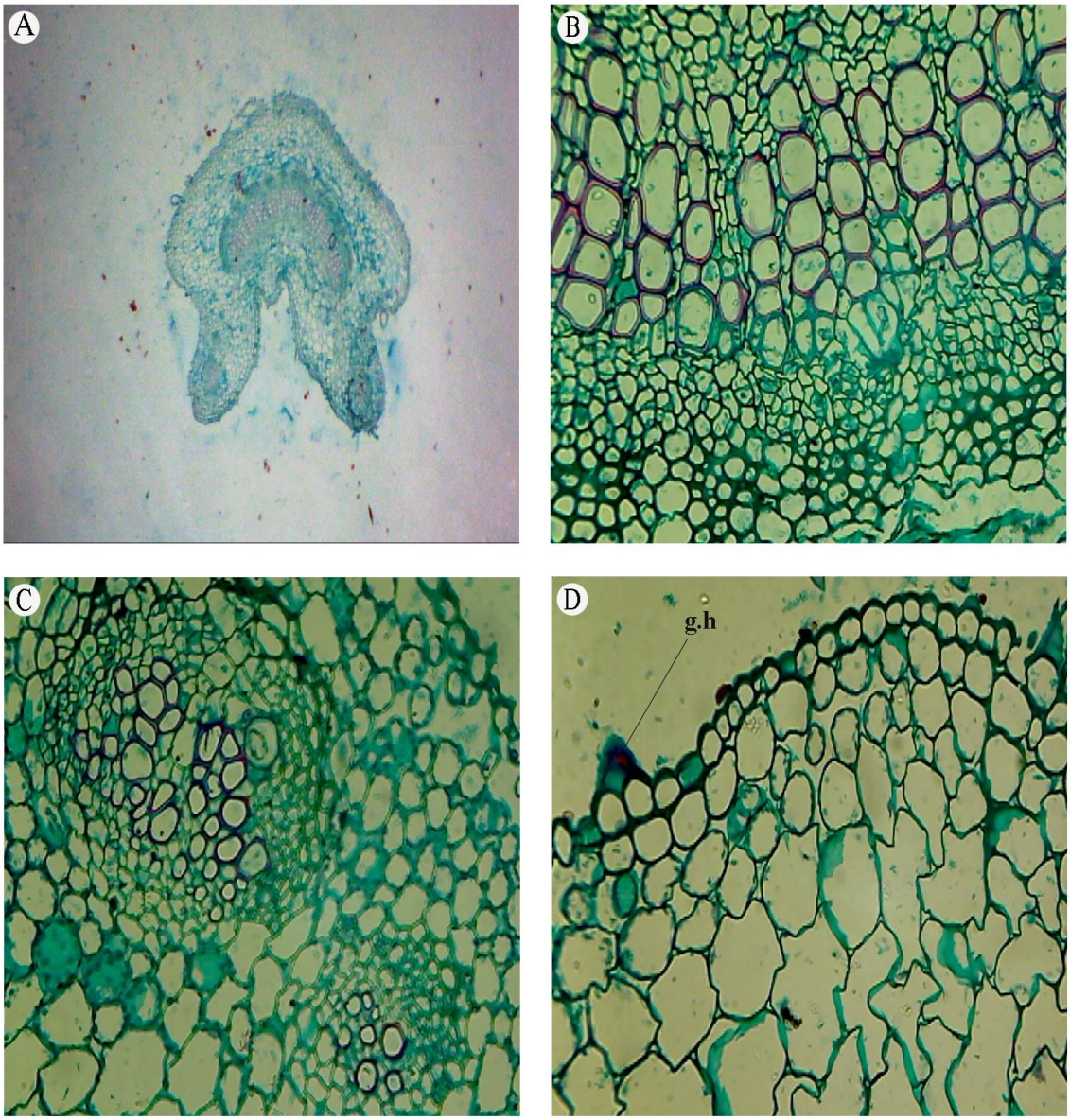

Plate 25: Light microscopic photographs of petiole section of *S. judaica* showing the general out line, epidermis, parenchyma cells, large and small vascular bundles.

A. Petiole out line, (30x).

B. Petiole section showing the large single vascular bundles, (400x).

C. Petiole section showing small vascular bundles at the end of petiole, (400x).

D. Petiole section showing capitate glandular hair (g.h), (400x).

3.4.2. Discussion and Conclusion

The petiole anatomy of 7 species of ***Salvia*** recorded in West Bank/Palestine have been investigated of fresh petiole transverse sections 6 µm thickness. The petiole characters, which were examined are the following:

3.4.2.1. Vascular Bundles

The size of undivided central vascular bundle of studied taxa, ranging from the largest; 178×712 (l.×w.) µm of ***S. judaica*** to the smallest; 94×290 (l.×w.) µm of ***S. lanigera***.

The central vascular bundles of ***S. viridis***, ***S. verbenaca*** and ***S. hierosolymitana*** are divided into lobes, while, in the other species are undivided. This out finding corresponds with what has been stated by Nakipoğlu and Oğuz 1990. They have subgrouped ***Salvia*** species into two groups. The first, with basal leaves in which the central vascular bundles are subdivided into lobes, while, the second group without basal leaves, have single large undivided central vascular bundle. (Özdemir et al., 2009).

In spite of that, ***S. viridis***, ***S. verbenaca*** and ***S. hierosolymitana*** have basal leaves, they vary in the number of the central vascular bundles lobes as they are 3 in the first two spices and 5 in the last one. In addition to that, they vary in the small vascular bundles number at the petiole margins, as it is 4, 2 and 5 respectively. Moreover, they vary in size. As small vascular bundles size at the end of petiole range from the largest; 225×170 (l.×w.)

µm of ***S. hierosolymitana*** to the smallest; size 33×21 (l.×w.) µm of ***S. viridis***.

The other ***Salvia*** species under study, ***S. fruticosa , S. dominica, S. lanigera*** and ***S. judaica***, which do not have basal leaves, have undivided large central vascular bundles. They all have 2 small vascular bundles at the petiole margins, except, ***S. judaica,*** which has 4 small vascular bundles at the end of petioles.

3.4.2.2. Petiole Thickness

The petiole thickness of the studied taxa varies from 206 µm of ***S. viridis*** to1261µm of ***S. hierosolymitana,*** which can be used as a tool to differentiate between studied species.

As a conclusion, the petiole comparative anatomy of the ***Salvia*** species under study has a taxonomic value in discriminating among species. Providing, with the previous other biosystematic evidences a reliable taxonomic information to classify and identify different ***Salvia*** species.

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جامعة النجاح الوطنية
كلية الدراسات العليا

دراسة تصنيفية لجنس *القصعين* (العائلة الشفويه) في الضفة الغربية / فلسطين

إعداد
محمد إبراهيم عبد الله عودة

إشراف
د. غدير عمر

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية
بكلية الدراسات العليا في جامعة النجاح الوطنية نابلس- فلسطين
2014

دراسة تصنيفية لجنس *القصعين* (العائلة الشفويه)

في الضفة الغربية / فلسطين

اعداد

محمد ابراهيم عبد الله عودة

اشراف

د.غدير عمر

الملخص

لقد تمت مراجعة شاملة للجنس القصعين (***Salvia***) في الضفة الغربية / فلسطين، مع الاخذ بعين الاعتبار كافة الدلائل التصنيفية المتنوعة.

واعتمد في هذا العمل على دراسة عينات نباتية في موطنها الطبيعي اضافة الى العينات المحفوظة في المعشبة ، قسم العلوم الحياتية، جامعة النجاح الوطنية.

ودرست الخصائص الظاهرية لأنواع الجنس القصعين. وتضمن البحث كتابة ملخص ومفاتيح الانواع ووصف تفصيلي كامل لكل منها. وقد تم توثيق ورود هذه الانواع حسب اعتمادها في المراجع العلمية ، وكذلك التوزيع الجغرافي كما هو موضح في الخرائط المرفقة.

وقد تبين ان الخصائص الظاهرية يجب ان تكون مرتبطة مع الدلائل الاخرى للكشف الدقيق عن جنس القصعين في الضفة الغربية / فلسطين.

وتمت دراسة الاشكال الظاهرية لحبوب اللقاح لجميع الانواع جنس القصعين ، باستخدام المجهر الضوئي و المجهر الالكتروني الماسح. وقد سجلت المعلومات الناتجة عن التماثل ، والقطبية، والشكل والحجم وفتحات الانبات وزخرفة السطح. اظهرت النتائج ان بعض الفوارق بين الانواع

المدروسة يمكن استخدامها كأداة مساعدة ، اضافة الى الدلائل الاخرى في تصنيف جنس *القصعين*.

فقد وجد ان كل الانواع تمتلك حبوب لقاح ذو شكل يتراوح من شبه المفلطح الى المفلطح- الكروي. حيث يتراوح حجمها من 30/27 ميكروميتر الى 55/45 ميكروميتر (P/E)، متماثلة شعاعيا وسداسية الفتحات الطولية، وقد تبين ان حبوب اللقاح في الغالب متماثلة قطبيا ونادرا ما تكون غير متماثلة قطبيا. اما الزخرفة على السطح فإنها تتراوح من الشبكي الى الشبكي-المثقب بدرجات متفاوتة من حيث التثقيب وظهور الشكل الشبكي. وتبين ان التشابهات والاختلافات في خصائص حبوب اللقاح لا يمكن استخدامها بشكل منفرد في المقارنة بين الانواع المدروسة.

وقد تمت دراسة تشريح الاوراق دراسة تفصيلية عن طريق عمل مقاطع عرضية في اوراق خضراء حديثة الجمع وتم رصد الاختلافات في الخصائص التشريحية لها.

لقد اظهرت النتائج ان كافة المقاطع العرضية للورقة عند الانواع المدروسة تتميز بوجود طبقتين، طبقة علوية وطبقة سفلية تظهران اختلافا واضحا فيما بينهما. كما وأظهرت النتائج تنوعا في سماكة الطبقة الوسطى ما بين الانواع المدروسة حيث تتراوح مابين اكثرها سماكة 200-230 ميكروميتر عند **S. verbenaca** وأقلها سماكة 90-112.5 ميكروميتر عند **S. viridis**، وقد تبين ايضا ان الطبقة الوسطى لجميع انواع الجنس المدروس تتألف من طبقتين، الطبقة العمادية والطبقة الاسفنجية الغنيتين بمادة الكلوروفيل.

بالإضافة الى ذلك، هنالك تنوع في حجم الحزمة الوعائية المركزية للأنواع المدروسة والتي تتراوح ما بين الاكبر حجما 122-644 ×161-763 ميكروميتر عند ***S. heirosoloymitana*** الى الاصغر حجما 74-61× 51-58 ميكروميتر عند ***S. viridis***.

اضافة الى ظهور الشعيرات الغدية او الشعيرات الغير غدية او كلاهما عند الانواع المدروسة.

وأظهرت النتائج وجود فروقات مميزة بين الانواع ساهمت في اثبات النتائج المتحصل عليها من الدلائل التصنيفية الاخرى لإمكانية الفصل بين انواع الجنس المدروس في الضفة الغربية/فلسطين.

بالإضافة الى ذلك، لقد تمت دراسة تشريحية عن طريق عمل مقاطع لأعناق اوراق حديثة الجمع و تبين من خلال النتائج امكانية استخدامها كوسيلة تصنيفية بين الانواع المختلفة وخاصة تركيب الحزمة الوعائية المركزية من حيث انقسامها الى فصوص او عدم انقسامها. أظهرت النتائج أن كل من الانواع ***S. hierosolymitana*** ، ***S. verbenaca*** و ***S. viridis*** كانت مقسمة الى فصوص بينما باقي الانواع المدروسة كانت غير مقسمة الى فصوص.