eISSN: 2357-044X *Taeckholmia 39 (2019): 103-129*

Morphological and Genetic Characteristics of *Sophora secundiflora* and *Sophora tomentosa* (Fabaceae) cultivated in Egypt

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Abstract

Genus *Sophora* L. belonging to family Fabaceae comprises about 80 species. It is a heterogeneous genus, ranging from tall trees to small, herbaceous plants. Morphological and genetic characteristics of two *Sophora* species, *viz*. *S. secundiflora* (Ortega) DC. and *S. tomentosa* L. were subjected to detailed study to delimit these species, where such data are lacking. During this study comparative morphology and anatomy of stem, leaves and petioles of both species was carried out. Moreover, DNA fingerprinting of both plants was carried out using 11 decamer random primers. The obtained results revealed that, many differences in morphological characters *viz*. the color of the flower, shape of the petiole and leaf dimensions. While the anatomical study reflecting other differential characters among them: the non-glandular hairs and the dimensions of some microelements. The features of the powdered stems and leaves are also described. Moreover, the DNA fingerprinting; created a total of 232 bands, out of the 99 were polymorphic representing a level of polymorphism of 42.67%. The primer (OPO-02) showed the highest degree of similarities (85.71%), while the lowest (35.29%) was recorded by the primer (OPO-10). This study concludes that the retrieved morphological, anatomical and genetic features provide a powerful identification and characterization tool for both of the studied *Sophora* species; even when used as powdered material. This study is a pioneer regarding the detailed botanical structure of both species; in addition to, the DNA fingerprinting to distinguish these species; in different prescriptions.

***Key words*:** Morphological profiling, DNA fingerprint, Anatomy, *Sophora*

Introduction

Family Fabaceae is the third largest family of angiosperm; comprising approximately 9.4% of flowering plant with approximately 19 325 species, mostly distributed in tropical areas (Kirkbride 1987; Willis 1993; Lewis *et al*., 2005; Judd *et al*., 2009). The family is well known in temperate and tropical regions of the world, ranging from large trees to annual herbs (Abd El-Ghffar *et al*., 2017; El-Nashar *et al*., 2017). This family is divided into three sub-families: Papilionoideae, Caesalpinioideae and Mimosoideae (Trease and Evans 2009).

The genus *Sophora* is by far the largest and most diverse genus of tribe Sophoreae of the subfamily Papilionoideae (Polhill 1981). Genus *Sophora* has an old history of traditional uses. It is commonly used to treat hematuria, hemorrhoids, scabies and hypertension along with diarrhea and gastrointestinal disorders (Krishna *et al*., 2012; Aly *et al*., 2019). The Arabian name is Sophera for a pea-flowered tree from which it derives its generic name (Gledhill 2008). This is a heterogeneous genus including 80 species of trees and herbs; that spread throughout much of the world, parti cularly the subtropics of the New World, warmer temperate regions of North America and Asia, and many Pacific islands (Andrews 1914; Cumbie and Mertz 1962).

Received on 05/12/2019 and accepted on 09/01/2020. Edited by: Prof. Dr. Wafaa Amer

Sophora secundiflora (Ortega) DC. is an evergreen shrub or occasionally a small tree up to 11 m high with 3 m crown diameter. Because of its tolerance to alkaline soils and moderate drought; it is considered an excellent native plant fo r landscaping in Texas (Hatfield *et al*., 1977; Meyer and Meola 1978; Ruter and Ingram 1991). In early spring, the plant produces terminal racemes of very fragrant showy violet-blue flowers (Cory 1935; Ruter and Ingram 1991). *Dermatophyllum secundiflorum* Ortega was the synonym of *S. secundiflora* (Ortega) DC. (Gandhi and Reveal 2011; Turner 2012). The English name is mescal bean and the common name is Texas mescal bean, Texas Mountain beans (Gilman and Watson 1994; Vines 1960; Zavala-Cháveza *et al*., 2006). *Sophora secundiflora* seeds are large, oblong, rounded and bright red or scarlet in color, seeds considered hallucinogenic in some Native American rituals due to their historical use (Izaddoost 1975; Keller and Hatfieldt 1979). Due to the presence of the quinolizidine alkaloids *S. secundiflora* is considered a toxic species and seeds stated as poisonous to livestock (Cory 1935; Murakoshi *et al*., 1986).

Sophora tomentosa L. is shrub or small tree up to 3m. It is the natural occurrence has been recorded on the Pacific Islands, Oceania, East Asia and Mexico and has been spread in China, Tanzania, Sri Lanka and Queensland. Among its medicinal value, it cures cholera and diarrhea, as well as an antidote after consuming poisonous fish and other marine animals (Sykes and Godley 1968; Perry and Metzger 1980; Huang and Redmann 1995; de Arruda and Nogueira 2006). Yellow *Sophora*, Eve's necklace, necklace pod, yellow necklace pod and silver bush are the common names of *S.tomentosa* (Lonard *et al*., 2015).

Phytochemical investigations of genus *Sophora* lead to the isolation and identification of plethora of secondary metabolites. Its major secondary metabolites including quinolizidine alkaloids, flavonoids and isoflavonoids (Krishna *et al*., 2012).

Lack of extensive botanical investigation on various plant organs promoted the authors to carry out a comparative study in order to discover the main diagnostic characteristics of the different organs of these two species in order to allow their identification. The earlier investigations carried out on various relevant species as DNA profiling, documented as a powerful tool for medici nal plant species authentication of various plant species (Rogl *et al*., 1996; Kojima *et al*., 1998).

Therefore, DNA fingerprinting and the macro-& micro-morphological studies of two species' leaf, petiole and stem will have carried out to obtain the diagnostic characteristics for their recognition and differentiation in both entire and powdered forms.

Materials and Methods:

1. Plant materials

Samples of *Sophora secundiflora* (Ortega) DC. and *S. tomentosa* L. cultivated in Egypt were collected from Orman Botanical Garden and El Zohreya Botanical garden in December 2017. The identification of the plants was accomplished by using the available taxonomic literature (Chestnut 1898; Meyer and Meola 1978; Tucker 1994; Perveen and Qaiser 1998). Additionally, the plants were kindly authenticated by Agricultural Engineer, Therease Labib, consultant of Plant Taxonomy at the Ministry of Agriculture and El-Orman Botanical Garden, Giza, Egypt. Specimens were dried according to the standard herbarium techniques and voucher samples (PHG-P-SS-206 for *S. secundiflora* and PHG-P-ST-207 for *S. tomentosa*) were kept in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ain

Shams University. Fresh samples were kept in 70% alcohol containing 5% glycerol and dried powdered materials were reduced to mesh size 36.

2. Botanical Profiling

Morphological and anatomical studies carried out using 10 individuals/species (3 specimens / individual plants). Photographs were taken using a Samsung Digital Camera (12 Megapixels, f/1.7, 26mm (wide), 1/2.55", 1.4µm, OIS, dual pixel PDAF). Fresh plant materials of different organs were fixed in ethyl alcohol (70%) containing glycerin 5%. Anatomy sections were cut using a manual microtome (American Optical Company) model 900 and stained using Safranin and Malachite green. Anatomical investigations were performed on the cross sections of the old and young stems. Petiole and leaves were air-dried, to obtain the powdered samples. The photographs were taken using the AmScope optical microscope fitted with an AmScope digital camera 8.0 Megapixels.

3. Genetic Profiling and DNA Fingerprinting

DNA was extracted from 10 g leaf tissue under liquid nitrogen in 1.5 ml microfuge tubes using the DNA extraction method reported (Williams *et al*., 1990; Azmat *et al*., 2012). A total of 11 random decamer oligonucleotide primers from C, G and O kits (Operon Technologies Inc.) were used to amplify *Sophora* genomic DNA having the following sequences:

OPO-02 (5'-ACGTAGCGTC-3'),
OPO-04 (5'-AAGTCCGCTC-3'),
OPO-08 (5'-CCTCCAGTGT-3'),
OPO-09 (5'-TCCCACGCAA-3'),
OPO-10 (5'-TCAGAGCGCC-3'),
OPC-08 (5'-TGGACCGGTG-3'),
OPC-12 (5'-TGTCATCCCC-3'),
OPC-13 (5'-AAGCCTCGTC-3'),
OPC-14 (5'-TGCGTGCTTG-3'),
OPC-15 (5'-GACGGATCAG-3') and
OPG-02 (5'-GGCACTGAGG-3').

Primers were obtained from C, O, and G kits (Operon Technologies Inc.) Almeda, California, USA**.**

Polymerase Chain Reaction (PCR) amplification was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM primer, 1 U *Taq* DNA polymerase and 25 ng templates DNA (Martins-Lopes, *et al.*, 2007). PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (*PE* Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 36ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts and then visualized on UV light and photographed using a Polaroid camera.

4.RAPD Analysis

Bands were treated as presence or absence, without considering their percentage. For estimating genetic distance among the tested samples; each of the DNA bands was treated as a unit character. Genetic similarity (GS) was analyzed according to the equation of Jaccard (Jaccard 1908; Milad *et al*., 2013): GS = 2 Nab/ (Na + Nb)

Where, Nab is the number of shared fragments between plants a and b; Na is the number of scored bands in plant a and Nb is the number of scored bands in plant b.

Results and Discussion

1. Macromorphology of *S. secundiflora* (Ortega) DC.

Sophora secundiflora (Plate 1 and Fig. 1) is a perennial evergreen slow growing typically multi-trunked shrub or small tree, smooth bark, pubescent young shoots and its average mature height is 4.5-6 m.

1. leaflet (X=0.75)

2. The upper surface of the leaf

3. The lower surface of the leaf

4. The flower (X=0.44)

5. The fruit (X=0.67)

6. The seed

Plate 1. (Figs. 1-6). Macromorphology of the different parts of *S. secundiflora*.

Around February to March the plant flowers. It is native from central Texas west to New Mexico and south to San Luis Potosi in Mexico.

1.1. Stem

The main trunk of the plant is erect, cylindrical, monopodially branched, multi-trunked shrub; its usual height at maturity is 4.5-6 m. Young branches are with fine longitudinal striations carrying whorled leaf with opposite leaflets. While the old branch is brown, hard, woody, erect, cylindrical, and longitudinally wrinkled with a rough surface. Old branches are hard to break, breaking with a fibrous fracture, the outer bark is dark gray to black, broken into shallow fissures separating flattened ridges, holding short internodes measuring of 2 cm in length. Branches have a faint characteristic odor and a slight bitter taste (Meyer and Meola 1978; Ruter and Ingram 1991), see Plate (1) and Fig. (2).

1.2. Leaf

The leaves compound imparipinnate, dense, alternate, measures 10-14 cm length, with 3-4 pairs of shiny-glossy, leathery, opposite leaflets. The leaflets are coriaceous, sessile, and exstipulate with ovate, obovate broad lamina and a retuse apex, entire with symmetric base. The upper surface is dark green; the lower surface is lighter glabrous. The leaflets in the middle portion are 2.5–5 cm x 1.8-2.8 cm, banchidodrome venation; midrib on the lower side is prominent. Petiole is 2.1- 4 cm, green, cylindrical to sub cylindrical. Leaf has a characteristic odor and a bitter taste, see Plate (1) and Figs. (3-6).

1.3. Inflorescence

The purple flowers are clustered in raceme inflorescence; with solid conical peduncle. The inflorescence having green, cylindrical, erect, and hard pedicle, the pedicle length is between 1.5 to 1.7 cm and between 2 to 3 mm in diameter. The whole inflorescence is 5 -7.5 cm long; with distinctly aromatic odor, and aromatic-slightly bitter taste, see Plate (1) and Fig. (7). Flower is hermaphrodite, zygomorphic, and sessile or sub sessile, purple, positioned in the same direction of the bract. They are small in size from 1.8 -2 x 7 - 8 mm. Bract is green, concave ovate to lanceolate, sessile with broad base; membranous texture, entire margin, and acuminate apex. Hairy surface, ranges between 5 to 7 mm in length and 1 to 1.2 mm in width.

Calyx deciduous, gamosepalous, ascending with imbricate aestivation. Sepals are green, with 1 - 1.3 x 4 -6 mm. The surface texture is hairy and membranous, margin is entire and apex is obtuse.

Corolla formed of 5 purple petals, papilionaceous form with a single, large, upper petal known as the banner (Standard petal). The semi cylindrical base of the banner compresses 2 similar and smaller lateral wings, the wings in turn enclose a pair of small keel petals that are inside the wings. The concave sides and the two keel petals are fused at their bases to form a boat shaped structure that enclose the essential parts of the flower (androecium and gynoecium). Usually, the flower has a vexillary aestivation; petals are of 1.5 -1.7 x 6 - 7 mm.

Androecium is diadelphous, 10 fertile stamens are basally united and stand in two alternating sub equal whorls, forming a sheath around the ovary. Filaments are filiform, yellowish green. Anthers are small and rising, orange, obovate to rotundate in shape, bi-lobed and attached to the filament along its back. The stamens measure up to 8 mm in length.

Gynoecium is uni-locular, monocarpellary; ovary is brownish yellow, hairy, sessile to sub sessile, superior and oblong to sub cylindrical. Ovules numerous on the ventral suture, with marginal placentation. The style is slightly bent at the apex, tube like flattened, hairy, pale yellowish together with a minute, simple, terminal, concave, oval, brown, and

papillosed, simple and obtuse stigma. Ovary is 1.0 x 7 –8 mm while the length of the style is 3 to 4 mm. Pollen grains are tricolpate and prolate spheroidal.

1.4. Fruit and seed

The fruit is legume, a semi-woody elongated compressed between the seeds, 2.5 - 7.5 cm x 1.2-1.9 mm, pubescent. Fruit stay on the tree for long time. Seeds bright red poisonous. Mature seeds called mescal beans, red beans or big drunk beans and have a diameter of about 11 to 12 mm, see Plate (1) and Figs. (8 & 9).

2. Macromorphology of *S. tomentosa* L.

Sophora tomentosa (Plate 2 and Fig. 1) is an erect shrub, native to southern Florida and South Texas, 1 to 3 m tall. From March to October the plant flowers and from September to December the fruiting.

2.1. Stem

The main trunk of the plant is erect, cylindrical, monopodially branched. Young branches are green showing fine longitudinal striations carrying whorled leaf with opposite leaflets. The branches have a faint characteristic odor and a slight bitter taste, see Plate (2) and Fig. (2).

2.2. Leaf

The Leaves are alternating, compound imparipinnate, up to 15 cm long, and have 13 - 21 leaflets; the rachis is pubescent; leaflets are usually ovate, 1.0- 2.5 x 1.0- 1.6 cm, leathery, densely pubescent below, glabrous in upper epidermis; apex acute-rounded; stipules deciduous, 1.0-1.5 mm long. Petioles measures 1.7-2.0 cm long and they are tomentose; see Plate (2) and Figs. (3-7).

2.3. Inflorescence

Inflorescence is 10 -40 cm long, terminal, elongated raceme, with yellow flowers in cluster form. Pedicels are pubescent and 8 to 9 mm long. Calyx is gamosepalous five- lobed above the hypanthium and is 6 to 8 mm long. Corolla formed of 5 bright yellow petals, bilaterally symmetrical. The upper petal is 1.8 to 2.5 cm long and the laterally arranged petals are fused. Androecium includes 10 nearly identical stamens free above the hypanthium apex and stand in two alternating whorls. Anthers are about 1.5 mm long and are copper-colored; filaments are about as long as the ovary.

Gynoecium is hypogenous, one carpel, with marginal placentation. The ovary is densely pubescent. Pollen grains are monads, prolate, spheroidal, and tricolporate, and have a thin-walled, a microreticulate patterned exine (e Silva and dos Santos 2009; Hurr *et al*., 1999), see Plate (2) and Fig. (8).

2.4. Fruit and the seed

The fruit is a semi-woody, tomentose legume strongly constricted between the marginal arranged seeds. Legumes are subtended by the persistent calyx and are 7-10 cm x 0.9-1 cm. The slowly dehiscent legume produces five to 10 seeds; they are hard-walled, subglobose, glossy, dark brown and have a diameter of about 5 to 8 mm (Hnatiuk 1979; Nakanishi 1988; Wickens 1979), see Plate (2) and Fig. (9).

The Major macromorphological differences between the two species are recorded in (Table 1).

Table (1). Major morphological differences between *S. secundiflora* and *S. tomentosa*

Item	*S. secundiflora*	*S. tomentosa*
Height	4.5-6 m	1.0-3 m
Leaf length	10-14 cm	12-20 cm
Number of leaflets	3-4 pairs	8-9 pairs
The leaflets L x W	2.5–5 x 1.8-2.8 cm	1.5-2.5 x 0.9-1.5 cm
Petiole length	2.1 – 4.0 cm	1.7 – 2.0cm
Inflorescence length	5.0 - 7.5 cm	10 - 40 cm
Fruit L x W	2.5 -7.5 x 1.2-1.9 cm	7.0- 10 x 0.9–1.0 cm

1. Leaflet (X=1.2)

2. The upper surface of the leaf

3. The lower surface of the leaf

4. The flower(X=0.16)

5. The fruit (X=0.54) & seed.

Plate 2. (Figs.1-5). Macromorphology of the different parts of *S. tomentosa*

3. Anatomical features of *S. secundiflora* (Ortega) DC.

3.1. Young stem branch

A transverse section in a young stem branch (Plate 3, Figs. 1 & 2) is almost rounded in outline. The epidermis consists of one layer of sub-rectangular cells in transverse section. In surface view, the cells are polygonal usually isodiametric sometimes axially elongated with more or less straight anticlinal walls. The cells are covered with thick smooth cuticle showing oval to rounded sunken paracytic stomata surrounded usually by 3-4 subsidiary cells. Numerous unicellular covering trichomes are present with broad bases and acute to acuminate apices; some are long and curved.

Cortex is composed of an outer 2-3 rows of thick walled cellulosic, rounded to ovoid collenchyma cells, followed by inner parenchyma region consisting of 7-9 rows, the cells contain prismatic calcium oxalate crystals and minute-simple starch granules. The endodermis is formed of one row of tangentially elongated cells containing minute starch granules.

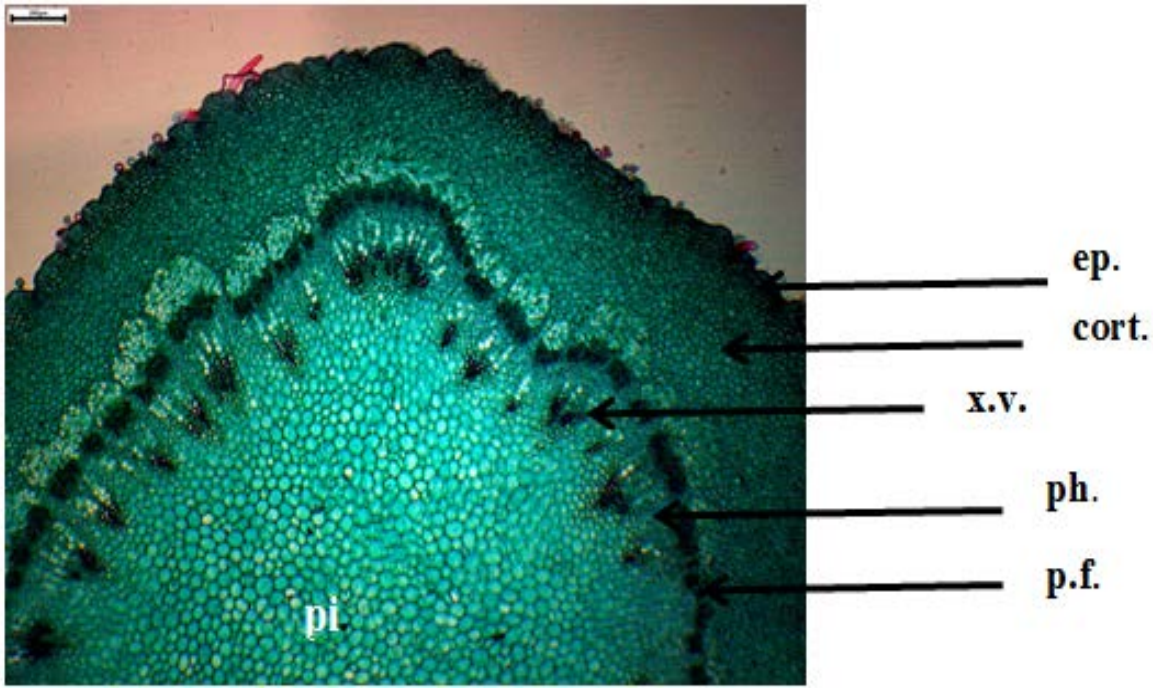
The pericycle consists of more or less complete ring of lignified fibers which is interrupted with parenchyma. The fiber has a lignified thick wall with narrow sometimes fairly wide lumen and acute to acuminate apex. The parenchyma cells surrounding the fibers contain prisms of calcium oxalate forming well developed crystal sheathes. While, the vascular system shows a continuous ring of collateral vascular bundles traversed by narrow uni- to biseriate medullary rays filled with starch.

The phloem is formed of a narrow ring, consisting of thin walled soft, cellulosic elements formed of sieve tubes, companion cells and phloem parenchyma which mostly contain calcium oxalate. The phloem region is traversed by uni- to biseriate medullary rays consisting of thin walled, somewhat radially elongated cells and filled with starch. Cambium zone is indistinct.

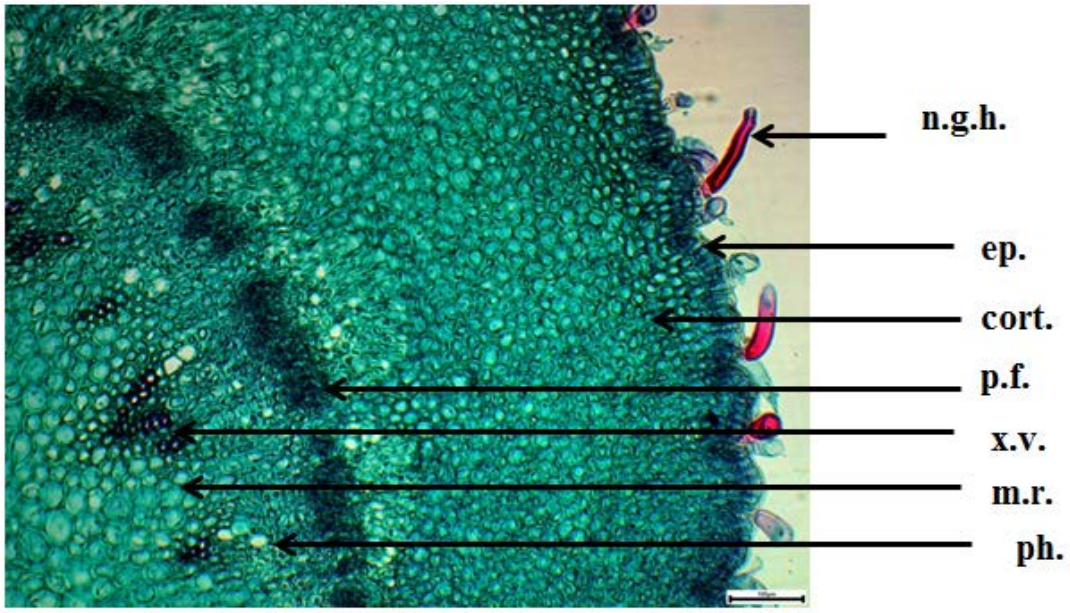
The secondary xylem consists of lignified vessels, tracheids, fibers, xylem parenchyma and is traversed by uni- to biseriate narrow lignified medullary rays. Vessels are arranged in radial rows. The primary xylem is formed of few lignified xylem vessels and cellulosic thin walled parenchyma. The pith is comparatively wide, parenchymatous, consisting usually of large, polygonal to rounded, pitted cells with moderately thin wall; where calcium oxalate prims and starch granules are not observed.

3.2. Old stem branch

A transverse section in the old stem branch (Plate 4, Figs. 1 & 2) is nearly circular in outline. It consists of somewhat wide layer of cork followed by a wide region of secondary cortex surrounding the well-developed vascular system. The cork arises lately in the pericycle and consists of several, usually irregular, rows of subrectangular cells with lignified brown and thick walls. The inner boundary of the cortex is well differentiated into an endodermis. The pericycle is formed of more or less continuous circle of lignified fibers interrupted with parenchyma cells. The vascular tissue is comparatively wide representing about 1/3 of the diameter forming a ring traversed by medullary rays. The phloem consists of thin walled soft, cellulosic elements formed of sieve tubes, companion cells, phloem parenchyma which mostly contain calcium oxalate and isolated groups of phloem fibers arise in the secondary phloem. The phloem fibers have relatively narrow lumens and acute tapering ends. The phloem region is traversed by uni- to biseriate medullary rays consisting of thin walled, somewhat radially elongated cells and filled with starch. The xylem is formed of lignified vessels, tracheids, fibers, wood parenchyma and is traversed



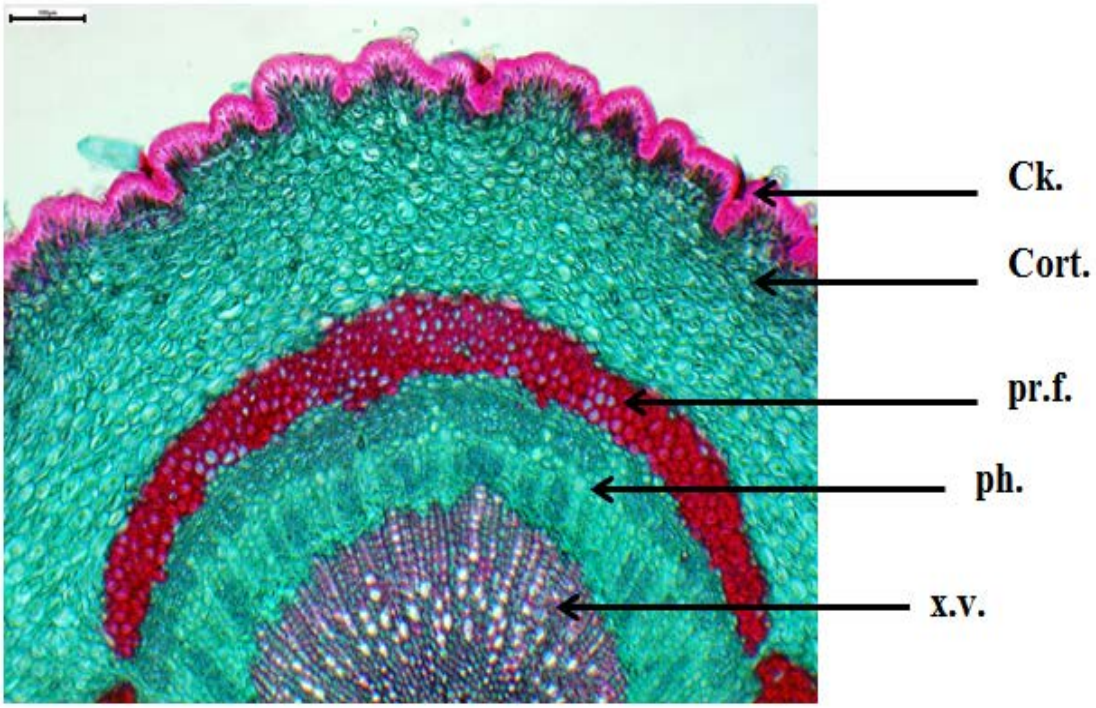
1. Low power view of the young stem branch T.S (40X)



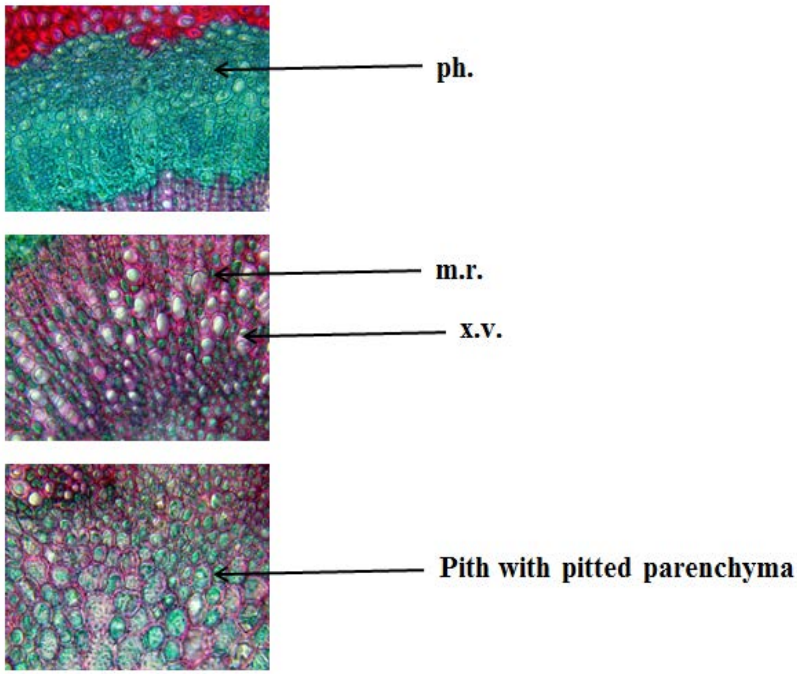
2. High power view of the young stem branch T.S (100X)

Plate 3. (Figs. 1-2). Micromorphology of the young stem of *S.secundiflora*

cort.= cortex; ep.= epidermis; m.r. = medullary rays; p.f.= pericyclic fiber; ph.= phloem; pi.= pith; v.= xylem vessels; n.g.h.= non-glandular hair.



1. Low power view of the old stem branch T.S (100X)



2. High power view of the old stem branch T.S (400X

Plate 4. (Figs. 1-2). Micromorphology of the old stem branch of *S. secundiflora*
cort.= cortex; ck.= cork; m.r. = medullary rays; ph.= phloem; pi.= pith; x.v.= xylem vessels.

by uni- to biseriate narrow lignified medullary rays. The fibers are fusiform having straight, moderately wide to narrow lumina and acute tapering apices and also, tortous with undulating walls, slightly acute to acuminate apices and with narrow lumina. The vessels are arranged in radial rows usually with pitted, spiral and annular thickening. The central pith relatively wide constitutes about 1/2 of the diameter and formed of pitted parenchyma. It differs from the young stem branches in the presence of a typical lignified cork (rhytidoma) dead tissues formed of tangentially elongated brown cells with lignified thick walls, the presence of irregular rectangular cells beneath the cork. The vessels are either single or arranged in radial rows.

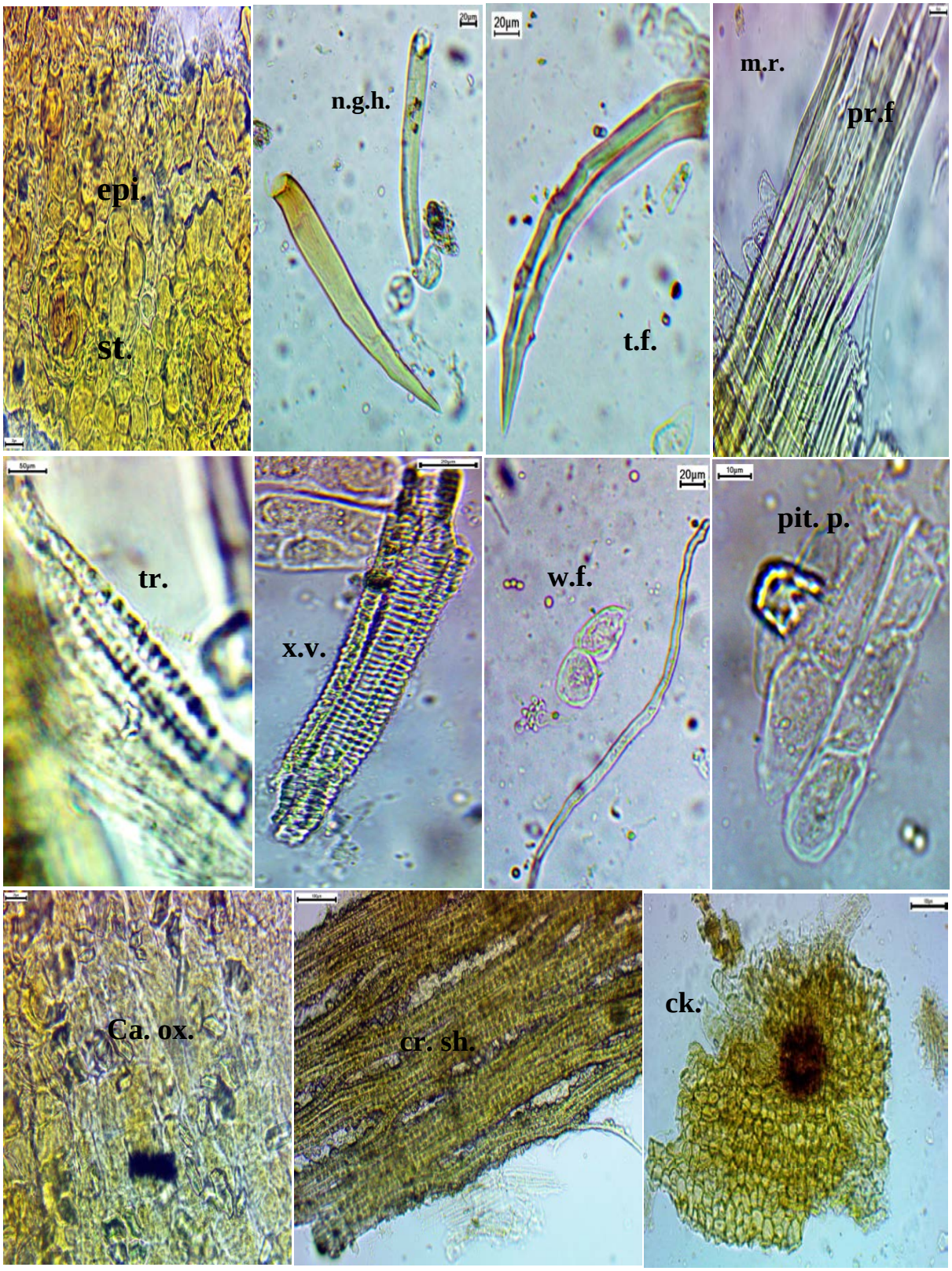
The powdered young stem of ***S. secundiflora*** is yellowish brown in color with characteristic odor and bitter taste. Microscopically, it is characterized by the presence of fragments of polygonal axially elongated epidermal cells with more or less straight to slightly wavy anticlinal walls and covered with thin smooth cuticle. Stomata are of the paracytic type. Trichomes are abundant, they are of non-glandular, unicellular rarely bicellular, short covered with faint warty or smooth cuticle, sometimes they are long and curved. Scattered entire or fractions of non-glandular hairs. Fragments of thin or thick-walled pitted parenchyma of the cortex containing prisms of calcium oxalate. Fragments of lignified pericyclic fibers which are relatively thin refractive walls and comparatively wide to narrow lumens. Their ends are acute to acuminate. Tortous fibers are with undulating walls, slightly acute to acuminate apex and with narrow lumen. Lignified fragments of xylem vessels showing spiral, annular and pitted lignified thickenings. Fragments of lignified pitted tracheids with blunt ends and fragments of pitted lignified walled wood parenchyma. Fragments of lignified fibers with straight or undulating walls having wide lumina and acute to acuminate apex and fragments of the rectangular medullary rays with pitted and lignified walls. Numerous fragments of crystal sheath where the pericyclic fibers are mostly surrounded by parenchyma cells containing prisms of calcium oxalate Fragments of parenchyma cells from the pith, see Plate (5). The microscopically measurements of the different elements of the young and old branches of *S. secundiflora* are shown in (Table 2).

3.3. Leaf

A transverse section in the leaf (Plate 6, Figs. 1 - 3) appears more or less biconvex outline with prominent midrib on the lower surface. It shows a dorsiventral structure with two rows of the upper palisade and 2-3 rows of spongy tissue; the outer row is slightly longer and regular than the inner one. Two groups of collenchyma mass support above and below the vascular bundle. The large main vascular bundle is shown in the mid rib region and accompanied with two to six subsidiary vascular bundles smaller ones distributed in the lamina. The central vascular bundle is rounded to oval in shape and consisting of a radiating upper xylem and a lower soft phloem and is surrounded by a pericycle formed of smaller upper arc and a larger lower one in the shape of crescent. The pericycle consists of cells that are parenchymatic. Starch granules and numerous styloids and prisms of calcium oxalate are scattered in the mesophyll and forming crystal sheathes around the fibers of the pericycle.

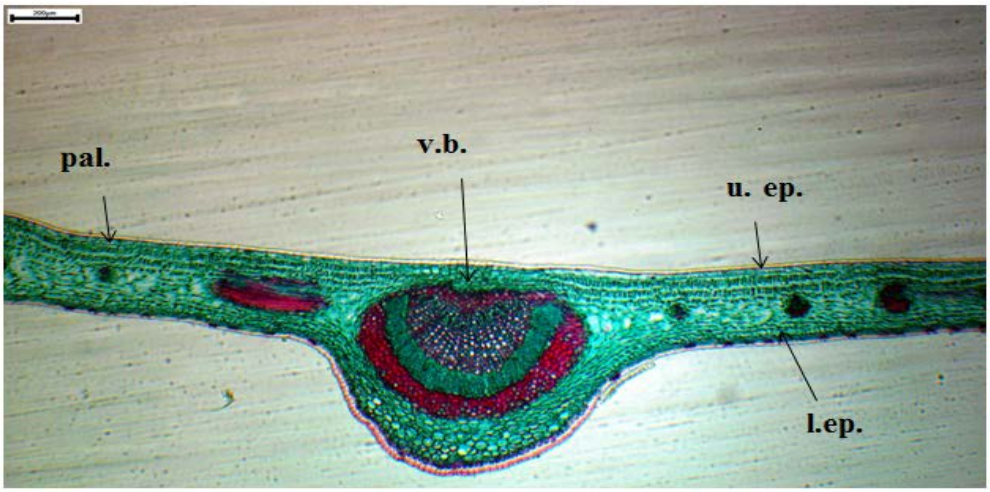
The upper epidermis consists of one row of tangentially elongated cells seen in transverse section. The surface-viewed cells appear polygonal, isodiametric to subrectangular with straight to curved or slightly wavy anticlinal walls. Also, rod-shaped crystals are present. Thick smooth cuticle covers the cells. In the upper epidermis, trichomes are absent.

The lower epidermis consists of one row of square cells with short, hemispherical papillae in transverse

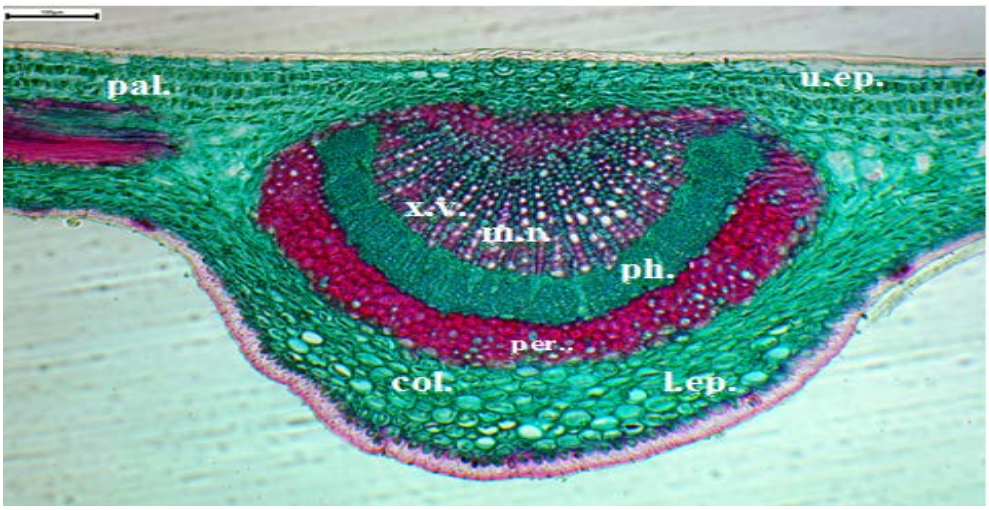


(Plate. 5). Isolated elements of the powdered stem of *S. secundiflora*

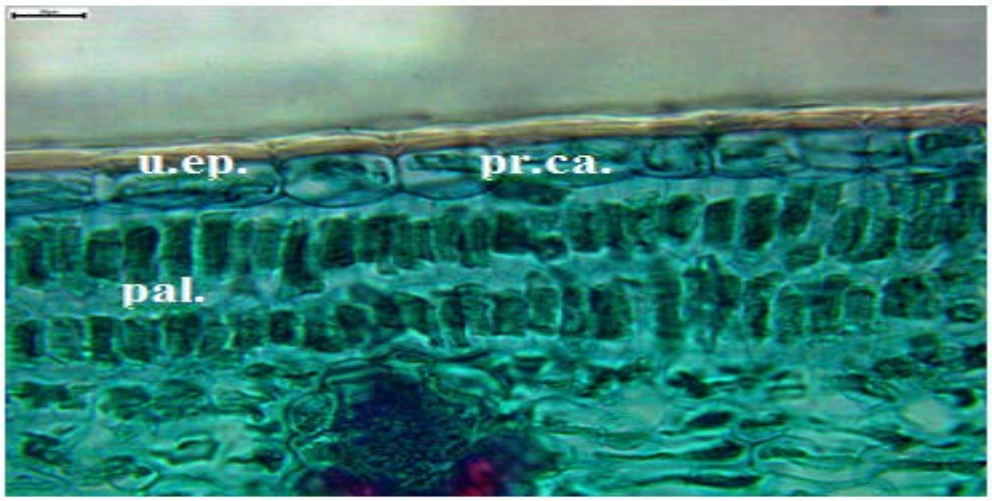
ep.= epidermis (400X); n.g.h.= non-glandular hair (100X); m.r.= medullary rays (400X); p.f.= pericyclic fiber (400X); t.f.= tortous fiber (100X); tr.= tracheids (100X); x.v.= xylem vessels (400X); w.f.= wood fiber (400X); pit.p.= pitted parenchyma (400X); pr.ca.= prism of Ca-oxalate (400X); cr. sh.= crystal sheath (100X); ck.= cork (100X).



1. Low power view of the entire T.S. (40X)



2. High power view in the midrib T.S. (100X)



3. High power view in the lamina T.S. (400X)

Plate 6. (Figs. 1-3). Micromorphology of the leaf of *S. secundiflora*

col.= collenchyma; l.ep.= lower epidermis; m.r.= medullary rays; pr.ca.= prisms of Ca- oxalate; per.= pericycle; ph.= phloem; u.ep.= upper epidermis; pal.= palisade; x.v.= xylem vessels; v.b.= vascular bundle.

section. The cells appear polygonal in surface view, mostly isodiametric with straight anticlinal walls. The cells are smaller than those of the upper epidermis. They are usually covered with smooth cuticle. The trichomes are very rare. They are unicellular covering trichomes rarely bi cellular that is covered with warty cuticle. The unicellular covering trichomes are conical in shape with broad lumen and acute to acuminate bent apices. In the transverse section in the midrib region, the epidermal cells of lower surface are elongated cells.

Mesophyll tissue is heterogeneous consisting of an upper zone of palisade layer separated by a mass of collenchymatous cells in midrib region. The palisade layer consists of two rows of columnar, cylindrical thin walled cells with intercellular spaces. The spongy tissue consists of 2-3 rows of more or less rounded thin-walled parenchyma cells with wide intercellular spaces. Subsidiary vascular bundles were embedded in the spongy tissue. Many cells contain prisms of calcium oxalate specially those surrounding the pericyclic fibers forming a crystal sheath. Their presence confirmed by mounting a transverse section in concentrated sulphuric acid, all crystals had been changed into needle crystals of calcium sulphate.

The cortical tissue cells surrounding the vascular bundle are rounded to sub rectangular parenchyma with narrow intercellular spaces. Many of them containing prisms of calcium oxalate especially those abutting on the fibers of pericycle forming crystal sheathe. There are two masses of sub epidermal collenchyma, one above the vascular bundle and formed of 2-3 rows of rounded shining small cells and the other below the bundle, abutting on the lower epidermis consisting of 4-6 rows.

The vascular system is represented by a large central crescent shape vascular bundle and from two to six subsidiary vascular bundles distributed in the lamina sides. The bundles are collateral formed of an upper xylem and a lower phloem and it is surrounded by a pericycle formed of upper and lower arcs and traversed by uni- to biseriate medullary rays. The xylem consists of mainly of vessels, tracheids, fibers and parenchyma; it is traversed by narrow medullary rays. Xylem vessels are pitted, spiral and reticulated lignification grouped in radial rows with the metaxylem towards the lower surface and the protoxylem towards the upper surface**.** The fiber is straight in outline and has lignified wall, a narrow lumen and an acute apex. Tracheids are lignified and show pitted thickness. They are stained red with phloroglucinol and concentrated hydrochloric acid. The xylem parenchyma is rectangular in shape having pitted lignified walls.

The phloem forms a narrow zone and consists of thin walled shining soft cellulosic elements. Some small prisms of calcium oxalate are present in phloem parenchyma and traversed by uni- to biseriate medullary rays consisting of thin walled, somewhat radially elongated cells. Phloem fibers are absent. The lower pericycle arc consists of 3-4 rows of pericyclic fibers while the upper arc is formed of 2-3 rows of parenchymatous cells and fibers. The fiber has a thick lignified wall, a narrow somewhat fairly wide lumen and an acute to acuminate apex. The fibers are fusiform with straight or tortuous lignified walls.

The Powdered leaf of *S. secundiflora* (Ortega) DC. is dark green in color, with characteristic odor and bitter taste and it is characterized by the presence of fragments of upper and lower epidermises of the lamina which appear polygonal, isodiametric to subrectangular with straight to curved or slightly wavy anticlinal walls. The cells are covered with thick smooth cuticle. The stomata are rare but if present it is of paracytic type. Trichomes are absent. Fragments of the lower epidermis of the lamina which appears polygonal,

mostly isodiametric with straight anticlinal walls. The cells are smaller than those of the upper epidermis. They are covered with usually smooth cuticle. Numerous are rounded to oval in shaped stomata of the paracytic type are present, surrounded by 5 to 7 epidermal cells. The trichomes are very rare; they are unicellular covering trichomes rarely bi cellular and covered with warty cuticle. The unicellular covering trichomes are conical in shape with wide lumen and acute to acuminate bent apices. Fragments of the mesophyll tissue with green columnar palisade cells and spongy parenchyma containing prisms of calcium oxalate. Lignified xylem vessels with pitted, spiral and reticulated thickenings, as well as tracheids with lignified pitted and reticulated thickenings. Abundant pericyclic or wooden lignified fibers either entire or broken, surrounded by crystal sheath with prismatic crystals of calcium oxalate, see (Plate 7).

The microscopical measurements of the different elements present in the leaf of *S. secundiflora* are recorded in (Table 2).

3.4. The petiole

The transverse section of the petiole (Plate 8, Figs. 1 & 2) is U-shaped, but dorsally compressed and with strongly incurved ends. The epidermis consists of one row of square to rectangular cells axially elongated cells with straight thick anticlinal walls and covered with thick smooth cuticle, stomata are rare but if present, they are of the paracytic type and similar to those of lamina in shape and size. The cells contain prisms of calcium oxalate crystals.

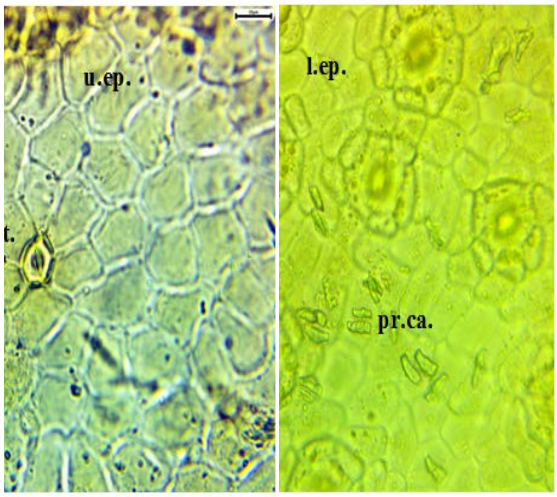
The cortical tissue is formed of 3-5 rows of angular collenchymatous cells. They are followed by 3-5 rows of rounded to sub rectangular parenchymatous cells with narrow intercellular spaces. The cells contain starch and calcium oxalate crystals. These contents are similar to those of the lamina. The cells above the two additive vascular bundles usually contain chlorophyll (chlorenchyma).

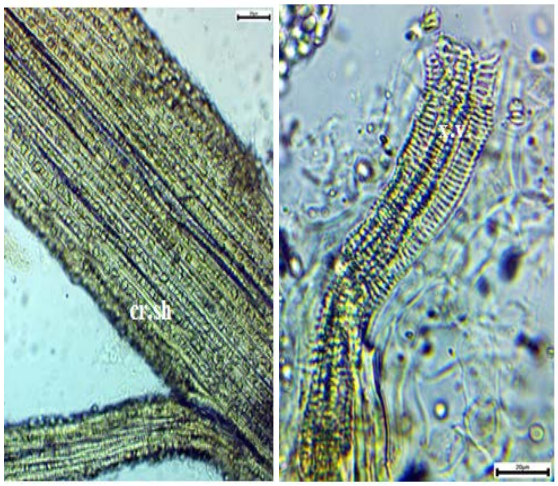
The vascular system is similar to that of the lamina and no great difference between the two vascular systems. Vascular bundle is formed of a large collateral vascular bundle. In addition, two smaller vascular bundles are suited at the upper side in the incurved ends. The pericycle is formed of continuous ring (4-6 rows) of pericyclic fibers. The fibers are elongated, fusiform with straight, moderately thick lignified walls. Those fibers are surrounded by parenchyma cells containing prismatic crystals of calcium oxalate forming crystal sheath. The vascular tissue consists of a wide circular vascular bundle occupying about 1/2 of the diameter. They are separated by uni and diseriate medullary rays which consist of lignified polygonal slightly elongated cells. The xylem consists of radial rows of lignified pitted and spiral vessels, parenchyma and fibers. The phloem is formed of a narrow zone of soft, small and thin walled elements below the xylem. It consists of sieve elements and phloem parenchyma containing calcium oxalate prisms and starch granules.

The pith: It is central, scanty and is formed of rounded to polygonal thin-walled parenchymatous cells, usually with lignified pitted walls and showing intercellular spaces and calcium oxalate prism.

4. Anatomical features of *S. tomentosa* L.

The anatomical structure of different organs of *S. tomentosa* is more or less similar to their respective ones in *S. secundiflora* (Ortega) DC., but could be differentiated and characterized by the anatomical structure of the young stem branch such as the presence of numerous bent, non-glandular, unicellular to bicellular, uniseriate hairs covered with warty cuticle; fewer number of stomata. The long lignified tracheids and the prisms of calcium oxalate are larger in size than that of *S. secundiflora*. The medullary rays





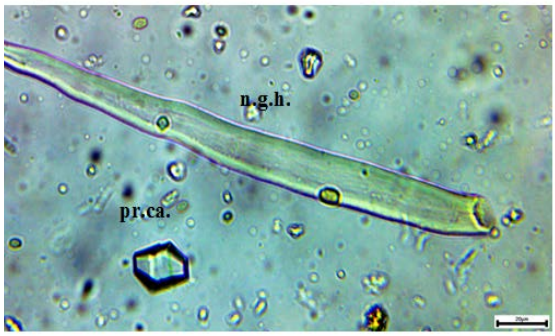
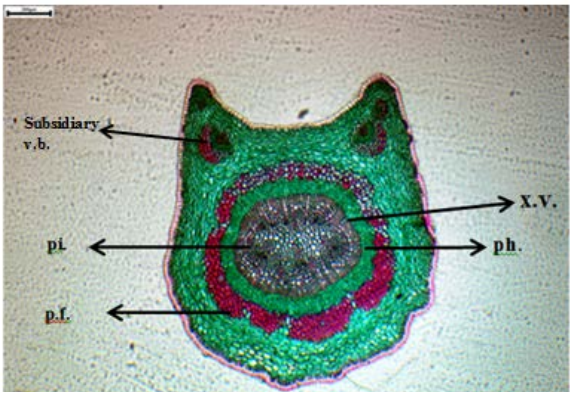
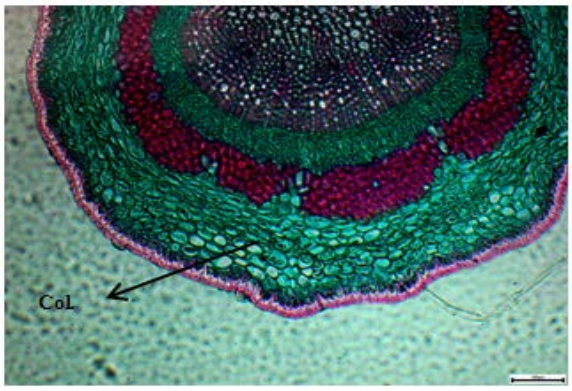


Plate 7. Isolated elements of powdered leaf of *S. secundiflora*

l.ep.= lower epidermis (400X); u.ep.= upper epidermis (400X); pr.ca.= prisms of Ca- oxalate (400X); st.= stomata(400X); n.g.h.= non-glandular hair (400X); pal.= palisade (400X); cr.sh.= crystal sheath (400X); x.v.= xylem vessels (400X).



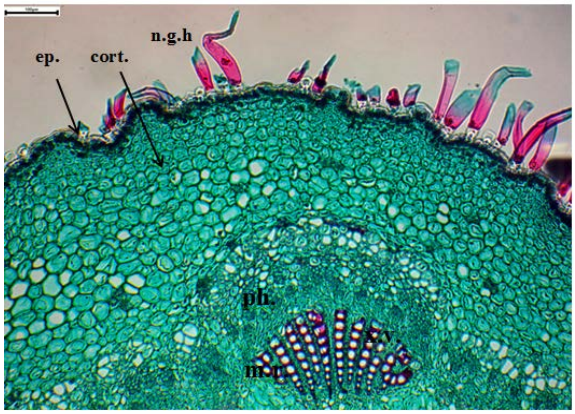
1. Low power view of the entire petiole



2. High power view of the petiole (100X)

Plate 8. Micromorphology of the petiole of *S. secundiflora*

Col.= collenchyma; p.f.= pericyclic fiber; ph.= phloem; pi.= pith; v.= xylem vessels; v.b.= vascular bundle



The young stem branch T.S (100X)

Plate 9. Micromorphology of the young stem of *S. tomentosa*

cort.= cortex; ep.= epidermis; m.r. = medullary rays; p.f.= pericyclic fiber; ph.= phloem; pi.= pith; v.= xylem vessels; n.g.h.= non-glandular hair

contain less starch granules; see Plates (9) & (10**)**

Many variations exist between the two species concerning the microscopical characters of the old stem branch being less lignified in *S. tomentosa*. The absence of calcium oxalate prisms beneath the cork and presence of non-glandular, unicellular, uniseriate hairs; covered with warty cuticle, see Plate (11).

There are also many differences in the leaf where, the trichomes are numerous. Including non-glandular type. They are covered with warty cuticle. The hairs consisting of an elongated cell and 2 basal cells are sunken in the epidermis. The covering trichomes are conical in shape with wide lumen and acute to acuminate bent apices. The upper and lower epidermises are straight with cicatrix. Stomata and prisms of calcium oxalate are less in number; see Plates (12) & (13).

Concerning the petiole**,** the transverse section is the same but less dorsally compressed and with less incurved ends. The vascular system is formed of main central circular vascular bundle and absence of the lignified pericyclic fibers. Presence of the covering trichomes, they are non-glandular type and covered with warty cuticle. They are conical in shape with wide lumen and acute to acuminate bent apices, see Plate (14) and Figs. (1 &2). The dimensions of the different elements of the stem and leaf of *S. tomentosa* are displayed in (Table 2).

5. DNA fingerprinting of the studied species

Genetic diversity as revealed by RAPD using eleven decamer primers was used to detect the genetic variability. Each of the eleven primers had successfully directed the amplification of a genome specific fingerprint of DNA fragments. The eleven primers **(OPO-02, OPO-04, OPO-08, OPO-09, OPO-10, OPC-08, OPC-12, OPC-13, OPC-14, OPC-15, and OPG-02)** of arbitrary sequences used for RAPD-PCR analysis generated totally 112 amplified DNA bands for *S.secundiflora*, and 120 fragments in *S. tomentosa* with a total of 232 bands (Table 3). The obtained RAPD-PCR products for the two *Sophora* species using eleven decamer primers are represented in Plate (15).

The eleven primers had produced multiple band profiles with a number of amplified DNA bands ranging from 15 when **OPC-15** was used with *S. secundiflora* and 17 bands with *S. tomentosa* whereas the least number of bands was 7 being produced by **OPO-02**, and **OPO-08** in *S. secundiflora* and 7 being produced by **OPO-02** in *S. tomentosa.* However, the total bands were 232 bands, out of them 99 were polymorphic representing a level of polymorphism of 42.67%. The highest degree of similarities (85.71%) was recorded using primer **OPO-02** and the lowest degree of similarity (35.29%) was recorded using primer **OPO-10** (Table 3).

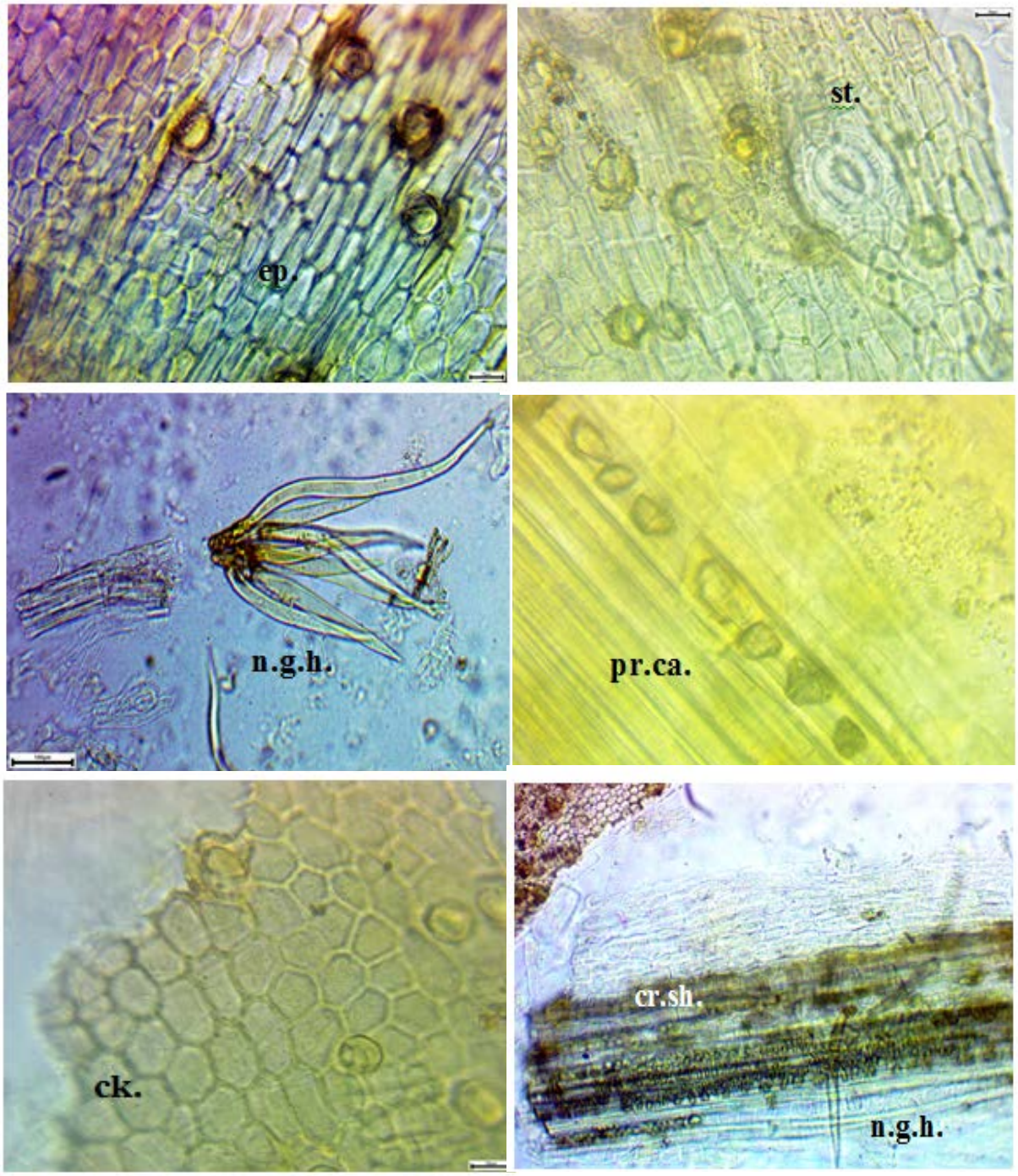
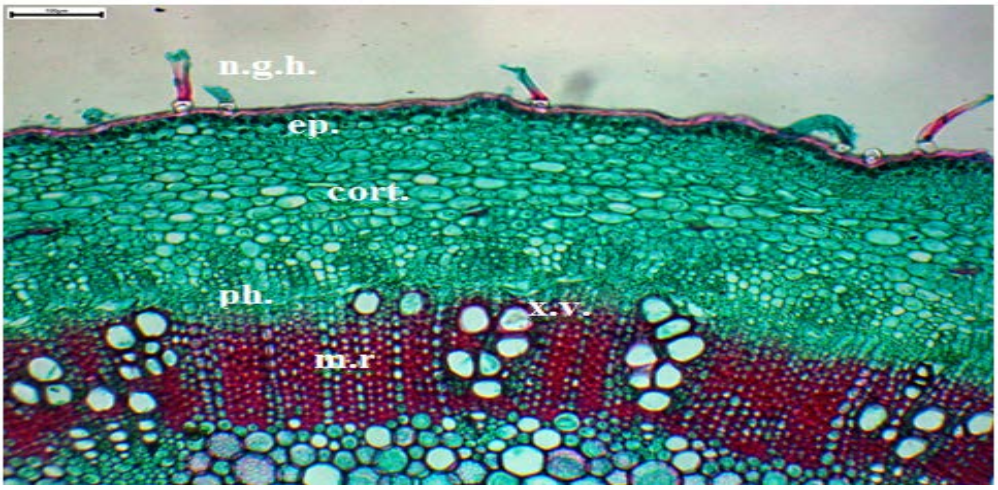
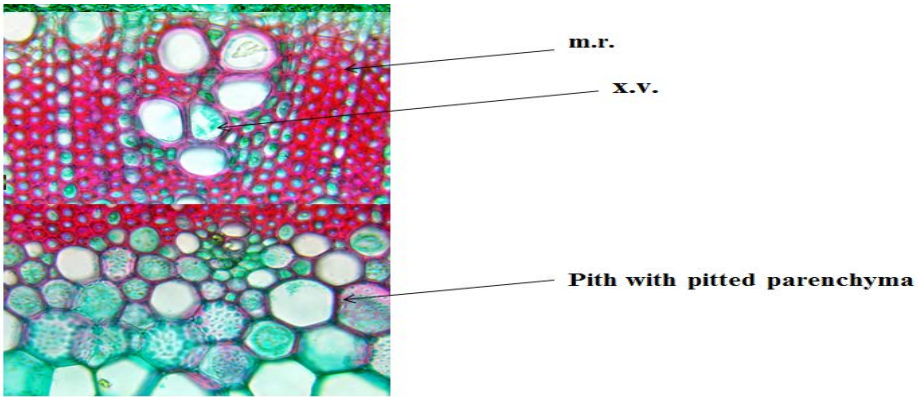


Plate 10. Isolated elements of the powdered stem of *S. tomentosa*

ep.= epidermis (400X); st.= stomata (400X); n.g.h.= non-glandular hair (100X); tr.= tracheids (400X); x.v.= xylem vessels (400X); pr.ca.= prism of Ca-oxalate (400X); cr. sh.= crystal sheath (100X); ck.= cork (400X).



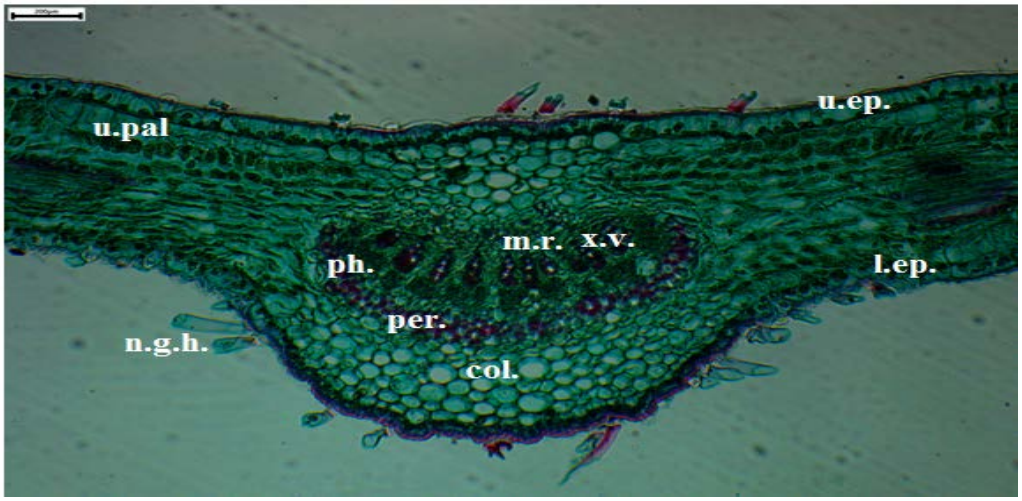
1. Low power view of the old stem branch T.S (100X)



2. High power view of the old stem branch T.S (400X)

Plate 11. Micromorphology of the old stem branch of *S. tomentosa*

cort.= cortex; ck.= cork; m.r.= medullary rays; ph.= phloem; pi.= pith; x.v.= xylem vessels



High power view in the midrib T.S. (100X).

Plate 12. (Micromorphology of the leaf of *S. tomentosa*

col.= collenchyma; l.ep.= lower epidermis; m.r.= medullary rays; pr.ca.= prisms of Ca- oxalate; per.=pericycle; ph.= phloem; u.ep.= upper epidermis; u.pal.= upper palisade; x.v.= xylem vessels; v. b.= vascular bundle; n.g.h.= non- glandular hair.

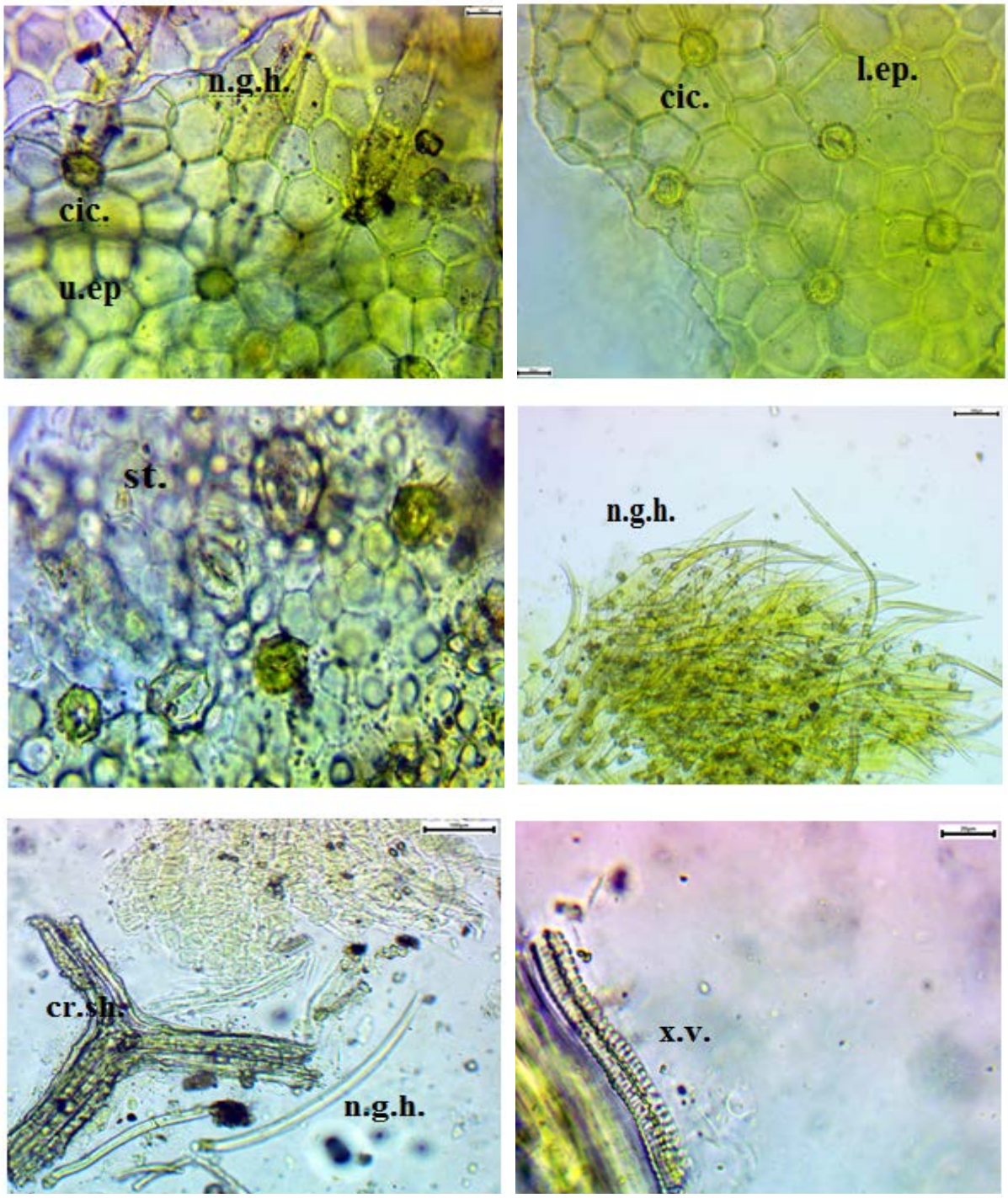
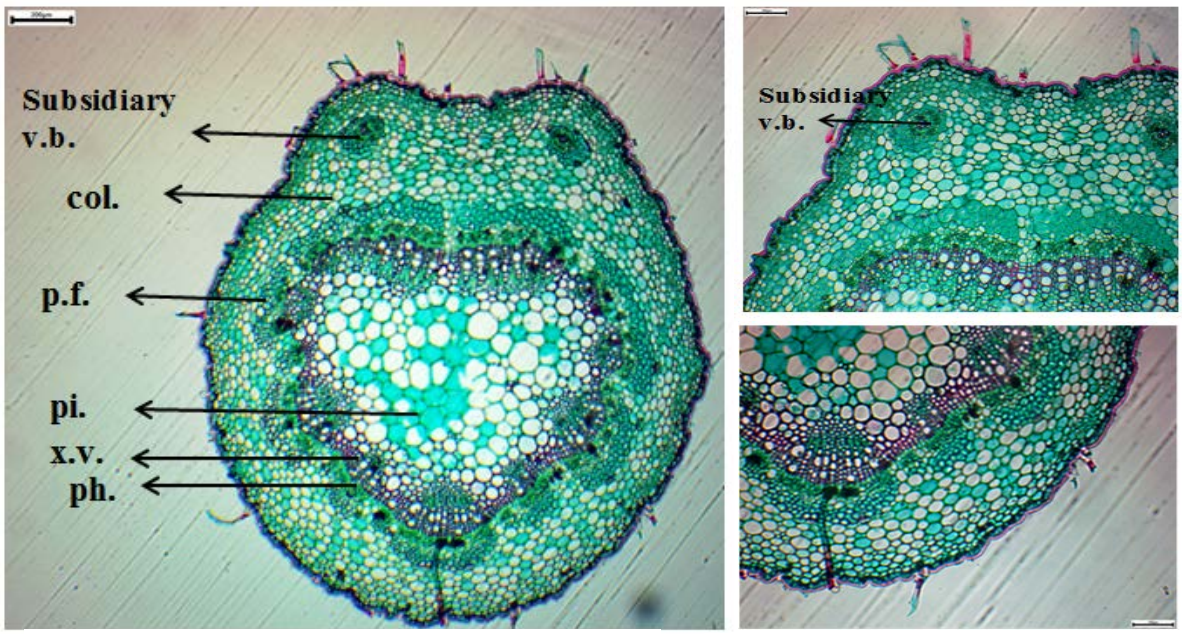


Plate 13. Isolated elements of powdered leaf of *S. tomentosa*

l.ep.= lower epidermis(400X); u.ep.= upper epidermis with cicatrix (cic.) (400X); st.= stomata (400X); n.g.h.= non-glandular hair(100X); cr.sh.= crystal sheath(100X); x.v.= xylem vessels (400X)



1. Low power view of the entire petiole (40X)

2. High power view of the petiole (a & b) (100X).

Plate 14. (Figs. 1-2). Maicromorphology of the petiole of *S.tomentosa*

Col.= collenchyma; p.f.= pericyclic fiber; ph.= phloem; pi.= pith; v.= xylem vessels; v.b.= vascular bundle

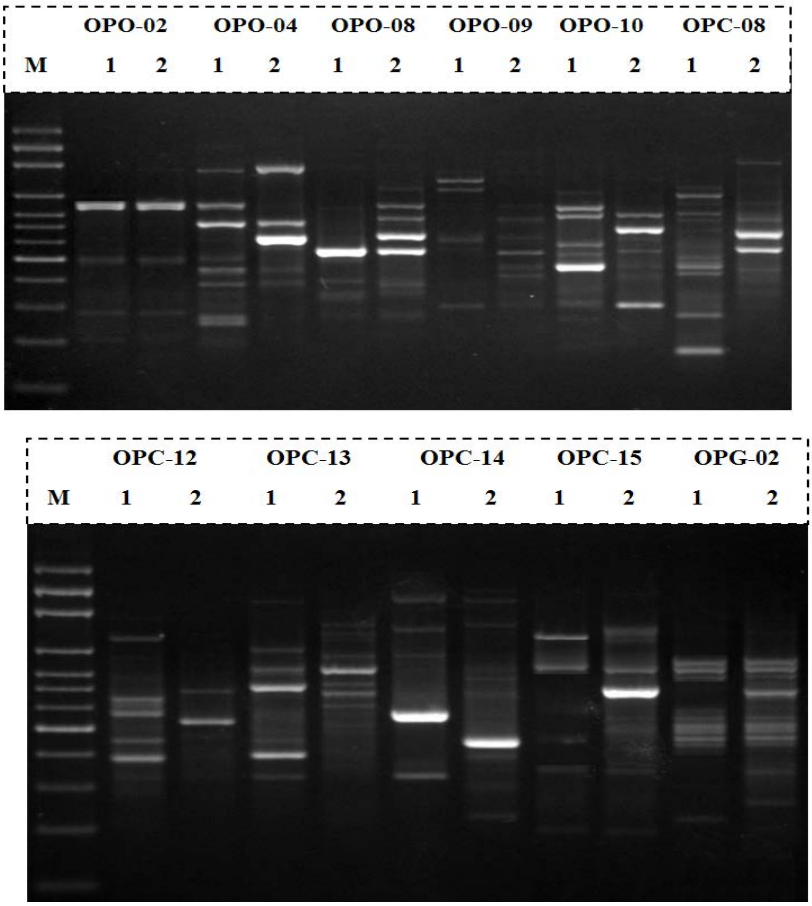


Plate 15. The obtained RAPD-PCR products for *S. secundiflora* (1) and *S. tomentosa* (2) using eleven decamer primers

Table (2). Microscopically measurements of the different elements of the organs under investigation of *S. secundiflora* and *S. tomentosa* are displayed as follows: (In microns)

Plant name	*S. secundiflora*				*S. tomentosa*			
Item	**L**	**W**	**H**	**D**	**L**	**W**	**H**	**D**
The Leaf								
Upper epidermal cells	24.36-34.89*-45.42	15.49-22.3*-29.20	14.66-16.47*-18.29		20.95-34.215*-47.48	25.92-34.58*-43.24	11.56-18.36*-25.16	
Lower epidermal cells	23.71-31*-38.48	13.09-17.82*-22.55	8.30-10.38*-12.46		23.26-30.38*-37.54	26.44-36.08*-45.73	26.99-21.84*-16.69	
Stomata	25.89-27.86*-29.83	16.78-20.17*-23.56			15.63-18.31*-20.99	15.66-17.44*-19.22		
Palisade cells	30.46-33.97*-37.48	8.69-9.68*-10.68			30.30-37.71*-45.12	10.74-13.9*-17.06		
Collenchyma				12.97-19.38*-25.80				9.87-20.76*-31.66
Non-glandular hairs	211.60-274.4*-337.20	12.70-14.16*-15.63			156.82-290.44*-424.06	17.76-21.97*-26.19		
Prisms of Calcium oxalate	10.79-15.06*-19.33	2.06-5.1*-8.14			6.70-9.35*-12.93	7.95-10.1*-12.24		
Xylem vessels				4.73-5.84*-				5.98-7.1*-
Pericyclic fibers				1.14-1.85*-2.56				13.38-15.79*-18.20

L: length, W: width, H: height, D: diameter

*= The mean value of the largest and the smallest measurements

Table (2). Cont.

Plant name	*S. secundiflora*				*S. tomentosa*			
Item	**L**	**W**	**H**	**D**	**L**	**W**	**H**	**D**
The stem								
Epidermis	13.95-19.03*-24.12-	11.45-14.88*-18.32	21.36-25.33*-29.31		13.41-17.22*-21.03	14.70-17.60*-20.51	17.90-20.51*-23.13	
Stomata	30.23-31.58*-32.94	32.06-30.64*-29.22			19.86-27.18*-34.51	14.24-21.27*-28.31		
Parenchyma of cortex				8.58-17.64*-26.71				13.24-24.51*-35.78
Non-glandular hairs	124.49-190.45*-256.4.2	12.68-27.03*-41.39			327.06-397.72*-468.39	19.61-19.9*-20.24		
Xylem vessels				6.96-9.03-11.11				12.20-18.85*-25.50
Tracheids	80.36-94.02*-107.68	5.26-7.45*-9.65			112.17-117.06*-121.95	39.15-40.87*-42.60		
Pericyclic fibers	453.07			11.82	729.2			19.21-26.45*-33.69
Wood fibers	306.62-388.64*-470.66			5.18-9.13*-13.09	1137.20			19.12-21.89*-24.67
Pitted parenchyma	25.88-30.05*-34.22	9.88-12.81*-15.74			53.59-67.20*-80.82	42.97-55.25*-67.53		
Prisms of calcium oxalates	12.70-16.03*-19.36	4.30-7.07*-9.84			41.54-48.57*-55.6	28.84-38.29*-48.75		
Old stem								
Cork cells	13.64-20.0*-26.37	15.43-24.02*-32.61			14.10-27.7*-41.30	15.63-19.23*-22.84		
Prisms of calcium oxalates	10.59-15.11*-19.64	3.73-8.16*-10.92			Absent			

L: length, W: width, H: height, D: diameter

*= The mean value of the largest and the smallest measurements

Table (3). Total numbers of RAPD-PCR bands, distribution of monomorphic (common) and polymorphic bands and similarity coefficients generated by eleven decamer arbitrary primers in *S. secundiflora* and *S. tomentosa*

Primer code	*RAPD Fragments*			*Monomorphic Fragments*	*Polymorphic Fragments*	*% Monomorphism*	*% Polymorphism*	*Similarity Coefficient (%)*
	S.S.	*S.T.*	*Total*					
OPO-02	**7**	**7**	**14**	**6**	**2**	42.85	14.28	85.71
OPO-04	**12**	**8**	**20**	**7**	**6**	35	30	70
OPO-08	**7**	**10**	**17**	**5**	**7**	29.41	41.17	58.82
OPO-09	**8**	**12**	**20**	**6**	**8**	30	40	60
OPO-10	**9**	**8**	**17**	**3**	**11**	17.64	64.70	35.29
OPC-08	**14**	**12**	**26**	**6**	**14**	23.07	53.84	46.15
OPC-12	**11**	**8**	**19**	**5**	**9**	26.31	47.36	52.63
OPC-13	**11**	**14**	**25**	**7**	**11**	28	44	56
OPC-14	**10**	**12**	**22**	**6**	**10**	27.27	45.45	54.54
OPC-15	**15**	**17**	**32**	**6**	**13**	18.75	40.62	37.5
OPG-02	**8**	**12**	**20**	**4**	**8**	20	40	40
Total	**112**	**120**	**232**	**61**	**99**	26.29	42.67	52.58

S.S. = S. secundiflora, S.T. = S. tomentosa

Discussion

Macro and micromorphological (anatomical) features of the studied *S. secundiflora* and *S. tomentosa* were investigated thoroughly. Their characters are highly similar to those of the family Fabaceae, subfamily Faboideae (Perveen and Qaiser 1998; Singh 2016). They occur as shrubs or trees with alternately arranged and sessile odd-pinnately compound leaves. Lamina is broad lamina and a retuse apex. Flowers are, grouped in raceme racemose inflorescence, hermaphrodite, zygomorphic and polypetalous corolla and deciduous, gamosepalous calyx, fruit is legume (Singh 2016).

Stomata of rubiaceous type that are present on both sides of the leaf that are surrounded by two subsidiary epidermal cells which are parallel to the long axis of the pore and guard cells, the existence of calcium oxalate crystals (mainly prisms) in the different organs and the presence of fibers in the pericycle forming the crystal sheath, all reveal their resemblance to the family Fabaceae (Singh 2016)

In addition, a great similarity exists between the morphological characters of both plants. Most of the reliable criteria which can be used for distinction between the two species are prominent in fresh samples, anatomy in transverse sections and as well as in the powdered stem and leaf ones.

Morphologically, they could be differentiated by the color of the flower. Being purple in *S. secundiflora* and yellow in *S. tomentosa*. They also differ in the dimensions of the leaf being larger in the former and smaller in the later. While, the later have more leaflets on the branch that the former. On the other hand, many anatomical variations exist between the two species. The presence of more prism of calcium oxalate in the former, while numerous non-glandular hairs exist in the later. They also differ in the dimensions of the microelements (cells & tissues) which are precisely displayed in (Table 2).

On the genetic level, it can be concluded that the most relevant fragment resulting from the successful combination of template and primer was that produced by **OPO-10** and **OPC-15** RAPD primers. Such primers could be used to discriminate between the two *Sophora* species depending on their low values of similarity coefficients and high level of polymorphism. However, the other estimated RAPD primers, which produce high values of similarity coefficient and

low levels of polymorphism, could be used in the identification of different *Sophora* species especially **OPO-02** RAPD primer which produces 85.71 % similarity coefficient.

References

Abd El-Ghffar, E.A.; El-Nashar, H.A.; Eldahshan, O.A. & Singab, A.N.B. (2017). GC-MS analysis and hepatoprotective activity of the n-hexane extract of *Acrocarpus fraxinifolius* leaves against paracetamol-induced hepatotoxicity in male albino rats. *Pharm. Biol.* 55, 441-449.

Aly, S.H.; Elissawy, A.M.; Eldahshan, O.A.; Elshanawany, M.A.; Efferth, T. & Singab, A.N.B. (2019). The pharmacology of the genus *Sophora* (Fabaceae): An updated review. *Phytomedicine.* 64, 153070.

Andrews, E.C. (1914). The development and distribution of the natural order Leguminosae. *Royal Society of New South Wales*, pp. 333-407.

Azmat, M.A.; Khan, I.A.; Cheema, H.M.; Rajwana, I.A.; Khan, A.S. & Khan, A.A. (2012). Extraction of DNA suitable for PCR applications from mature leaves of *Mangifera indica* L. J. Zhejiang Univ. Sci. B. 13(4):239-43.

Chestnut, VK. (1898). *Preliminary Catalogue of Plants Poisonous to Stock.* Department of Agriculture, Bureau of Animal Industry, 15, 387-420.

Cory, V. (1935). *Sophora secundiflora: poisonous for livestock.* Texas Agricultural and Experimental station, Bulletin No. 519.

Cumbie, B. & Mertz, D. (1962). Xylem anatomy of *Sophora* (Leguminosae) in relation to habit. *Am. J. Bot*. 49, 33-40.

de Arruda, V.L.V. & Nogueira, E.M.L. (2006). Fructification and damage to fruits and seeds of *Sophora tomentosa* L. on a sandbank of Joaquina beach, Florianópolis, SC. *Biotemas.* 19, 41-48.

e Silva, F.H.M. & dos Santos, F.D.A.R. (2009). Pollen morphology of the shrub and arboreal flora of mangroves of Northeastern Brazil. Wetl. *Ecol. Manag*. 17, 423.

El-Nashar, H.A.; Eldahshan, O.A.; Elshawi, O.E. & Singab, A.N.B. (2017). Phytochemical Investigation, Antitumor Activity, and Hepatoprotective Effects of *Acrocarpus fraxinifolius* Leaf Extract. *Drug Dev. Res*. 78, 210-226.

Gandhi, K.N.; Vincent M.A. & Reveal J.L. (2011). *Dermatophyllum*, the correct name for *Calia* (Fabaceae). *Phytoneuron*. 57, 1-4.

Gilman, E.F. & Watson, D.G. (1994). *Sophora secundiflora* (Texas mountain laurel). *Florida Cooperative Extension Service*, Fact Sheet ST-597, University of Florida.

Gledhill, D.(2008). *The names of plants. Cambridge University Press*, 202..

Hatfield, G.M.; Valdes, L.J.; Keller, W.J.; Merrill, W.L. & Jones, V.H. (1977). An investigation of *Sophora secundiflora* seeds (Mescalbeans). *Lloydia*. 40, 374-383.

Hnatiuk, S.H. (1979). A survey of germination of seeds from some vascular plants found on Aldabra Atoll. *J. of Biogeogr.* 6, 105-114.

Huang, J. & Redmann, R. (1995). Responses of growth, morphology, and anatomy to salinity and calcium supply in cultivated and wild barley. *Can. J. Bot.* 73, 1859-1866.

Hurr, K.A.; Lockhart, P.J.; Heenan, P.B. & Penny, D. (1999). Evidence for the recent dispersal of *Sophora* (Leguminosae) around the Southern Oceans: molecular data. *J. Biogeogr*. 26, 565-577.

Izaddoost, M. (1975). Alkaloid chemotaxonomy of the genus *Sophora*. *Phytochemistry.* 14, 203-204.

Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.* 44, 223-270.

Judd, W.S.; Campbell, C.S.; Kellogg, E.A.; Stevens, P.F. & Donoghue, M.J. (2009). *Sistemática Vegetal-: Um Enfoque Filogenético. Artmed Editora.*

Keller, W.J. & Hatfieldt, M. (1979). 11-Allylcytisine and other minor alkaloids from unripe *Sophora secundiflora* fruits. *Phytochemistry*. 18, 2068-2069.

Kirkbride, J. (1987). Phylogeny and classification of Pakistani legumes. *Pak. J. Bot*. 18, 287-299.

Kojima, T.; Nagaoka, T.; Noda, K. & Ogihara, Y. (1998). Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. *Theor. Appl. Genet.* 96, 37-45.

Krishna, P.M.; KNV, R. & Banji, D. (2012). A review on phytochemical, ethnomedical and pharmacological studies on genus *Sophora*, Fabaceae. *Rev. Bras. Farmacogn.* 22, 1145-1154.

Lewis, G.; Schrire, B.; Mackinder, B. & Lock, M. (2005). *Legumes of the World. Royal Botanic Gardens, Kew Richmond, UK.*

Lonard, R.I.; Judd, F. & Stalter, R. (2015). The biology of the subtropical and pantropic shrub, *Sophora tomentosa* L.(Fabaceae), in Coastal Dune Environments. *Int. Biol. Rev.* 2, 1-16.

Martins-Lopes, P.; Lima-Brito, J.; Gomes, S.; Meirinhos, J.; Santos, L. & Guedes-Pinto, H. (2007). RAPD and ISSR molecular markers in *Olea europaea* L.: Genetic variability and molecular cultivar identification. *Genet. Resour. Crop Ev.* 54(1):117-128.

Meyer, R.E. & Meola, S.M. (1978). *Morphological characteristics of leaves and stems of selected Texas woody plants*. Department of Agriculture, Agricultural Research Service.

Milad, R.; Youssef, F.S.; Ashour, M.L. & Singab, A.N. (2013). Botanical and genetic characteristics of *Eremophila Maculata* and *Eremophila Purpurascens* (Scrophulariaceae). *World J. Pharm. Res.* 2, 2711-2736.

Murakoshi, I.; Kubo, H.; Ikram, M.; Israr, M.; Shafi, N.; Ohmiya, S. & Otomasu, H. (1986). (+)-11-Oxocytisine, a lupine alkaloid from leaves of *Sophora secundiflora*. *Phytochemistry*. 25, 2000-2002.

Nakanishi, H., 1988. Dispersal ecology of the maritime plants in the Ryukyu Islands. *Japan. Ecol. Res.* 3, 163-173.

Perry, L.M. & Metzger, J. (1980). *Medicinal plants of east and southeast Asia:* attributed properties and uses. MIT press, Cambridge, Massachusetts, London.

Perveen, A. and Qaiser, M. (1998). Pollen Flora of Pakistan-VIII Leguminosae (subfamily: Papilionoideae). *Turk. J. Bot*. 22(2): 73-92.

Polhill, R. (1981). *Sophoreae. Advences in Legume Systematics*. Polhill, R. M. and Raven, P. H. (Eds.), Royal Botanic Gardens, Kew 1, 23-230.

Rogl, S.; Javornik, B.; Sinkovic, T. & Batic, F. (1996). Characterization of oak (*Quercus* L.) seed proteins by electrophoresis. *Phyton-Horn*. 36, 159-162.

Ruter, J.M. & Ingram, D.L. (1991). Germination and morphology of *Sophora secundiflora* seeds following scarification. *HortScience*. 26, 256-257.

Singh, G. (2016). *Plant Systematics, 3^{ed}.: An Integrated Approach. CRC Press.*

Sykes, W.R. & Godley, E.J. (1968). Transoceanic dispersal in *Sophora* and other genera. *Nature.* 218, 495.

Trease and Evans (2009). *Textbook of Pharmacognosy*. Sixteen ed., William Charles Evans, London, UK, p. 29, 30.

Turner, B.L. (2012). New names in *Dermatophyllum* (Fabaceae). *Phytoneuron*. 3, 1-4.

Tucker, S.C. (1994). Floral ontogeny in Sophoreae (Leguminosae: Papilionoideae): II. *Sophora* sensu lato (Sophora group). *Am. J. Bot*. 81(3):368-80.

Vines, R.A. (1960). *Trees, shrubs and woody vines of the southwest.* University of Texas, Austin,1, 104.

Wickens, G.E. (1979). *The propagules of the terrestrial flora of the Aldabra Archipelago, Western Indian Ocean*. Atoll Res. Bull. 229, 1-39.

Williams, J.G.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. & Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18, 6531-6535.

Willis, J.C. (1993). *A dictionary of the flowering plants and ferns*, 8th edn., revised by H.K. Airyshow ed. Cambridge University press, London.

Zavala-Cháveza, F.; García-Mateos, R.; Soto-Hernández, M. & Kited, G. (2006). Phytochemical differences between *Calia secundiflora* (Leguminosae) growing at two sites in Mexico. *Z. Naturforsch.* C. 61, 155-159.