



Comparative morphological and anatomical studies between a native species (*Prosopis cineraria* (L.) Druce) and an introduced species (*Prosopis juliflora* (sw.) dc.) in south west region of Arabian Peninsula

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Abstract

This study shows the similarity and differentiation features between *Prosopis cineraria* and *Prosopis juliflora* based on morphological and anatomical characters. *Prosopis cineraria* is a native species while *Prosopis juliflora* is an introduced one. The data includes qualitative and quantitative characters. It assures on the importance of plant morphology and anatomy in determining the identification, classification and phylogeny between species.

Keywords: prosopis, leaf, stem, mesophyll, Arabian Peninsula

Introduction

The genus *Prosopis* L. belongs to the family Leguminosae, subfamily Mimosoideae, tribe Mimosae (Burkart, 1976; Sherry *et al.*, 2011) [3, 28]. It comprises 44 species and five sections based on observed morphological differences among studied taxa (Burkart, 1976) [3]. The five sections included: *Prosopis*; *Anonychium*; *Strombocarpa*; *Monilicarpa* and *Algarobia* (Burkart, 1976; Landeras *et al.*, 2005; Elmeer and Almalki, 2011) [3, 17, 7]. Section *Prosopis* included 3 species which have internodal prickles. Each of section *Anonychium* and *Monilicarpa* included 1 species. Section *Strombocarpa* included 9 species which have spiny stipules. Section *Algarobia* included 30 species, which have axillary thorns (Burkart, 1976) [3].

The genus *Prosopis* L. is thought to have originated in tropical Africa at the end of the Mesozoic or the beginning of the Tertiary period (Burkart, 1976) [3]. Owing to its fast growth and drought hardiness with extraordinary ecological amplitude (Tewari *et al.*, 2000) [29], *Prosopis* has a wide distribution, occurring in South, North and Central America, Africa and Western Asia (Burkart, 1976; Llanes *et al.*, 2011; Sherry *et al.*, 2011) [3, 18, 28].

Prosopis species were introduced to various areas primarily to combat desertification and improve the quality and quantity of the arid regions (El Fadl and Lukznen, 2003; Mosweul *et al.*, 2013) [6, 20]. Some of the species are of major economic importance; pods are utilized as food and fodder and wood as fuel and timber (Pasiiecznik *et al.*, 2001; Sherry *et al.*, 2011) [24, 28]. Introduced alien species can destroy natural pasture and reduce grazing potential of natural rangeland and can directly or indirectly affect the food security (Van Wilgen *et al.*, 2008; Van den Berg *et al.*, 2013) [31, 30].

P. cineraria is native to dry and arid regions of Arabia and India (Khan, 1999) [1]. Its main population is center on the Thar Desert of India and Pakistan, with less dense populations occur in the Arabian Peninsula, Iran, and Afghanistan (Pasiiecznik *et al.*, 2004) [25]. It is considered the national tree of the United Arab Emirates (Jongbloed *et al.*, 2003) [14]. *P. cineraria* is known as Ghaf in Arabic, Khejri in Indian, and Jand in Pakistan. *Prosopis*

cineraria has been valued by different communities and cultures for the versatility of all its parts which and have been used in many ways as food, i.e., flour, drink, vegetable, and gum (Liu *et al.*, 2012) [19]. *Prosopis cineraria* extensively used in traditional medicine to cure many diseases such as ailments like leprosy, dysentery, asthma, leucoderma, dyspepsia and earache (Al-Aboudi and Afifi, 2010) [2]. Barks are used for non-nutritional purposes, i.e., wood, tanning, fuel, firewood and charcoal (Garg and Mittal, 2013) [9].

Prosopis juliflora is an introduced plant that presents as a native plant from America to Peru (GISP 2004) [10]. It was first introduced in India as an introduced plant and spread to Pakistan, Myanmar, Sri Lanka, Thailand, Cambodia, Brunei, Vietnam, Philippines and Indonesia and reached the Arabian peninsula in the 1950s in Yemen, Bahrain, United Arab Emirates, Qatar, Saudi Arabia, Kuwait, Iraq and Jordan (Burkart, 1976; Ahmad *et al.* 1996) [3, 1]. It was cultivated by man during the last 100-150 years for the perceived value of the trees' products as a rich and delicious flour from pods, a major honey source in Bolivia, Jamaica and Pakistan, a good firewood with excellent charcoal, a source for fibre in the production of paper, for furniture and crafts, used for preparing various medicinal syrups, particularly for expectorants, used to arrest wind erosion in addition to stabilize sand dunes on coastal areas and for ornamentation (Hunde and Thulin, 1989) [13], (Habit and Saavedra, 1990) [12], (Poynton, 1990) [27], (Felker and Moss, 1996) [8] and (Perry, 1998). It is considered as a "Royal tree" in India (Muthana and Arora, 1983) [26].

Prosopis species have been shown differences in their leaves and fruits due to exposure to variable stresses (Villagra *et al.*, 2010) [32]. The introduced and naturalized or invasive species devise different strategies for environmental management (Nel *et al.*, 2004) [22]. The recorded taxonomic identity of introduced and invasive *Prosopis* taxa remains questionable (Zimmermann, 1991) [33].

The aim of this work will be useful for clarifying the differences between introduced and native *Prosopis* taxa (*Prosopis juliflora* and *P. cineraria* respectively) through morphological and anatomical characterizations for understanding plant invasions and the processes underlining their success.

Materials and Methods

The site selected for the collection of *Prosopis* sp. is located in the city of Jazan, Kingdom of Saudi Arabia and Aden of Yemen. Plant specimens were identified at the herbarium of Science faculty – Jazan University. The plant materials were washed with distilled water and used or fixed in FAA (Formalin-Acetic Acid-70% ethanol) solution until processing.

1. Morphological Studies

Characterization and observations on morphology of the two studied plant species including plant height leaflet and stem morphology as well as flowering and fruiting were recorded and discussed.

2. Anatomical Studies:

The epidermis analysis

It was performed by using fragments of the middle third of leaves fixed in 70% alcohol. The selected fragments were boiled in a 10% nitric acid solution (Ghouse and Yunus, 1972)^[11] up to the dissociation of the epidermis. Subsequently, the epidermis was washed 3 × in distilled water and placed in a 50% sodium hypochlorite solution for clarification; washed 3 × in distilled water and in a solution of distilled water and acetic acid 1:500 (v/v) and mounted between slide and cover slip in 50% glycerin medium.

The histological analysis

The fourth node of the stem and leaf blade (midrib and intercostal region) and proximal, medial and distal petiole samples were used and three individuals from both species were selected. The materials were fixed in 2.5% glutaraldehyde in phosphate buffer pH 7.2, 0.1 M (Karnovsky, 1965)^[15] and later preserved in 70% alcohol.

The sections obtained by a rotary microtome at and were stained with fast-green safranin (Dizeo de Strittmatter, 1979)^[5]. Excess stain was washed off with water before a drop of glycerin was added. Slides were observed with an Olympus microscope and photographs were taken with a digitized camera (Nikkon (O'Brien *et al.*, 1965)^[23].

In addition to the general observations, size of parts or cell length and width or layers were recorded or measured using a calibrated ocular scale in stomata length and width and pore size, epidermis, mesophyll, ground tissue and vascular bundle.

Results and Discussion

Stem and Leaf Morphology

The two studied *Prosopis* are ever green tree, up to 10m, green or brown red in the apical stems and brown to gray in the lower parts, with spikes arise from the epidermis (Fig. 1). They have alternate leaves, stipulate with small size with dimensions 10 x 5 mm (Fig. 2), swollen leaf base with pale green, petiolate 9-28 mm in length with red green color, paripannate 20-50 mm in length, composed of 2-3 pair leaves 27-83mm in length, each pair is composed of 7-16 pair leaflets with dimensions 7-15 mm in length and 3-6 mm in width, dark green, smooth, oblong, entire,

mucronate, the distance between leaflets 6.1-8mm (Fig. 3). Secretary gland is present in the middle of leaf petiole 11mm from leaf base, wide conical like shape, wide secretory opening and there are number of glands between leaflets (Fig. 4).



Fig 1: The whole *Prosopis* plant



Fig 2: Stipule and petiole 1-stipule, 2- petiole

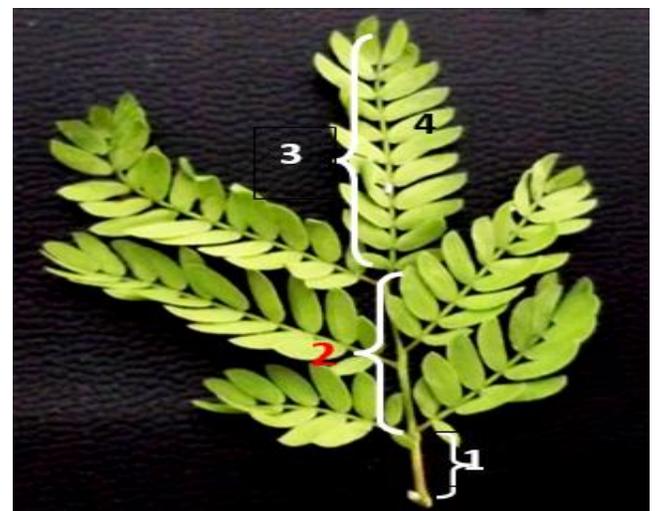


Fig 3: Leaf shape 1- Petiole, 2- rachis, 3- paripannate, 4- leaflet

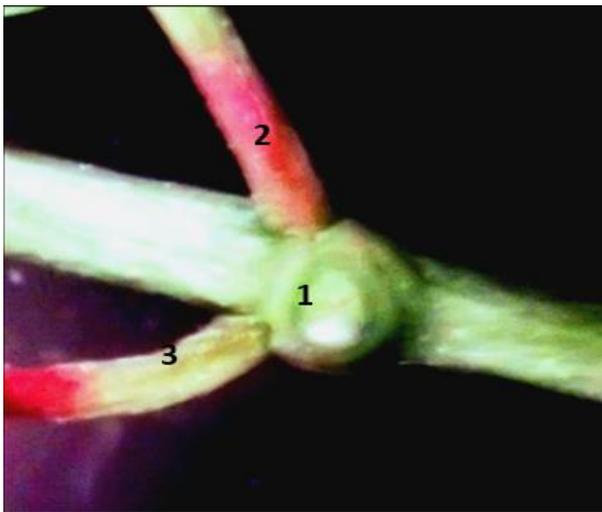


Fig 4: Leaf gland 1- gland, 2&3- petioles

Inflorescence and Fruit Morphology

Inflorescence is spike, raceme, 125 mm in length, red color in half spike stalk, little separated florets (Fig. 5). Flower is ebractate, hermaphrodite, actinomorphic, smooth. Calyx is five united sepals, imbricate, opposite sepal edges. Corolla is five united petals, imbricate, opposite petal edges. Androecium is 10 free stamens, nectar gland presents on apical lobe of stamen. Gynoecium is one carpel, marginal placentation (Fig. 6). Floral symbols (Fig. 7) are:

$Br, +, \overset{\sigma}{\text{♂}}, K_{(5)}, C_5, A_{10}, G_1$ Fruit is smooth legume indehiscent, simple depression between seed positions, yellowish white, 245 mm in length and 8 mm in width.



Fig 5: Inflorescence morphology

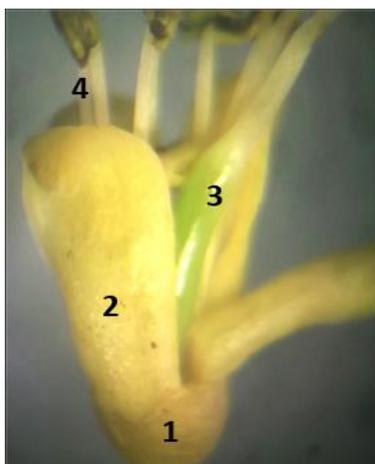
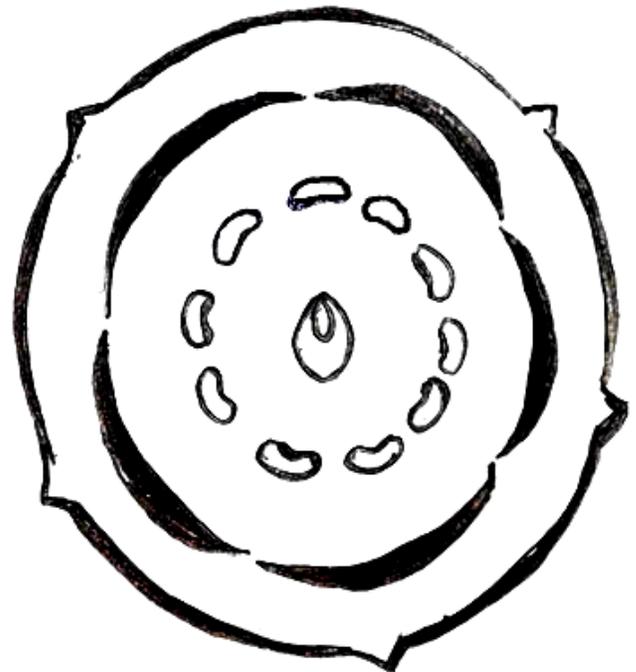


Fig 6: Flower parts

1- Calyx, 2- corolla, 3- carpel, 4- stamens



$Br, +, \overset{\sigma}{\text{♂}}, K_{(5)}, C_5, A_{10}, G_1$

Fig 7: Floral diagram and formula

Stem Anatomy

Epidermis has one layer of polygon parenchyma cells covered with a layer of cuticle 1 μm in thickness. Cortex: It is made up of 6-7 cell layers with 2-3 laminar collenchyma present under the epidermis directly, the rest are round large parenchyma cells with intracellular spaces, some of them are storage, there are two layers of cells contain chloroplasts and rhombic crystals at the end of cortex, endodermis (starch sheath) is composed of one layer of cells containing number of starch granules and some rhombic crystals. Sclerenchyma tissue: lignified thick tissue, composed of fibers present in the outer part of phloem as connected strips (Fig. 8) and (Fig. 9).

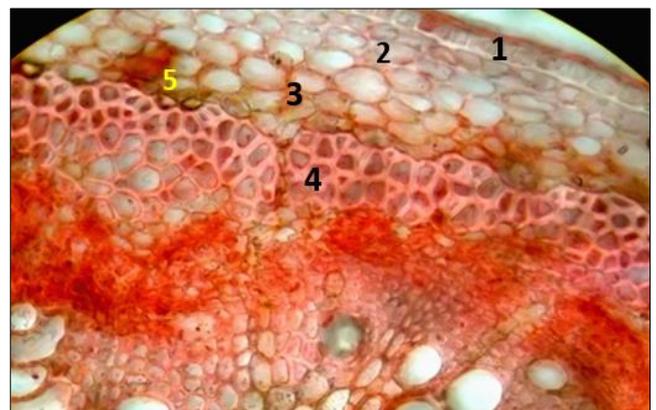


Fig 8: T.S. in *Prosopis* stem 1- epidermis, 2- collenchyma, 3- parenchyma 4- sclerenchyma 5- crystals

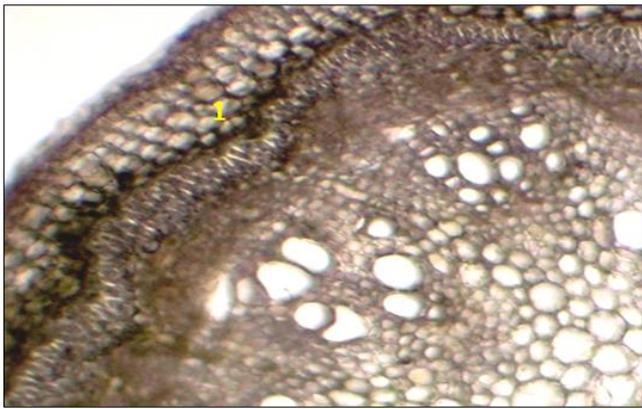


Fig 9: T.S. in *Prosopis* stem 1- endodermis (starch sheath)

Vascular cylinder has eight different opened collateral vascular tissues in size, connected in cylinder shape, composed of sclerenchyma tissue, phloem, cambium and xylem. Phloem tissue is composed of compound tissue with large size, present upper xylem. Xylem tissue is complicated tissue connects water and salts. Pith is star like shape, composed of storage parenchyma cells with thin walls, enlarge in size towards the center, intracellular spaces present without any crystals (Fig. 10).

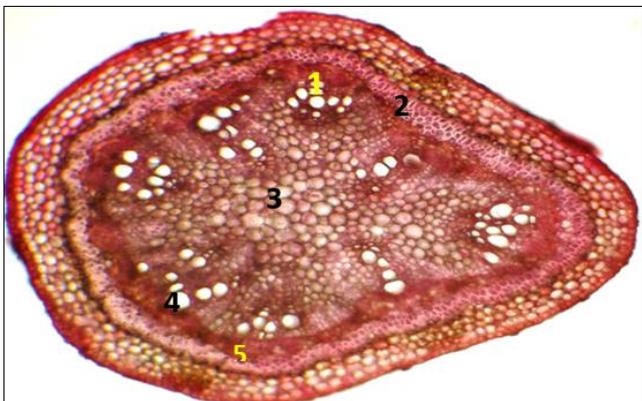


Fig 10: T.S. in *prosopis* stem

1- Vascular bundle, 2- sclerenchyma, 3- pith, 4- xylem, 5- phloem

Stem Node Anatomy

There is no difference between node and stem in structure. It is composed of the same tissues (epidermis, cortex, vascular tissues and pith). The only difference is present appendages of bundles in the node out from vascular cylinder of the stem which regards as leaf trace. Vascular cylinder of the node has 9 essential vascular bundles and two secondary vascular bundles, variety in size, arranged in a connected cylinder, composed of sclerenchyma, phloem, xylem and pith. These bundles are arranged inside stem cylinder. In addition to the cylinder, there are three essential vascular bundles with large cylindrical in shape exit little from the stem cylinder. This feature reflects

Developmental and structural between stem and lateral extensions (Leaves). This extension of vascular tissue from a plant's stem into a nearby leaf, resulting in a leaf gap in the plant's stele is Called leaf trace. The node has rich of rhombic crystals especially in the fibers around vascular cylinders (Fig. 11).

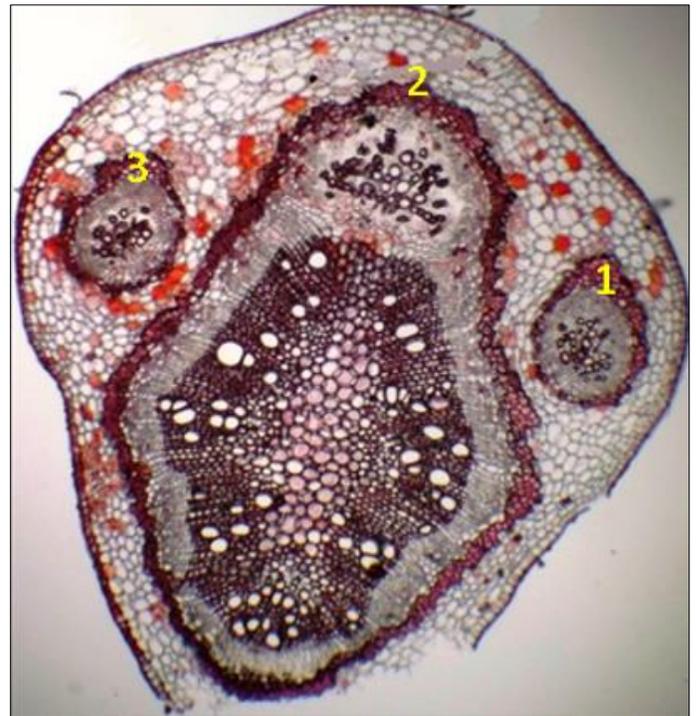


Fig 11: T.S. in Stem node 1-3 vascular bundles for leaf trace

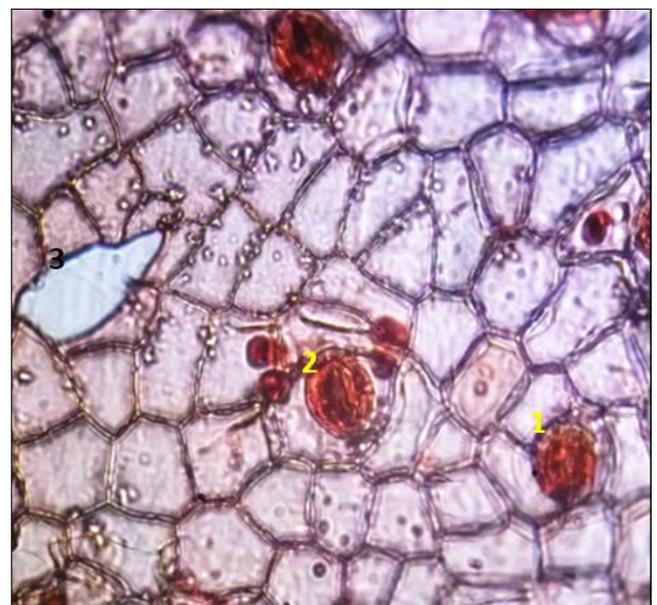


Fig 12: Upper epidermis of *Prosopis* leaf 1- Anisocytic stomata, 2- Paracytic stomata, 3- rupture from strip



Fig 13: lower epidermis of *Prosopis* leaf 1- epidermis, 2- guard cells, 3- subsidiary cells, 4- Paracytic stomata

Leaf Anatomy

Upper epidermis has one layer of rectangle parenchyma contains secretory substances, nearly irregular size thick wall, covered with cuticle with 2µm in thickness, glabrous. Lower epidermis: one thick layer of rectangle parenchyma with the same size. It's covered with a thick cuticle 2.5 µm. There are some solitary unicellular trichomes with medium in length 15 µm and 0.15% density. The both epidermis contain number of stomata with high density at the upper one which reach 5.97% for upper epidermis while 6.46% for lower one. There are two types of stomata (Fig. 12 and 13):

Paracytic: the guard cells are accompanied with two or more subsidiary cells while the axis of subsidiary cells is parallel to the axis of guard cells and stomata opening. Its density is high for both epidermises 97.12%

Anisocytic: the guard cells are surrounded with three subsidiary cells with irregular size where one is small and the two others are large. Its density is very low for both epidermises 2.44%.

Mesophyll

Upper palisade contains 3-4 layers of rectangle cells 11.5 µm length. They are close and present under the upper epidermis directly. It contains also number of chloroplasts. Lower palisade contains 2-3 layers of rectangle cells 8 µm length. They are close and present under lower epidermis directly. It contains also number of chloroplasts. Spongy tissue contains 1-2 layers of parenchyma, with chloroplasts. It is present between upper and lower palisade. There are number of rhombic crystals present in the cells which are close to fibers of midrib (Fig. 14).

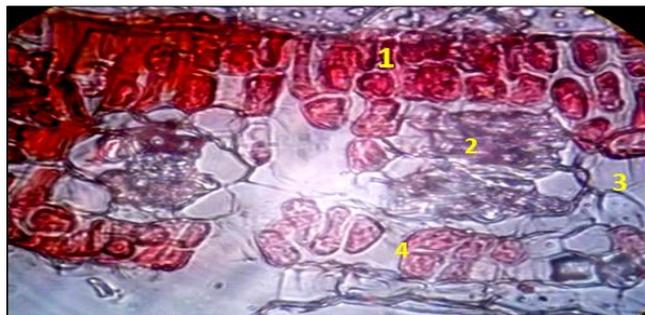


Fig 14: T.S. in *Prosopis* leaf

1- Upper palaside tissue, 2- secondary bundle, 3- spongy tissue, 4- lower palaside tissue

Fundamental bundle is composed of vascular tissue. It's present in midrib. It's large in size with comparison to secondary bundle. It's surrounded by a layer of upper epidermis and two layers of palaside from the upper side of the leaf and a layer of lower epidermis from the lower side of the leaf besides a layer of parenchyma covers the whole vascular bundle with large cells on both sides of bundle. The midrib appears outward on the both levels of upper and lower sides of the leaf. The vascular bundle is composed of xylem tissue which is directed to the upper surface of the leaf. Phloem tissue which is present under xylem tissue and directed to the lower surface of the leaf. Sclerenchyma tissue which covers the bundle as cutting masses shaped. It's present under wards as U letter. It is rich with heavy masses in the upper direction from the bundle. Finally, secondary bundle which is small in size with xylem and phloem. It's present in the area where upper palaside connects with spongy tissue (Fig.14).

Leaf Base

At the region far from the stem

It is nearly circular in shape and composed of the epidermis which has cubical closely cells covered with a layer of cuticle 1 µm thick. The cortex which is large in size. It's composed of 6-9 large parenchyma which contain very low number of rhombic crystals. The vascular cylinder which is composed of vascular tissue which contains 8 basic vascular bundles where nearly one bundle is separated and the rest arranged as U letter with different sizes from xylem and phloem. Finally, the pith which is very narrow, present as U letter and composed of parenchyma without crystals (Fig. 15).

At the adjoining base leaf to the stem or the node

This part is oval in shape resemble the previous description with minor differences; there are 7 basic vascular bundles separated from each other. There are no glandular trichomes but stellate crystals and deposit materials are present (Fig. 16).



Fig 15: T.S. in *Prosopis* leaf base 1- cortex, 2- phloem, 3- xylem, 4- pith

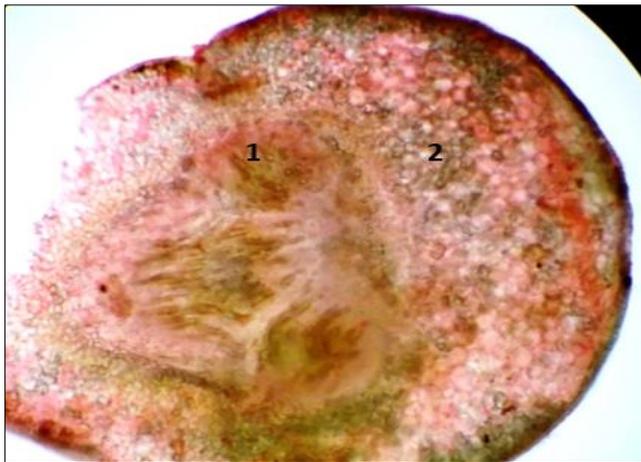


Fig 16: T.S. in *Prosopis* adjoining leaf base 1- Vascular bundles, 2- secretory substances

Leaf Petiole

The shape of leaf petiole in transverse section is oval and concave something at the upper direction and composed of The epidermis which is composed of a layer of parenchyma polygon in shape covered with a thick cuticle layer with 2.5 μm. The cortex which is composed of two types of external cells with 3-4 chlorenchyma layers with low number of chloroplastids while the second type of internal cells with 5-6 layers of parenchyma have some cells with secretory materials. The vascular cylinder which is composed of the vascular tissues: They are composed of 8 vascular bundles with 6 essential vascular cylinders in the petiole having scattered xylem and 2 bundles in the concave area from the upper direction of the petiole which are surrounded with fibers as incomplete circle shape. There are little number of rhombic crystals present in upper vascular fibers. The pith which is like very narrow lamellea shape and composed of 3-4 small parenchyma layers with large interspaces (Fig. 17).

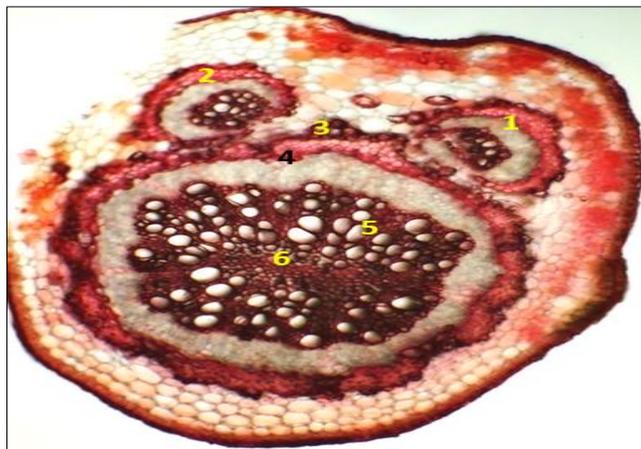


Fig 17: T.S. in leaf petiole 1&2- vascular bundles coming from vascular cylinder, 3- fibers, 4- phloem, 5- xylem, 6- pith

Leaf Gland

It looks like bowl. Its height is little over the petiole height. The internal cavity is little concave or depressed and more enlargement so the secretory surface becomes large (Fig. 18).

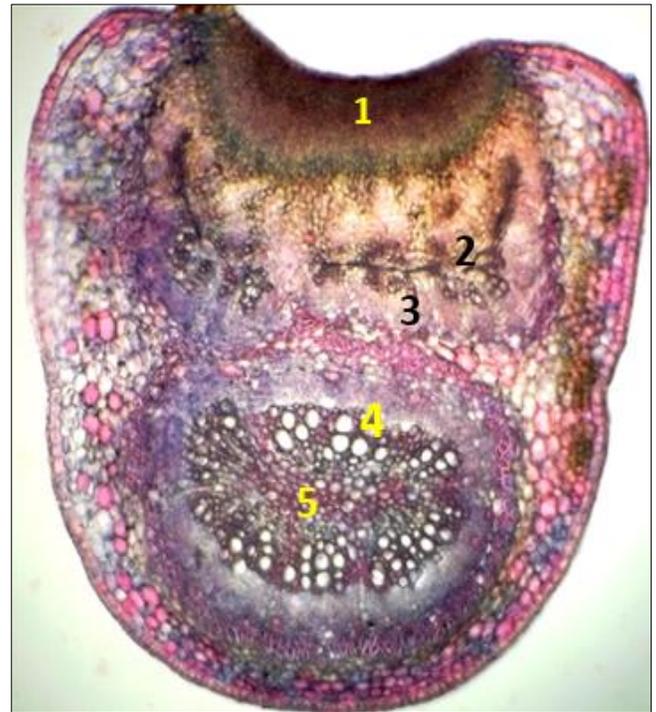


Fig 18: T.S. in leaf gland 1- Secretory surface, 2- xylem, 3- phloem, 4- vascular cylinder, 5- pith

The two investigated *Prosopis* species reveal some variations in their morphological and anatomical features. The morphological differentiations are like in internode and stipule forms, rachis and flower surface in addition to pod color and shape. Moreover, the anatomical differentiations are like in leaf petiole and base in addition to midrib anatomy. Mesophyll type is Equifacial for *P. cineraria* while Dorsiventral for *P. juliflora* (Table 1 and 2). These features show so diagnostic where they can be confined to use in classification, identification or phylogeny between introduced and native species taxa (Clement *et al.*, 2015). *Prosopis cineraria* is belonged to the section *Prosopis* which contains two other species and they are resemble in having prickles like found on roses (*Rosa* spp.) while *Prosopis juliflora* is belonged to the section *Algarobia* which have other twenty nine species having axillary thorns (Burkart, 1976)^[3]. *P. juliflora* is likely to be of sub-tropical North American origin where it survives prolonged cold periods so when it is introduced into other regions particularly in the Middle East, the adaptation of morphological and anatomical features is changed. The complex variation has become referring to biology, ecology, silviculture and management of habits.

Table 1: Comparison of the various morphological characters of *P. cineraria* and *P. Juliflora*

Species	<i>P. cineraria</i>	<i>P. juliflora</i>
Habit		
1.shape	Perennial tree	Perennial tree
2.branching	Non	Zigzag

3.internodes	Prickly	Non
Stem		
4. length (m)	10	8
Leaves		
5.stipule form	Leafy	Spiny
6. stipule length (mm)	10	70
7. stipule width	5	2
8.shape	Paripinnate	Paripinnate
9.petiolo length (mm)	9-28	44-103
10. base color	Red or green	green
11. base shape	Bulbs without curves	Bulbs with curves
Rachis		
12.length (mm)	20-50	20-61
13.surface	Glabrous	Pubescent
PINNAE		
14.number	2-3 pairs	1-2 pairs
15. length (mm)	27-83	51-235
Leaflets		
16.petiolo	Sessile	Subsessile
17.pairs	7-16	12-16
18.length (mm)	7-15	9-43
19. width (mm)	3-6	2-7
20. shape	Oblong	Oblong
21. apex	Obtuse	Obtuse
Glands		
22. petiolo	At the middle	At the end
23. Rachis	Regular arrangement	Irregular arrangement
24. height	Below petiolo level	Above petiolo level
25. shape	Large secretory surface	Narrow secretory surface
FLOWERS		
26. flower surface	Glabrous	Pubescent
27. pedicelo length (mm)	125	122
28. color	Whitish	Yellowish
29. calyx (mm)	10-15	2-3
30. calyx shape	Truncate	Bell-shaped
31. petal (mm)	3-4	3-4
32.bracts	Absent	present
PODS		
33. shape	Cylindrical, low distinctive	Straight, distinctive
34. color	Yellowish	Yellowish
35. stalk	Present	Present
36. length (mm)	245	275
37. width (mm)	8	10
38. seeds number	10-15	10-18

Table 2: Comparison of the various anatomical characters of *P. cineraria* and *P. Juliflora*

Species	<i>P. cineraria</i>	<i>P. juliflora</i>
Stem		
1. cuticle	1 μ m	2 μ m
2. collenchymas	2-3 layers	2-3 layers
3. number of vascular bundles	20	20
Leaf		
4. cuticle	2 μ m in upper epidermis and 2.5 μ m in lower one	4 μ m in upper epidermis and 2 μ m in lower one
5. stomata frequency	5.97%	9.36%
6. hair ratio	0.15%	0.44%
7. hair length	15 μ m	26 μ m
8. Mesophyll type	Equifacial	Dorsiventral
9. no. pallaside layers	3-4 upper and 2-3 lower	2-3 upper
10. pallaside length	11.5 μ m upper and 8 μ m lower	12.5 μ m
11. no. spongy layers	1-2	5-7
12. Stomata type	Paracytic and Anisocytic	Paracytic and Anisocytic
13. stomata ratio	6.46%	4.91%
14. Paracytic stomata ratio	97.12%	98.43%

15. Anisocytic stomata ratio	2.44%	1.57%
Midrib		
16. shape	Exhibit on both adaxial and abaxial sides	Exhibit crescent on abaxial side
17. fibers	As 2 dissected masses like U shape at the distal part and condensed one at the proximal portion	As condensed cylindrical shape connected surround the midrib.
Base		
18. cuticle	1 μ m	2 μ m
19. vascular bundle	Circular	Ellipsoid
20. glandular hairs	Absent	Present
Petiole		
21. cuticle	2.5 μ m	2 μ m
22. shape	Heart	Distinct from both sides
23. no. of bundles	8	7
24. vascular bundle	Circular, irregular	Square, Distinct from both sides
25. pith	Closed very narrow with small parenchyma cells	Opened on both sides, very narrow with small parenchyma or collenchymas cells

Conclusion

The study enhances the importance of plant taxonomy via morphological and anatomical characters which play vital role in the knowledge of species in terms of identification, classification and phylogeny.

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