Bangladesh J. Bot. **54**(1): 83-90, 2025 (March) DOI: https://doi.org/10.3329/bjb.v54i1.80286

COMPARATIVE ANATOMY OF *SALVADORA OLEOIDES* DECNE. AND *SALVADORA PERSICA* L. FROM DERA GHAZI KHAN, PAKISTAN

YEHIA HAZZAZI, UZMA HANIF[1*], ZAINIB BINTE ZIA[1], JOTYAR J MUHAMMED[2], NIDAL SHABAN[3] AND A EL-SHABASY

Department of Biology, College of Science, Jazan University, P.O. Box. 114, Jazan 45142, Kingdom of Saudi Arabia

Keywords: Salvadora oleoides, *Salvadora persica* , Anatomical adaptation

Abstract

Comparative anatomical studies of *Salvadora oleoides* Decne. and *S. persica* L. were performed for their better adaptations and survival. The results showed that hairs were only present on the outer surface of epidermis in *S. oleoides.* due to its xerophytic conditions. In *S. oleoides.* vascular bundles were arranged in circle while in *S. persica,* they were arranged in rays. In transverse section of stem, length and width of parenchyma were bigger in *S. oleoides* while other tissues were bigger in *S. persica.* In T.S. of petiole, the length and width of metaxylem and protoxylem were larger in *S. oleoides* Decne. while other tissues were larger in *S. oleoides*. In leaf epidermis, the length of epidermal cells was larger on the adaxial surface of *S. persica* . These differences on the basis of anatomical characters and statistical analysis can be helpful for their identification and classification of species.

Introduction

Salvadoraceae is a small family, having three genera and about ten species. Two species of this family, *Salvadora oleoides* Decne. and *Salvadora persica* L. are medicinal plants. They are commonly known as miswak and toothbrush tree In Pakistan, it is also known as Jall (jhaal and pilu). Pilu is useful in the treatment of many diseases and its distinct parts are also used therapeutically such as *Arsh*, *Anaha* and *Gulma* etc. In *S. persica*, the size of leaf is smaller and greater in number as compared to *S. oleoides* (Goriya *et al*. 2016). Both of these species are deep rooted mesomorphic xerophytes. These are also facultative halophytes adapted for high salt tolerance (Korejo *et al.* 2010).

Salvadora oleoides is a small tree of huge ethno-medicinal, ecological and economical importance (Barman *et al*. 2018). It is very well adapted to the arid conditions such as aridity, high temperature and salinity. It can tolerate distinct varieties of soil like saline soil and sodic or alkaline soil and geographical conditions like loamy or pure sand and hard rocky foothills. According to IUCN and Red Data List, it has become regionally vulnerable endangered tree due to its excessive utilization and extensive collection in respective areas as well as deserts. In desert environment, it is adapted to physiological and phenological mechanisms for its survival. Phenological mechanism raises the water stress and alters its structural and functional features structurally. Its xeromorphic features are multilayered epidermis, abundant storage parenchyma and thick waxy coating on the surface of plant. Some other features such as osmotic regulation, deep root penetration, turgor maintenance, leaf exposure and stomatal appearance also assist in its survival (Iqbal *et al*. 2021, Iqbal *et al*. 2022).

*Author for correspondence: <uzmahanif@gcu.edu.pk>. [1]Department of Botany, Government College University, Lahore Pakistan. [2]Department of Forestry, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan region of Iraq. [3]University of Forestry Sofia.

Salvadora. persica is a small upright shrub. Its height is approximately 3 m and diameter is 30 cm. It is used for oral hygiene because it contains antioxidants. Miswak extract contains biological properties such as antifungal effects, amelioration of gum inflammation and antibacterial activity (Sawidis 2013). The extract of this plant can treat piles, intestinal worm, rheumatic joint pain, polio and gall bladder disease (Sukkarwalla *et al.* 2013, Parida *et al.* 2016). It is threatened to extinction due to its overutilization by humans and global climate change (Mansour *et al*. 2020).

Comparative anatomical characterization among different species related to the same genus can give the opportunity to help them to know the taxonomic interrelationships with more understanding of ecological adaptation especially they occur at different habitats (Saima *et al.* 2024). Salvadoraceae share several morphological and developmental synapomorphies. So this family needs more investigation by anatomical studies to indicate the phylogeny of its genera to determine the close and distant species on standard taxonomic basis (Louis and Livia 2009).

The objective of this study is to identify the anatomical differences by comparing two species *Salvadora oleoides* Decne. and *Salvadora persica* L.

Materials and Methods

Salvadora oleoides and *S. persica* were collected from D.G Khan and samples were stored in Formalin-Acetic acid-ethanol fixative: Formalin 100 ml + acetic acid 50 ml + ethanol 500 ml and distilled water 360 ml.

Fine transverse sections were obtained by using sharp blade. Fine selected sections were placed in 30% alcohol for 2 min. Placed these sections in 50% alcohol for 2 min and then transferred in toluidine blue stain for 2 min. These sections were placed in 70% alcohol for 1 min and then put on glass slide and observed under microscope.

The Pearson Correlation uses *P* values for significance tests based on degrees of freedom for the coefficient among stem, petiole and leaf traits for both species. To determine the two-way analysis of variance (ANOVA), the parameters for each anatomical part were statistically analyzed. The difference between them based on standard error > 0.5 *P* was tested using the *F*-test. The linear regression techniques that investigated the extent effect for each anatomical trait were used to depict the correlated data in the form of Simple Linear Regression (SLR) equations by scattered plot graphs (Al Faifi *et al*. 2023).

Results and Discussion

Transverse section of *S. oleoides* stem was normally round in shape. Epidermis was 3-5 layers thin cutinized. Cells were compactly arranged. Hypodermis was 3-4 layers of hypodermis along with starch grains, oil globules and chlorophyll pigmentation. In pericycle, there was a continuous layer as well as oil globules and pericyclic fibres. Vascular bundles displayed the secondary development of phloem and xylem. Just below the pericyclic region, the phloem was situated along with phloem fibers. Thin walled and small polygonal celled characteristic inter xylary phloem was present in all vascular bundles. The xylem vessels were situated beneath the phloem radially arranged in a single file. Medullary rays were few, uniseriate to diseriate and almost straight. These rays were ascending from central pith and extended up towards the inner layers of phloem region containing starch grains. The primary xylem was tetrarch. Pith located at the central part of stem contained pith of parenchyma cells along with xylem vessels. The parenchyma cells were pitted. They were largely containing starch grains and oil globules. Trichomes were also present on outer surface of epidermis (Plate 1B,C,D).

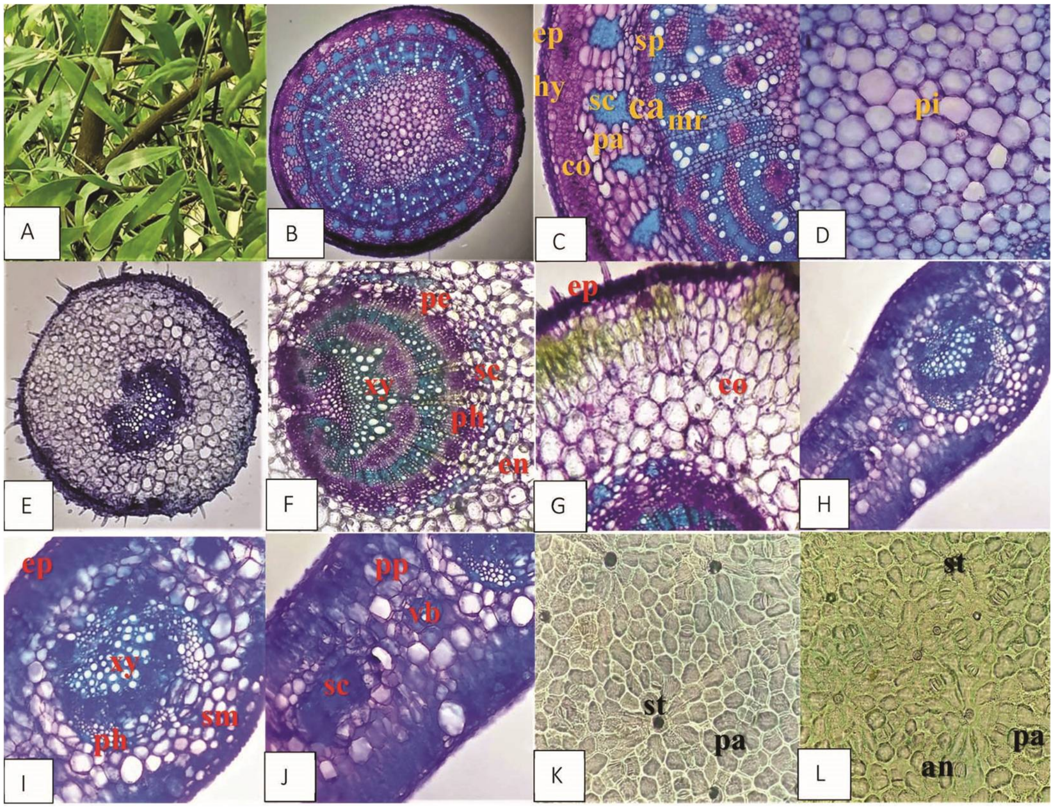

Plate 1. Morphological and Anatomical sections of different parts of *Salvadora oleoides*. A. Part of Plant, B. Stem section C. Epidermis, Hypodermis, chlorenchyma, parenchyma, Sclerenchyma, cambium, Secondary phloem, Inter xylary phloem, pericycle and medullary rays of stem, D. stem pith, E. Petiole section, F. Pericycle, endodermis, vascular bundles, sclerenchyma of petiole, G. Epidermis and cortex of petiole, H. Leaf section, I. Midrib (epidermis, spongy mesophyll, Xylem & phloem), J. Vascular bundles, palisade parenchyma and sclereids of leaf K. Leaf epidermis of adaxial surface (staurocytic stomata and Paracytic stomata) L. Leaf epidermis (10X). M. Leaf epidermis of abaxial surface (staurocytic stomata, paracytic stomata and anisocytic stomata).

Transverse section of *S. persica* stem showed that epidermis was 2-4 thin, cutinized outermost layers of the epidermis with compactly arranged cells. Hypodermis was 3-5 layers, having chlorophyll pigmentation as well as starch grains and oil globules. Pericycle located below the hypodermis wherer a continuous layer along with pericyclic fibers as well as oil globules. There was a secondary development of xylem and phloem at vascular bundles. The phloem was present beneath the pericyclic region having phloem fibers. All vascular bundles contained characteristic intraxylary phloem having small, thin walled polygonal cells. Below the phloem, the xylem vessels were arranged in a single file and were not wide. Medullary rays were few, uniseriate to triseriate and straight. These rays were originating from central pith and expand towards the inner layers of phloem consisting of starch grains. There was tetrarch primary xylem. Pith contained parenchyma cells that were present mostly adjacent to the xylem vessels. These cells were pitted containing starch grains and oil globules. (Plate 2B,C,D).

Transverse section of *S. oleoides* petiole was circular in shape. Epidermis was single layer covering with thick cuticle. It was stomatiferous. The size of epidermal cells was small and their shape was squarish-rectangular. On the outer surface of epidermis, many trichomes were present.

The cortex of petiole was compact and multilayered. Many small intercellular spaces were present. The cortical cells were parenchymatous and thin-walled. Their shape was round to irregular and their size increase near pericycle. The pericyclic region was sclerenchymatous and discontinuous. Endodermis was single layered. Vascular bundles were present in the center of the petiole and circular in shape. There was thicker rim of xylem. Phloem was present outside the xylem. In the xylem tissues, the patches of intraxylary phloem were present. There was also round lumen of vessels. (Plate 1E,F,G).

Transverse section of *S. persica* petiole was round in shape. Epidermis was a single layered. It was covered with thick cuticle and stomatiferous. Epidermal cells were small in size and squarish-rectangular in shape. The cortex was compact and multilayered having small intercellular spaces. The cells were thin-walled and parenchymatous. They were round to irregular in shape. Their size was increased towards pericycle where it was discontinuous and sclerenchymatous. Endodermis was a single layered. Vascular bundles were round in shape. They were present in the center of petiole having thicker rim of xylem. Phloem was situated external to the xylem but the patches of intraxylary phloem were randomly present in the xylem tissue. Round lumen of vessels was generally present. (Plate 2E,F,G).

In case of leaf of of *S. oleoides*, epidermis was 1-2 layers with thin cutinized epidermal cells. The shape of epidermal cells was oval to oblong. Palisade parenchyma was composed of four or more layers of columnar cells. The columnar cells were arranged closely containing chlorophyll along with sunken stomata. Spongy parenchyma had starch grains, rosette crystals, cluster crystals and oil globules. The arrangement of xylem was towards the upper epidermis while the arrangement of phloem was towards the lower epidermis. On the adaxial surface of leaf, the epidermal cells were cuticularized. Staurocytic stomata were under depression with radiating striations. Paracytic stomata were also present. On the abaxial surface of leaf, there was highly cuticularization in the epidermal cells. There was leaf cuticular encrustation due to increase in age making the epidermal cells masked and showing the stomata like eye-balls. Few staurocytic stomata were under depression. Paracytic and anisocytic stomata were also present. (Plate 1H, I, J, K, L).

At transverse section of *S. persica* leaf epidermis was single layered with thin cutinized epidermal cells. The cells were oval to oblong in shape. Palisade parenchyma was columnar cells that arranged closely in four or more layers forming palisade parenchyma. In spongy parenchyma, oil globules, rosette crystals, cluster crystals and starch grains were present. At vascular bundles, xylem was arranged towards the upper epidermis while phloem was arranged towards the lower epidermis. Pericycle was towards the lower epidermis as discontinuous chains of pericyclic fibers. On the adaxial surface of leaf, the epidermal cells were irregular to polygonal in shape. Many radiating striations were present near the stomata. Few paracytic stomata were present in depression. On the abaxial surface of leaf, the shape of epidermal cells was irregular to polygonal. These cells were papillose and cuticularized. There was also cuticular encrustation on the epidermal surface showing the stomata in depression like eye-balls. Many striations were present around the staurocytic stomata. Few paracytic and anisocytic stomata were also present. (Plate 2, H,I,J,K,L).

ANOVA analysis displayed the highest values with stem length (F test 3.56, P value <0.1) and width (F test 3.72, P value < 0.1) parameters against two studied species. However, leaf length (F test 1.05, P value 0.5) and width (F test 1.27, P value <0.5) were lower than petiole length (F test 1.50, P value <0.5) and width (F test 1.62, P value < 0.5) parameters. On the other hand, correlated data was the highest value at leaves of two studied species while both stems scored the lowest one. Simple Linear Regressions (SLR) represented highly positive regressed data for both leaves and petioles for both studied species while moderate regressed data was obtained at stems level (Table 1, Figs 1 and 2).

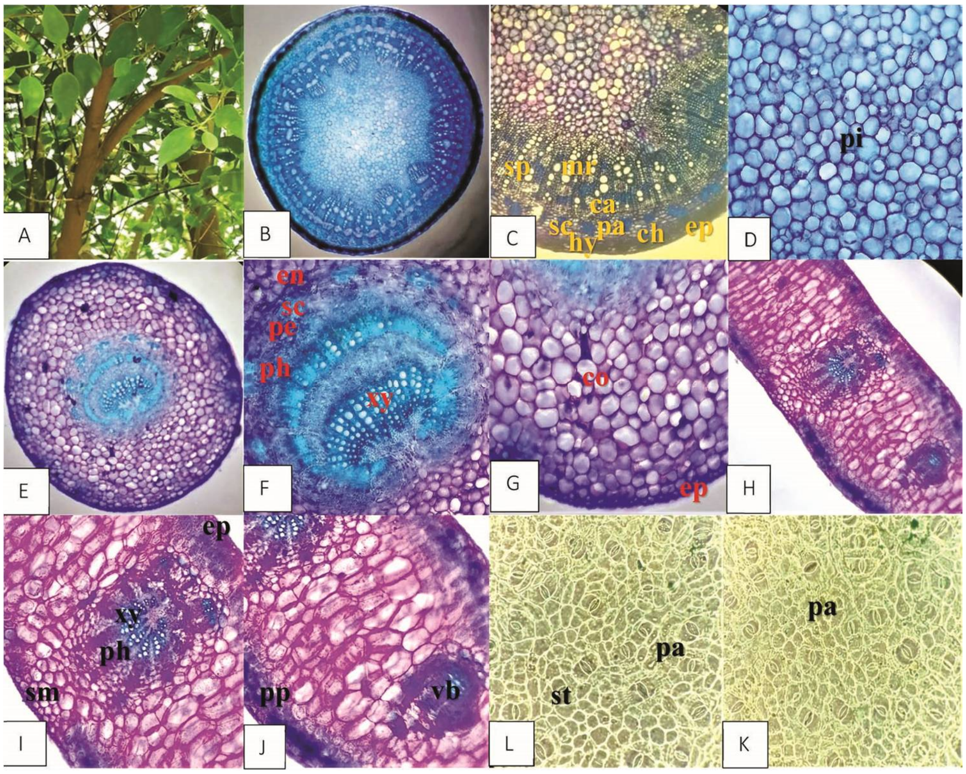

Plate 2. Morphological and Anatomical sections of different parts of *Salvadora persica,* A. Part of plant, B. Stem section, C. Epidermis, hypodermis, chlorenchyma, parenchyma, sclerenchyma, secondary phloem, Interxylary phloem and cambium of stem, D. Stem pith, E. Petiole section, F. Endodermis, pericycle, sclerenchyma, phloem and xylem of petiole, G. epidermis and cortex of petiole, H. Leaf section I. Midrib (epidermis, spongy mesophyll, xylem and phloem), J. Vascular bundles and Palisade parenchyma of leaf, K. Leaf epidermis of adaxial surface (paracytic stomata and few stomata with radiating striations), L. Leaf epidermis of abaxial surface (paracytic and anisocytic stomata, staurocytic stomata with striations).

Salvadoraceae is a small family having 3 genera and 10 species. The need of this research was to find out the correct microscopic authentication and identification for their better adaptations and survival. There was also need to observe differences and similarities between both species. In the transverse section of stem of both species, thin cutinized epidermal layers were present. The hypodermis contains chlorophyll pigmentation, starch grains and oil globules. Pericyclic region was present beneath the hypodermis adjacent to oil globules and pericyclic fibres. The vascular bundles were present below the pericyclic region. Tetrarch primary xylem and medullary rays were present. Pith consisted of pitted parenchyma cells. All these were similarities between stems of both species. While the differences were that trichomes was present on the outer surface of *S. oleoides*. In *S. oleoides,* all vascular bundles contained characteristic intra xylary phloem while in *S. persica*, characteristic Inter xylary phloem were present. Medullary rays were uni to triseriate in *S. oleoides* while these were uni to diseriate in *S. persica*. The similar results were also reported by Goriya *et al.* (2016) and Khatak *et al.* (2010).

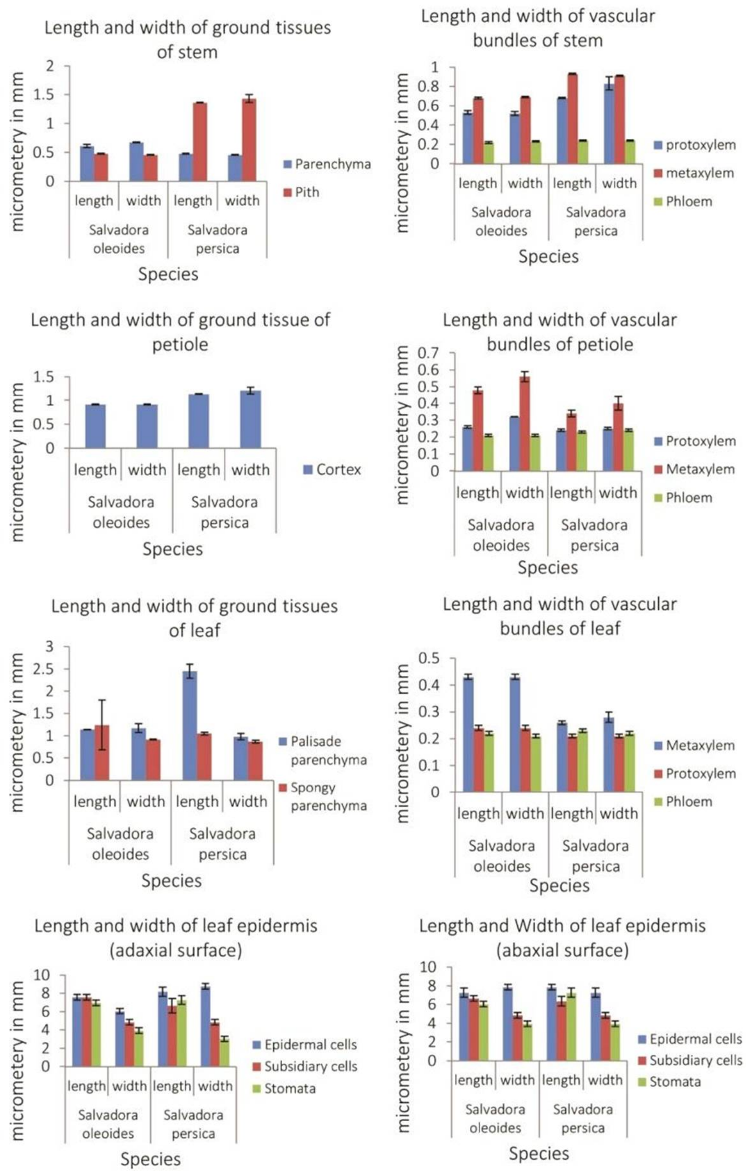

Fig. 1. Comparitive graphs of stem, petiole and leaf of *Salvadora oleoides* and *Salvadora persica.*

Table 1. Correlation among stem, petiole and leaf of *Salvadora oleoides* and *Salvadora persica*

	Stem *Salvadora oleoides*	**Petiole *Salvadora oleoides***	**Leaf *Salvadora oleoides***
Stem *Salvadora persica*	**0.652**		
Petiole *Salvadora persica*		**0.962**	
Leaf *Salvadora persica*			**0.970**

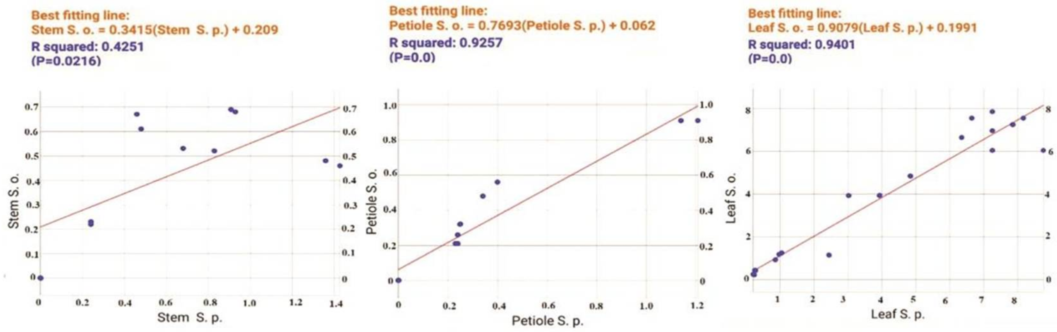

Fig. 2. Simple Linear Regression (SLR) graphs of stem, petiole and leaf of *Salvadora oleoides* and *Salvadora persica.*

In the transverse section of petiole of both species, thick cuticularized and stomatiferous single layer of epidermis was present. Multilayered cortex was present and the cortical cells were compact. These were thin walled and parenchymatous containing small intercellular spaces. Discontinuous pericycle was present. It was sclerenchymatous. Single layered endodermis and vascular bundles were situated in the center. Phloem was situated outside the xylem. The difference between the species was on the outer surface of petiole of *S. oleoides*, the presence of trichomes. These similar results were also observed by Khan *et al*. (2020). In case of leaf of both species, the similarities were that thin cutinized epidermis was present. The epidermal cells were oval to oblong. The palisade parenchyma cells were arranged as columnar cells in four or more layers. Spongy mesophylls having starch grains, crystal and oil globules were present. Xylem was situated adjacent to the upper epidermis and phloem towards upper epidermis. The difference between these species was that discontinuous chain of pericyclic fibres towards lower epidermis. These similar results were also predicted by Goriya *et al*. (2016). In the leaf epidermis of both the species, the epidermal cells were encrusted with cuticle due to increase in age of the plant. Due to this, the epidermal cells were masked and stomata were shown eye-balls. Staurocytic stomata were under depression and the stomata around them were very small. Anisocytic and paracytic stomata were present. The similar results were also explained by Khan *et al.* (2020).

Simple Linear Regressions confirmed that all the anatomical differences are significant and leaf is more responsive in terms of anatomical differences

The current study on *S. oleoides* compared to *S. persica* confirms that these species exhibits adaptations in their anatomical features to survive in diversified environments. More specifically, these adaptations sclerenchyma, increased pith area and large vessels. All These modifications contribute to the plant’s survival in water scarcity and their overall success in various habitats.

References

Al faifi T, Abdurrahman S, Masrahi A and El-Shabasy 2023. Mechanical structures of sidewalk plants: Anatomical evaluation. Saudi J. Biol. Sci. **30**(6): 103647.

Arora M, Siddique AA, Paliwal S and Sood P 2014. A phyto-pharmacological overview on *Salvadora oleoides* Decne. Indian J. Nat. Prod. Resou. **5**(3): 209-214.

Balakrishnan M, Dhanapal M, Vasmi MLM and Sekhar KBC 2010. Studies on pharmacognostical specifications of *Azima tetracantha* Lam. Int. J. Phytopharma. **1**(1): 35-42.

Barman C, Singh VK and Tandon R 2018. Reproductive biology of *Salvadora oleoides* Decne. (Salvadoraceae). Int. J. Plant Reprod. Biol. **10**(1): 69-76.

Bast F and Kaur N 2017. Nuclear and plastid DNA sequence-based molecular phylogeography of *Salvadora oleoides* (Salvadoraceae) in Punjab, India reveals allopatric speciation in anthropogenic islands due to agriculture expansion. J. Phylogen. Evolution. Biol. **5**: 180.

Dagar JC, Bhagwan H and Kumar Y 2004. Effect on growth performance and biochemical contents of *Salvadora persica* when irrigated with water of different salinity. Indian J. Plant Physiol. **9**(3): 234-238.

Devi HM 1972. Salvadoraceae- A study of its embryology and systematics. J. Indian Bot. Soc. **51**: 56-62.

Garg A, Mittal SK, Kumar M, Gupta V and Singh M 2013. Phyto-pharmacological study of *Salvadora oleoides*-overview. Int. J. Bioassays **3**(1): 1714-1717.

Goriya P, Ladani S and Fichadiya G 2016. Comparative micromorphological and microscopical evaluation of white and red variety of Pilu. Indian J. Ethnophytopharma. **2**(4): 16-23.

Iqbal U, Hameed M and Ahmad F 2021. Water conservation strategies through anatomical traits in the endangered arid zone species *Salvadora oleoides* Decne. Turkish J. Bot. **45**: 140-157.

Iqbal U, Hameed M, Ahmad F, Ahmad MSA, and Ashraf M 2022. Unraveling the survival potential of a desert halophyte Salvadora oleoides Decne. across heterogenic environments. Trees, 36(3): 1085-1104.

Khan D, Zaki MJ and Ali SV 2020. Some leaf characteristics of *Salvadora persica* L. (family Salvadoraceae) from fringes of supra-littoral zone of sands pit (Hawkes bay), Karachi, Pakistan. Int. J. Biol. Biotechmol. **17**(4): 789-805.

Khan MT, Azhar I, Shehzadi N, Hussain K, Parveen S and Hanif U 2020. Morphological, microscopic, and physiochemical studies of Diospyromontana. Micros. Res. Tech. **83**: 1260-1281.

Khatak M, Khatak S, Siddqui AA, Vasudeva N, Aggarwal A and Aggarwal P 2010. *Salvadora persica.* Pharmacognosy Reviews **4**(8): 209-213.

Korejo F, Ali SA, Tahir S, Rajput MT and Akhter MT 2010. Comparative morphological and biochemical studies of Salvadora species found in Sindh, Pakistan. Pakistan J. Bot. **42**(3): 1451-1463.

Louis Ronse De Craene and Livia W 2009. Floral development and anatomy of Salvadoraceae. Ann. Bot. **104** (5): 913-923.

Mansour H, Alsamadany H and Al-Hasawi ZM 2020. Genetic diversity and genetic structure of *Salvadora persica* L., rare plant species in Rabigh province, Saudi Arabia: implications for conservation. J. Taibah Univ. Sci. **14**(1): 881-888.

Parida AK, Veerabathini SK, Kumari A and Agarwal PK 2016. Physiological, Anatomical and metabolic implications of salt tolerance in the halophyte *Salvadora persica* under hydroponic culture conditions. Front. Plant Sci. **7**: 351.

Ramzan S, Alsolami W , Hanif U, Sardar A, Jan H, Mustafa A, Bilal M, Sharma K, and El-Shabasy A 2024. Cultural Significance and Indigenous Knowledge of Medicinal Plants in Tehsil Arifwala, Punjab, Pakistan: A Comprehensive Study. Catrina: The International Journal of Environmental Sciences, **32**(1): 29-43.

Sawidis T 2013. Anatomy and ultrastructure of *Salvadora persica* stem: Adaptive to arid conditions and beneficial for practical use. Acta Biol. Cracov. **55**(2): 7-17.

Sukkarwalla A, Ali SM, Lundberg P and Tanwir F 2013. Efficacy of Miswak on oral pathogens. Den. Res. J. 10: 314-319.

(Manuscript received on 30 September, 2024; revised on 05 January, 2025)