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Foliar epidermal anatomy of some ethnobotanically important species of wild edible fruits of northern Pakistan

Mamoona Munir*, Mir Ajab Khan, Mushtaq Ahmed, Abida Bano, Sidra Nisar Ahmed, Kanwal Tariq, Saira Tabassum, Tehmeena Mukhtar, Madhia Ambreen and Shazia Bashir

Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

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The present study is focused on the foliar epidermal anatomy of some ethnobotanically important species of wild edible fruits that is, *Berberis lycium* Royle, *Diospyrous lotus* L., *Morus alba* L., *Morus leavigata* Bureau, *Morus nigra* L., *Morus laevigata var. rubra* L., *Myresine africana* L., *Pistacia integerrima* J. L. Stewart, *Prunus persica* L., and *Zanthoxylum armatum* DC., Prodr. The objective of the present study is to use the foliar epidermal characteristics for the identification and differentiation of wild edible fruits. The study used light microscopic (LM) for the characterization of foliar epidermis. Shape of epidermal cells, presence and type of stomata and trichomes and stomatal index plays a key role in the identification of these species. This study also highlights the ethnobotanical uses of these wild fruits against different diseases and as a nutrient supplement. Although, detailed characterization of these species at molecular and genetic level is still needed.

Key words: Epidermis, ethnobotany, wild, fruits, northern Pakistan.

INTRODUCTION

The Pakistani Himalaya range is located south and east of the Indus River, which originates close to Mount Kailash in Western Tibet, marking the ranges true western frontier. The Himalaya is a totally separate range to the Karakoram which runs parallel to the north. The Himalaya in Pakistan is green and fertile as compared to the arid Karakoram and Hindukush. The Lesser Himalaya is a prominent range 2,000 to 3,000 m (6,600 to 9,800 ft) high formed along the main boundary Thrust fault zone, with a steep southern face and gentler northern slopes. These Himalayas lie north of the sub-Himalayan range and south of the Great Himalayas. The climate of the Lesser Himalayas ranges from tropical at the base of the mountains to permanent ice and snow at the highest elevations. The amount of yearly rainfall increases from west to east along the front of the range. This diversity of climate, altitude, rainfall and soil conditions generates a variety of distinct plant and animal communities (Anonymous, 2011).

Traditional fruits represent inexpensive but high quality nutrition sources for the poor segment of the population, especially where malnutrition is widespread. Traditional fruits grow wild and are readily available in the field as they do not require any formal cultivation wild edible plants (fruits and vegetables) are necessary for the well being of the populations, not only as source of supplemental food, nutritionally balanced diets, medicine, fodder and fuel, but also for developed methods of resource management, which may be fundamental to the conservation of some of the world's important habitats (Chweya and Eyzaguirre, 1999).

Leaf epidermal tissue characteristics have an important role in taxonomy and determining the number of plant genera and species (Scatena et al., 2005; Uphof, 1962). The epidermis possesses a number of important diagnostic character that offer valuable clues for identification, like size, shape and orientation of stomata, guard cells and subsidiary cells, structural peculiarities of epidermal cell walls, distinctive or specialized form of

*Corresponding author. E-mail: mamoonam@yahoo.com or m.munir87@gmail.com.

trichomes (Dickison,2000). Leaf epidermal features like shape of epidermal cells, stomata and trichomes are useful anatomical tools. Length and width of epidermal cells is a useful aid in distinguishing verities.

The plant epidermis consists of three main cell types: epidermal cells, guard cells and their subsidiary cells that surround the stomata and trichomes, otherwise known as leaf hairs. The present study confined to leaf epidermal features of same ethnobotanically important species of wild edible fruits (Table 1). Some species have been investigated first time for anatomical characters that might be useful for plant biologist for the identification of important wild fruits at global level. Stomatal guard cells are essential to keep one particular component inside the plant that is, water. However, they must also allow the gaseous exchange essential for photosynthetic activity. Stomata and associated epidermal cells are an important source of taxonomic characters. The pattern and frequency of stomata on any leaf surface are under conservative genetic control, but may be modified by environmental parameters such as the availability of CO_2 (Croxdale, 2000; Glover, 2000).

METHODOLOGY

Fresh samples of each species were collected from different localities of Northern Pakistan (Murree, Hazara, Gilgit and Galyat). Plants species were determined by Dr. Mir Ajab Khan and Dr. Mushtaq Ahmad and also comparing with preserved specimens in the Herbarium of Pakistan, Quaid-i-Azam University Islamabad.

Foliar epidermal anatomical study

Using the methodology of Ahmad et al. (2010) fresh leaves were infused in 30% nitric acid and boiled with 2.0 g of potassium chloride in a test tube for 2 to 3 min. These leaf pieces were then washed own with distill water twice. Epidermis was peeled off and kept in 60% potassium hydroxide solution for 1 to 2 h. Finally, thin sections of epidermis were suspended in lactic acid on glass slide for LM study. Microphotographs were taken by using CCD digital camera (Model: DK 5000) fitted on Leica light microscope (Model: DM 1000).

Stomatal index (SI)

The stomatal index (SI) was calculated using the formula of Salisbury (1972).

$$SI = \frac{S}{S+E} \times 100$$

S donates the number of stomata per unit area and E the number of epidermal cells in the same unit area.

RESULTS AND DISCUSSION

The foliar epidermal anatomy is one of the most useful taxonomic characteristics for plant systematics studies on numberous families (Bhatia, 1984; Stace, 1984; Jones, 1986). In the present study, foliar epidermal anatomy of 10 selected species of wild edible fruits was investigated. Both qualitative and quantitative characteristics of adaxial and abaxial foliar epidermis were evaluated (Tables 2 and 3). During this study, light microscopy has made it possible to evaluate leaf surface features such as shape of epidermal cells, stomata and trichomes types.

The present study proved very helpful and resulted in exploration of valuable variations in the configuration of foliar epidermal anatomy that can be used as an important taxonomic tool for the identification and differentiation of different species of wild fruits. Anatomical studies revealed clear cut differences in size, shapes of epidermal cells, stomata and trichomes etc.

Epidermal cells

Epidermal cells in all the examined species of wild edible fruits varied in their shapes. Most of the species possess characteristically shaped epidermal cells that is, tetra-, penta- and hexagonal, rectangular to polyhedral and a very few species have undulating-irregular shaped epidermal cells. The shape of epidermsl cells on both surfaces that is, abaxial and adaxial surfaces show slight difference in their shape and length (Tables 2 and 3). Only two species of wild fruits that is *Diospyrous lotus* (Figure 1C and D) and *Myresine africana* (Figure 1Q and R) possess undulating to irregular shaped epidermal cells. While rest of the species of wild fruits that is, *Berberis lycium* (Figure 1A and B), *Morus alba* (Figure 1E and F), *Morus leavigata* (Figure 1H and I), *Morus nigra* (Figure 1K and L), *M. laevigata var. rubra* (Figure 1N and O), *Pistacia integerrima* (Figure 1S and T), *Prunus persica* (Figure 1U and V) and *Zanthoxylum armatum* (Figure 1W and X) have variously shaped epidermal cells.

In *D. lotus* the margins of epidermal cells is not too much wavy (Figure 1C and D). The length and width of epidermal cells is presented in Tables 2 and 3. Contreras and Lerste (1984) studied the anatomy, morphology and distribution of extrafoliar nectories in different species of *Diospyrous*.

They concluded that the nectariferous tissue in all species studied was of the benincasa type, consisting of a discoid mass composed of many small, thin-walled polyhedral cells lacking visible intercellular spaces. The existing literature is very poor regarding the leaf epidermal anatomy of *D. lotus.* While in *M. africana* margins of epidermal cells are wavy (Figure 1Q and R). The data regarding the micromorphological features of epidermal cells are explicitly displayed in Tables 2 and 3. To the best of our knowledge, this plant has been studied for the first time with reference to leaf epidermal anatomy.

B. lycium has variously shaped epidermal cells that is, tetra-, penta- and hexagonal, rectangular to polyhedral (Figure 1A and B). The length and breadth of cells on

Table 1. Botanical diversity and ethnobotanical uses of wild fruits.

S/N	Botanical name	Local name	English name	Family	Status	Ethnobotanical uses
1.	*Berberis lycium* Royle	Simblu	Berberry	Berberidacee	Common	Fruits are edible. Leaves are used as fodder for goats, and sheep. Plant used for hedges and fencing and also as fire wood when dry. Spines are used by young girls to pierce their ears and noses for ornaments. The plant is also used to cure jaundice, eye diseases, mouth gums and throat pain.
2.	*Diospyrous lotus* L.	Kala Malook	Date plum	Ebenaceae	Common	The ripe fruit is dried and eaten. Leaves are used as fodder for goats and sheep. It is also used for the treatment of constipation.
3.	*Morus alba* L.	Chitta Toot	White mulberry	Moraceae	Common	Fruits are edible and are collected, dried and used as winter food. This tree is grown for rearing silk worms. Leaves are used as cattle feed for milk production because of high protein content. Fruits are used for making wine, while the seeds are used for making jam. The fruits are used to cure several diseases like sore throat and dyspepsia.
4.	*Morus leavigata* Bureau	Shah Toot	Long black mulberry	Moraceae	Rare	The fruits are edible. Leaves juice keeps skin smooth, healthy and prevent throat infections. Leaves are also used as fodder for goats and cattle.
5.	*Morus nigra* L.	Kala Toot	Black mulberry	Moraceae	Common	Fruits are edible. The leaves are used for feeding silkworms. It is used to cure several diseases like diabetes, and to reduce blood sugar level.
6.	*Morus laevigata var. rubra* L.	Shah Toot Kala	Black mulberry	Moraceae	Rare	Fruits are edible. Fruits are dried and are sold in market. Leaves are used as fodder for cattle and goats. The plant and fruits are used as anti-dysenteric and urinary problems weakness.
7.	*Myresine africana* L.	Khukhan	African boxwood	Myrsinaceae	Common	Ripe fruits are edible and are sold in market to make money. Leaves are used as fodder for cattle, goats, and sheep. Stem and branches are used in mud roofing hatching, and as fire wood. Ropes are also made from young elastic stem. The plant is used to treat intestinal worms, skin diseases (pimples and allergy), and urinary disorders. It is also used to purify blood.
8.	*Pistacia integerrima* J. L. Stewart	Khangar	Galls/ Pistacia galls	Anacardiaceae	Common	The fruits are edible. Leaves are used as fodder for goats and sheep. Wood yields timber and used for making furniture. It is also used as fire wood and for hatching. Plant is used to treat several diseases such as, cough, asthma, diarrhea, phlegm and gleets (Jiryan).
9.	*Prunus persica* L.	Jangli aru	Peech	Rosaceae	Common	Fruit is edible. Unripe fruit is used in chutnies. Leaves are used as fodder for cattle, goats and sheep. The plant is also used as fire wood when dry. The plant is used to kill intestinal worms and to remove maggots from wounds in cattle and dogs.

Table 1. Contd.

10	*Zanthoxylum armatum* DC., Prodr.	Timbar	Bamboo-Leaved prickly ash	Rutaceae	Common	Fruits and flowers are used in chutnies, curries, as aromatic and flavoring agent and to improve taste. Leaves are used as fodder for goats and sheep. Young twigs are used as toothbrush (miswak). The plant is also used as fire wood when dry and also used in fencing. The plant and the fruits are used to treat gas trouble, cholera, piles, and mouth gums

Table 2. Qualitative characters of foliar epidermal cells of wild fruits.

S/N	Taxa	Leaf epidermis (Ad/Ab)		Stomata		Trichomes	
		Shape	Margin/wall morphology	P/A (Ab/Ad)	Type	P/A (Ab/Ad)	Type (glandular/non glandular)
1.	*Berberis lycium* Royle	Variously shaped	straight	Ab	Paracytic	Absent	Absent
2.	*Diospyrous lotus* L.	undulating to irregular	Wavy	Ab/Ad	Anomocytic	P on both	Both
3.	*Morus alba* L.	Variously shaped	Straight	Ab	Anomocytic	P on both	Both
4.	*Morus leavigata* Bureau	variously shaped	Wavy on Ab Straight on Ad	Ab	Anomocytic	P on Ad	Glandular
5.	*Morus nigra* L.	Variously shaped	Straight	Ab	Anomocytic	P on both	Both
6.	*Morus laevigata var. rubra* L.	Variously shaped	Straight	Ab	Actinocytic and Anomocytic	Both	Both
7.	*Myresine africana* L.	undulating to irregular	Wavy	Ab	Anomocytic	Absent	Absent
8.	*Pistacia integerrima* J. L. Stewart	Variously shaped	Straight	Ab	Anomocytic	Ab	Non-glandular
9.	*Prunus persica* L.	Variously shaped	Straight	Ab	Paracytic	Absent	Absent
10.	*Zanthoxylum armatum* DC., Prodr.	Variously shaped	Straight	Ab/Ad	Anomocytic	Absent	Absent

Key: P: Present; A: Absent; Ab: Abaxial; Ad: Adaxial.

adaxial and abaxial surface are explicitly displayed in Tables 2 and 3. To the best of our knowledge, little literature is reported for the leaf epidermal anatomy of *B. lycium.* Metcalfe and Chalk (1989) and Hurrell and Bazzano (2003) mentioned that this taxon shows sclerophyllous characteristics, which was one adaptation to plain areas and stronger insolation situations or perhaps to the sandy and pedregous soils where this species grows.

M. alba, M. leavigata and *M. nigra* variously shaped epidermal cells that is, tetra-, penta- and hexagonal, rectangular to polyhedral and undulating in all the 4 examined species of family Moraceae. All the four species of genus *Morus* possess peltate glands (Figure 1E, F, H, I, K, L, N and O). Length and width of epidermal cells are presented in Tables 2 and 3. Biasiolo et al. (2004) devoted his efforts to the study of micro-morphological characters that is, wax ornamentation, wrinkling of the epidermis cuticle and flower hairiness in *M. alba* with the help of Scanning electron microscope. They recorded that these features are found to be very useful to distinguish the different species of genus *Morus*. Although, no reference exist about the leaf epidermal anatomy of understudied species of

Table 3. Quantitative characters of foliar epidermal cells of wild fruits.

S/N	Taxa	Surface Ab/Ad	Leaf epidermis (µm) Length × Width	Stomatal complex (µm) Length × Width	Trichomes (µm) Length × width
1.	*Berberis lycium* Royle	Ab	26.25 (21.25 - 32.5) × 12.66 (12.5 - 13)	31.25 (30 - 32.5) × 24.33 (22.5 - 25.5)	Absent
		Ad	33.33 (23.75 - 38.75) × 19 (17.5 - 20)	Absent	Absent
2.	*Diospyrous lotus* L.	Ab	72.33 (69.5 - 75) × 28 (24.5 - 34.5)	56.66 (55 - 60) × 43 (35 - 50)	195 (120 - 0.250) × 21.25 (15 - 30)
		Ad	81 (60 - 90) × 40 (25 - 45)	53 (40- 55) × 43.16 (40 - 45)	Absent
3.	*Morus alba* L.	Ab	20.83 (12.5 - 27.5) × 10.08 (7.5 - 12.75)	17.5 (12.5 - 22.5) × 9.58 (6.25 -12.5)	43.33 (37.5 - 47.5) × 7.58 (7.5 -7.75)
		Ad	33.41 (27.5 - 37.75) × 18.58 (13.25 - 22.5)	Absent	165 (20 - 250) × 22.66 (12.5 - 25.5)
4.	*Morus leavigata* Bureau	Ab	41.25 (23.75 - 62.5) × 13.41 (10 -17.5)	20.75 (17.5 - 24.75) × 14.25 (10 - 17.5)	35 (26-38) × 2.2 (1 - 3)
		Ad	36.66 (30 - 42.5) × 21.66 (17.5 - 25)	Absent	45 (43.5 - 46.25) × 3.125 (3 - 3.25)
5.	*Morus nigra* L.	Ab	21.75 (17.5 - 25.25) × 11.58 (10 -12.5)	18.41 (12.75 - 22.5) × 6 (5.25 - 7.5)	17.58 (12.25 - 27.5) × 10.75 (7.5 - 12.5)
6.	*Morus laevigata var. rubra* L.	Ad	25 (22.5 - 27.5) × 16.75 (12.75 - 20)	Absent	40.91 (40 - 42.5) × 9.16 (7.5 - 12.5)
		Ab	30 (27.5 - 32.5) × 14.25 (12.5 - 15.25)	19.88 (19.75 - 20) × 18.16 (17 - 20)	29.91 (27.5 - 32.25) × 13.66 (12.25 - 16.25)
7.	*Myresine africana* L.	Ad	33.33 (30 - 37.5) × 21.75 (20.25 - 22.5)	Absent	127.91 (112.5 - 157.5) × 26.58 (24.75 - 30)
		Ab	53.12 (50 - 57.5) × 35 (22.5 - 47.5)	29.16 (25 - 32.5) × 20.83 (17.5 - 25)	Absent
8.	*Pistacia integerrima* J. L. Stewart	Ad	54.3 (52.5 - 60) × 33.75 (25 - 42.5)	Absent	Absent
		Ab	19.33 (17.5 - 22.75) × 10.91 (7.75 - 15)	23.33 (20 - 27.5) × 14.16 (12.25 - 17.5)	65 (40 - 69) × 4 (2 - 7)
		Ad	32.5 (20 - 40) × 22.58 (21.25 - 23.75)	Absent	Absent
9.	*Prunus persica* L.	Ab	26.25 (21.25 - 32.5) × 12.66 (12.5 - 13)	31.25 (30 - 32.5) × 24.33 (22.5 - 25.5)	Absent
		Ad	33.33 (23.75 - 38.75) × 19 (17.5 - 20)	Absent	Absent
10.	*Zanthoxylum armatum* DC., Prodr.	Ab	55 (45 - 70) × 20.83 (22.5 - 37.5)	34.91 (32 - 37.5) × 25 (20 - 22.25)	Absent
		Ad	60 (40 - 75) × 22 (21 - 23.75)	32.41 (27.5 - 37.25) × 19.66 (18.75 - 20.25)	Absent

Key: Ab: Abaxial; Ad: Adaxial.

genus *Morus*. *P. integerrima*, *P. persica* and *Z. armatum* possess variously shaped epidermal cells (Figure 1S and T, U, V, W and X). The cross different species of *Pistacia* was examined by Saghir et al. (2006). They confirmed isobilateral leaves and section of leaf let lamina of

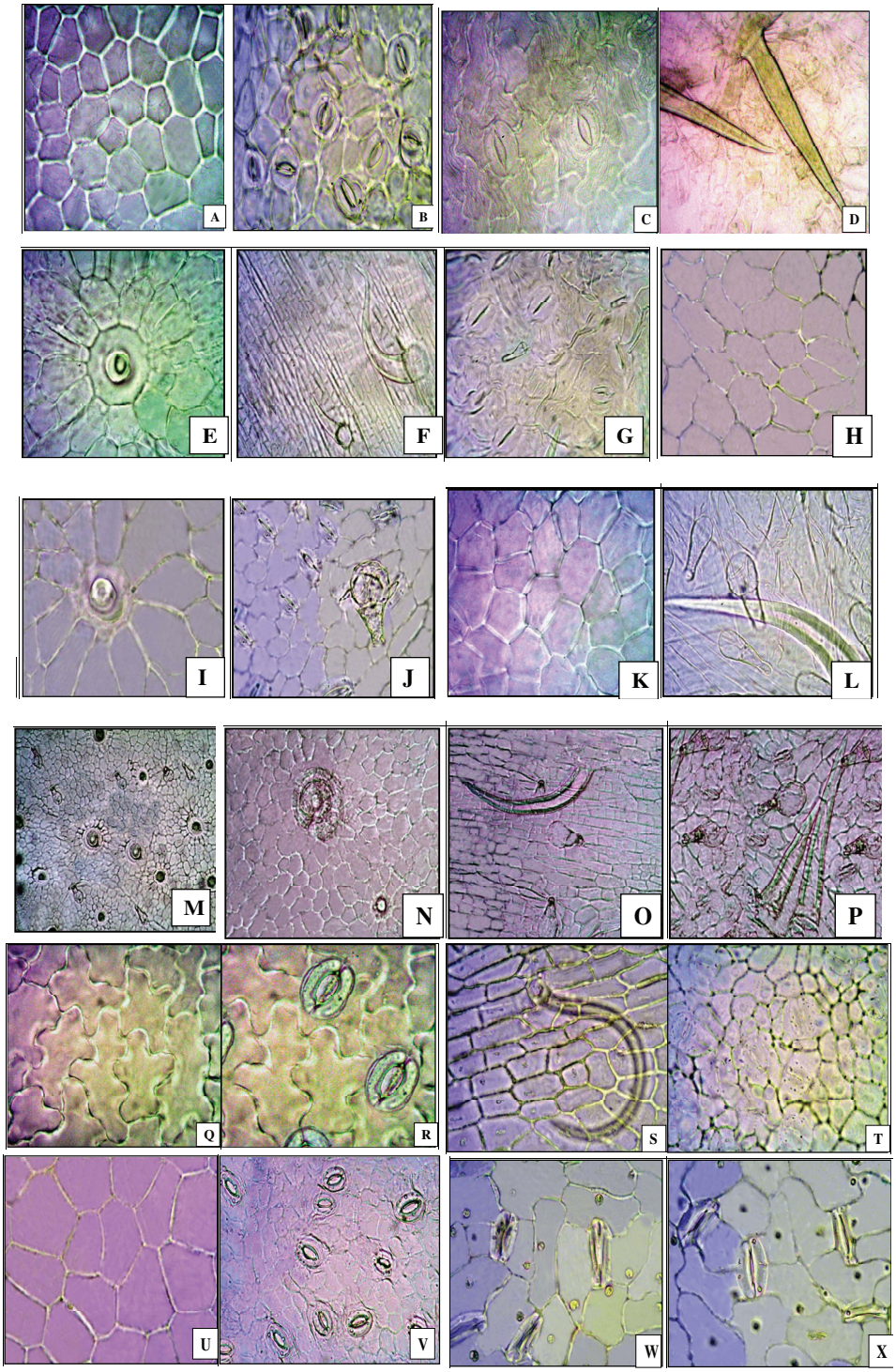

Figure 1. Epidermal cells, different types of stomatas and trichomes; A: *Berberis lycium* Adaxial surface (40×), B: *Berberis lycium* Abaxial surface (40×), C: *Diospyrous lotus* Adaxial surface (20×), D: *Diospyrous lotus* Abaxial surface (20×), E and F: *Morus alba* Adaxial surface (20×), G: *Morus alba* Abaxial surface (10×), H and I: *Morus leavigata* Adaxial surface (20×), J: *Morus leavigata* Abaxial surface (10×), K and L: *Morus nigra* Adaxial surface (20×), M: *Morus nigra* Abaxial surface (10×), N and O: *Morus laevigata var. rubra* Adaxial surface (20×), P: *Morus laevigata var. rubra* Abaxial surface (10×), Q: *Myresine africana* Adaxial surface (40×), R: *Myresine africana* Abaxial surface (40×), S: *Pistacia integerrima* Adaxial surface (20×), T: *Pistacia integerrima* Abaxial surface (20×), U: *Prunus persica* Adaxial surface (40×), V: *Prunus persica* Abaxial surface (20×), W: *Zanthoxylum armatum* Adaxial surface (20×), X: *Zanthoxylum armatum* Abaxial surface (20×).

Table 4. Stomatal index of wild fruits.

S/N	Texa	Family	No. of epidermal cells		No. of stomata		Stomatal index (%)	
			Ab	Ad	Ab	Ad	Ab	Ad
1.	*Berberis lycium* Royle	Berberidaceae	115	123	42	-	26.75	-
2.	*Diospyrous lotus* L.	Ebenaceae	60	50	12	7	16.66	12.28
3.	*Morus alba* L.	Moraceae	89	120	40	-	31.0	-
4.	*Morus leavigata* Bureau	Moraceae	105	96	27	-	20.45	-
5.	*Morus nigra* L.	Moraceae	82	65	18	-	18.00	-
6.	*Morus laevigata var. rubra* L.	Moraceae	45	32	25	-	35.71	-
7.	*Myresine africana* L.	Myrsinaceae	58	52	12	-	17.14	-
8.	*Pistacia integerrima* J. L. Stewart	Anacardiaceae	320	210	24	-	6.97	-
9.	*Prunus persica* L.	Rosaceae	185	170	30	-	13.95	-
10.	*Zanthoxylum armatum* DC., Prodr.	Rutaceae	108	85	23	16	17.5	15.84

Key: Ab: Abaxial; Ad: Adaxial.

relatively thick layer of cutin in different species of *Pistacia*. Although, studies about the leaf epidermal anatomy of genus *pistacia* with special reference to *P. integerrima* has not been reported so far. The margins of epidermal cells are straight in *P. persica*. Balsamo et al. (2003) examined the leaf anatomy of *P. serrulata*. They reported the presence of thin cuticle on a small uni-layered adaxial epidermis with numerous epidermal hairs that were also present on the abaxial epidermis. These trichomes were embedded in columns of collenchyma cells (epithem) that branched off from the secondary veins to both the abaxial and adaxial epidemal layers. A single layer of palisade mesophyll cells was observed and spongy mesophyll appeared isodiametric and loosely packed.

In *Z. armatum* ellipitical to rounded papillae appears on both surfaces and have straight margins cells (Figure 1W and X). Length and width of stomatal aperture and epidermal cells are displayed in Tables 2 and 3. Mbagwu et al. (2007) examined the leaf epidermal characteristics of four species of genus *Citrus* (Rutaceae). Although, studies about the leaf epidermal anatomy of genus *Zanthoxylum* with special reference to *Z. armatum* has not been reported so far.

Stomata

Three different types of stomata were observed in all the examined species of wild edible fruits. These types comprises of Anomocytic, Paracytic and Anisocytic. In all these species stomata are present mostly on abaxial surface and a very few species possess stomata on both surfaces (Table 2).

B lycium (Figure 1B) and *P. persica* (Figure 1V) possess paracytic type of stomata on abaxial surfaces while anomocytic type of stomata were observed among *D. lotus* (Figure 1D), *M. alba* (Figure 1G), *M. leavigata* (Figure 1J), *M. laevigata var. rubra* (Figure 1P), *M. nigra* (Figure 1M), *M. africana* (Figure 1R), *P. integerrimam* (Figure 1T) and *Z. armatum* (Figure 1X). All the examined species of wild edible fruits were reported for the first time in literature with reference to stomatal diversity. Qualitative and quantative features of stomatal type, stomatal complex and stomatal apparture are presented in Tables 2 and 3.

Stomatal index

The values of stomatal index among of all the examined species of wild edible fruits show a wide variation. This character is found to be very useful for differentiation of hybrid plants from parental type. It is also useful to determine the level of atmospheric CO_2. Therefore, this character is used to declare the degree of atmospheric pollution. Variation of stomatal index on adaxial and abaxial surfaces is presented in Table 4. The highest rate of stomatal index is an indicative of highest transpiration rate, metabolism and highest rate for the absorption of mineral and water. Stomatal index of species can also be used as a geographical indicator.

On adaxial surface stomatal index is found to be high in *M. laevigata var. rubra* (35.71) and lowest in *P. integerrima* (6.97). While on abaxial surface stomatal index is reported only in two species *Z. armatum* (15.84) *and D. lotus* (12.28). These values indicate that *P. integerrima* grow in CO_2 rich environment (mostly along road sides) and on the other hand *M. laevigata var. rubra* grows in environment with low air pollution or CO_2 concentration as this species found only in Northern areas of Pakistan. Although, no literature exist regarding the stomatal index of wild edible fruits.

Trichomes

Indumentum characterstics, composition, distribution and

density of trichomes appear to be especially informative for the systematics (Aliero et al., 2006; Ma et al., 2004; Selvi and Bigazzi, 2001).

Two main types of trichomes were observed in all the examined species of wild edible fruits that is, glandular and non-glandular. Mostly trichomes are present either on abaxial surface or adaxial surfaces, present on both surface and in some cases these are absent. In the present study trichomes are present on both surfaces in among *D. lotus, M. alba, M. leavigata, M. laevigata var. rubra and M. nigra* (Figure 1D, F, G, J, L, M, O and P) while in *B. lycium* and *P. persica*, *M. africana* and *Z. armatum* (Figure 1B, R and X), trichomes are absent. *P. integerrima* possess non-glandular trichome only on adaxial surface (Figure 1S). The quantitative data related to trichomes are also presented in (Tables 2 and 3).

Conclusion

Leaf epidermal anatomy with special reference to stomata type, types of trichomes and stomatal index are very useful features form taxonomic point of view. Stomatal index is found to be very useful tool for determining the level of atmospheric CO_2. This characteristic therefore indicates that these species of wild fruits can be used as a geographical indicator. Although, there is a need to integrate and analyze this data at molecular level. This will provide a pathway for future workers to identify and recognize wild culinary vegetables in order to overcome the micronutrient deficiency and food crises.

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