SYSTEMATIC STUDIES OF FAMILY BERBERIDACEAE

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APPROVAL CERTIFICATE

This is to certify that the dissertation entitled **"Systematic Studies of Family Berberidaceae"** submitted by **Saeed ur Rahman** is accepted in its present form by the Department of Plant Sciences, Quaid-i-Azam University Islamabad, as satisfying the thesis requirement for the degree of Master of Philosophy in Plant Sciences, (Plant Systematics and Biodiversity)

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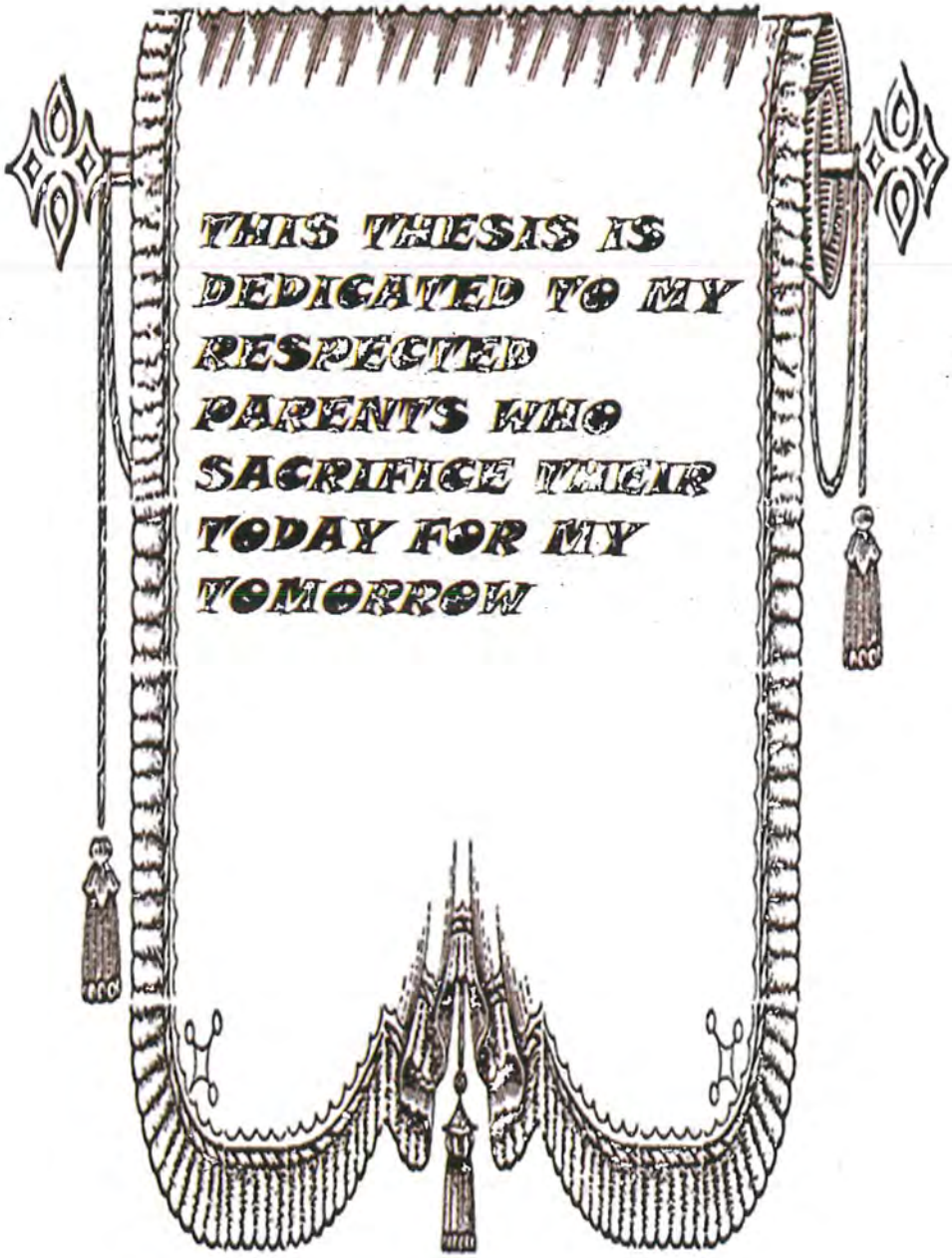
THIS THESIS IS
DEDICATED TO MY
RESPECTED
PARENTS WHO
SACRIFICE THEIR
TODAY FOR MY
TOMORROW

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I offer my deepest sense of gratitude to Almighty 'Allah' the most beneficent and merciful, who enables me to complete my research work.

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ABSTRACT

The present study was carried out to analyze comparative morphology, leaves epidermal anatomy and palynology of 10 species of genus *Berberis*of family Berberidaceae from Pakistan. A reasonable variation was observed both in qualitative as well as quantitative aspects in comparative morphology, leaf epidermal anatomy and palynology and among the investigated species. All the studied species were shrubs varies in height from 2 to 5meter. Epidermal micro-morphological characters were studied through light microscope. Considerable variation in cell wall pattern, epidermal cells and types of stomata were observed. Epidermal cell shape varies both on adaxial as well as abaxial surface from irregular to polygonal and oval to polygonal shape. Cell wall pattern was observed with great variation and most of the species possess straight to curved wall pattern. Among the conspicuous characters one was stomatal presence or absence.*Berberis aitshisonii* and *Berberis baluchistanica* was observed having stomata on both adaxial and abaxial surfaces while the rest of the species were recorded having stomata only on adaxial surface. Out of 10 studied species, 6 possess paracytic type of stomata while rest of the species has anomocytic and anisocytic type of stomata. Two types of stomatal aperture were noted in the investigated species. Spindle type of aperture was in 5 species and the rest of the 5 were with eleptic type.Palynologically 10*Berberis* speciesfrom family Berberidaceae were studied, analyzed and documented in which *Berberis aitchisonii, Berberis calliobotrys Berberis glaucocarpa, Berberis chitria* and *Berberis parkeriana* were studied first time from Pakistan. For each pollen grain, morphological features such as pollen shape, size, presence or absence of colpi, colpi length and width, exine thickness and polar-equatorial ratio were investigated. Variation was seen in all above mention characters in pollen morphology of the studied species. The most conspicuous and common character was the presence of colpi, varies from tricolpate to pantacolpate.

CHAPTER 1

INTRODUCTION

1. General Introduction and Distribution of Family Berberidaceae

Being a heterogeneous family, Berberidaceae consists of 12 genera and 600 species, cosmopolitan in distribution. Among them the *Berberis* is the major one, most established and woody plant genus with 500 species (Rao *et al.*, 1998; Ahrendt, 1961;Schneider, 1905; Mokhber-Dezfuli *et al.*, 2014; Rounsaville and Ranney, 2010; Ghavipanje *et al.*, 2016; Frodin, 2004). Due to morphological diversification, some authors split the family into small families like Podophyllaceae (*Podophyllum*) and Nandinaceae (*Nandina*); (Chaloner *et al.*, 1970; Hutchinson, 1959). In Pakistan, the family is represented by 3 genera (*Berberis*, *Epemedium* and *Mahonia*) and 29 species (Aydemir and Bilaloğlu, 2003).

1.1.Genus Berberis

The genus *Berberis* is characterized by deciduous, mostly spiny ever green shrubs or small trees with pale flowers larger than leaves (Malik *et al.*, 2014) and woody stem (Sastri, 1950) while roots are usually stiff (Ahmed *et al.*, 2009) knotty and surrounded by thin and delicate brittle bark (Das *et al.*, 2015). The genus is characterized by androgynous flowers pollinated by insects but self-pollination also takes place (Irshad *et al.*, 2013) Usually inflorescence-racemose, dense, 10-20 flowers, drooping, (Tiwari *et al.*, 2012). Some *Berberis* species may act as secondary host for fungus (*Puccinia graminis triticii*) particularly that of wheat and other cash crops (Jin *et al.*, 2010; Negi, 2013; Jin, 2011; Naef *et al.*, 2002; Barbu-Diaconescu, 1961; Watson and Luig, 1958). It is well known that habitat changes along with environmental conditions bring about severe modifications in active compounds, metabolism and plant growth that is why difficulties may occur due to extraordinary variable characters in *Berberis* identification (Rodov *et al.*, 2010). The largest genus *Berberis* is mostly native to temperate regions, Africa, Asia, India, Bhutan, Vietnam, Nepal, Myanmar, Java, China, Sumatra, Taiwan, Luzon (Harber, 2012). The important centers of the genus diversity are Eurasia and South America with ca. 300 and ca. 200 species respectively (Ahrendt, 1961; Kim *et al.*, 2004).

In Pakistan, species of *Berberis* is commonly distributed in North West Himalayan regions and up to some extent in mountainous areas of Kashmir (Sood *et al.*, 2010), Baluchistan, Punjab, NWFP, Diamer, Astor, Ghizer, Gilgit, Baltistan (Alam and Ali, 2010), Swat at 900-2900m elevation (Ahmed *et al.*, 2009), Chitral, Dir, Hazara, and Murree (Ivanovska *et al.*, 1999). Flowering season may range from March to august, depend upon species and location while some other factors may also involve (Srivastava *et al.*, 2006).

1.2 Leaf Epidermis Micro Morphology

Epidermal features of leaf like stomata, trichomes epidermal cells are helpful taxonomical tools (Kadiri *et al.*, 2005). A number of diagnostic characters which are helpful in identification like shape and size of stomata, guard and subsidiary cells etc. are associated with anatomical study of epidermis (Dickison, 2000; Moore *et al.*, 2008). With the amazing variation in species *Berberis* is problematic and complex genus taxonomically; further, hybridization and polyploidy makes the boundaries difficult to differentiate the species (Sodagar *et al.*, 2012). Leaf epidermal anatomy of few taxa of genus *Berberis* is studied so far. Munir *et al.*, (2011) observed foliar epidermal features of *Berberis lyceum* and observed polyhedral, hexagonal and pentagonal to rectangular epidermal cells with paracytic stomata. Ellis and Fell, (1963) observed irregular shaped epidermal cells and anomocytic stomata in *Podophyllum peltatum*.

1.3 Palynological Studies

Pollen characters have long been and still are in use for solution of problems occurs in various taxonomic groups (Castro *et al.*, 2009; Panajiotidis *et al.*, 2000; Pardo *et al.*, 2000; Mott, 1978; Myoung and Yuon, 2012; Abu-Asab and Cantino, 1994; Spiridonova *et al.*, 2008; Noor *et al.*, 2004). It has not only important in taxonomic and phylogenetic study but also in paleobotany, aeropalynology, pollination biology, pollen-pistil interaction, potential source of nectar, pollen allergy and in the recognition

of bee plants, hence a tremendous work has been done by various scientists on palynology (Saensouk *et al.*, 2009; Theilade *et al.*, 1993; Paul *et al.*, 2014; Yuanhui, 1988).

Blackmore and Hath (1984) carried pollen morphology of three species of Berberidaceae and observed 3- colpate, microreticulate pollen in *Epimedium alpinum* and syncolpate, psilate or punctate in *Mahonia aquifolium* and *Berberis vulgaris*.

Perveen and Qaiser (2010) studied pollen grain morphology of 12 species representing 2 genera of Berberidaceae from Pakistan using light and scanning electron microscope. Their results show that the pollen was radially symmetrical, spheroidal or sub-prolate, isopolar or apolar, mostly foveolate- fossulate or sub-psilate often regulate-reticulate.

1.4 Morphological Studies

Plant morphology was originated by (Goetghebeur, 1998) and the concern discipline is 211 years old (Kaplan, 2001). The term morphology is combination of two Greek words: *morphe* and *logos* means structure or form and investigation or discourse respectively. The term can be used in both broad and narrow sense (Sattler and Rutishauser, 1997). Morphology in narrow sense means only confined to external form while morphology in broad sense refers to all organizational levels i.e. whole plant structure, molecules, tissues, cells and organelles (Bell and Bryan, 2008). Family Berberidaceae is morphologically variable and based on various characters like chromosomes number, fruit type, floral anatomy and floral morphology (Terabayashi, 1985). Efforts have been made up to some extent in the field of taxonomy (Ahrendt, 1961), palynology, floral anatomy (Rao *et al.*, 1998) and DNA barcoding but still taxonomic problems exist in the genus (Kaplan, 2001). Mabberley, (1997) elaborated the importance of floral characters of genus *Berberis* along with the number of ovules, mature fruit's style distinctness, leaf morphology viz shape and venation.

(1997) elaborated the importance of floral characters of genus *Berberis* along with the number of ovules, mature fruit's style distinctness, leaf morphology viz shape and venation.

1.5 Justification of The Study

Berberidaceae is heterogeneous family and members of the family are cosmopolitan in distribution. In Pakistan, some species are rare and restricted to particular areas. Various species of genus *Berberis* are in flora of Pakistan but are not collected and still remains unexplored. Leaf epidermis has important taxonomic characters such as stomata, trichrome and papillae, such kind of features play pivotal role in resolving taxonomic problems. From Pakistan or even in there is no detailed account on leaf epidermal micro morphology of Berberidaceae.

Being an important taxonomic aspect, palynology had and still is a role in species identification. Like epidermal anatomy no satisfactory work has been done in the field of palynology. This study will also cover this important aspect of taxonomy up to some extent from Pakistan.

For introduction of new species as well as resolving taxonomic problems morphological variations are always helpful. Due to lack of important plant organs viz flowers, seeds and fruits etc. which may get damage or spoil with the passage of time herbarium materials sometimes does not provide important information. Geographically flowering period of *Berberis* species are also vary greatly and that mentioned in floras are usually based on single area. So, this study will provide flowering period and morphological revision of the family.

1.6 Aims and Objective of the Study

- To provide implications of epidermal micro morphological features in *Berberis* taxonomy
- To highlight taxonomic revision and exploration of some taxonomic features of family Berberidaceae

- To find out amplification of palynology and leaf epidermal micromorphology in the taxonomy of Berberidaceae

CHAPTER 2

MATERIAL AND METHOD

The current study was carried out from March to December 2016 at the Department of Plant Sciences Quaid-i-Azam University Islamabad Pakistan to investigate morphology, leaf epidermal anatomy and pollen grain morphology of selected species of family Berberidaceae.

2.1Sampling of Taxon

Ten (10) plants were collected during present study from different phytogeographical zones of Pakistan. For this purpose, regular field trips were conducted during flowering period. Collected specimens were dried, preserved and identified using standard herbarium techniques. Plant name, locality and flowering period are write down (Table 2.1). Voucher specimens were deposited in herbarium of Pakistan Quaid-i-Azam University Islamabad for reference and further study.

2.2 Morphological studies

For morphological studies, fresh plant samples collected in the field were used while some characters such as plant height, color and habitat were noted on the spot. Plant parts, especially flowers for the sake of recovery to more or less its original shape on need bases were boiled in water and were studied with the help of forceps, needle and hand lenses. The morphological studies of the collected species were done by examining with naked eye. Measurements were taken in centimeters. In the study, different characters related to external morphology leaf length, width, shape, internodes distance, spines length, number of spines, stem color, ridges on stem, pedicle length, sepals and petals length and width and plant height were focused. Flowers were also studied under a binocular stereo zoom light microscope (Model SF2 Kyowa Japan) using different eye piece lenses.

Table 2.1:List of collected plant's name its location and accession numbers

S. No	Taxa	Collection site	Collected by	Flowering period	Accession No.
1	*B. aitchisonii* Ahrendt	Chitral (Boni)	Saeed ur Rahman	May-June	129521
2	*B. baluchistanica* Ahrendt	Balochistan	Raees khan, Zain ul Abidin and Siraj khan	June	129522
3	*B. calliobotrys* Bien	Chitral (Ziarat)	Saeed ur Rahman	April-June	129523
4	*B. chitria* Buch. - Ham	Murree	Saeed ur Rahman, Benazir Abbasi	June-July	129524
5	*B. glaucocarpa* Stapf	Kashmir (Rawlakot)	Saeed ur Rahman, Sajad Hussain	April-May	129525
6	*B. kunawurensis* Royle	Kashmir (Ponch)	Saeed ur Rahman	May-July	129526
7	*B. lycium* Royle	Dir Upper, Dir Lower, Murree	Saeed ur Rahman, Fazal Ullah	April-June	129527
8	*B. orthobotrys* Bien	Kashmir (Rawlakot)	Saeed ur Rahman	May-June	129528
9	*B. parkeriana* C.K. Schneid	Dir Upper	Saeed ur Rahman	June	129529
10	*B. psodoumbellata* R.Parker	Gilgit	Saeed ur Rahman, Siraj khan	June-July	129530

2.3 Protocols for Leaf Epidermal Anatomy

Apart from manual (the simplest one and usually common) a total of 3 protocols were used to study leaf epidermal anatomy of the collected plant specimens.

2.3.1 By Free-Hand Using scalpel blade method

For anatomical study (both adaxial and abaxial) a total of 20 randomly selected both fresh and dried, healthy and fully expanded leaves were used. The leaves were kept on white tile (8×4 inches) and the leaf surface was rubbed slowly and carefully with sharp razor blade/camel hair brush until the opposite epidermis remains intact on tile surface. Other than removed, the rest of the portion (mostly chlorophyll) was placed in a plate half filled with commercial detergent/bleach (for 10-15 minutes) for the purpose to remove chlorophyll. Thereafter, peeled off epidermis was twice washed with distilled water and transferred to microscope slides. A few drops of lactic acid were put on slide and was sealed with transparent nail varnish to prevent drying up of the slides.

2.3.2 Nitric acid method

By applying this method, Shltze's techniques of softening with enhanced methods were followed (Subrahmanyam, 1996). 2-3 leaves were taken in test tube and 4 ml nitric acid, 4 gm potassium chloride and 1 ml distal water were added and boiled till epidermal peel was removed. The strip of leaves having chlorophyll was treated with commercial bleach in order to remove chlorophyll. There after epidermis was washed twice with distal water and sections were transferred to slides and 1-2 drops of lactic acid were added and make permanent by applying transparent nail polish. Samples were studied under different objective lenses (10, 40 and 100 μm) but the measurements were taken under 40 μm.

2.3.3 Lactic acid method

Botanical material was passed through different stages and sections were made according to the usual techniques used by (Cotton, 1974). In a test tube half filled with 88% lactic acid, dried leaves were placed; tube was kept for 50-60 minutes in hot water

bath chamber. Leaf tissues soften with lactic acid and were ease to peel off epidermis. For abaxial surface preparation, leaf was placed on tile having adaxial surface upward and mounted in 88% lactic acid. Same was done for adaxial surface preparation. The removed epidermis was put on slide and was permanent with cover slip using transparent nail polish.

2.3.4 Combination of nitric acid and lactic acid method

A combination of 70% lactic acid and 30% nitric acid was used in a test tube to peel off epidermis from fresh and dried leaves. Less time (3-5 minutes) need in this method as compare to above discussed methods for boiling and was the most effective. After boiling leaves were washed twice with distilled water and slides were made.

2.3.5 Observation of characters under microscope

Different characters like cells shape, cells wall pattern and stomata shape were observed under light microscope (MT 4300H) while photography was done by using Leica light microscope connected with CCD digital camera (Model: HDCE- 50B).

2.3.6 Stomatal index (SI)

Stomatal index was calculated by using formula that of (Salisbury, 1928).

$$SI = S/(S + E) \times 100$$

S = Number of stomata per unit area

E = Number of epidermal cells per unit area

2.4 Palynological study

By applying this method with more or less modifications that of (Harley, 1992) method was used for light microscopy. Anthers from already opened flowers were separated carefully with the help of forceps and a piercing needle for further investigation. The removed anthers were put on microscope slide a few drops of acetic acid were put and crushed. A few drops of glycerin jelly was applied on slides for visibility of pollens under light microscope. For SEM, pollens were shed on small

slides and a few drops of acetic acid were added and crushed. After this, slides were placed and fixed with double sided adhesive tape on metallic stub and coated with gold in a sputtering chamber. Pollens were clearly observed and photographs were taken at different magnifications with a JEOL-(Model-JSM5910) scanning electron microscope at Physics Department University of Peshawar, Pakistan. The terminologies used were followed that of (Erdtman, 1952; Faegri & Iversen, 1964; Kremp 1965 and Walker & Doyle 1975).

CHAPTER 3

RESULTS

3.1 Morphological Study

3.1.1 *Berberis aitchisonii*Ahrendt

Habit: Shrub 2 m in height, usually deciduous

Habitat: Bare dry slope and hill foot.

Stem color reddish to dark red, parallel groves present on stem, internodes 25 mm long, spines 3-fid, 15 mm long, middle one longer than side ones. Leaves are dark green in color and 30 mm long while 12 mm broad, obovate, petiolate (petiole 3 mm long), rounded at the tip and reticulate. Flowers; 7 mm across, pedicles 7 mm long, sepals and petals same in size. Berries; grey to dark red in color, 6 mm long, 4 mm broad.

3.1.2 *Berberis baluchistanica*Ahrendt

Habit: Semi deciduous shrub, 3.5 m tall, dense branched.

Habitat: Plains, slopes.

Stem color varies from brown to red rarely grayish in color, glabrous, sub-sulcate, spines usually 3-fid, 2.5 mm long, narrow, tip very sharp, smooth, average internode distance 35 mm long. Leaves; usually tough, thick, obovate, short stalked or sessile, vary in size on the same plant or even on the branch, 50 mm long, 20 mm broad, petiole 6 mm in length, veins reticulate, oblong to entire, rarely spinulose, 6 in number, serrate, on leaves apex spine is larger as compare to side ones. Raceme 25 mm long, peduncle 10 mm long. Flowers; yellowish in color, 10 mm across, pedicles 10 mm long, bracts 1.7 mm long, outer sepals are smaller than the inner ones, petals 5 mm long, 3 mm broad, stamens 4.5 mm long. Berries; reddish to black in color, ovoid, 6 mm long, and 3 mm broad.

3.1.3 *Berberis calliobotrys*Bien

Habit: Shrub usually 3 m tall, semi deciduous.

Habitat: Found on hills foot and slopes.

Stem reddish to pale brown in color, glabrous, spines 3- fid, and 20 mm long, sulcate, internodes distance 22 mm. Leaves; variable in size and shape, oblong, 30 mm long, 10 mm broad, mostly sessile, sometime petiolate (7 mm long), entire/ 5 spinose,

usually green from upper side and whitish from beneath, reticulate, sub obtuse, spinose of tip longer than the side ones. Flowers; 7 mm across, pale yellowish in color, glabrous, pedicles, 10 mm long, inner sepals are longer than outer, petals are same size of sepals. Berries; ovoid, blackish in color, 7 mm long, 3 mm broad.

3.1.4 *Berberis chitria*Buch. -Ham

Habit: Shrub or small tree up to 4 m tall, usually deciduous.

Habitat: Found on hills.

Stem blackish to dark red, sub-glabrous, terete, average internode distance 50 mm long, spines usually 3 fids, 30 mm long. Leaves; very different from other species, mostly elliptic, 70 mm long, 30 mm broad, brightly green, sessile to sub sessile, if petiolate
(4 mm in length), 10 spinulose on the margins, reticulate, rarely entire. Flowers; pale yellow in color, 16 mm across, mostly in three groups, peduncle 7 cm long, 18 flowered, pedicles 16 mm long. Sepals; outer smaller than inner ones, petals 10 mm long, subacute at the apex, Stamens; 7 mm long. Berries; dark brown in color, 12 mm long, 6 mm broad.

3.1.5 *Berberis glaucocarpa*Stapf

Habit: Shrub 4 m tall.

Stem yellowish in color, glabrous, verruculose, average internode distance 45 mm long, spines 3-fid, and 8 mm long. Leaves; elliptic, bright green on upper side while grey on lower side, 70 mm long, 35 mm broad, sessile or rarely small petiolate (8 mm long), spinose on margin may vary in number 8, reticulate. Racemes covered with 23 flowered. Flowers; 10 mm across, bright yellow in color, inner petals are smaller than the outer sepals, pedicles stout, 10 mm long, petals 7 mm long, 4 mm broad, stamens 5 mm long. Berries; 8 mm long, oblong, 6 mm broad, dark in color.

3.1.6 *Berberis kunawurensis*Royle

Habit: Very small shrub as compare to other *Berberis* species, 2 m tall.

Habitat: Slopes and hills.

Stem yellowish to brown in color, sulcate, younger shoots red in color, puberulous, brown in color, spines 3 fid, 30 mm long, average internodes distance 28 mm long. Leaves; somewhat very long, 43 mm long, 15 mm broad, obovate to elliptic, sessile or sub sessile, sometimes petiolate (3.5 mm in length), spinose at the margin 10 in number, reticulate venation, dark green in color, panicles are in size that of leaves, peduncles, 13 mm long. Flowers; 6 mm across, pedicles 4 mm long, bracts 1 mm long, inner petals and sepals are same in size. Berries 7 mm long, ovoid or oblong, 4 mm broad, dark red in color.

3.1.7 *Berberis lycium*Royle

Habit: Woody perennial shrub, monoecious, much branched, 5 m tall.

Habitat: Mostly found in crop fields, hills and plains.

Stem erect or sometime sub erect, deciduous, stem and branches may vary in color e.g. yellowish to greyish, sometime irregular ridges on stem, glabrous, large branches (terete to sub-sulcate) 3 mm while large branches range from 2 mm long, internodes 38 mm long, spines 3-fid, yellowish in color, middle one is large (27 mm) as compare to side (24 mm) ones and arranged alternate on stem. Leaves thick as compare to other *Berberis* species, bright and dark green in color, oblong-obovate to oblanceolate, toothed or entire, 50 mm long, 12 mm in width, papillose, spinose on margins 4 may be increase or decrease, acute, veins can be seen clearly on leaves, petiole 4 mm long. Flowers; pale yellow in color, 8.5 mm across, pedicles 13 mm long, sometime very thin, bracts 2.6 mm long, outer sepals 3 mm long while middle and inner ones 5 mm long. Berries; red in color early while blackish on ripening, 9 mm long and 5 mm broad.

3.1.8 *Berberis orthobotrys*Bien

Habit: Shrub range from 2.5 m tall.

Habitat: Alpine hills, slopes.

Stem pale-brownish to dark red in color, spines 3-fid, and 22 mm long, orange to brownish in color, internodes 35 mm long. Leaves; vary in size, 35 mm long, 18 mm broad, petiole 5 mm long, spinose 18 in number at the margins, green on upper side while grey beneath, sessile or sub-sessile. Inflorescence highly variable in size.

Flower; 8 mm across, bright yellow in color, pedicles 10 mm long, upper are smaller than the lower ones, petals, sepals and stamens are same in size, convolute. Berries; sub-obovoid to oblong, 9 mm long, 5 mm broad, red when unripe and dark on ripened.

3.1.9 *Berberis parkeriana* C.K. Schneid

Habit: Shrub, 3.5 m tall, deciduous.

Habitat: Northern hills

Stem grayish to light dark in color, ridges found on lower portion of the stem (starts from ground level up to 1 meter), glabrous, sulcate, spines 3-fid, 35 mm long, middle larger than the sider ones, very narrow on branches upper portion while thicker on lower or middle portion, internodes up to 40 mm in length. Leaves; greenish, 45 mm long, 30 mm broad, petiole 1.5 mm long or rarely sessile. Flowers; 8 mm across, pale yellow in color, pedicle 4 mm long, petals are larger than sepals, stamens 3 mm long. Berries 10 mm long, 5 mm broad, dark red in color ripening. Suborbicular to broadly obovoid.

3.1.10 *Berberis psodoumbellata*R.Parker

Habit: Shrub ranges up to 3 m tall, usually deciduous.

Habitat: Hills, slopes.

Stem is usually terete or sometime sulcate, yellowish in color, glabrous, slender, spines 3-fid, 16 mm long, smooth, tightly fixed on the stem and branches, distance among internodes reach up to 60 mm in length. Leaves; 33 mm in length and 20 mm in width, petiole 10 mm long, obovate, entire, 7 spinose at the margins, apex pointed rarely round, dull green in color and reticulate venation. Flowers; 7 mm across, pedicle long, 35 mm, bracts 1.5 mm long, inner sepals are longer than the outer ones, petals 6 mm long, 3 mm broad. Berries; 10 mm long, 6 mm broad, color changes to dark red on ripening.

Table 2.2: Quantitative morphological characters of the studied plants

S. No.	Taxon	Habit	Plant height in meter	Internodes distance in mm	Leaf features		Petiole length in mm	Spines length in mm	Berries features		Pedicles length in mm	Flowers across in mm	Berries color on ripening
					Length mm	Width mm			Length mm	Width mm			
1	*B. aitshisonii* Ahrendt	Shrub	2-2.5	15-25	20-30	8-12	2-3	8-15	2-6	2-4	4-7	3-7	Grey to dark red
2	*B. baluchistanica* Ahrendt	Shrub	3-4	20-35	35-50	15-20	4-6	4-10	3-6	1-3	5-10	5-10	Black
3	*B. calliobotrys* Bien	Shrub	2.5-3	10-22	20-30	7-10	3-7	15-20	3-7	2-3	7-10	4-7	Blackish
4	*B. chitria* Buch. - Ham	Shrub	4-5	30-50	40-70	20-30	2-4	20-30	5-12	3-6	10-16	10-16	Dark brown
5	*B. glaucocarpa* Stapf	Shrub	3-5	20-45	35-70	25-35	5-8	4-8	3-8	4-6	5-10	6-10	Dark
6	*B. kunawurensis* Royle	Shrub	2-3.5	15-28	30-43	10-15	1-3.5	15-30	2-7	2-4	20-43	3-6	Dark red
7	*B. lycium* Royle	Shrub	4-5	25-38	30-50	7-12	2-4	15-24	5-9	3-5	5-13	4-8.5	Blackish
8	*B. orthobotrys* Bien	Shrub	2.5-4	20-35	20-35	10-18	2-5	10-22	4-9	2-5	6-10	5-8	Red
9	*B. parkeriana* C.K. Schneid	Shrub	3.5-5	25-40	30-45	15-30	1-3	25-35	4-10	3-5	2-4	4-8	Dark red
10	*B. psodoumbellata* R.Parker	Shrub	3-5	35-60	20-33	15-20	5-10	5-16	6-10	1-3	25-35	4-7	Dark red

Plate 3.1: Plants photographs (A) *Berberis parkeriana*; (B) *Berberis chitria*

Plate 3.2: (A)*Berberis kunawurensis*; (B) *Berberis psodoumbellata*

Plate 3.3: (A) *Berberis orthobotrys*; (B) *Berberis calliobotrys*

Plate 3.4: (A) *Berberis glaucocarpa*; (B) *Berberis baluchistanica*

Plate 3.5: (A) *Berberis lycium*; (B) *Berberis aitchisonii*

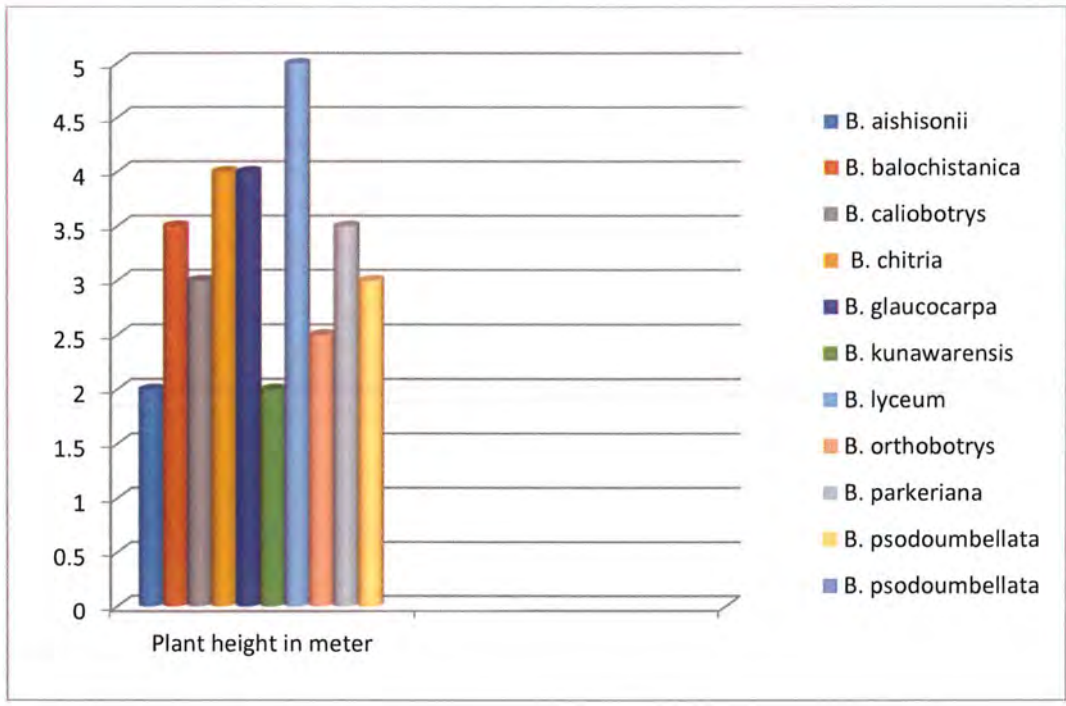

Figure 3.1: Comparative plant height

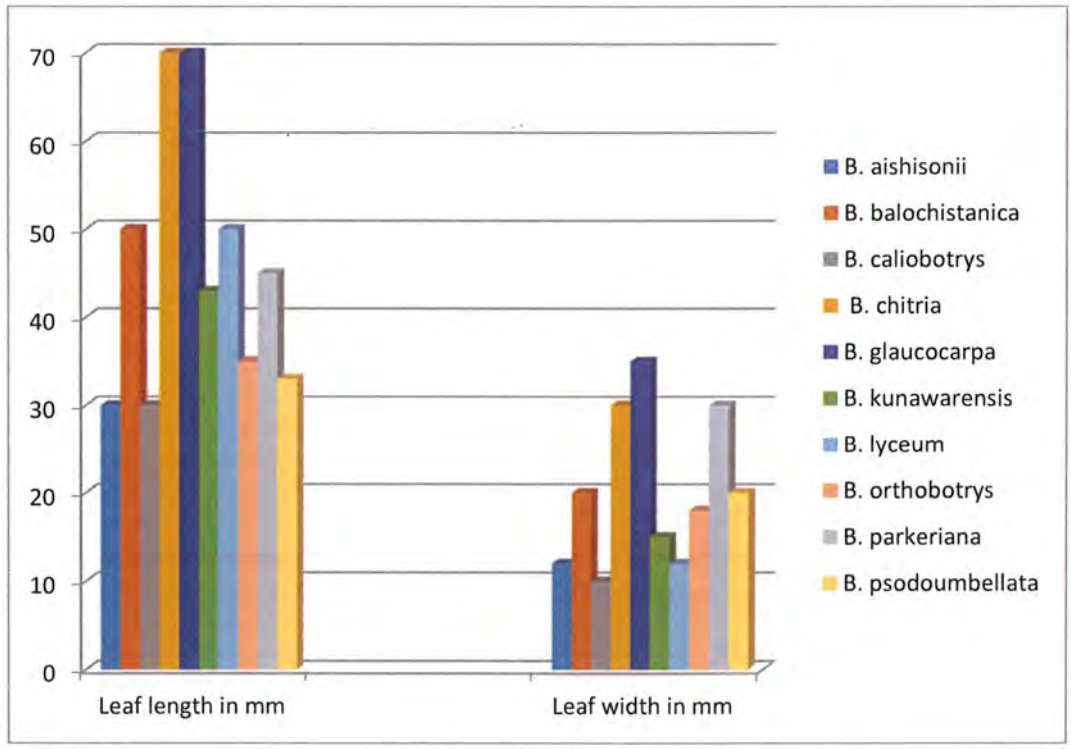

Figure 3.2: Comparative leaf length and width

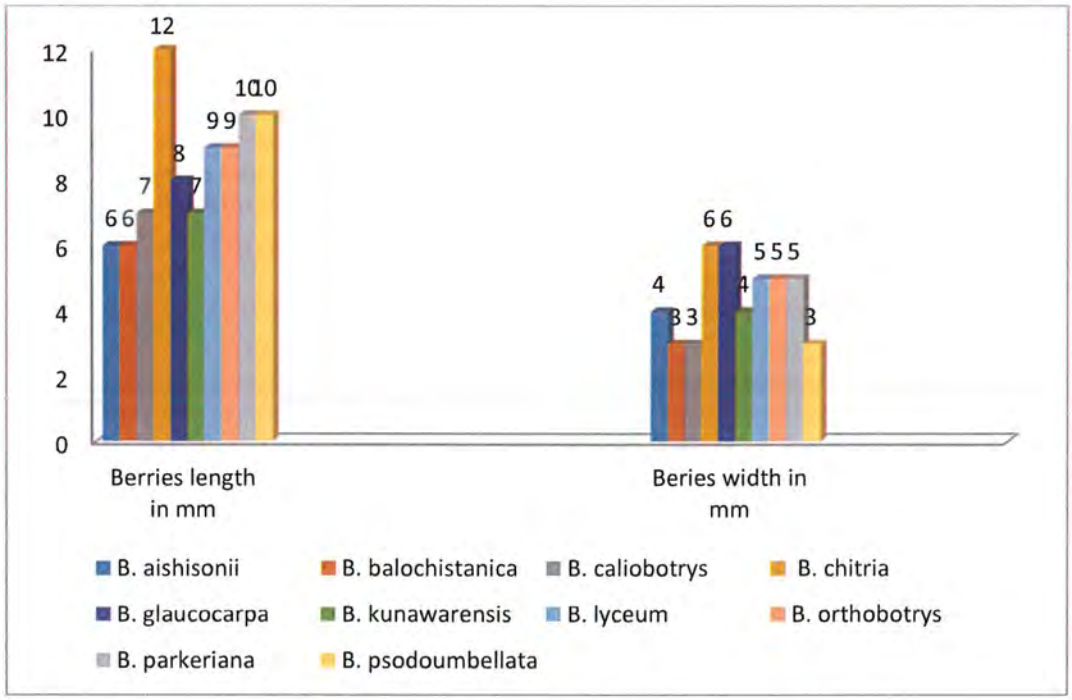

Figure 3.3:Comparative berries length and width

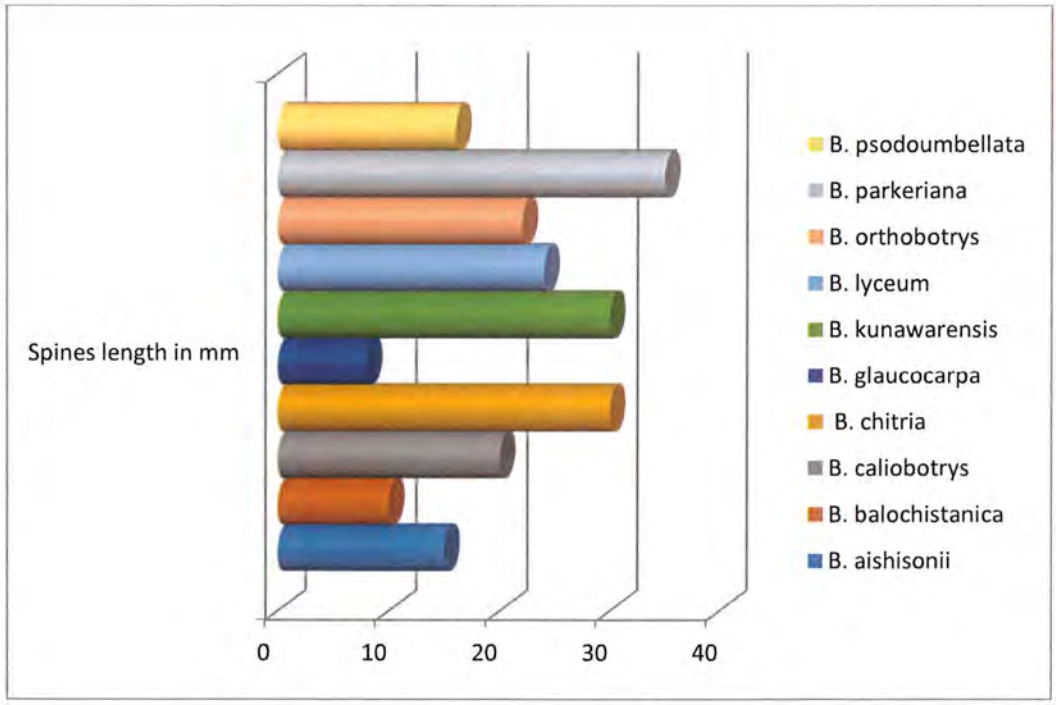

Figure 3.4: Comparative spines length

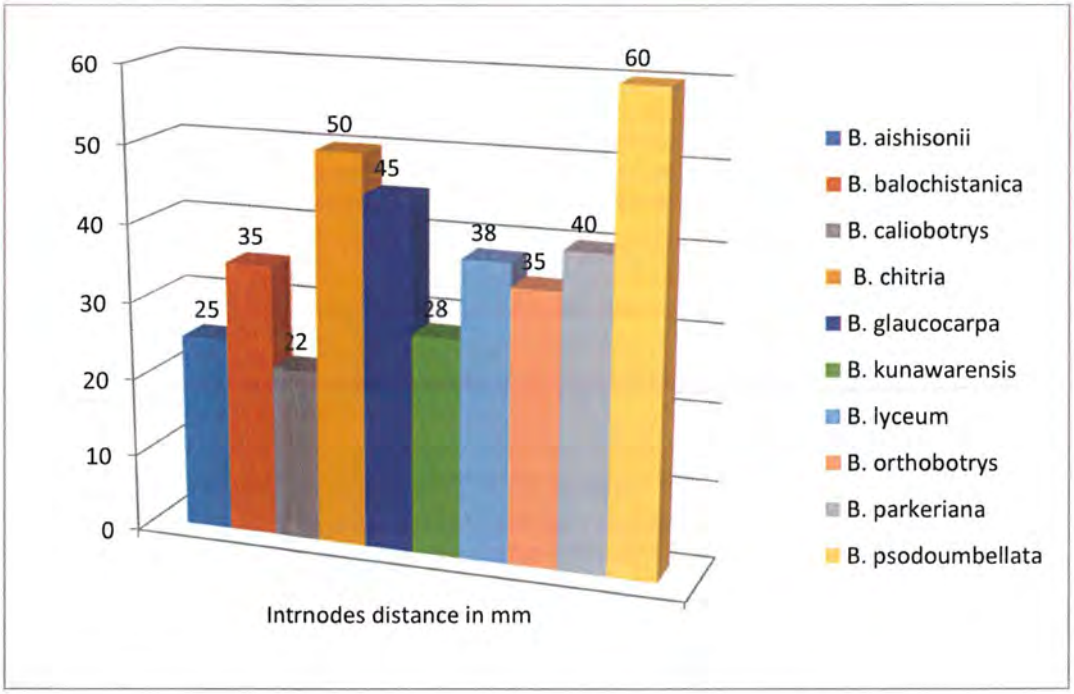

Figure 3.5:Comparative internode distance

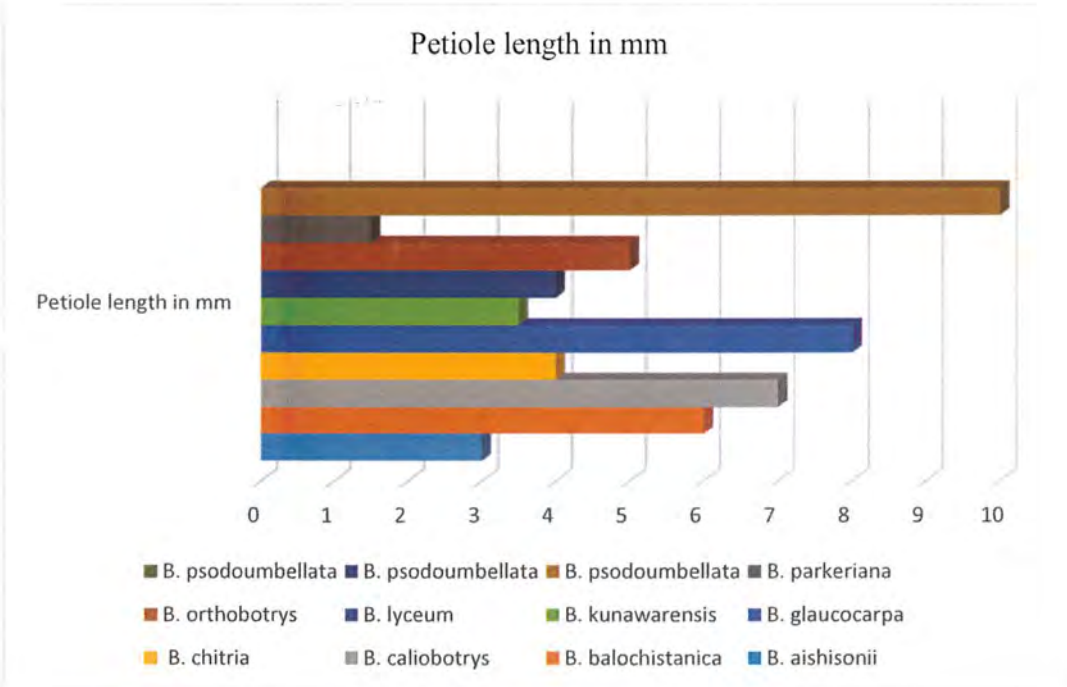

Figure 3.6: Comparative petiole length

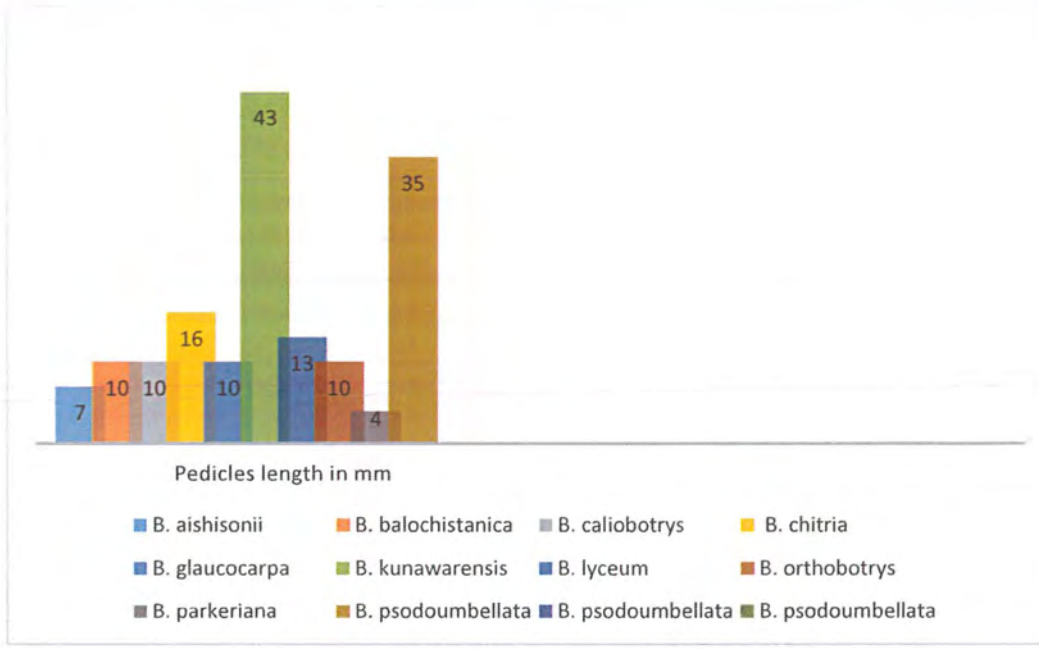

Figure 3.7:Comparative pedicles length

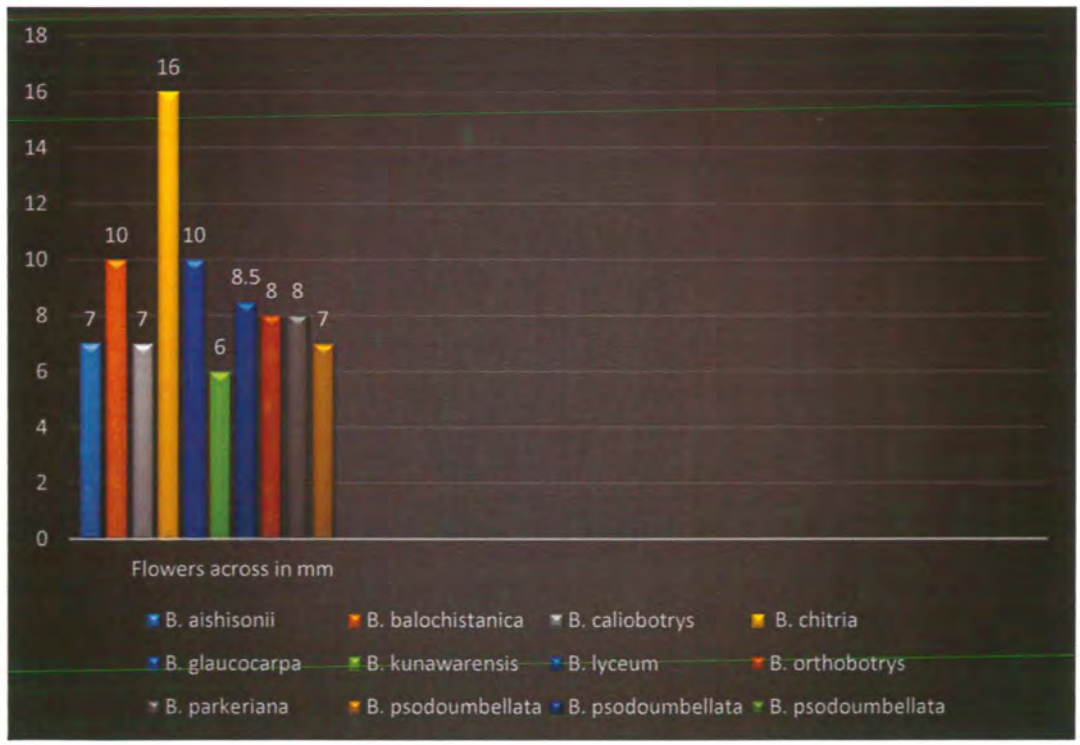

Figure 3.8: Comparative flower in across

3.2 Leaf Epidermal anatomy

3.2.1 Epidermal Cells Morphology

Among the studied taxa great morphological differences were noted, qualitative as well as quantitative modifications were studied especially in the leaf epidermal cells, cells wall pattern and stomata shape. The studied species usually possess characteristically tetra, Penta and hexagonal epidermal cells shape. Irregular to polygonal cell shape occurs only in two species named *B. aitchisonii*(both on abaxial and adaxial) and *B. lycium*(abaxial) while polygonal on adaxial surface, *B. baluchistanica* possess polygonal to irregular cells on both adaxial as well as abaxial surface, *B. calliobotrys* has polygonal on adaxial while polygonal to irregular on adaxial surface.*B. chitria* with polygonal to irregular on both surfaces, *B. glaucocarpa* possess polygonal on adaxial while oval to polygonal on abaxial surface, *B. kunawurensis* has polygonal to irregular on adaxial and spherical to polygonal on abaxial surface.*B. orthobotrys*possess polygonal to irregular on adaxial and spherical to irregular on abaxial surface, *B. parkeriana*has polygonal on adaxial while irregular, polygonal, elongated to spherical on abaxial surface and *B. psodoumbellata*has polygonal to irregular on adaxial and irregular to spherical on abaxial surface.Cells wall morphology also tend towards great variation e.g. *B. aitchisonii*possess straight to slightly curved cell wall pattern both on adaxial as well as abaxial surface, *B. baluchistanica* with straight to curved both on adaxial and abaxial surface, *B. calliobotrys* shows straight on adaxial and straight to slightly curved on abaxial surface, *B. chitria* possess straight to curved cells wall pattern both on adaxial and abaxial surfaces.*B. glaucocarpa*possess straight to slightly curved wall pattern both on adaxial and abaxial surfaces,*B. kunawurensis*has straight to slightly curved on adaxial while straight on abaxial surface.*B.lycium*with straight on upper surface and straight to curved pattern on lower surface, *B. orthobotrys*with straight to slightly curved on both surfaces, *B. parkeriana* has straight on adaxial and straight to curved on abaxial on abaxial surface, *B. psodoumbellata* possess straight to curved both on adaxial and abaxial surfaces. Mostly polygonal cells were observed having 5-7 corners. The epidermal cells not only show variation in cell wall pattern but also depicted a reasonable variation in length and width among different species. *B. baluchistanica* was found with largest epidermal cells [Adaxial: length = 45 (53.9) 62.5 µm; and width = 22.5 (26.3) 30 µm; Abaxial: length = 37.5 (43.25) 50 µm; and

width = 20 (22.6) 25] followed by *B. aitshisonii* [Adaxial: length = 37.5 (43.15) 50 µm; and width = 20 (27) 32.5 µm; Abaxial length = 30 (35.2) 42.5 µm; and abaxial width = 20 (29) 37 µm;] and the smallest ones were noted in *B. calliobotrys* [Adaxial: length = 20 (29.5) 37.5 µm; width on adaxial = 15(19.1) 25 µm; Abaxial: length = 20 (22) 25 µm; abaxial width = 20 (12.6) 25 µm;] followed by *B. orthobotrys* [Adaxial: length = 25 (36) 50 µm; adaxial width = 17.5 (24) 30 µm; Abaxial length = 17.5 (20.7) 25 µm; abaxial width = 10 (20.7) 25 µm. Large number of cells per unit area were present on adaxial surface in *B. calliobotrys* with number of 245 (252.4) 260 followed by *B. parkeriana* with 209 (227.8) 250 on abaxial surface while lesser number of epidermal cells per unit area were found in *B. baluchistanica,* 101 (107.2) 112 on adaxial surface followed by *B. aitshisonii* with 101 (107.8) 115. Guard and subsidiary cells were also found variable in length and width among the species. In guard cell length *B. baluchistanica* was observed with longest cells of 27.75 µm on abaxial surface followed by *B. aitshisonii* with 25 µm on adaxial and *B. psodoumbellata* with also 25 µm on abaxial surface while *B. orthobotrys* whichwas recorded with the smallest cell length that of 8.4 µm on abaxial surfaces and *B. calliobotrys*with 12 µm value on adaxial surface was on 2nd number. Similarly, guard cell width was noted in *B. kunawurensis*with the value of 13.5 µm on abaxial surface followed by *B. aitshisonii* and *B. psodoumbellata* with 12 µm on abaxial surfaces.

3.2.2 Stomatal Occurrence and Density on Epidermis

Stomata were present mostly on abaxial surfaces of the studied specimens. Stomata were present both on abaxial as well as adaxial surfaces in *B.baluchistanica* and *B.aitchisonii* while the rest of 8 species possess stomata only on abaxial surfaces. Three types of stomata were observed in anatomical studies i.e. paracytic types of stomata were found only in *B.lycium* while anomocytic and anisocytic type of stomata were found in rest of the species. In most of the species stomata were confined to abaxial surface. The observed characters were variable among the species and the resulted variability is greatly reflected in their stomatal index (SI) values. In different specimens of the same species or even a single slide of the same specimen's stomatal index was observed variable. Most species were noted having randomly 1 mm square portion avoided completely of stomata on abaxial surface. In such a portion, stomatal index was calculated as zero. *B. kunawurensis* was noted with highest number of SI

value 31.9 followed by *B. glaucocarpa* with 28.9. Lowest number of SI value was counted 2.6 in *B.baluchistanica* followed by *B. calliobotrys* with SI value of 9.8. *B. baluchistanica* (abaxial), *B. psodoumbellata* (abaxial)and *B.aitchisonii* (adaxial)having largest stomatal aperture length 6 µm followed by *B. parkeriana* and *B. glaucocarpa* with 5 µm while the smallest aperture was observed in *B. orthobotrys* 3 µm. Largest value for width of stomatal aperture was noted in *B. psodoumbellata* and *B. baluchistanica* with the value of 3.5 µm and the lowest number of value was counted for *B. orthobotrys* 1.5 µm.

Table 2.3: Qualitative foliar epidermal features of genus *Berberis*

S. No.	Plant name	Surface	Epidermal cell shape	Cell wall pattern	Lobes per cell	Stomatal type	Stomatal aperture type
1	*B. aitshisonii* Ahrendt	Adaxial	Irregular to polygonal	Straight to slightly curved	3-5	Anomocytic	Spindle
		Abaxial	Irregular to polygonal	Straight to slightly curved	4-5	Anisocytic, anomocytic	Spindle
2	*B. baluchistanica* Ahrendt	Adaxial	Polygonal to irregular	Straight to curved	3-4	Paracytic	Spindle
		Abaxial	Polygonal to irregular	Straight to curved	3-5	Paracytic	Spindle
3	*B. calliobotrys* Bien	Adaxial	Polygonal	Straight	3-4	-	-
		Abaxial	Polygonal to irregular	Straight to slightly curved	3-4	Paracytic	Spindle
4	*B. chitria*Buch. - Ham	Adaxial	Polygonal to irregular	Straight to curved	3-5	-	-
		Abaxial	Polygonal to irregular	Straight to curved	3-4	Paracytic	Eleptic
5	*B. glaucocarpa* Stapf	Adaxial	Polygonal	Straight	3-4	-	-
		Abaxial	Oval to polygonal	Straight	3-5	Paracytic	Eleptic
6	*B. kunawurensis* Royle	Adaxial	Polygonal to irregular	Straight to slightly curved	3-4	-	-
		Abaxial	Spherical to polygonal	Straight	3-5	Paracytic	Spindle
7	*B. lycium* Royle	Adaxial	Polygonal	Straight	2-3	-	-
		Abaxial	Irregular to polygonal	Straight to curved	3-6	Paracytic	Eleptic
8	*B. orthobotrys*Bien	Adaxial	Polygonal to irregular	Straight to slightly curved	3-4	-	-
		Abaxial	Spherical to irregular	Straight to slightly curved	4-5	Anomocytic	Eleptic
9	*B. parkeriana* C.K. Schneid	Adaxial	Polygonal	Straight	2-4	-	-
		Abaxial	Irregular, polygonal, elongated to spherical	Straight to curved	3-5	Anomocytic, anisocytic	Spindle
10	*B. psodoumbellata* R.Parker	Adaxial	Polygonal to trigonal	Straight to curved	3-4	-	-
		Abaxial	Irregular to spherical	Straight to curved	3-5	Anomocytic	Eleptic

Table 2.4: Quantitative foliar epidermal features of genus *Berberis*

S. No	Taxa	Surface	Epidermal cell features Min (mean ± SE) Max			Stomatal features Min (mean ± SE) Max			Stomatal aperture features Min (mean ± SE) Max			Guard cells Min (mean ± SE) Max		Subsidiary Cells Min (mean ± SE) Max	
			Length (um)	Width (um)	N0 of cells/Unit area	Length (um)	Width (um)	No of stomata /Unit area	Index	Length (um)	Width (um)	Length (um)	Width (um)	Length (um)	Width (um)
1	*B. aitshisonii* Ahrendt	Adaxial	37.5 (43 ± 2.15) 50	20 (27 ± 4) 32.5	101 (107.8) 116	18 (20 ± 1) 23	9 (10 ± 0.7) 12	11	10.2	11 (13 ± 0.4) 15	7 (9.2 ± 1.5) 11	22 (25 ± 1) 28	10 (11 ± 0.5) 12	19 (24 ± 1.5) 27	5 (8 ± 1) 10
		Abaxial	30 (35.2 ± 2.1) 42.5	20 (29 ± 2) 37	98 (104.2) 115	17 (18.5 ± 0.5) 20	8 (9.5 ± 0.4) 10	22	20.4	12 (13.5 ± 0.4) 16	6 (8 ± 1) 10	22 (23.5 ± 0.5) 25	8 (9.2 ± 0.4) 10	17 (22.5 ± 1.3) 25	5 (9.5 ± 1.2) 12
2	*B. baluchistanica* Ahrendt	Adaxial	45 (53.9 ± 3.6) 62.5	22.5 (26.3 ± 1.3) 30	101 (107.2) 112	15 (17 ± 0.9) 20	10 (9.5 ± 0.3) 12	8	2.6	10 (12.5 ± 1) 15	8.5 (9 ± 0.7) 11	23 (25 ± 1) 27	5.5 (6 ± 0.5) 7	40 (42 ± 1) 46.5	17.3 (20.5 ± 1.5) 23.5
		Abaxial	37.5 (43.25 ± 2.15) 50	20 (22.6 ± 0.8) 25	110 (117.6) 130	25 (30 ± 1.7) 35	10 (19.5 ± 2.4) 23.75	15	12.8	12.5 (14.9 ± 0.8) 17.5	7.5 (8.5 ± 0.5) 10	25 (27.75 ± 1) 30	6.25 (7.75 ± 0.4) 8.75	33 (34.6 ± 1.25) 37	15 (19.5 ± 1.2) 22.5
3	*B. calliobotrys* Bien	Adaxial	20 (29.5 ± 3.2) 37	15 (19.1 ± 1.6) 25	245 (252.4) 260	-	-	-	-	-	-	-	-	-	-
		Abaxial	20 (22 ± 0.8) 25	12 (14 ± 0.8) 17	196 (202.2) 210	10 (11.5 ± 0.4) 12	5.8 (6.4 ± 0.2) 7.5	20	9.8	8 (9 ± 0.3) 10	4 (4.8 ± 0.2) 5.4	10 (12 ± 0.6) 13.5	7.5 (8.11 ± 0.2) 8.75	20 (21.7 ± 0.8) 25	7 (8.6 ± 0.4) 01
4	*B. chitria* Buch. - Ham	Adaxial	37 (42.5 ± 2.1) 50	12.5 (14.5 ± 0.8) 17.5	145 (159.2) 170	-	-	-	-	-	-	-	-	-	-
		Abaxial	24 (25.2 ± 0.5) 27	12 (15 ± 1.5) 17	199 (214.6) 235	10 (11.5 ± 0.5) 12.5	6 (6.2 ± 0.3) 7.5	45	20.9	7.5 (7.2 ± 0.2) 8.5	4 (4.7 ± 0.2) 5.5	15 (16.1 ± 0.4) 17.5	7.5 (8.6 ± 0.4) 10	25 (28.8 ± 1.1) 32	12.5 (15.5 ± 1.3) 20
5	*B. glaucocarpa* Stapf	Adaxial	25 (39 ± 4.2) 50	20 (24 ± 1.8) 30	171 (185.8) 196	-	-	-	-	-	-	-	-	-	-
		Abaxial	22 (26.2 ± 1.2) 30	12 (16.2 ± 1.2) 20	210 (213.8) 220	15 (19 ± 1.3) 22	12 (14 ± 0.8) 17	62	28.9	10 (11.3 ± 0.4) 12	6 (7.2 ± 0.3) 8	12 (14 ± 1.3) 18	7 (8.5 ± 0.4) 10	16 (18 ± 0.6) 20	5 (6 ± 0.4) 7
6	*B. kunawurensis* Royle	Adaxial	37.5 (41 ± 2.0) 50	12.5 (18 ± 2.1) 25	198 (204) 206	-	-	-	-	-	-	-	-	-	-
		Abaxial	25 (29 ± 1.8) 35	12.5 (15 ± 1.3) 20	165 (172.4) 180	20 (23 ± 0.8) 25	11 (12 ± 0.3) 13	55	31.9	12.5 (14 ± 0.8) 17.5	7.5 (8 ± 0.4) 10	17.5 (18.5 ± 0.5) 20	12.5 (13.5 ± 0.5) 15	20 (22.5 ± 1.0) 25	7.5 (8.5 ± 0.4) 10
7	*B. lycium* Royle	Adaxial	32 (44 ± 4) 55	20 (25 ± 2.6) 35	135 (140.4) 146	-	-	-	-	-	-	-	-	-	-
		Abaxial	20 (24 ± 2.5) 35	12 (14 ± 1.5) 20	173 (183.2) 190	10 (13.5 ± 0.9) 15	6 (7 ± 0.3) 8	38	20.7	6 (6.8 ± 0.2) 8	3 (4 ± 0.2) 5	12 (15 ± 0.9) 17	7 (8 ± 0.2) 9	15 (17 ± 0.8) 20	7 (7.8 ± 0.3) 9
8	*B. orthobotrys* Bien	Adaxial	25 (36 ± 4) 50	17.5 (24 ± 2.3) 30	165 (171.4) 180	-	-	-	-	-	-	-	-	-	-
		Abaxial	17 (20.7 ± 1.3) 25	10 (13.3 ± 1.3) 17.5	205 (215.4) 230	6 (6.6 ± 0.2) 7.5	4 (4.5 ± 0.7) 5	50	23.2	4 (5 ± 0.4) 6	4 (4.5 ± 0.1) 5	5 (8.4 ± 1.2) 12.5	4.2 (6 ± 0.7) 7.5	12 (14 ± 0.6) 16	5 (6 ± 0.4) 7
9	*B. parkeriana* C.K. Schneid	Adaxial	30 (36 ± 2.5) 45	7.5 (12.5 ± 1.7) 17.5	205 (212.8) 224	-	-	-	-	-	-	-	-	-	-
		Abaxial	25 (31 ± 2.1) 37.5	15 (17.3 ± 0.9) 20	209 (227.8) 250	21 (24 ± 1.5) 30	13 (14 ± 0.3) 15	44	19.3	10 (11 ± 0.3) 12	6.5 (7.2 ± 0.1) 7.5	18 (20 ± 1) 22	7.5 (8.6 ± 0.4) 10	19 (21 ± 0.6) 22	7 (8.5 ± 0.4) 10
10	*B. psodoumbellata* R.Parker	Adaxial	25 (33 ± 2.8) 41	12 (18 ± 2.4) 25	135 (180) 246	-	-	-	-	-	-	-	-	-	-
		Abaxial	25 (28 ± 1.8) 35	12.5 (14.8 ± 1) 17.5	171 (176.8) 185	21.5 (22.9 ± 0.4) 24	15 (16.1 ± 0.4) 17.5	33	18.6	12 (13 ± 0.5) 15	6 (7 ± 0.2) 8	22 (25 ± 1.3) 30	10 (12 ± 1) 15	20 (23 ± 1) 25	12 (14 ± 0.6) 15

3.2.3 Taxonomic key to the species

+ 1 Stomata on both surfaces.....2

- Stomata only on lower surface.....3

+ 2 Stomatal type anomocytic and anisosytic with 3-5 lobes per cell on upper surface.....*Berberis aitchisonii*

-Stomatal type paracytic, cell wall pattern straight to curved on both surfaces.....*Berberis baluchistanica*

+ 3 + Stomatal apperture spindle.....4

- Stomatal apperture eleptic.....6

4 + Stomata anomocytic and anisocytic, epidermal cells polygonal on upper surface.....*Berberis parkeriana*

- Stomatal type paracytic.....5

5 + Epidermal cell shape spherical to polygonal on lower surface.....*Berberis kunawurensis*

- Epidermal cell shape polygonal to irregular on lower surface.....*Berberis calliobotrys*

6 + Stomatal type anomocytic 7

- Stomatal type paracytic.....8

7 + Epidermal cell shape spherical to irregular on lower surface, lobes per cell 4-5.....*Berberis orthobotrys*

- Lobes 3-5.....*Berberis psodoumbellata*

8 + Epidermal cell shape oval to polygonal, cell wall pattern straight on both surfaces.....*Berberis glaucocarpa*

- Epidermal cell shape polygonal to irregular.....9

9 + Lobes per cell 3-4 0n lower surface.....*Berberis chatria*

- Lobes per cell 3-6 on lower surface .....*Berberis lycium*

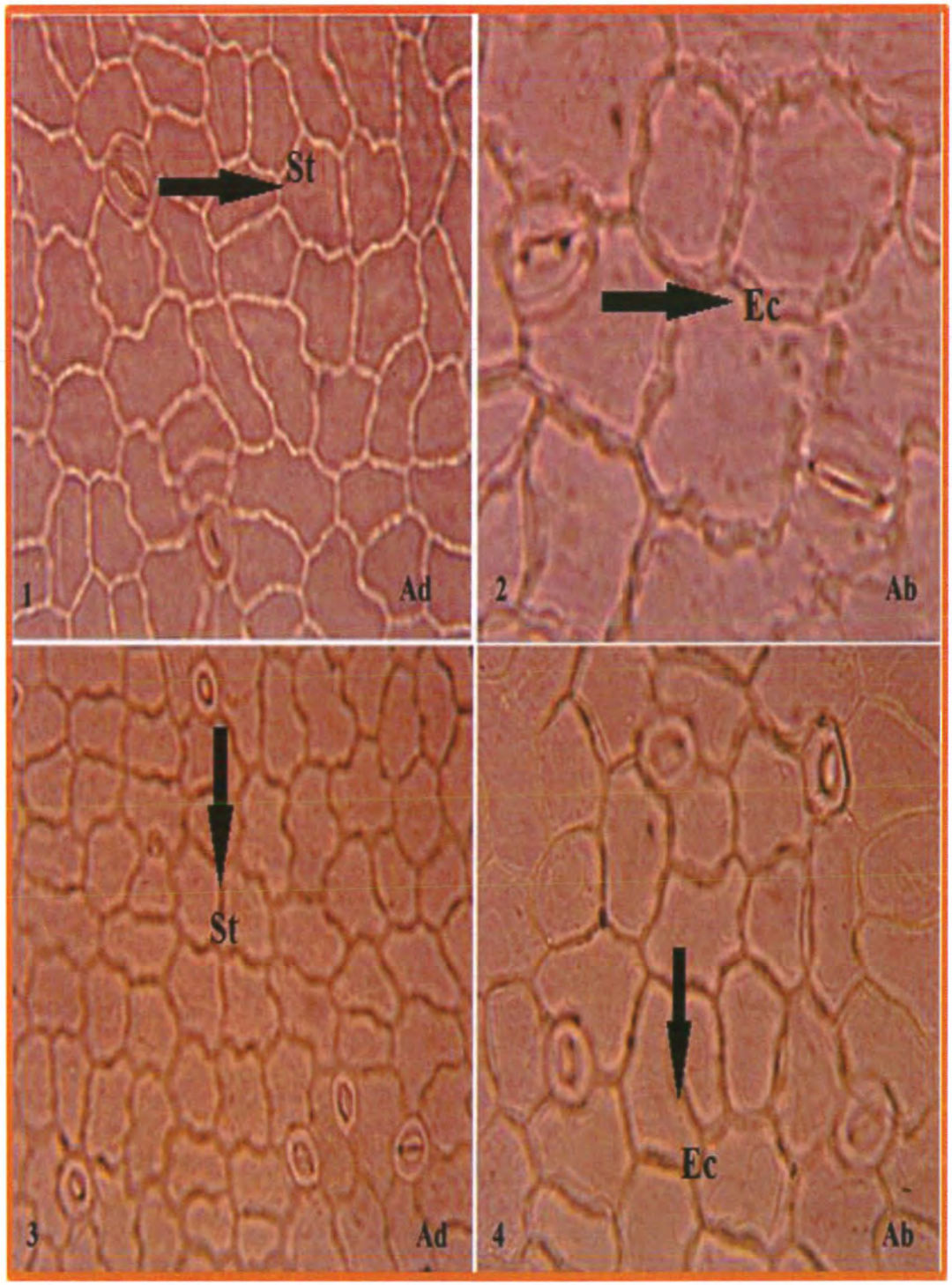

Plate 3.6: Microscopic photographs of epidermal cells and stomata (1, 2) *Berberis aitchisonii*; (3, 4)*Berberis baluchistanica*

Abbreviations: Ab: abaxial; Ad: adaxial; St: stomata; Ec: epidermal cell

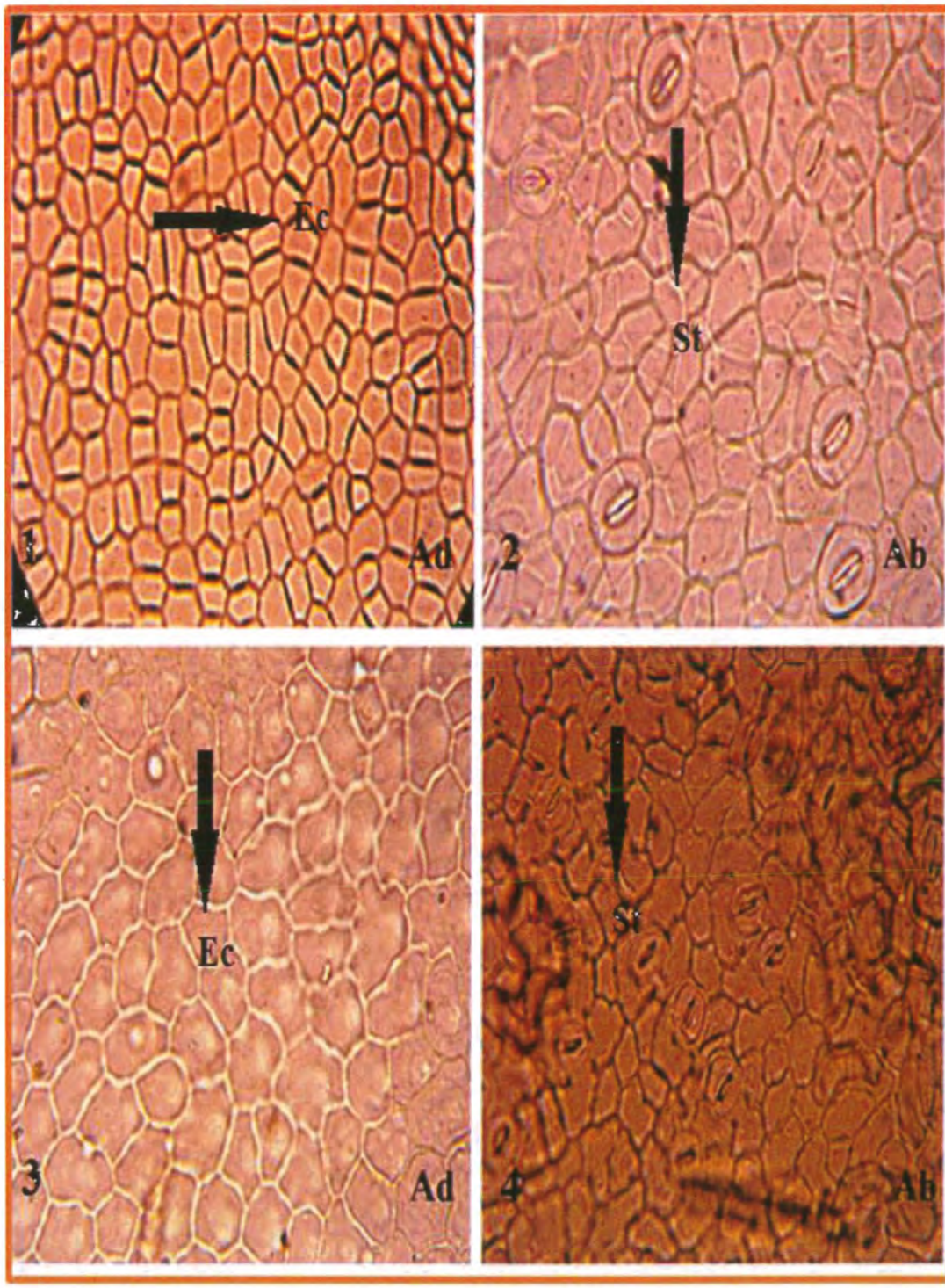

Plate 3.7: Microscopic photographs of epidermal cells and stomata (1, 2) *Berberis calliobotrys*; (3, 4) *Berberis chitria*

Abbreviations: Ab: abaxial; Ad: adaxial; St: stomata; Ec: epidermal cell

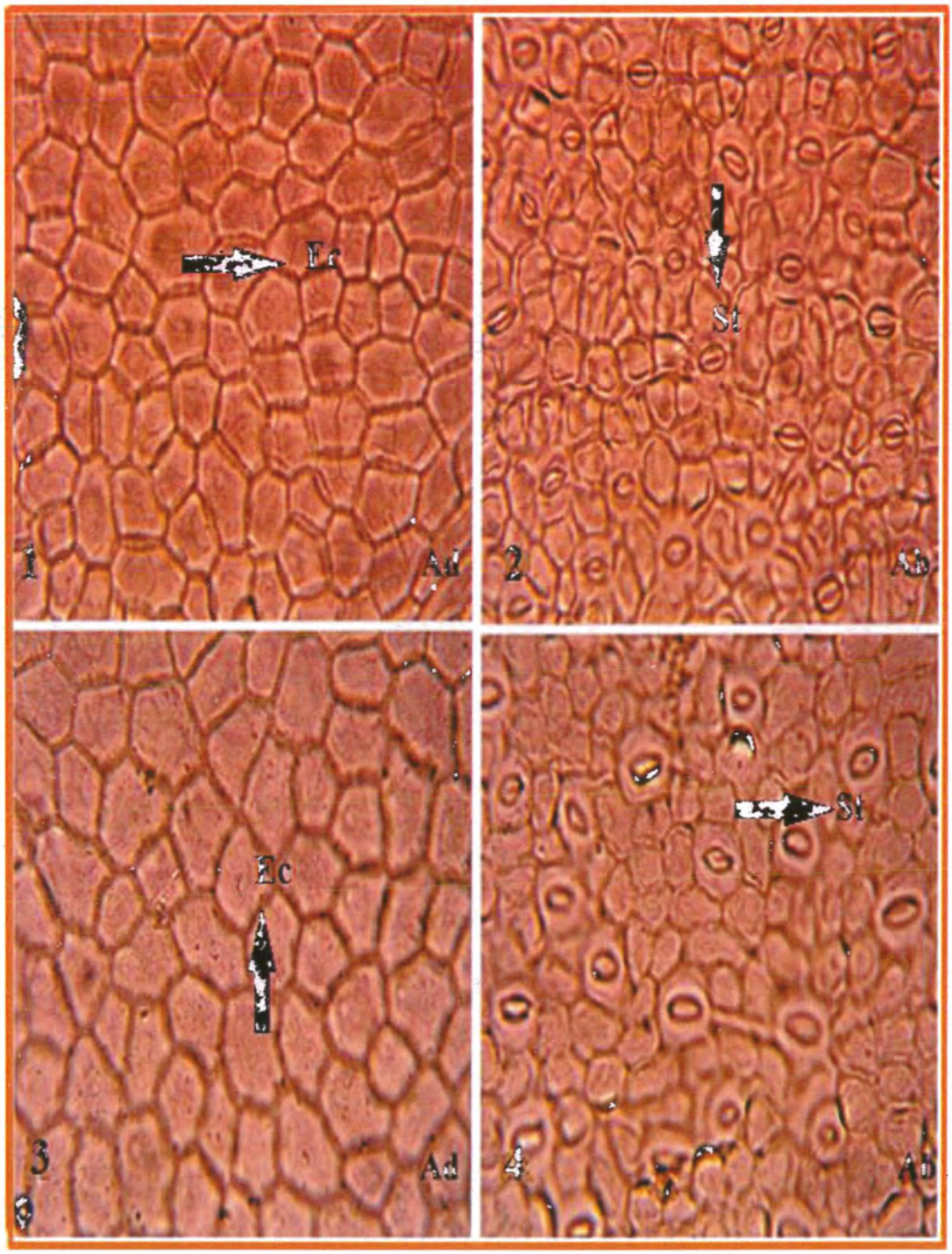

Plate 3.8: Microscopic photographs of epidermal cells and stomata (1, 2) *Berberis glaucocarpa*; (3, 4) *Berberis kunawurensis*

Abbreviations: Ab: abaxial; Ad: adaxial; St: stomata; Ec: epidermal cell

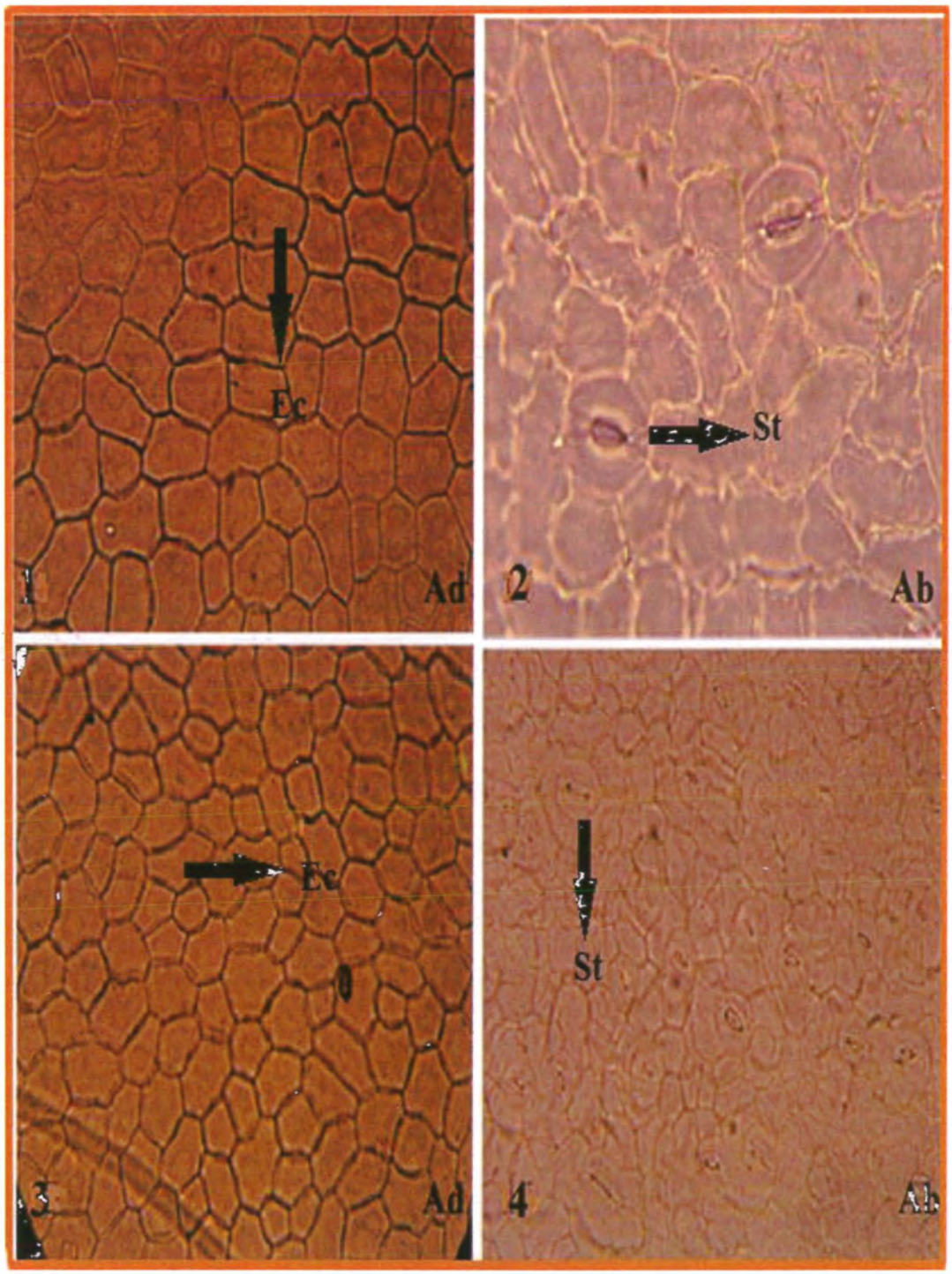

Plate 3.9: Microscopic photographs of epidermal cells and stomata (1, 2) *Berberis lycium*; (3, 4) *Berberis orthobotrys*

Abbreviations: Ab: abaxial; Ad: adaxial; St: stomata; Ec: epidermal cell

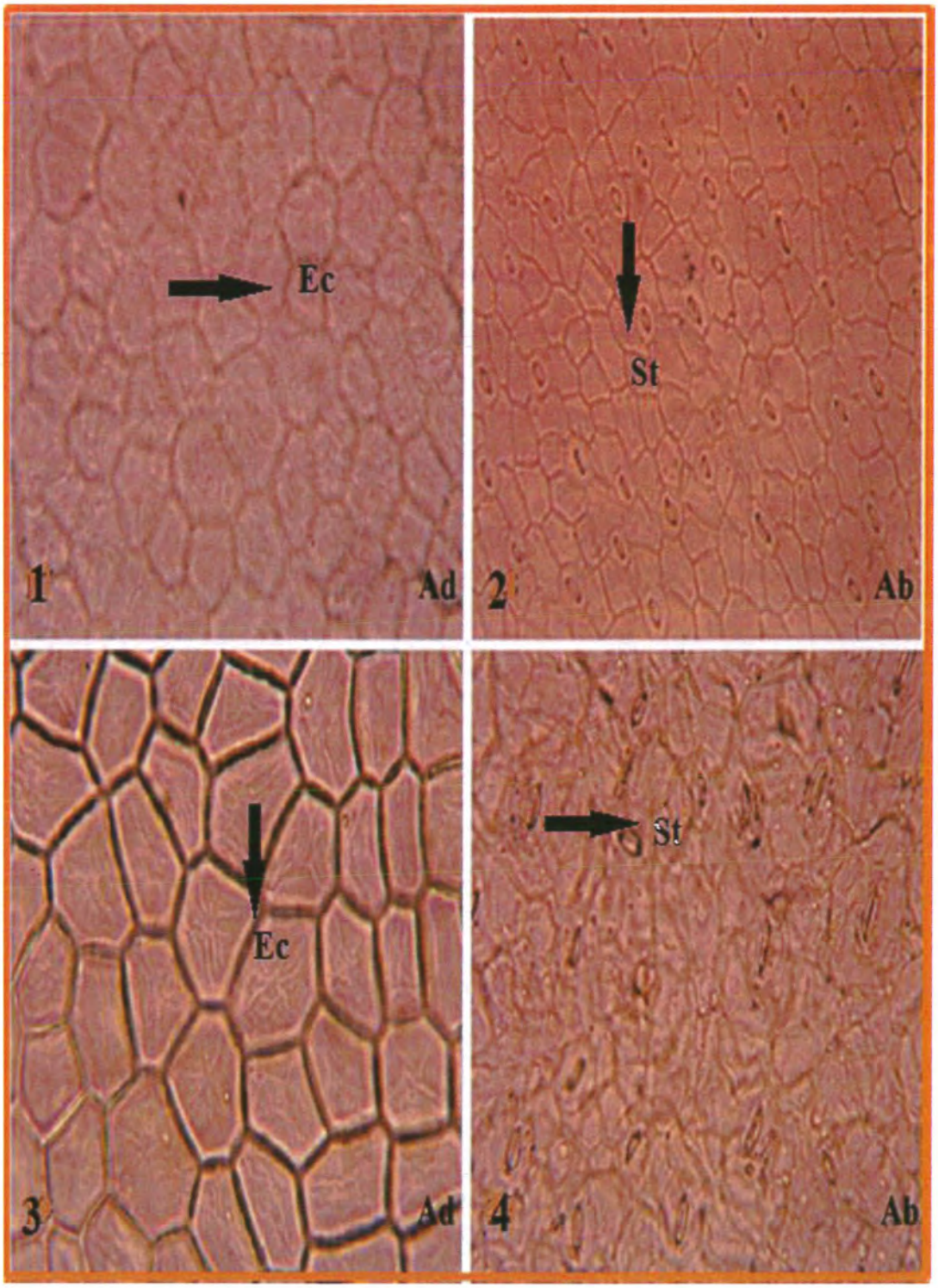

Plate 3.10: Microscopic photographs of epidermal cells and stomata (1, 2) *Berberis parkeriana*; (3, 4) *Berberis psodoumbellata*

Abbreviations: Ab: abaxial; Ad: adaxial; St: stomata; Ec: epidermal cell

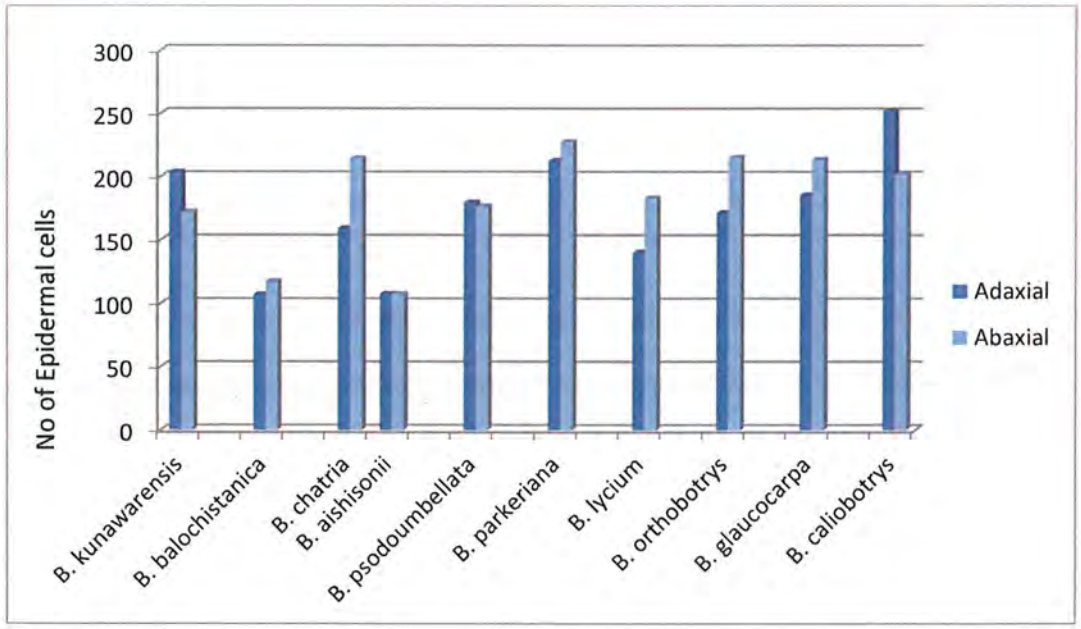

Figure 3.9:Comparative number of epidermal cells

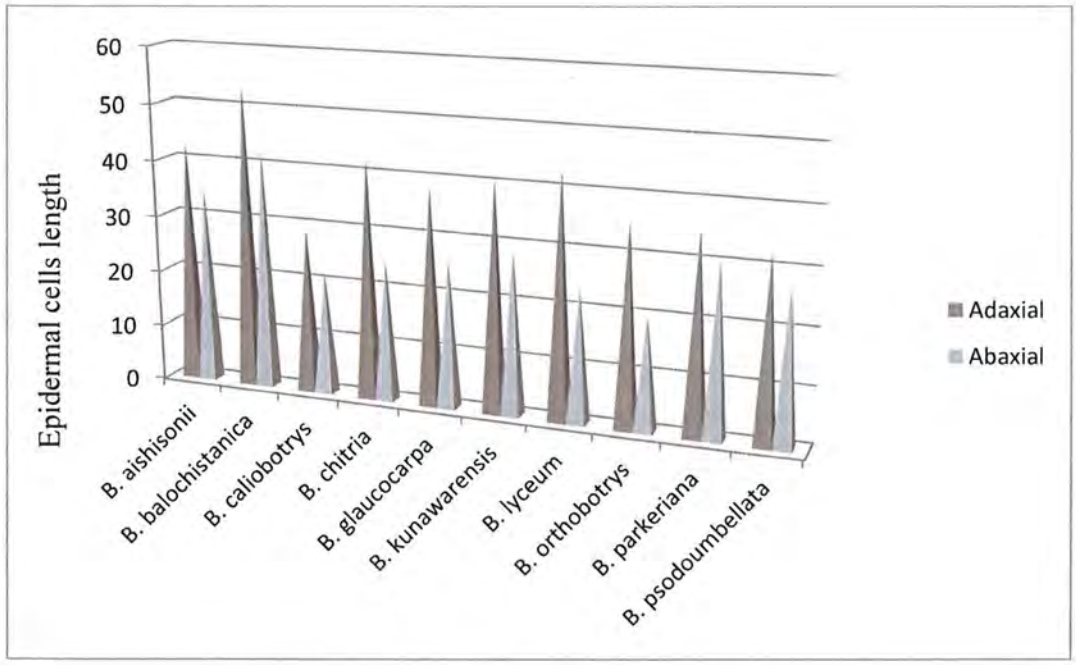

Figure 3.10: Comparative epidermal cells length

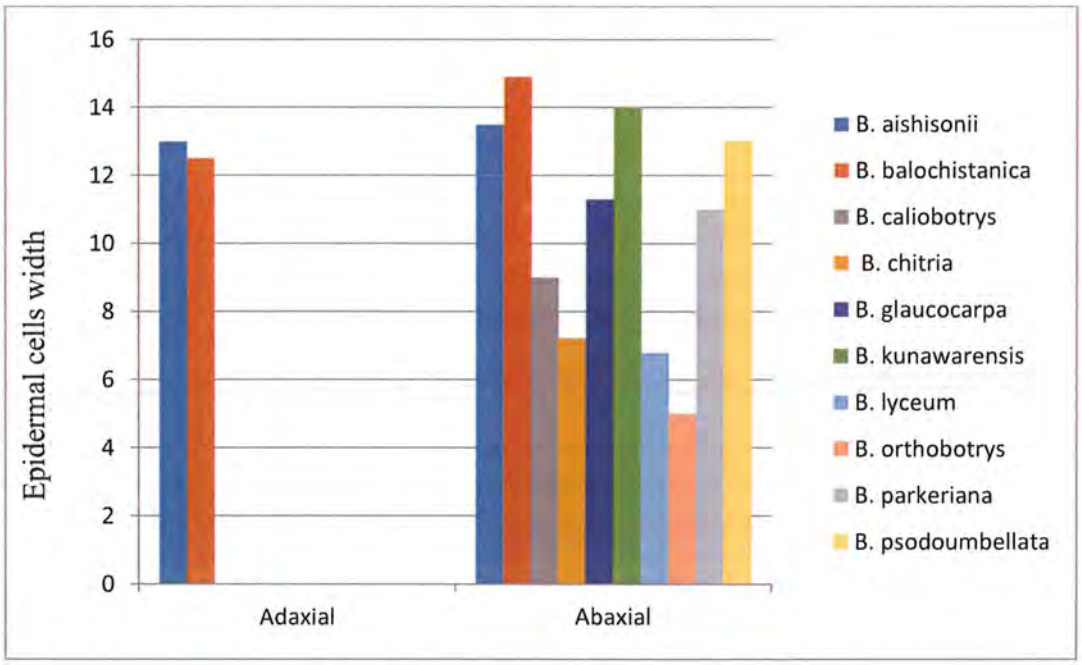

Figure 3.11: Comparative epidermal cells width

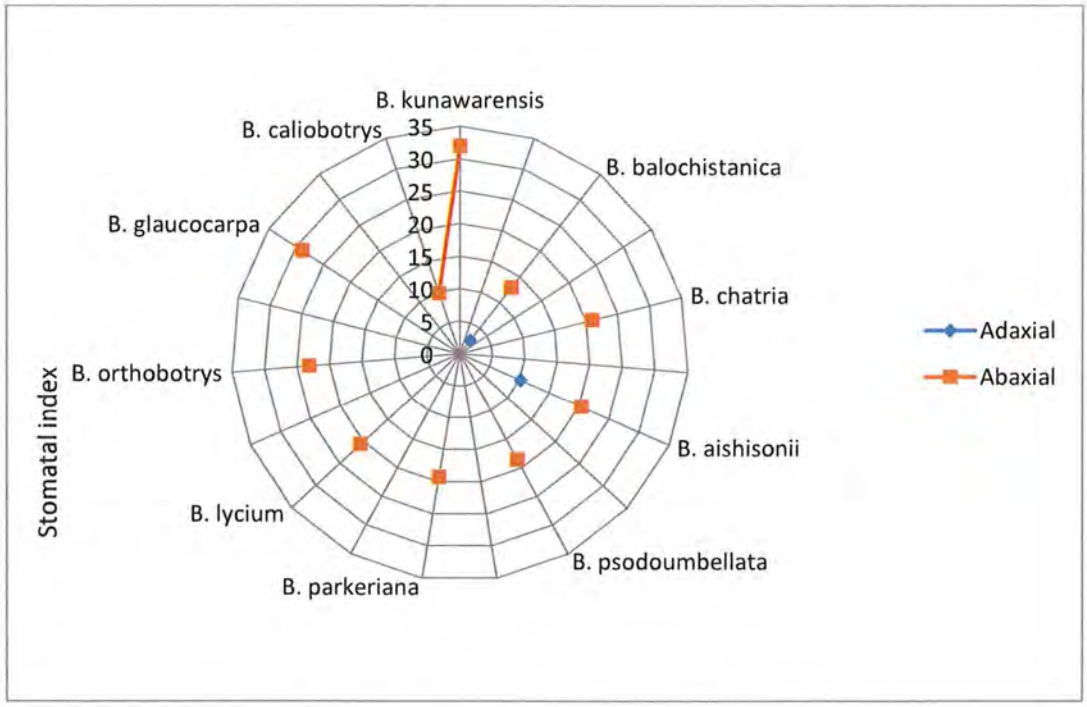

Figure 3.12: Comparative stomatal index

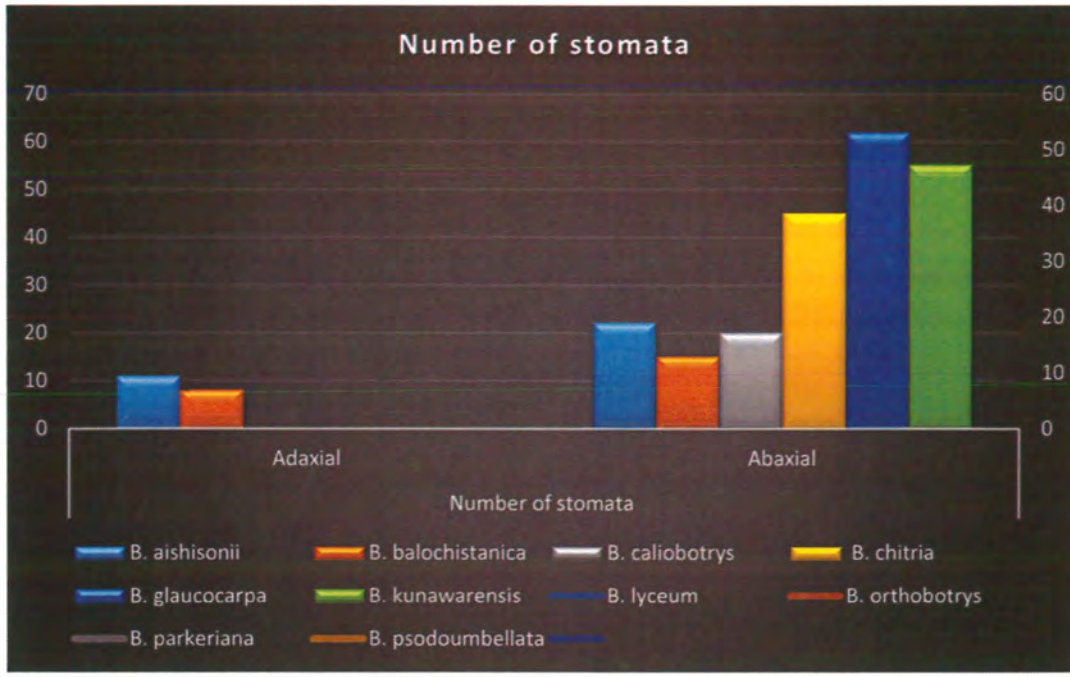

Figure 3.13: Comparative number of stomata

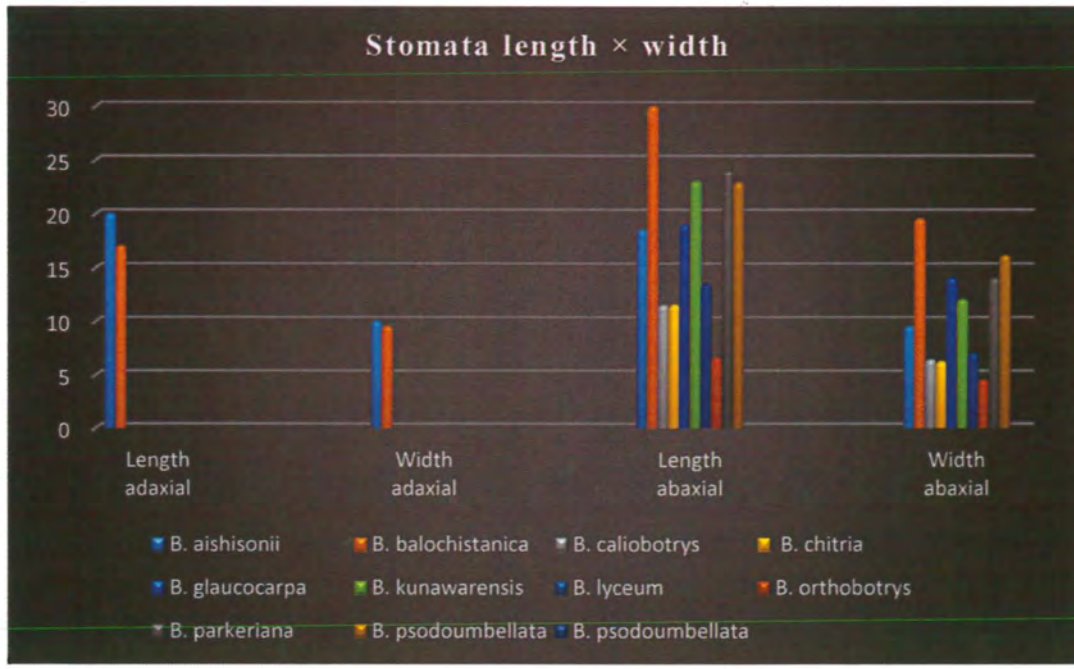

Figure 3.14: Comparative stomatal length and width

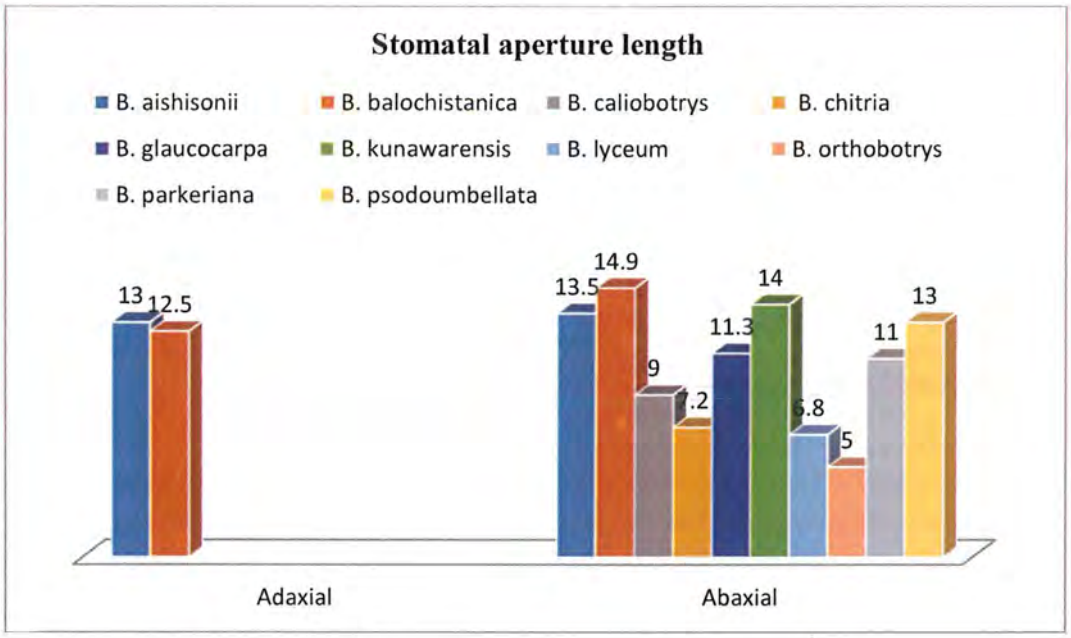

Figure 3.95: Comparative stomatal aperture length

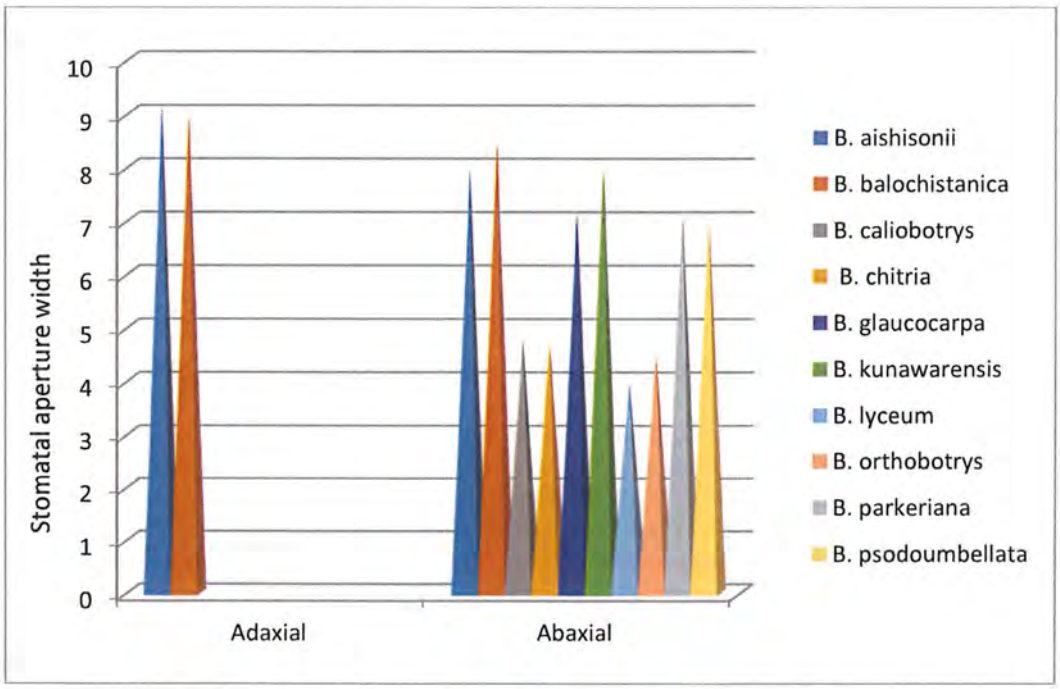

Figure 3.106: Comparative stomatal aperture width

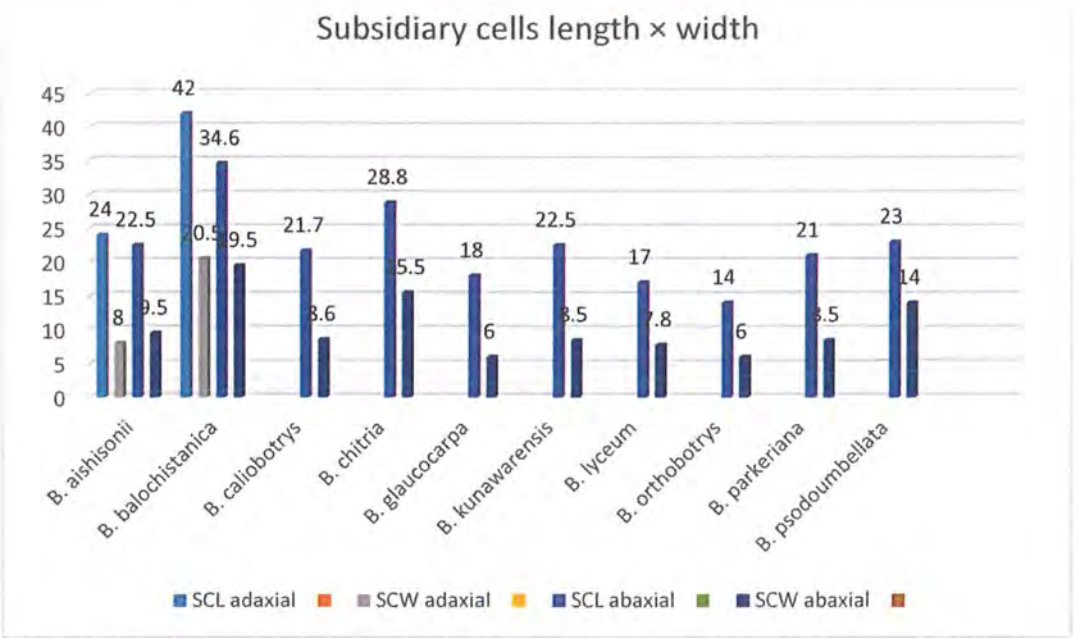

Figure 3.117: Comparative subsidiary cells length and width

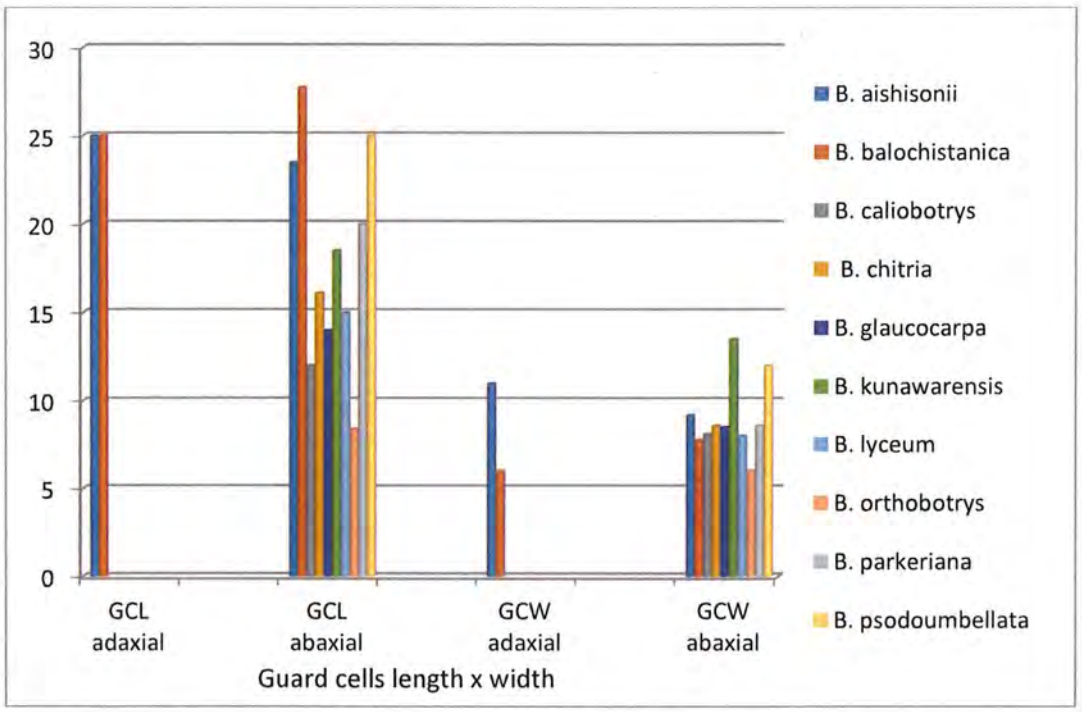

Figure 3.128: Comparative guard cells length and width

3.3 Palynological Study

Palynologically10*Berberis* species namely *B.aitshisonii, B. baluchistanica, B. chitria, B. calliobotrys, B. glaucocarpa, B. kunawurensis, B. lyceum,B. orthobotrys, B. parkeriana* and *B. psodoumbellata*from family Berberidaceae were studied, analyzedand documented first time from Pakistan. For each pollen grain, morphological features such as pollen shape, size, presence or absence of colpi, colpi length and width, exine thickness and P/E ratio were investigated. Variation was seen in all above mention characters in pollen morphology of the studied species. Both polar and equatorial measurements were recorded and among the species largest pollen was observed in *B.psodoumbellata*(zonocolpate)with an average value of 62.4 µm followed by *B. glaucocarpa* with 57.8µm while the smallest pollen was recorded in *B. lyciumwith* a value of 32.2 µmfollowed by *B. chitria* with a value of 42.6 µm. Various sizes and shapes of the pollen were observed under light microscope. Pollen are usually monads; all the studied pollens were psilolophate. All pollenwas colpate range from tri to Penta-colpate. Colpi were usually elongated and narrowing at the end and pole. Colpus length ranges from 2 µm in *B. calliobotrys* to 3 µm in *B. psodoumbellat.* Colpus width in the studied species ranges from 1.2 µm in *B. glaucocarpa* to 2µm in *B.chitria*. The pollen grains were radially symmetrical and isopolar. The shape index (P/E ratio) varies from 1 µm in *B. chitria* to 2.6 µmin *B. lycium.* Shapes of pollen grains also varies from spherical as observed in *B. aitshisonii* to ovoid in *B. chitria.* Other types of pollens were oblate in *B. calliobotrys, B. lycium* and sub-prolate in *B. glaucocarpa,* sub-spheroidal in *B. kunawurensis*and prolate spheroidal in *B. psodoumbellata, B. parkeriana* and *B. orthobotrys*. Minimum number of colpi was observed in *B. glaucocarpa, B. baluchistanica, B. kunawurensis*and *B. lyciumas* 3followed by *B. aitchisonii,* and *B. parkerianaand B. orthobotrys*as 4while maximum number was recorded as 5 in *B. calliobotrys, B. chitria* and *B. psodoumbellata*. Exine thickness ranges from 1µm in *B. chitria* to 2.5µm in *B. glaucocarpa* and *B. kunawurensis*.

Table 2.5: Quantitative characters of studied pollens

S. No	Species	Polar diameter (µm)	Equatorial diameter (µm)	Shape of pollen	No of colpi	Length of colpi (µm)	Width of colpi (µm)	P/E ratio	Exine thickness (µm)
1	*B. aitshisonii*Ahrendt	44(46.9 ± 1.1) 50	32(33.3 ± 0.5) 35	Spherical	4	2.5	1.4	1.4	1.5
2	*B. baluchistanica* Ahrendt	37 (39± 1) 41	31 (32.5 ± 0.7) 34	Ovoid	3	2.3	1.5	1.2	2
3	*B. chitria*Buch. -Ham.	40 (42.6 ± 0.9) 45	28(29.5 ± 0.5) 31	Ovoid	5	2	2	1.0	1
4	*B. calliobotrys* Bien	50(51.6 ± 0.5) 53	30(32.3 ± 0.8) 35	Oblate	5	2	1.3	1.4	1.2
5	*B. glaucocarpa* Stapf	55 (57.8 ± 0.8) 60	33 (34.7 ± 0.5) 36	Sub-prolate	3	2.5	1.2	1.7	2.5
6	*B. kunawurensis* Royle	41 (44.2 ± 1.1) 48	21 (23 ± 0.7) 25	Sub-spheroidal	3	2.3	2	1.9	2.5
7	*B. lycium* Royle	29 (32.2 ± 1) 35	10.5 (12 ± 0.5) 13.5	Oblate	3	2	1.2	2.6	1.9
8	*B. orthobotrys* Bien	35 (37 ± 0.8) 40	25 (27 ± 0.8) 30	Prolate spheroidal	4	2.4	2.1	2.3	1.3
9	*B. parkeriana*C.K.Schneid.	49 (51.4 ± 0.9) 54	19 (21 ± 0.7) 23	Prolate spheroidal	4	2.8	1.4	2.4	1.3
10	*B. psodoumbellata* R. Parker	60 (62.4 ± 0.9) 65	29 (31.2 ± 0.8) 34	Prolate spheroidal	5	3	1.5	1.9	2

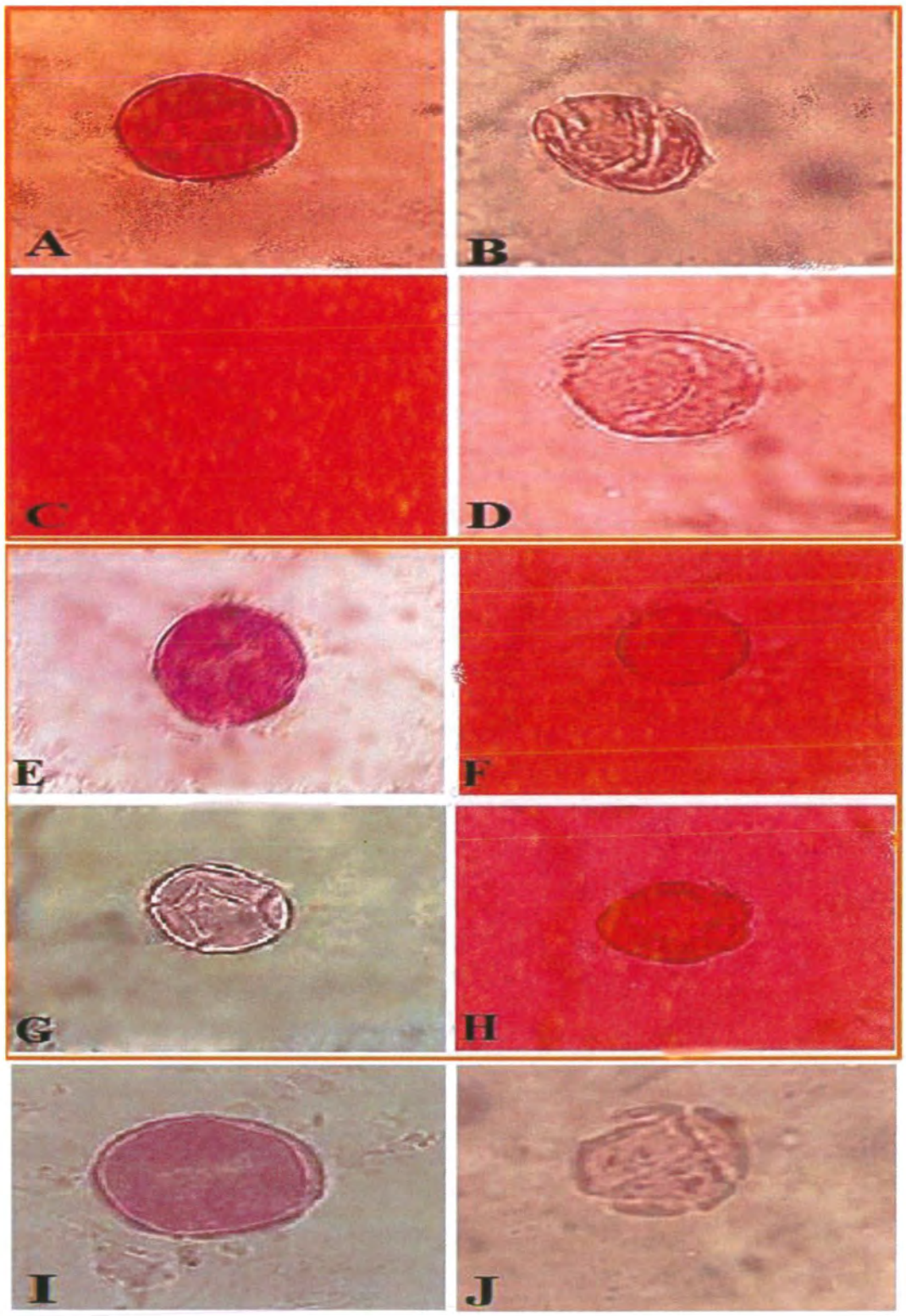

Plate 3.11: Light microscopic photographs of pollen(A) *Berberis aitchisonii*; (B) *Berberis baluchistanica*; (C) *Berberis chitria;* (D) *Berberis calliobotrys;*(E) *Berberis glaucocarpa*;(F) *Berberis kunawurensis*; (G) *Breberis lycium*; (H) *Berberis orthobotrys;* (I) *Berberis parkeriana;* (J) *Berberis psodoumbellata*

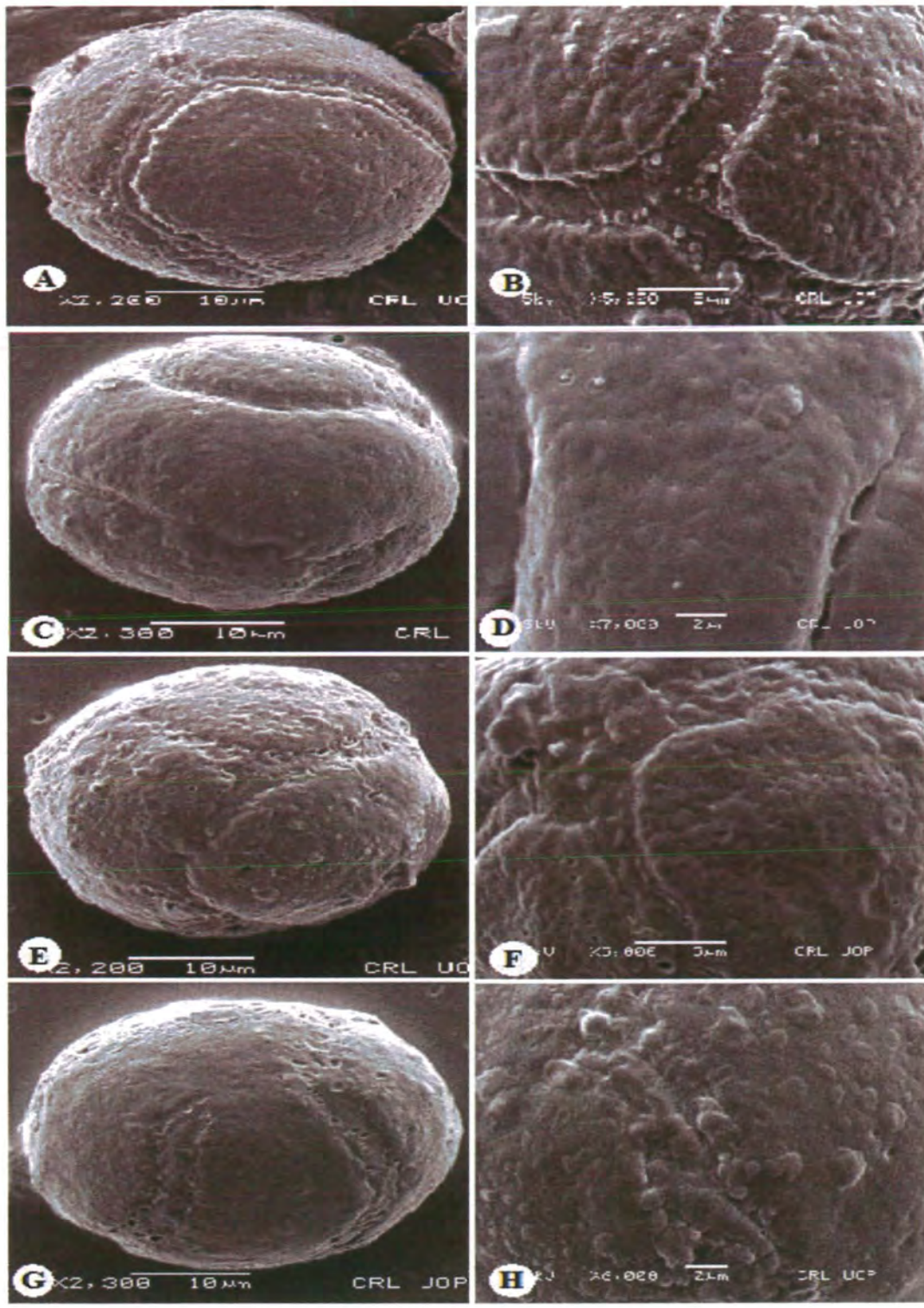

Plate 3.12: SEMmicrographs of (A-B) *Berberis aitchisonii* (C-D) *Berberis baluchistanica* (E-F) *Berberis chitria*(G-H) *Berberis calliobotrys*

Scale bar: A, C, E, G = 10µm. **Exine pattern:**B=5 µm; D = 2 µm; F = 5 µm; H = 2 µm.

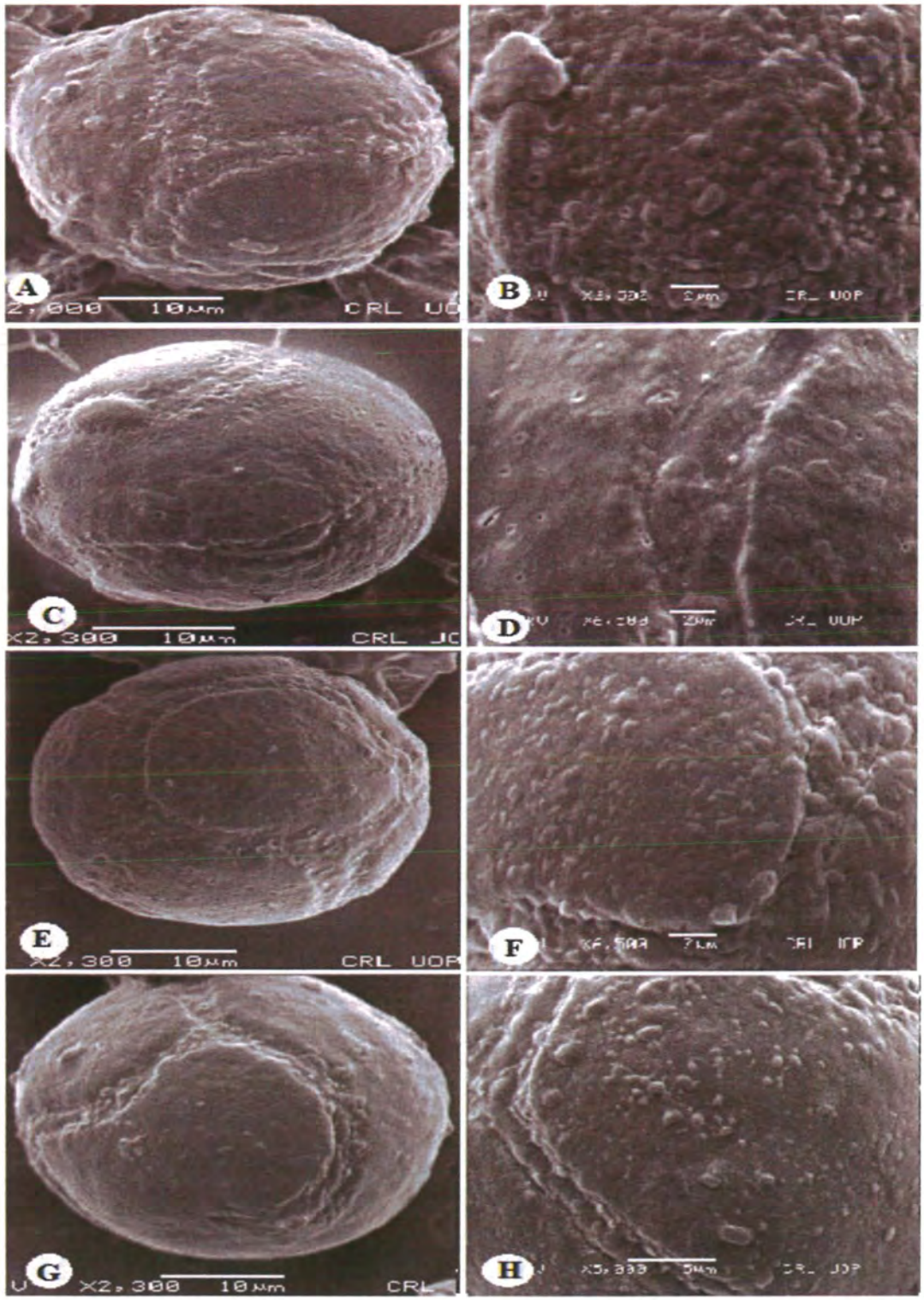

Plate 3.13:SEMmicrographs of(A-B)*Berberis glaucocarpa*(C-D) *Berberis kunawurensis*(E-F) *Berberis lycium*(G-H) *Berberis orthobotrys*

Scale bar:A, C, E, G = 10µm.**Exine pattern:**B = 2 µm; D = 2 µm; F = 2 µm; H = 5µm.

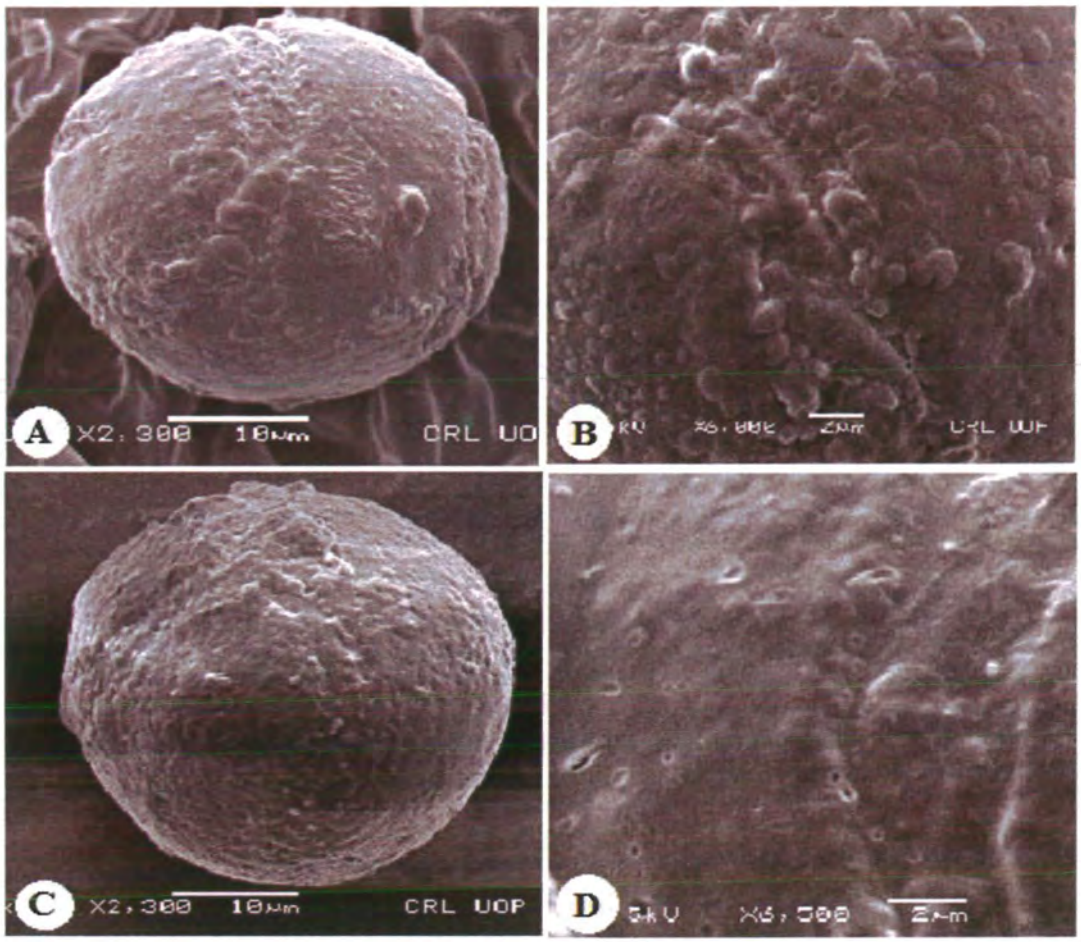

Plate 3.14:SEMmicrographs of(A-B)*Berberis parkeriana* (C-D) *Berberis psodoumbellata*

Scale bar: A, C = 10 µm. **Exine pattern:** B, C = 2 µm.

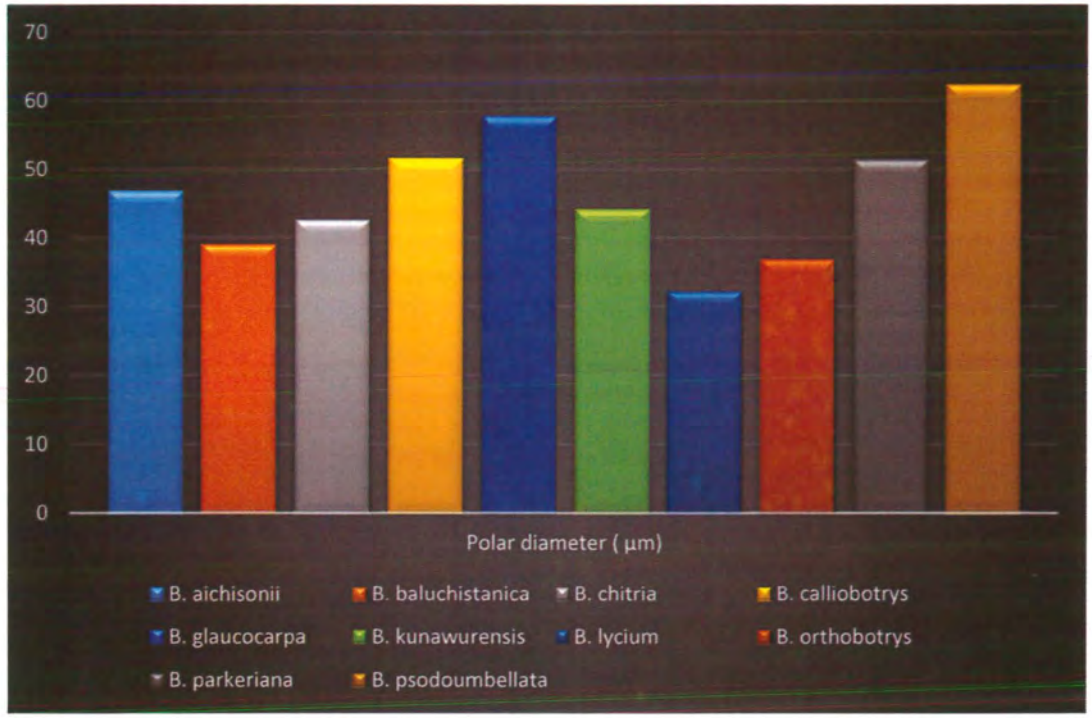

Figure 3.139: Comparative polar diameter of pollen (µm)

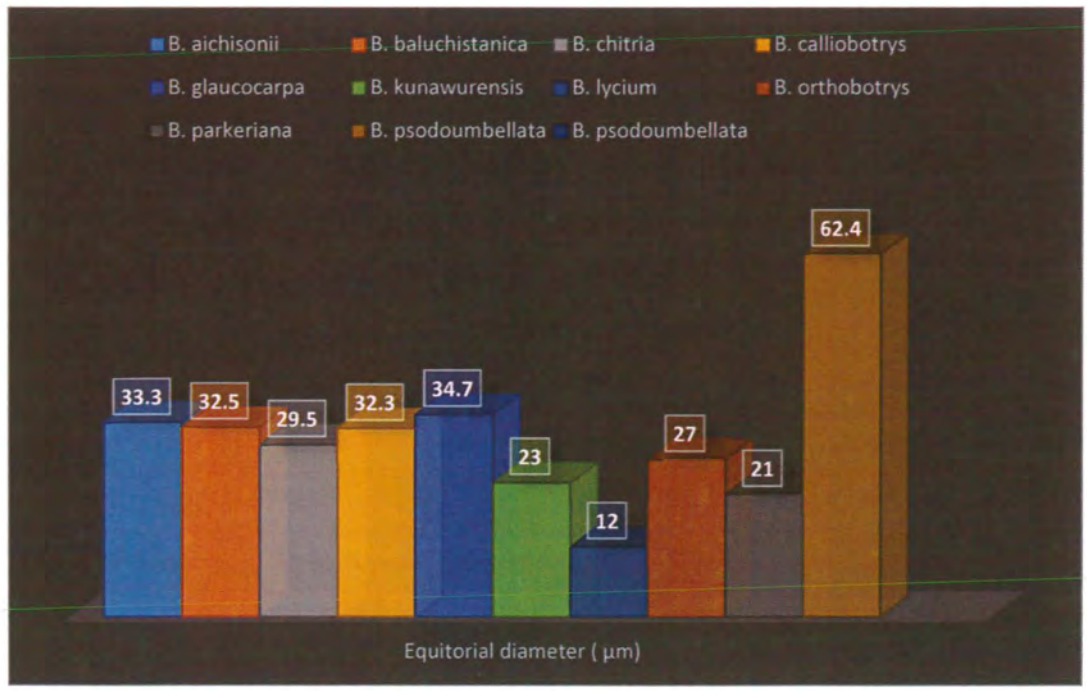

Figure 3.20: Comparative equatorial diameter of pollen (µm)

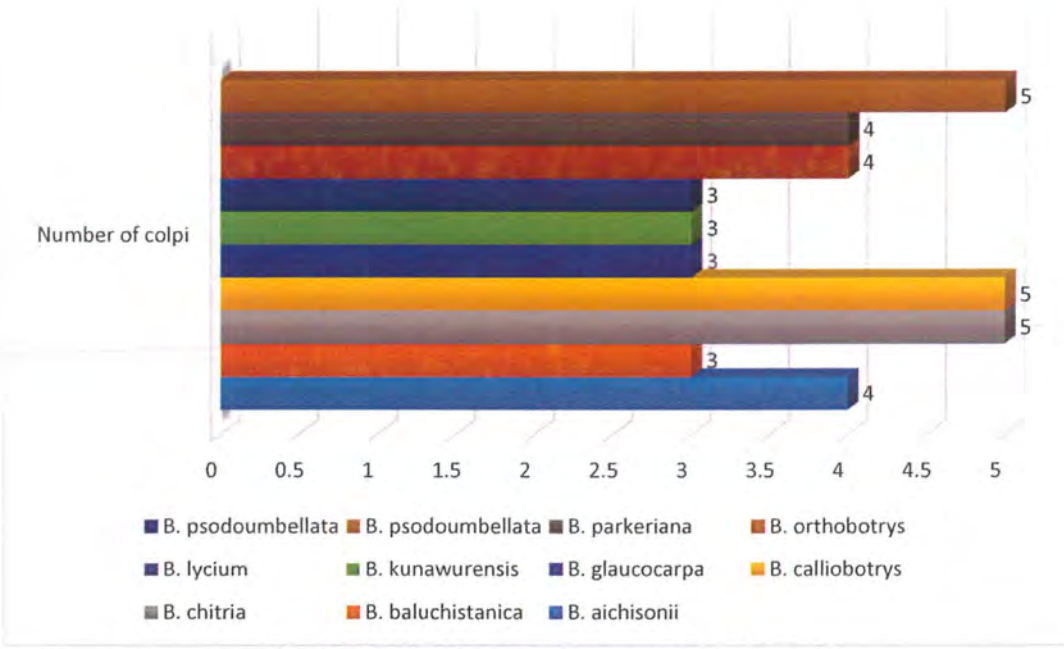

Figure 3.214: Comparative number of colpi

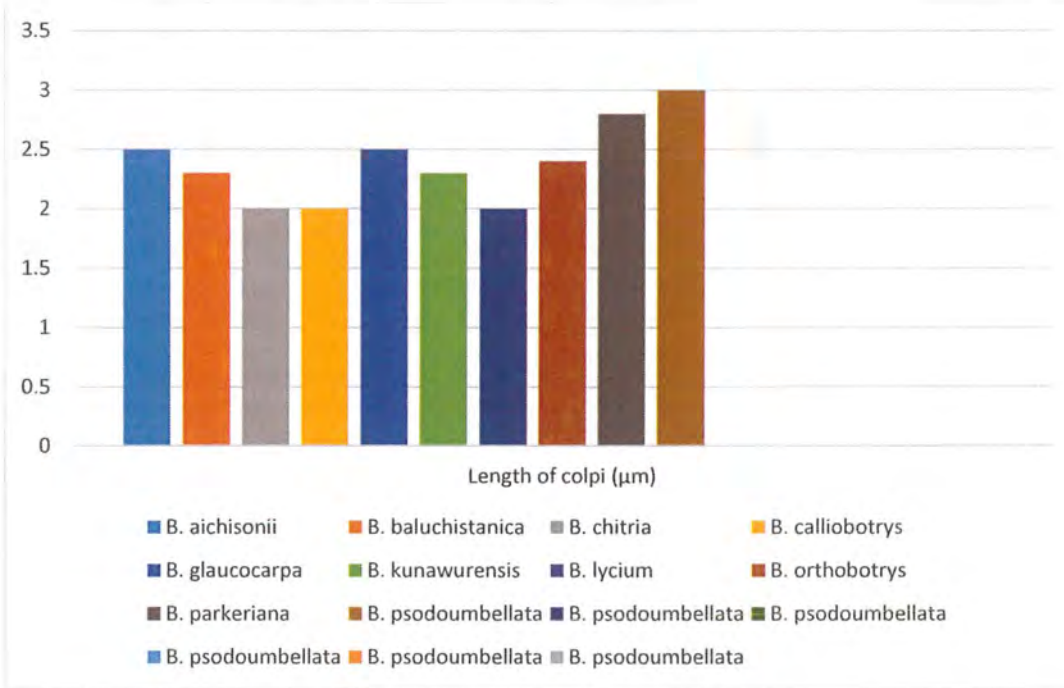

Figure 3.152: Comparative length of colpi (µm)

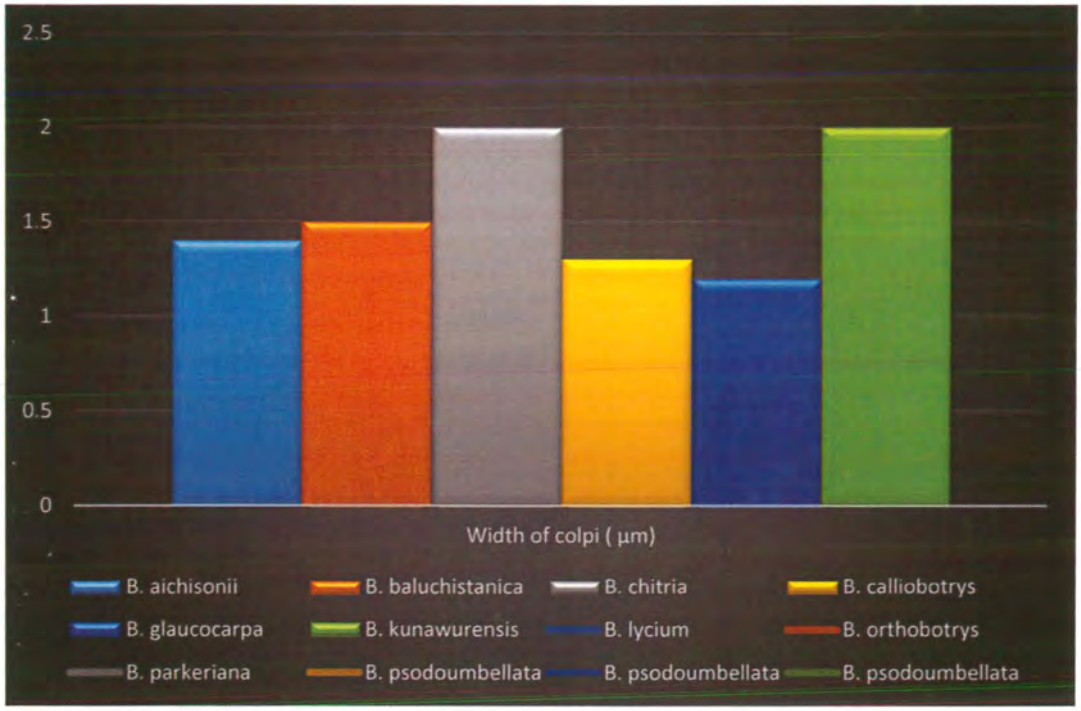

Figure 3.163: Comparative width of colpi (µm)

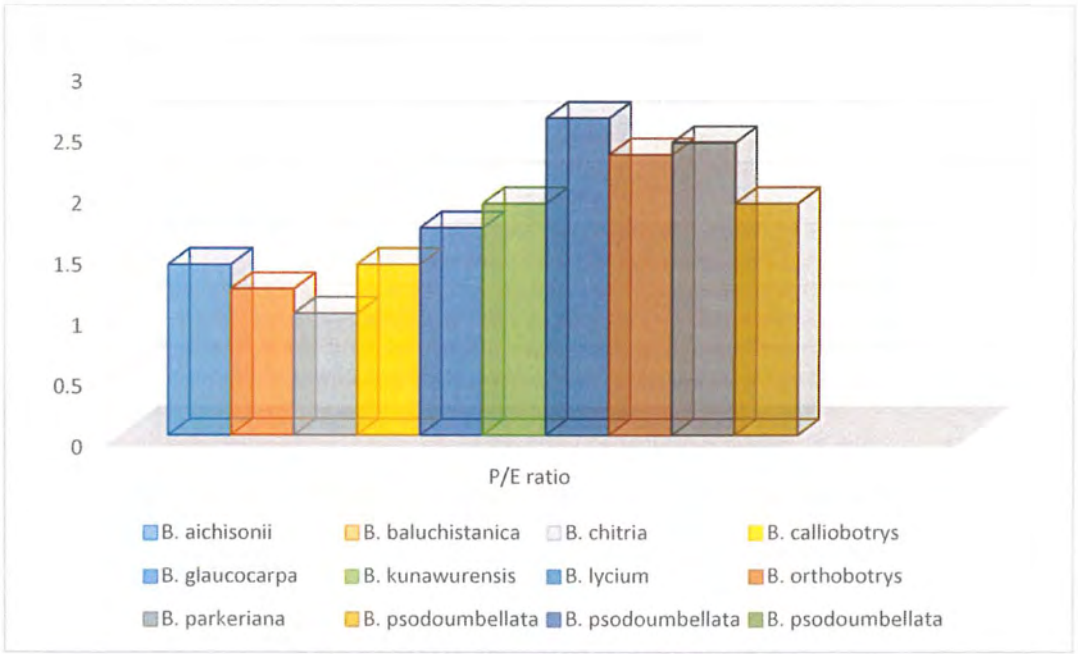

Figure 3.17: Comparative P/E ratio

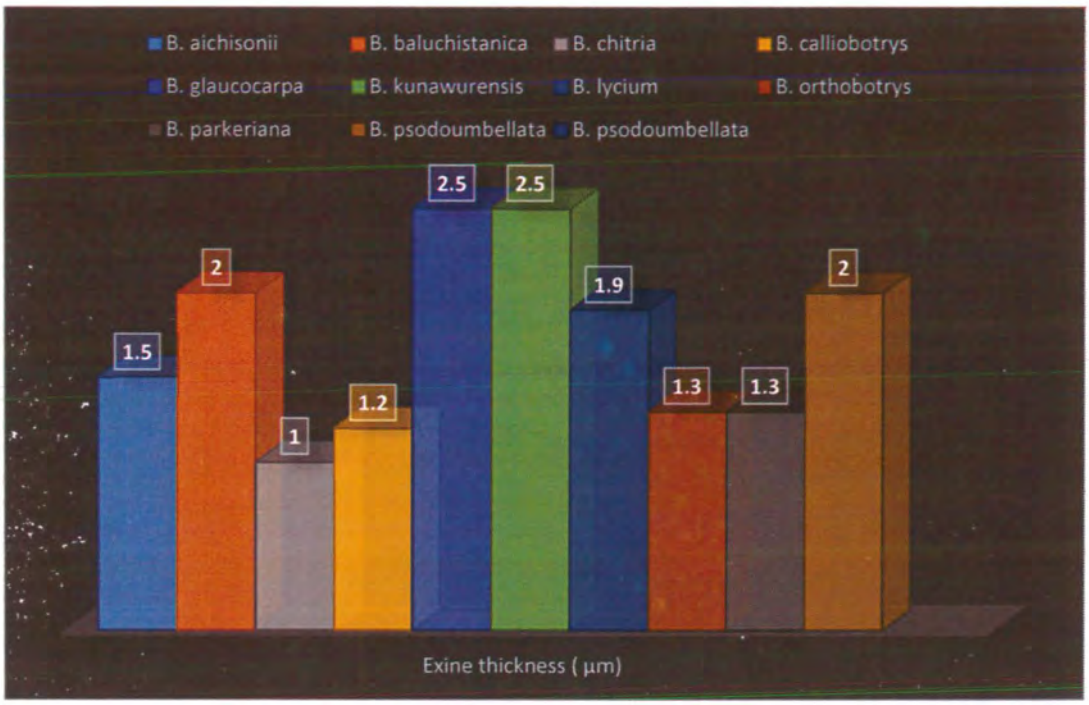

Figure 3.185: Comparative exine thickness (µm)

CHAPTER 4

DISCUSSION

4.1 Comparative morphological study

The comparative study of different plant structures viz anatomy and morphology has played a pivotal role in plant systematics and as a result explains plant phylogeny, biodiversity and evolution (Linnaeus, 1753). (Li *et al.*, 2010) stated that all the living members (500 in number) of genus *Berberis* are shrubs. Our results are agreed with Ying and Chen, as all the studies species were shrubs. Wide range of variation was showed by studied species in morphological features. Qualitative as well as quantitative characters were noted varying from species to species. (Klimko *et al.*, 2015) stated that for identification of individual species general leaf morphology is very important. In our comparative morphological study, a reasonable variation was noted in leaf form, length and width. The studied species were not only different in leaf size, shape and structure but also in plant height, internodes distance, berries size and shape, spines length and petiole length etc. Longest leaf was noted in *B. chitria* and *B. glaucocarpa* with a value of 70 mm followed by *B. baluchistanica* and *B. lycium* with 50 mm. Longest petiole was observed in *B. psodoumbellata* with a value of 10 mm while smallest was recorded in *B. parkeriana B. chitria* can be differentiated from other *Berberis* species by having leaves along with 5-12 large spinose on margins similar to that of oak tree while *B. baluchistanica* has entire leaves broad at the apex with no spinose. Both *B. baluchistanica* and *B. psodoumbellata* leaves were without spinose but distinguishing character was observed in leaf length. The farmer one with small and broad leaves and the later one with long and narrow leaves. The smallest leaf was recorded in *B. calliobotrys* that of 30 mm while leaf of *B. chitria* was two and half time longer than that of *B. calliobotrys*. Characterizing the phyllotaxis as whorled, each node possesses 4-9 leaves in the studied species. On young twigs leaves were arrange alternate while on old and long branches leaves were arrange in whorl. Great variation was observed in leaf color range from slightly red to dark green. In majority of the species leaves were glabrous, reticulate veined and petiolate rarely sessile or sub-sessile. Among the most conspicuous characteristics one was internodes distance ranges from 22 mm (smallest) in *B. calliobotrys* to 60 mm (longest) in *B. psodoumbellata*. All the plants possess woody stem and tallest one was recorded as *B. lyceum* that of 5 meter in length while smallest one was *B. aitshisonii* with a height of 2 meter. In early stages berries color, size and shape also play an important role in species differentiation but on ripening the color usually alter to dark red in every

species. In all studied species spines were of 3-fid more or less same in length except *B. lycium* in which middle on was noted longer than side ones.

4.2 Epidermal micro morphology of leaf

To clarify taxonomic status of plants, successfully anatomical studies have been done which helped in identification of different species (Evert, 2006). Anatomical studies along with morphological ones being used for taxonomical problems. For taxonomic purposes of grasses (Webster, 1983) studied anatomical characters. For distinguishing verities and basic taxonomic characters, silica bodies, papillae (Honaine *et al.*, 2009); (Mehra and Sharma, 1965); (Metcalfe, 1969); (Starr *et al.*, 2008) epidermal features of leaf like stomata, trichomes epidermal cells are helpful anatomical tools (Kadiri *et al.*, 2005). A number of diagnostic characters which are helpful in identification like shape and size of stomata, guard and subsidiary cells etc. are associated with anatomical study of epidermis (Dickison, 2000); (Moore *et al.*, 2008). Taxonomical as well as nomenclatural problems have been and still are in family Berberidaceae that is why difficulties may occur in collection and identification of the taxa. Because of this the family remains mostly neglected (Nickol, 1995). Morphological characters like arrangement of spines, coloration of berries, leaves and deciduous-evergreen nature of the genus *Berberis* have been widely revised by Taxonomists and was concluded that the above mention characters not only vary from population to population but within population and up to some extent within the same plan (Rao *et al.*, 1998), (Ahrendt, 1961). Efforts have been made up to some extent in the field of taxonomy , palynology, (Ahrendt, 1961) floral anatomy (Rao *et al.*, 1998) and DNA barcoding but still taxonomic problems exist in the genus (Kaplan, 2001). Being a problematic genus *Berberis* identification, collection and preservation for herbarium specimens is difficult due to its close resemblance and overlapping in morphological characters between species. The species of *Berberis* genus mostly blossom from March to august. An attempt was made to solve taxonomic problems in the species and to pave for further work, 10 *Berberis* species were sampled for leaf anatomical purposes from their natural habitats. In the current research work epidermal cells shape, cells wall pattern and stomatal features were revealed. Regarding foliar epidermis *Berberis* has not been investigated comprehensively still yet. In the genus, each species possesses a set of different characters on the basis of which it can be

distinguish from other species. For instance, as diversification has shown by epidermis and stomata were only confined to adaxial surfaces in *B. baluchistanica* and *B. aitshisonii*, largest epidermal cells in *B. baluchistanica*, maximum and minimum cells per unit area in *B. calliobotrys* and *B. aitshisonii* respectively. Munir *et al.*, 2011 used stomatal index values for differentiation of hybrid plant species from their parental ones an also states that stomatal index can be used as geographical indicator. As in most plant species stomatal index varies greatly and in case of our study was maximum in *B. kunawurensis* with the value of 31.9 (average) followed by *B. glaucocarpa* with an average value of 28.9 while minimum SI value was noted 2.6 in *B. baluchistanica*. Similarly, reasonable variation was observed in stomata length and width in all examined species. Including *Berberis* majority of angiosperms possess stomata on abaxial surface. The presence of stomata mostly on abaxial surface was the common character of all examined species; however, difference can be seen with *B. baluchistanica* and *B. aitshisonii*. Likewise, guard and subsidiary cells were found variable regarding length and width that is why based on foliar epidermal characters a taxonomic key was constructed which will not only help in identification but also in further investigation. (Gilani *et al.*, 2002) delimit *Digiteria* species by using comparative cells length and width of adaxial and abaxial surfaces. Different epidermal cells were found to be varied greatly in length and width in a single leaf, resulting in different cells number among the species. So, this detailed foliar epidermis analysis provided some additional features that can be used to identify and differentiate *Berberis* species. Quantitative as well as qualitative characters should not be neglected especially in foliar epidermal anatomy as these provide pivotal results in species identification. Collectively no work has been done on foliar epidermis on genus *Berberis*, so far however, (Munir *et al.*, 2011) investigate anatomical characters of *Berberis lycium* along with other wild fruits from northern Pakistan. We have no conflicts of interest and our results are same for *Berberis lycium* as they also revealed that *Berberis lycium* has paracytic stomata only on abaxial surface along with polygonal cells but for comparative studies or more or less identification at species level the details given are not sufficient. For upcoming studies, especially research work it would be fruitful and will provide a basis in resolving taxonomic complexes regarding new taxa. To the best of our knowledge, this is the first attempt based on foliar epidermis to resolve taxonomic confusion in *Berberis* identification and further investigation. We also

emphasis that the proper identification would only be possible with the aid of foliar anatomical characters. All the mentioned characters are meaningful to identify and analyzed species for further work. Despite this more work is needed on molecular level to further explore the gnus.

4.3 Palynological study

Because of identification problems the pollen morphology of family Berberidaceae is still poorly understood. Our study sought to provide important and useful information on palynology and is first report of the characters studied in the species. In this portion, we provide a discussion on various found variable characters noted during study which has implications in systematics. (Price and Ayers, 2008) stated that pollen shape can be influenced by various factors such as drying time, process and preparation. Pollen grains of 10 species of genus *Berberis* from family Berberidaceae shown a reasonable variation in various aspects such as size, shape and presence or absence of colpi etc. For systematic study of different genera pollen grains characters such as shape, size, number of apertures and exine ornamentation are not only important but also deciding factor for various systematic problems (Myoung and Yuon, 2012). Especially pollen shapes of the studied species could be categorized under different types which ranges from small to medium size. Pollen varies from spherical in *B. aitshisonii* to ovoid in *B. chitria*. Oblate type of pollens was observed in 2 species viz *B. calliobotrys* and *B. lycium* while prolate-spheroidal shape was recorded in *B. parkeriana* and *B. orthobotrys* and *B. psodoumbellata*. Sub-prolate and sub-spheroidal shape of pollen grains was noted in *B. glaucocarpa* and *B. kunawurensis* respectively. Earlier workers (Perveen and Qaisar, 2010) done pollen grains study of 12 *Berberis* species belongs to 2 genera and stated that pollens were tri and pantacolpate. In this regard our results are in accordance with them based on palynomorphological investigations for some species but we also observed tri and tetracolpate pollens in our studied species. As tricolpate pollens were observed in *B. kunawurensis*, *B. glaucocarpa, B baluchistanica* and *B. lycium* while tetra-colpate pollens were noted in *B. aitchisonii*, *B. orthobotrys*, and *B. parkerina*. Penta-colpate pollens were observed only in *B. psodoumbellata, B chitria* and *calliobotrys*. Our results are also agreed with Parveen and Qaisar regarding pollen shapes whose reported spheroidal and sub-prolate. The most conspicuous character common for all species was the presence of colpi.

4.4 Conclusion

Morphologically most of the *Berberis* species are similar and due to various overlapping characters identification of the species is a big problem. Therefore, an effort was made to solve the problem of identification in *Berberis* genus. A detailed account is provided by studying three important disciplines of systematics namely leaf epidermal anatomy, comparative morphology and palynology. It was concluded from leaf epidermal anatomy that with more or less variation epidermal cells were mostly same on adaxial surfaces of all species while a reasonable variation was noted on abaxial surface. Morphological study revealed that most *Berberis* species are same. Some detailed anatomical characters were investigated to delimit the species.

4.5 Recommendations

It is recommended that the family needs a detailed study on various aspects especially on anatomical as well as molecular level.

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SYSTEMATIC STUDIES OF FAMILY BERBERIDACEAE

by Saeed Ur Rahman

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