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Morpho-anatomical studies of *Monotheca buxifolia* (Falc.) A. DC. (Sapotaceae)

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Abstract

Thisresearch was aimed to explore the morphological and anatomical features of *Monotheca buxifolia* (Falc.) A. DC. (Sapotaceae) fruit, seed, leaf and barks of stem and root. The berry fruit of *M. buxifolia* has a single seed with ruminate endosperm and horizontally curved embryo. The simple bifacial leaf is covered by a single layer of upper epidermis followed by double layered palisade parenchyma with a ratio of 6.75± 0.5 to the epidermal cells. Lower epidermis is thoroughly furnished with simple and forked trichomes. The reticulate venation of leaf formed a vein islets number of 37.4±2.88 per mm^2 and vein termination number of 35.4±1.8 per mm^2. The stephanocytic stomata of leaf had a stomatal density of 77.4±3.57 per mm^2 on lower epidermis while the stomatal density on upper epidermis was comparatively very low i.e. 7.75± 0.97 per mm^2. The lower and upper epidermis had a stomatal index of 10.53±0.40 and 5.39±0.33 respectively. Stem and root bark show a characteristic arrangement of tissues. These features of *M. buxifolia* are important in tracing its taxonomic and phylogenetic affiliations, standardization and for pharmacognostic evaluation.

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Introduction

The evergreen, small statured, bushy tree of *Monotheca buxifolia* is around 10-12 meters long. It is commonly spread on the north facing slopes at altitudes as high as 2700-5300 ft. The slightly pubescent stem and branches are greyish brown in colour bearing small terminal and axillary pointed spines. The minute, bisexual, pentamerous flowers form clusters in the leaf and branch axils. Locally *M. buxifolia* is known as "Gwargurah, Gurgura, or Gargol". The ripe fruit is a small, tender, purplish-black, single seeded berry loved for its delicious taste. It is the only endemic species of the family Sapotaceae in Pakistan (Malik, 1984).Itis mostly confined to Northern mountain ranges of the Pak-Afghan region. In Pakistan it is widely distributed in Attock, Nowshehra, Nizampur, Cherat, Kohat, Kala Chitta hills, Chitral, Dir, Darra Adam Khel, Drosh, Loralai, Zhob, and Gorakh hills (Nasirand Ali 1972; Mirza *et al.*, 1992; Riaz *et al.*, 2010).

Anatomical studies are meant to expose the internal architecture of a plant which includes a look into the types, structures and spatial arrangements of various histological components of the conducting vascular tissue system, the embedding ground tissue system and the surrounding dermal system (Nancy and Dengler, 2002).

This serves as an additional lead towards correct identification of a plant (William, 2000). The characteristic arrangements and features of leaf tissues particularly those of epidermal layers are useful taxonomic tools since such features like the composition and thickness of cell walls, shape and size of the cells of epidermal layers, stomatal densities and types, epidermal appendages i.e. trichromes of different kinds can help in finding evolutionary track of a plant species and in tracing the links among various taxonomic groups (Taia, 2005; Babalola and Victoria, 2009).In raw powdered drugs, where the intact morphology of a plant has been lost, such anatomic features help in pharmacognosy, standardization and prevention of adulterations (Idu *et al.*, 2009).

In the present study morphological and anatomical studies of fruit, seed, leaf and barks of stem and root of *M. buxifolia* were carried out.

Methodology

Collection

Collections of fresh plant samples including leaves, fruit, stem bark and root bark of *M. buxifolia*were made in mid-July (fruiting season) from Dir, KP.

Morphological studies

Morphology of the selected plant parts was studied and their organoleptic features were noted following Evans (2002).

Anatomical studies

Stained permanent mounts of freshly cut fine sections of the plant specimens were prepared following the method of Johnson, (1940).

Their characteristic microscopic features were studied and photographed under microscope fitted camera.

Features of leaf surface

Occurrence of stomata and stomatal types

Both upper and lower epidermii of fresh *M. buxifolia*l eaf were peeled off and examined under microscope for stomatal occurrence and types following Dilcher, (1974).

Stomatal density and Stomatal Inde

The stomatal count in a square millimetre of epidermal tissue is simply called the Stomatal density. To determine the stomatal density of *M. buxifolia* leaf, epidermis was peeled from both sides, each was boiled with chloral hydrate, mounted on slide in a drop of glycerin and observed under microscope. 1mm² area was focused and the epidermal cells and stomata in that area were counted.

In a unit area of epidermal tissue the percent value of stomatal density is called its Stomatal index (Chaudhary and Imran, 1997). To find the stomatal index of *M. buxifolia* leaf following formula was used:

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

Where, (I= stomatal index; S= no. of stomata in a given defined area; E= no. of epidermal cells in that area (Evans, 2002)

Palisade cell ratio

Palisade ratio is an average count of palisade mesophyll cells attached to the underside of a cell of upper epidermis (Wallis, 1985). 2mm² pieces of leaf were decolorized by boiling with concentrated chloral hydrate solution and then observed under microscope. By slight rotation of the fine adjustment four adjacent epidermal cells were focused and the underlying palisade cells were counted. By dividing the sum of these values by 4 an average figure is obtained which is the palisade cell ratio (Evans, 2002).

Vein islets and vein termination number

A small area of leaf's photosynthetic tissue encircled all around by extremely fine veinlet is called vein islet. The average number of these vein islets in a square millimeter area (from mid-vein to the margin) of a leaf is its Vein islet number (Chanda *et al.,* 2010).

Vein termination number is the average count of terminal free ends of the finest ultimate veinlets occurring ina mm²area of leaf (Wallis, 1985). To determine these values, small pieces of *M. buxifolia* leaf were boiled in concentrated choral hydrate solution to remove pigments and decolorize the tissues. A square millimeter area of the decolorized tissue was focused under microscope to trace and count the vein islets and ultimate veinlet endings. Ten such readings were taken and their averages were calculated to obtain more objective and accurate values (Evans, 2002).

Results and discussions

Morphological studies

The alternate leaves of *M. buxifolia*are simple, petiolate (not exceeding 1.5-3 mm in length), expanded, elliptic, 3-3.5 cm in length and around 1.5 cm broad. The upper dark green surface of leaf is glabrous while the lower surface is glaucous and light green in colour. The leaf base is cuneate and the apex is blunt and rounded.

Table 1. Constant leaf surface values of *Monothecabuxifolia*.

Leaf surface feature	Range	Average/ (mm²)
Palisade cell ratio	6 to 6.75 to 7	6.75± 0.5
Vein islets number	35 to 38 to 42	37.4±2.88
Vein termination number	33 to 36 to 38	35.4±1.8
Stomatal number (lower epidermis)	72 to 78 to 82	77.4±3.57
Stomatal Index (lower epidermis)	10.03 to 10.4 to11.15	10.53±0.40
Stomatal number (upper epidermis)	7-9	7.75± 0.97
Stomatal Index (upper epidermis)	5.14 to 5.44 to 5.83	5.39±0.33

The entire margins are recurved and the leaf has a leathery texture. The reticulate veins of the leaf form an intricately obscure network. The round, dark blackish, single seeded fleshy fruit of *M. buxifolia*is a berry, 1-1.5 cm in diameter having a soft texture and a sweet fruity odour. Seed is rounded, light brown, 0.5-1 cm in diameter and endospermic. The fleshy fruit is a unique berry as it has a single seed. Similar berry fruits of larger size with one or two dicotyledonous seeds are found in *Mimusops elengi* (Gopalkrishnan *et al*., 2010).

The stem bark is greyish brown with a rusty tinge on outer side and light brown on inner side, irregular in shape, flat, rough and fibrous, having an uneven fracture, 7-9 cm long, around 3-4 cm wide and up to 0.5 cm thick. Root bark is dark brown on outer surface, light brown on inner surface, irregular in shape, curved but not quilled, 9-10 cm long, 2-3 cm wide and 3-4 mm thick, rough and fibrous (Fig. 1). The morphological characteristics of *Monotheca buxifolia* studied in the present work fairly coincide with the morphological details described by Nasir and

Ali (1980-2005) in the flora of Pakistan thus confirming our results. This set of observations provides detailed information of the plant which may be used as a significant taxonomic tool for its correct identification before any research based or medicinal exploitations.

Fig. 1. Morphology of various parts of *M. buxifolia*, **a**-Shoot with leaves and Fruits**, b-** Seeds, **c-** Stem bark**, d-** Root bark.

Various workers have reported more or less similar morphological observations in members of Sapotaceae like Gopalkrishnan and Ringmichon (2016) seeds of *Madhuca longifolia;* Moteriya *et al*., (2015) leaf and stem of *Madhuca indica;*Nagani *et al*., (2012) leaf of *Manilkara zapota*; Swenson and Munzinger, (2010) *Pycnandra subgenus* Sebertia shoot.

Anatomical studies

Anatomy of leaf lamina

In transverse section the dorsiventral leaf lamina has a distinct upper epidermis covered with thick cuticle and composed of rectangular, closely packed cells having few stomata and simple unicellular, non-glandular trichomes. The heterogeneous mesophyll tissue has an upper double layered palisade tissue composed of closely packed, narrow, elongated, cylindrical parenchyma cells. Oil globules and chloroplasts are frequently spread in these cells. Isodiametric cells of non-uniform sizes having large intercellular spaces form the spongy mesophyll tissue. Prismatic and cuboid crystals of calcium oxalate frequently occur in this tissue. Lateral vascular bundles traverse the mesophyll tissue. The collateral vascular bundle is arc shaped and is supported by layers of sclerenchyma cells. The much smaller cells of lower epidermis are compactly arranged and have numerous stomata. Several types of epidermal appendages are known to occur in family sapotaceae (Metcalfe and Chalk, 1950). The lower epidermis of *M. buxifolia* leaf is densely covered with unicellular, non-glandular, forked hairs/trichomes. Occurrence of such forked trichomes is a character specific to members of family Sapotaceae (Fig. 2a and 3b).Similar bifid long trichomes were found on the glabrous leaves of various Manikara species(de Almeida-Jr, 2013).

Fig. 2. Transverse anatomy of *M. foliabuxilia* leaf along lamina (a) (100µm)and midrib(b) (100µm).

iii. b. Midrib anatomy

In transverse section the mid rib region of the leaf of *M. buxifolia*has a planoconvex upper and a semi-circular lower surface. The single layered upper epidermis has compactly arranged, rectangular cells covered with a thin cuticle. It is followed by two to three layered thick hypodermis leading to thin walled parenchymatous cortex having a few oil cavities. The arc shaped vascular bundle surrounded by idioblasts has a patch of collenchyma cells above it and 3-5 layers of sclerenchyma cells below it. Xylem is composed of thick walled cells arranged in radial rows while small, compactly arranged, nearly circular cells form the phloem. There are fewer stomata and trichomes in the lower epidermis of this region (Fig. 2b).

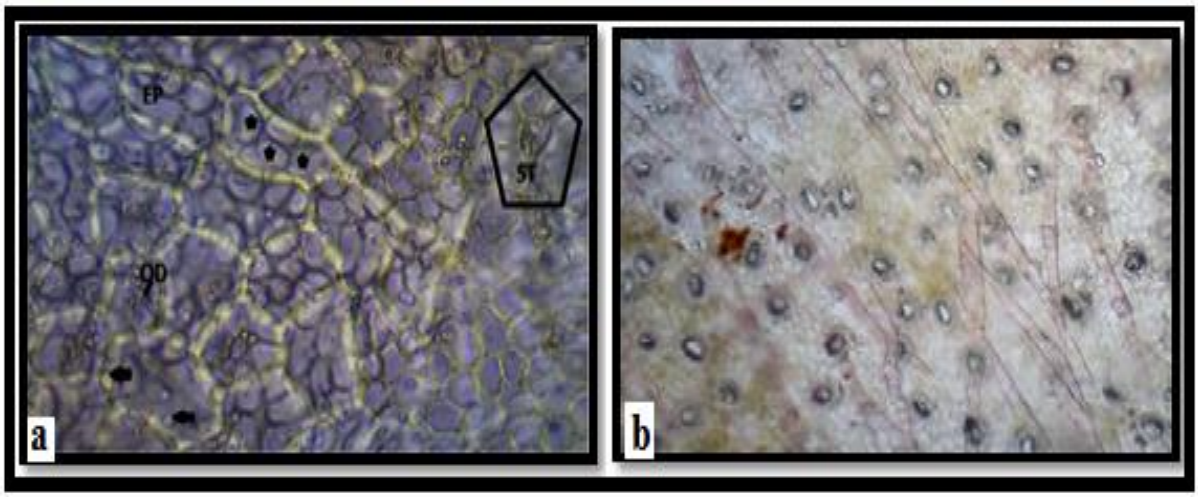

Fig. 3. *M. foliabuxilia* leaf (a) Portions of upper epidermis (100µm) having actinocytic stomata (ST); oil droplets (OD);starch grains (arrows); palisade parenchyma cells (stars)and (b) the lower epidermis (200µm),having stomata and non-glandular trichomes.

Leaf surface characteristics

Stomata of *M. buxifolia* leaf are mostly found confined to lower epidermis but the upper epidermis also hasa few small stomata. Such a condition where stomata are present on both upper and lower epidermis of leaf is called Amphistomatic. The lower epidermis of leaf had a stomatal density ranging from 72-82 (average 77.4±3.57) and stomatal index ranging

from 10.03 - 11.15 (average 10.53±0.40). A much lower stomatal density of around 7-9 stomata per mm² (average7.75± 0.97) was observed on the upper epidermis while the stomatal index of upper epidermis was found to be 5.39±0.33. A uniform orientation of stomata was noted on both the epidermii (upper and lower) with respect to the epidermal cells and each other. Each stoma on the lower epidermis was surrounded by radially elongated subsidiary cells forming a rosette of 7-9 cells(Actino stephanocytic stomata) (Fig. 4a) while only5-6 subsidiary cells with much pronounced radial elongations surrounded the guard cells of upper epidermis (Actinocytic stomata). These are both types of Stephanocytic stomata (Carpenter, 2005).Palisade cell ratio of *M. buxifolia*leaf was found to range from 6 to 6.75 to 7 (average 6.75± 0.5) (Fig 3a).

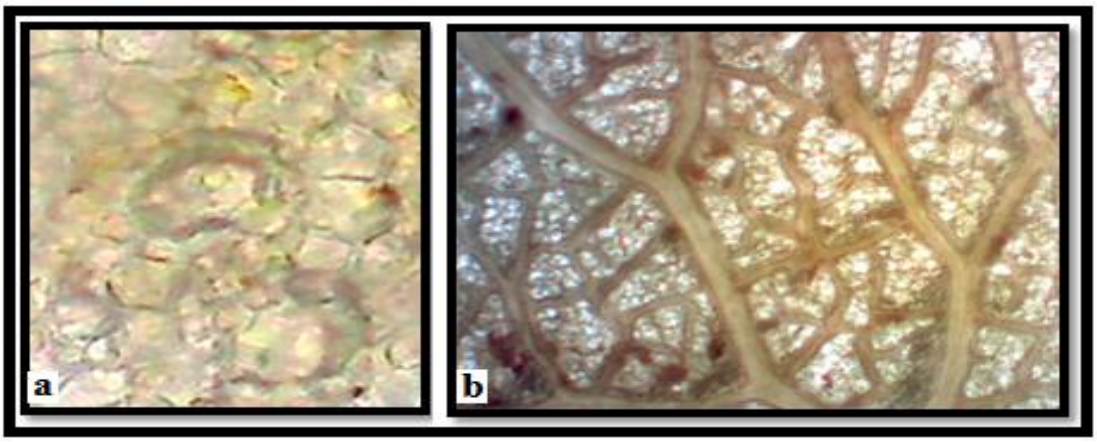

Fig. 4. *M. buxifolia* leaf epidermis; a-Actinostephanocytic stomata(200µm), b- a portion of leaf showing Vein islets and vein terminal endings (100µm).

The vein terminals were found to have single or forked ends while angled veinlets surrounded small chambers of photosynthetic tissue to form vein islets. The average vein islet number of *M. buxifolia* leaf was 37.4±2.88/mm²(ranging from 35 - 38 - 42 while the vein termination number ranged from 33 to 36 to 38 per mm²(Average 35.4±1.8) (Fig 4b; table 1).

Stomatal characteristics like the occurrence, types, arrangement and orientation of stomata on the epidermal layers; stomatal densities and indices are can help in finding evolutionary and taxonomic relationships among various plant species, in standardization, tracing origin and identification of leafy crude drugs (Hameed *et al.*, 2008; Evans, 2002). Palisade cell ratio is one such feature which remains constant in leaves of a particular species at all ages and habitats. This feature can also serve as a tool for correct identification, standardization and quality test of foliar herbal drugs (Shruthi *et al.*, 2010). Stomatal arrangement on epidermis is a manifestation of adaptive capabilities of plants to their surrounding environment. Thus provide clues for impact of various environmental factors on plants. Various members of family Sapotaceae have been standardized by determination of their leaf constants by researchers around the globe like the leaf surface characteristics of *Madhuca indica* were studied by Moteriya *et al.*, (2015); de Almeida-Jr *et al.*, (2013) determined the surface characteristics of *Manilkara dance* leaf; leaf constants of *Manikara zapota* were determined by Nagani *et al.*, (2012); Chanda *et al.*, (2010) *Manilkara hexandra* (Dubard) leaf.

The observations and findings of these researcherss how close similarities with our findings in the present research work which speaks for the relationship among the members of this family. It also elaborates the significance of these peculiar leaf constants in tracing origin and correct identification of foliar herbs and drugs.

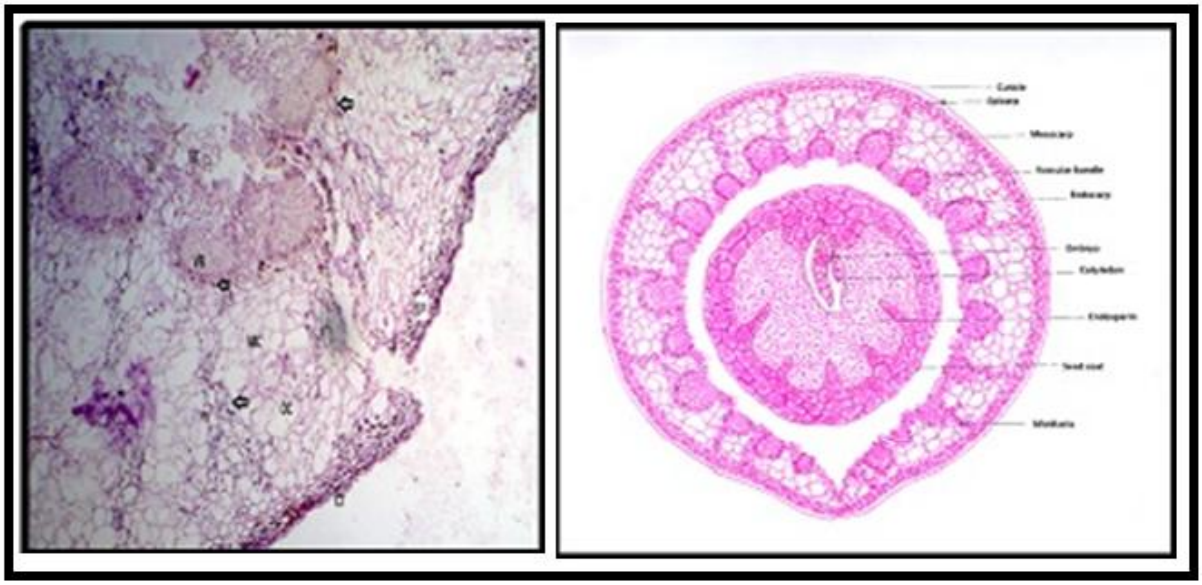

Fig. 5. Transverse fruitanatomy of *M.buxifolia* (Arrows: Idioblasts; CT: Cuticle; EC: Endocarp; EP: Epicarp/Exocarp; MC: Mesocarp; OC: Oil cavity; VB: Vascular bundle) (100µm).

Anatomy of fruit and seed

The Pericarp of mature *M. buxifolia* fruit in cross section was differentiated into a 3-5 layered Epicarp composed of thick walled rectangular pigmented cells, rich in flocculated contents, a few stomata and covered by a thin cuticle; a much broader parenchymatous Mesocarp composed of about50-60 layers of cells of varying size and shapes with a few intercellular spaces and interspersed with vascular bundles. Surrounding the vascular bundles are idioblasts and cells having thick lignified walls; an Endocarp of small, tightly packed cells filled with oil globules and starch grains. At instances the cells of inner layers of endocarp are collapsed and disintegrated.

The centrally placed single seed of *M. buxifolia* fruit is orbicular in outline. The thick seed coat is composed of 10-15 layers of polygonal, isodiametric, lignified sclerenchyma cells. Distinction between closely spaced testa and tegmen is not easy however, the cells of the inner layers of seed coat (tegmen) have intercellular spaces and are larger than the compactly arranged peripheral layers (testa). Endosperm which lies next to tegmen is divided into many incomplete chambers by invagination of the tegmen, thus called ruminate endosperm. The nucellus is collapsed to form perisperm. The endosperm is composed of parenchymatous cells of varying shapes having dense cytoplasm, contain aggregates of calcium oxalate, starch granules and oil droplets. A single layered epidermis composed of rectangular cells surrounds the endosperm.

The endosperm occupies major part of the seed. Two small cotyledons and a much reduced embryo which is curved horizontally are enclosed within endosperm. (Fig. 5).

Anatomy of stem bark

Stem bark in transverse section has an outermost Periderm differentiated into Cork composed of 2-5 rows of dark brown, radially arranged, narrow, rectangular cells; a compact Phellogen and a loosely arranged Phelloderm. Both phellogen and phelloderm are around 2-3 layered thick. Next is a much broader cortex the cells of which are rectangular in shape, thin walled, closely packed, interrupted by patches of secondary phloem separated by medullary rays. Cortical cells are rich in starch grains and calcium oxalate crystals (Fig. 6a).

Anatomy of root bark

The T.S of root bark shows an outer cork composed of 5-7 layers of compactly arranged, thick walled, rectangular cells followed by continuous layers of

small, tangentially elongated, thin walled cells of phellogen and phelloderm hardly distinguishable from each other. Next is the parenchymatous cortex leading to secondary phloem composes of sieve tubes, phloem fibres and phloem parenchyma. It is traversed by phloem rays (Fig. 6b).

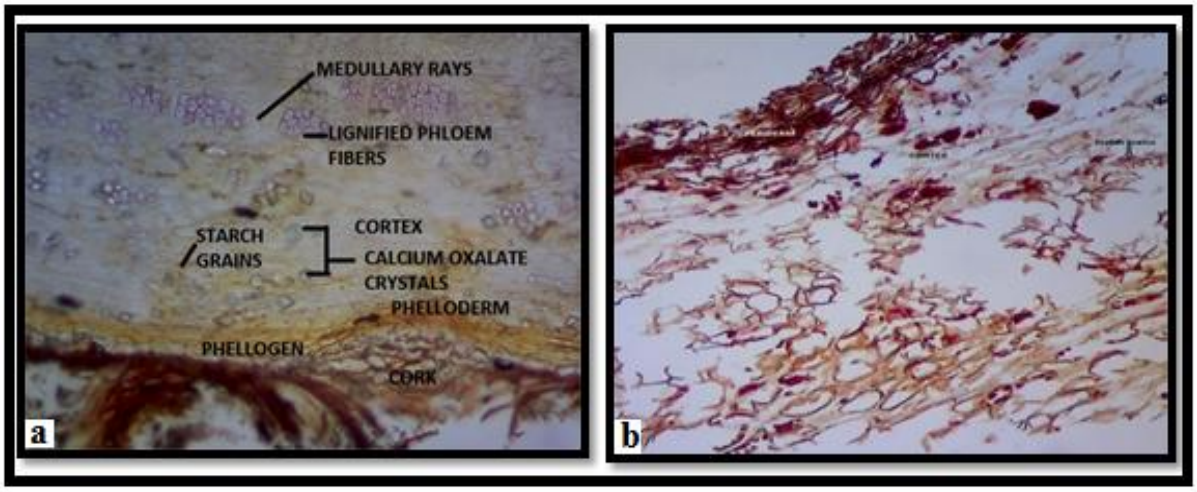

Fig. 6. Tranversesection of *M.buxifolia*a- stem bark (100µm); b- root bark (100µm).

The anatomical features are used for centuries as indices for taxonomic placement of plants (Al-Edany *et al.*, 2012). Wood anatomy is subject to change by the prevailing environmental conditions thus not very reliable for taxonomic handling but still there are orders and families that show similar anatomical peculiarities and can be used in tracing taxonomic relations among various plants and groups (Baas *et al.*, 2000).

The morphological and anatomical details reported here will prove useful in preparation of a comprehensive monograph for proper identification and future exploitation of *M. buxifolia*.

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