

# Morphological, anatomical and preliminary phytochemical characterization of *Buddleja madagascariensis* Lam.

Vaishali Dobriyal<sup>1</sup>, Saurabh Guleri<sup>1</sup>, Maneesha Singh<sup>2\*</sup>

<sup>1</sup>School of Basic and Applied Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun-248001, Uttarakhand, India,

<sup>2</sup>School of Agricultural Sciences, Shri Guru Ram Rai University, Pathri Bagh, Dehradun -248001, Uttarakhand, India

## ABSTRACT

*Buddleja madagascariensis* Lam. is a perennial invasive shrub distributed in tropical and sub-tropical regions of the world. The present study was conducted on morphological, anatomical and phytochemical characterization of *Buddleja madagascariensis* and the results revealed that plant height varied from about 188-191 cm, and the whole plant was covered with trichome which may be glandular and eglandular and unicellular or, multicellular. Inflorescence was terminal and axillary in position, thyrsoid panicle. Anatomical and histo-chemical studies of the transverse section of various parts of the plant revealed the presence of steller tissues, starch in cortical and pith region. Phytochemical analysis of the crude extract showed the presence of carbohydrates, proteins, saponins, flavonoids, phenolic compounds and tannins. But alkaloids, glycosides and free amino acids were absent. Thus, characterization of plants on the basis of these parameters could be used as tools to distinguish the crude drugs of plants from adulterants, used in the preparation of traditional medicines and used as diagnostic keys. Also, it is useful in the future for revealing the importance of plants and phytochemical resources for the conservation of resources.

**KEYWORDS:** *Buddleja madagascariensis*, morphological characters, phytoconstituents, histochemicals

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**\*Corresponding Author:**  
Maneesha Singh  
Email: singhmaneesha2@gmail.com

## INTRODUCTION

Biodiversity is the variety and variability of life which includes all the plants and animals that live and grow on the Earth, all the habitats in which they survive, and all the natural processes of which they are a part. The earth supports an incredible array of biodiversity. Globally around 1.75 million species have been described and about 14 million species are estimated to be on earth. India is the country with large ecological diversity (from sea levels to highest mountains- forests, grasslands, wetlands, coastal and marine and desert). India's total geographical area is 692,027 km<sup>2</sup> and contribute about 2.4% of world total landmass and comes under 17 mega diversity country in the world (Singh & Dash, 2013). About 7-8% of world biodiversity is contained by India, in which it contributes in the presence of 11.4% of world's flora (Karthikeyan, 2009). India is diverged with 47513 species of plants are known yet, 28% of which are endemic to the country. The Northern part of India harbors a great diversity of plants because of the majestic Himalayan range. The Himalayan region of India is bestowed with a variety of natural resources

which have been exploited by mankind since times immemorial. Samant and Pant (2003) revealed that 1748 species were native to Himalayas and about 493 species were exotic, it indicates that these species had the ability to establish in diverse environmental conditions. Out of 15,000 species of flowering plants about 17% are considered to be of medicinal value (Jain, 1968). Medicinal and Aromatic plants constitute great economic and strategic value for Asia and Pacific. It has been estimated that about 30% of pharmaceutical are derived from green plants and this percentage has raised considerable in recent years. India has about 8,000 species of known medicinal plants and about 1,000 plants have been used in the traditional system of medicine viz., Ayurveda, Unani and Siddha, while tribal use 7500 plant species for medicinal purposes (Anon,1998a,b). Medicinal plants possesses appetizers, emollient, cooling, astringent (*Ocimum basilicum*), hypertensive (*Rauvolfia serpentina*), analgenic, antipyretic (*Andrographis paniculata*), antioxidant (*Aloe barbadensis*) properties. Medicinal and aromatic plants are used in the pharmaceutical industries in the preparation of herbal products as well as value added and consumer articles like cosmetics, tooth paste, soap etc (Chopra ,1956). Using

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the current global rates of species extinction at around 10 to 12% of the plants *i.e.*, around 800-1000 species are likely to be threatened as estimated by the International Union for the Conservation of Native and Natural Resources (IUCN). The major concern is that about 95% of the medicinal plants are collected from the wild population and over 70% of the plant collections involve destructive harvesting mainly because of the use of the plant parts like roots, bark, wood and whole plant (Tiwari, 1999). Thus, there is an urge for conserving the plant biodiversity along with to search out new plants which can be alternatively used for the extraction of drugs.

*Buddleja madagascariensis* commonly known as smoke bush belongs to family Scrophulariaceae is native to Madagascar found in temperate, sub-tropical and tropical region of the world. The plant is characterized by scented yellow to orange flowers, whole plant covered with whitish stellated hairs, thyrsoid panicle inflorescence, basifixed stamens, and multi locular ovary with stellated hairs. *Buddleja madagascariensis* Lam. can reproduce from stem fragments and is capable of resprouting quickly after a fire (Flora Base, 2010). The species was identified by mimengoside B, a triterpenoid saponin, in its leaves and is useful to treat asthma, coughs, and bronchitis and for dyeing clothes (Emam *et al.*, 1997). The leaves show diuretic activity due to presence of the flavonoid and iridoid content (Houghton & Mensah, 1999). It is grown as a popular ornamental plant. Knowledge about diverse applications, the present study on *Buddleja madagascariensis* was conducted on characterization of *B. madagascariensis* traditional methods like morphological, anatomical, histochemical and phytochemical basis which can be valuable, and can be beneficial for determination of bioactive potential along with bio resource conservation.

## MATERIAL AND METHODS

### Experimental Site and Plant Material

The experiment was carried out in the Department of Botany, School of Basic and Applied Science of Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand. Fresh and disease free aerial parts of *Buddleja madagascariensis* Lam. (Family-Scrophulariaceae) were collected from different places of Doon valley, Uttarakhand and collected samples were cleaned. The half of plant sample was dried in shade at 25 °C to 30°C for 10-15 days and then crushed to coarse powder using grinder. The dried plant material was stored in paper bags for phytochemical analysis.

### Morphological Study

Plant samples were subjected for morphological studies which includes plant height, length of third inter node from the ground, stem girth, leaf length, leaf breadth, inflorescence length, number of primary and secondary branches, length of petiole, leaf arrangements etc.

### Anatomical Study

The fresh plant part were preserved in alcoholic acetic solutions which was prepared by the mixture of 50% of alcohol, 40% of

formalin, 2% of acetic acid. The preserved plant material was subjected for microscopic studies.

The transverse section was dehydrated in 30%, 50%, 70% and 95% solution of ethanol. For double staining safranin and fast green was used. Double staining was done so that the living tissues can be differentiated from the dead one and at last this double stained section was fixed in the slide by the use of Canada balsam and kept for further studies.

### Histo-chemical Analysis

The thin section of the preserved plant material was examined under microscope using different stains and to evaluate morphological and organoleptic characters.

- Safranin – for vascular bundles
- 20% H<sub>2</sub>SO<sub>4</sub> - Stele and calcium oxalate
- Gram's iodine – for starch in cortical medullary ray cells
- Phloroglucinol – for stellar tissue

### Phytochemical Analysis

Fifty gram of plant powder were soaked with 70% ethanol for 48 hours at room temperature. After that the contents were refluxed for 2 hours at room temperature, cooled and filtered. The extracted drugs were then taken in 100 ml beakers and their solvents were evaporated on the water bath till they were finally reduced to dryness to get dry extracts. The extract was then transferred to previously weigh airtight containers and stored in a refrigerator until they were screened for phyto-constituents and antioxidant activity.

Percentage yield of the crude extracts were calculated with the formula:

Percentage yield = weight of extract / weight of powdered drug taken X 10

The solvent extracts of *B. madagascariensis* were subjected to preliminary phytochemical investigation. The various test such as alkaloids (Dragendorff's reagent), proteins (Biuret test), carbohydrates (Molisch's test), flavonoids (Lead acetate test), glycosides, amino acids (Ninhydrin test), phenolic compounds (FeCl<sub>3</sub> solution), saponins (Foam test) were conducted (Yadav *et al.*, 2014; Singh & Naithani, 2014). The presence of phyto-constituents were analysed on the basis of colour changes. The values are expressed as mean ± standard error.

## RESULT AND DISCUSSION

*B. madagascariensis* commonly known as smoke bush, belongs to family Scrophulariaceae, is an aromatic shrub native to Madagascar, grown in tropical parts of the world. This plant is known as an aggressive invader of disturbed areas. Mostly plant is grown as ornamental with less economic values. Essential oil extracted from the aerial parts of the plants and used for the treatment of headache, burn, wounds, ulcers etc (Houghton & Mensah, 1999). In the present study, the plant was subjected

to morphological, anatomical, histo-chemical and preliminary phytochemical characterization collected from different locations of Uttarakhand. The findings of the present studies were as follows:

## MORPHOLOGICAL CHARACTERIZATION

### Vegetative Characters

The plant was the perennial shrub with height varying from 188 to 191 cm. It was branched from about 10-15 cm above the ground level. The whole plant was covered with glandular and eglandular trichome responsible for reduction in water lose making the plants drought resistance (Figure 1) (Emam *et al.*, (1997). Leaves were simple, lanceolate showed the opposite decussate phyllotaxy (Fig. 2). Leaves were petiolate with tapering base, serrate margin, acute apex with unicostate reticulate venation up to 15.5 cm-16 cm long and 2.5 cm-2.6 cm wide. Petiole was 0.8 - 1.2 cm long, slightly grooved stalk with continuous epipodium (Table 1). Both the surfaces of the leaves were covered with dense trichome. Hair density was higher on the abaxial surface than the adaxial surface at their young stage as compare to maturity (Fig.3).

Inflorescence was terminal and axillary in position, thyrsoid panicle (Fig. 4). About 6 to 8 flowers aroused from each node, sessile, ebracteate, epigynous, actinomorphic, hermaphrodite. Sepals were 4 in number, gamosepalous, valvate aestivation, persistent, inferior, calyx teeth absent. Petals were 4 in number, gamopetalous, valvate aestivation, actinomorphic, inferior and yellow in colour. Male reproductive part i.e., androecium consist



Figure 1: (a, b) Habit of the plant *Buddleja madagascariensis*

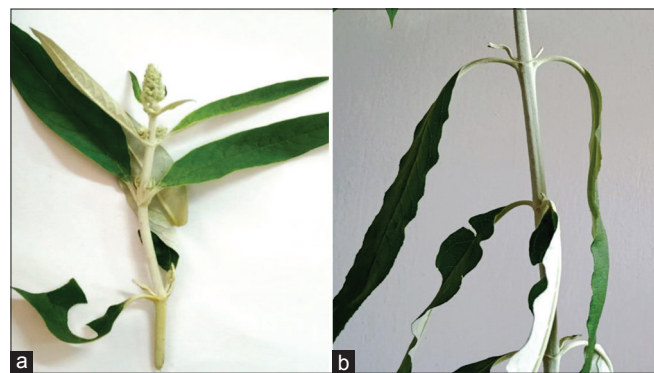


Figure 2: (a, b) Plant showing Phyllotaxy of leaves of *Buddleja madagascariensis*

of four stamens, basifixed, epipetalous, introse, ditheous. Gynoecium was monocarpellary, syncarpous. Ovary showed hypogynous condition, multilocular, axile placentation, topped with style and clavate shaped stigma (Fig. 5). Flowering occurs mostly during spring and summer, but flowering would also be reported throughout the year. The fruit were round or oblong berries (2.5-5 mm across) containing many small seeds. These fruit turned from whitish in colour to dark bluish, purplish or violet as they mature. The seeds are oval in shape. The present morphological characters were similar to the studies reported by Bhattacharya *et al.* (2015) and Oxelman *et al.* (2004) in *Digitalis* and *Verbascum* of Scrophulariaceae family.

### Anatomical Characterization

Anatomy has been considered as a potential source of systematically and phylogenetically significant data. Anatomical studies provide highly reliable and additional evidences for the taxonomic delimitations (Bhattacharya *et al.*, 2015).

### Anatomical Study of Root

In the transverse section of the older root, on the outer side the bark was present followed by cork (phellem) and cork cambium (phellogen) (Fig.6). Inside the cork cambium, secondary cortex (phellogen) of about 6-8 layers thick was present. The cortical region was followed by secondary vascular tissues in endarch condition showed similarities between study on *B.*



Figure 3: (a, b) Adaxial and abaxial surface of *B. madagascariensis* leaf

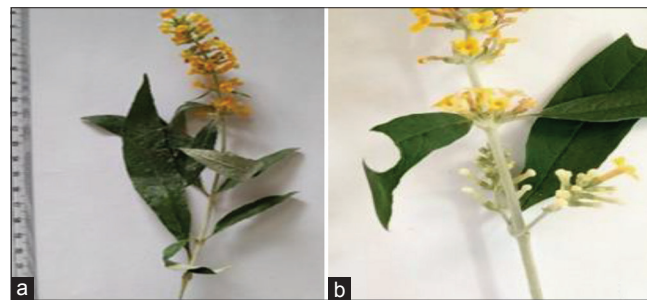
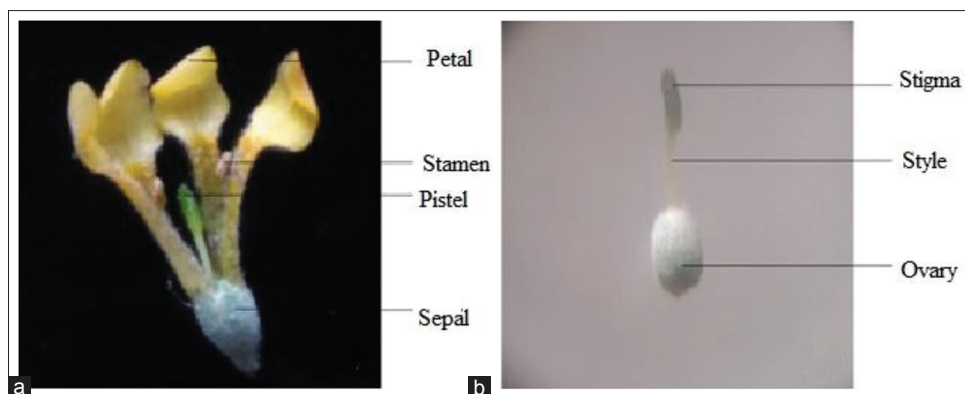
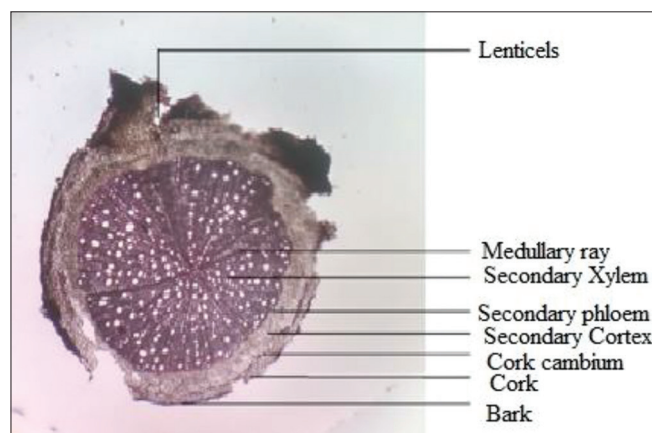


Figure 4: (a, b) Twig showing thyrsoid panicle inflorescence in *Buddleja madagascariensis*



**Figure 5:** (a, b) Single flower showed the position of anthers and carpel



**Figure 6:** Transverse section of root (Older)

*indica* by Oxelman *et al.* (2004) and in the present analysis of *B. madagascariensis* (Fig.7) and medullary rays were arranged in 40-45 in row without pith.

### Stem Anatomy

Transverse section of the stem showed a nearly circular outline in young part of the shoot but it became square in older shoot (Fig.8). Whereas, in *B. indica* quadrangular in younger and rounded shape in older stem section reported by Oxelman *et al.* (2004). A single layered epidermis was covered by dense hair like extensions, which were of different shapes and types. The epidermal layer was followed by the chlorenchymatous cell layer called hypodermis which was about three to four layered in thickness, followed by loosely arranged parenchymatous cells which were about three to four layered. Vascular bundles were arranged in a ring, collateral, open and have an endarch condition. The medullary rays varied in 20-40 in numbers. Phloem was present in the form of cluster in the inner cortical region and some of them were pressed by xylem (Fig.9). The nodal portion of stem showed two opposite leaf traces (Fig. 9 c). The center was occupied by parenchymatous pith.

### Petiole Anatomy

The petiole was sulcate with obtuse margins (Fig.10 a). In the transverse section, both the adaxial and abaxial epidermises were

consisting of single layered rectangular and oval cells. Both the surfaces were covered with dense trichome. Chlorenchymatous hypodermis was loosely packed present just below the epidermis (Fig.10 b). Vascular bundles were located in the parenchyma tissue. Petiole consisted of a total of three vascular bundles, with a big bundle in the middle and a single small vascular bundle at each corner. The big bundle was arc-shaped which were surrounded by parenchyma cells and the smaller ones with bundle sheath cells (Fig.10 c).

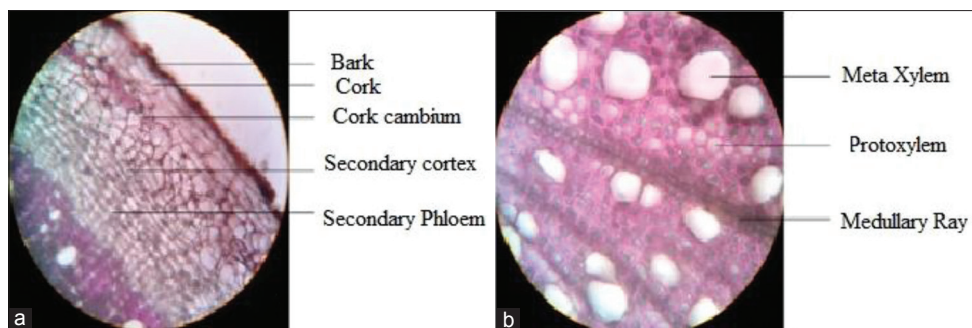
### Leaf Anatomy

Young leaf of the plant was covered with densely packed hair all around the surface, the density of trichome varies in both the surfaces, *i.e.*, adaxial surface of the leaf were less dense than that of abaxial surface (Fig.11). The trichome raised from the epidermal cells and also varied in shape and size, as some have globular head and some with triangular head, trichome were found in short, long, branched and unbranched forms (Fig.12). The outermost layer of the leaf was irregular epidermis which was covered with a cuticle layer. The epidermis was followed by 3-4 layered mesophyll cells densely packed and containing chlorophyll, the concentration of chlorophyll varies, *i.e.*, higher in lower surface (Fig.12b). In the midrib part the epidermal cells are followed by the loosely packed hypodermal cells. Phloem was pressed against each other and in middle conducting tissue was present in continuation (Fig.12c).

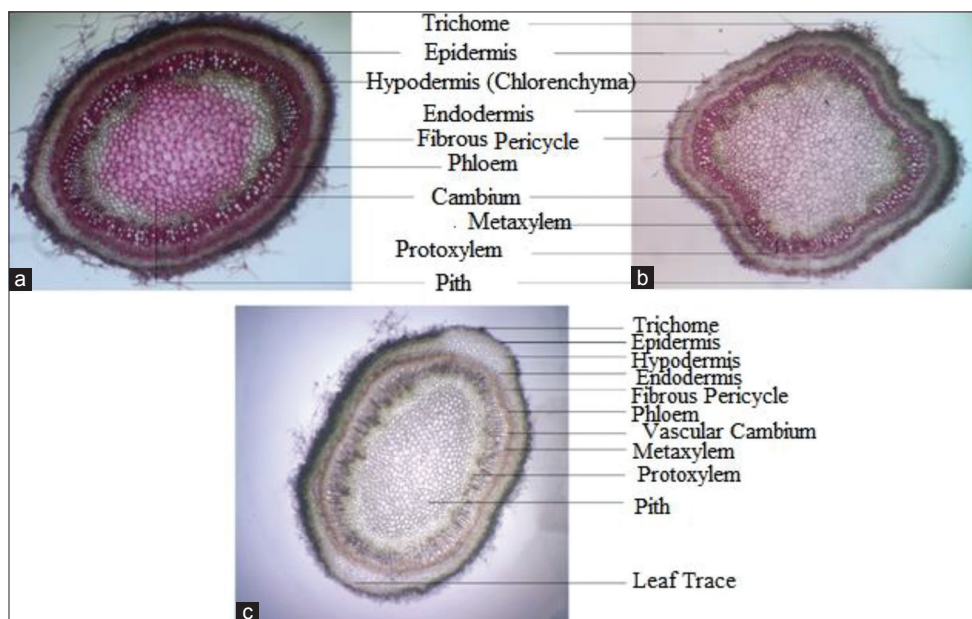
Stomata were present on both the surfaces. The lower surface contained more stomata than the upper, but the lower surface was densely packed with trichome. Stoma is anomocytic type surrounded by four to five subsidiary or accessory cell (Fig. 13). This work is similar as earlier reported by Oxelman *et al.* (2004) in *B. indica*.

### Trichome

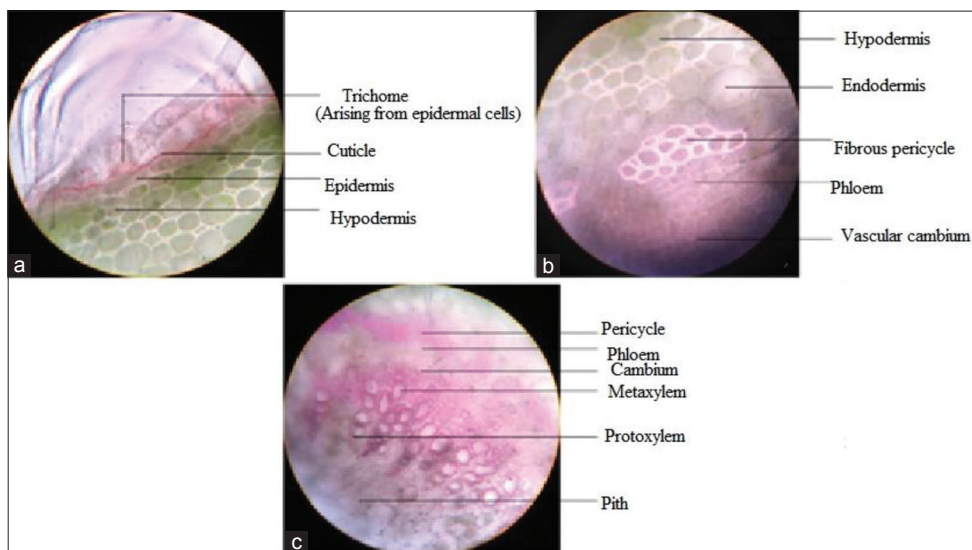
Trichomes were the white hair like structure present all over the surface of the plant with different shape and size. Trichomes were found unicellular as well as multicellular (Fig.14 a, b, c, d). Glandular and eglandular trichome were evident to be arising from the epidermis. Eglandular hairs were multicellular and long or unicellular, in contrast glandular hairs were capitate type (with 1-2 stalk cells and 1-3 head cells). The earlier report



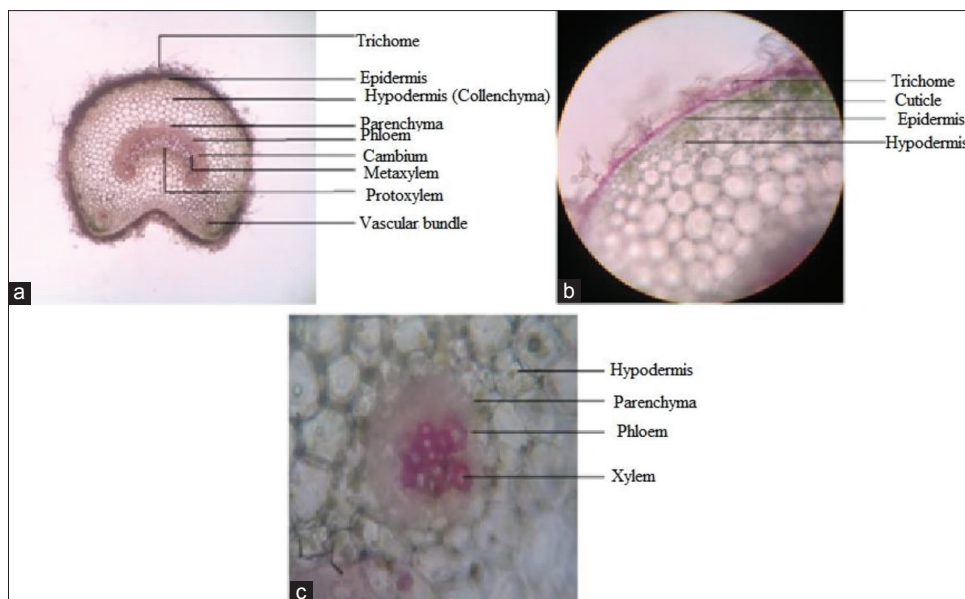
**Figure 7:** (a, b) Transverse section of root (Older) showing the position of cork, cork cambium and vascular tissues



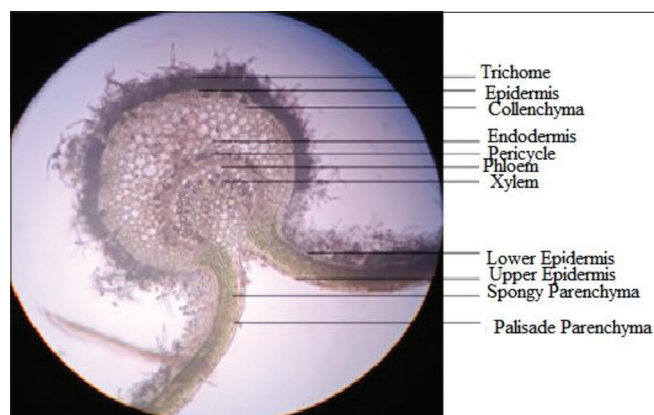
**Figure 8:** (a, b, c) Transverse section of stem, (a) Younger and (b) Older showing the position of various tissues and vascular bundles in internodal portion, (c) Stem through nodal portion



**Figure 9:** (a, b, c) Transverse section of stem, (a) Single layered epidermal tissue showing the position of trichome, (b) position of hypodermis, pericycle and phloem, (c) position of protoxylem and metaxylem



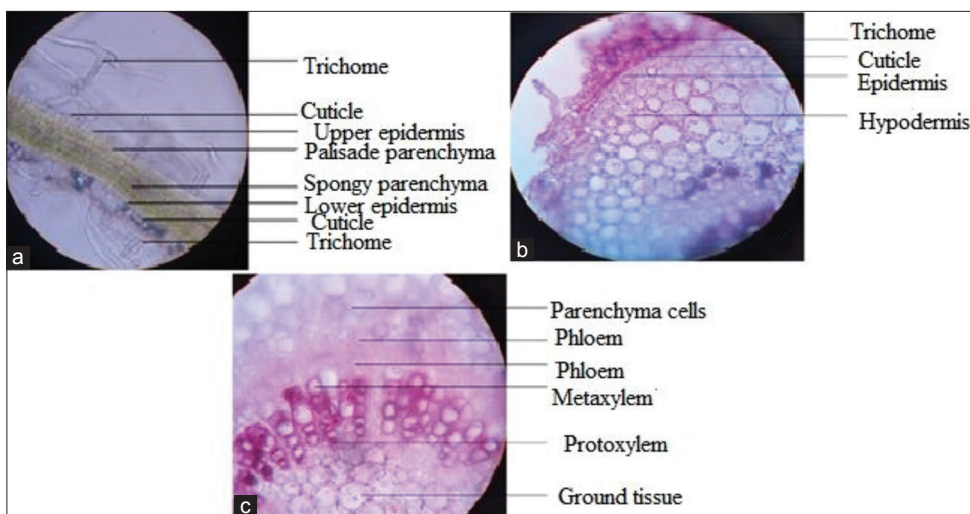
**Figure 10:** (a, b, c) Transverse section of petiole, (a) cellular organization of petiole, (b) position of hypodermis and trichome on epidermal layer, (c) arrangement of phloem and xylem in vascular bundle



**Figure 11:** Transverse section of leaf.

**Table 1:** Morphological characterization of the different plant samples of *Buddleja madagascariensis*

S.No.	Characters	Sample 1	Sample 2	Sample3	Mean ± SE
1.	Plant height (cm)	188	195	191	191.33 ± 1.65
2.	Length of third inter node (cm)	43	41	44	42.67 ± 0.73
3.	Stem girth (cm)	11.5	10.2	10.6	32.30 ± 0.13
4.	Number of primary branches	4	3	5	4.00 ± 0.47
5.	Number of secondary branches	12	13	15	13.33 ± 0.73
6.	Leaf length (cm)	15.1	15.2	15.6	15.30 ± 0.41
7.	Leaf breadth (cm)	2.6	2.6	2.5	2.57 ± 0.13
8.	Length of petiole (cm)	0.9	1	12	1.03 ± 0.26
9.	Length of inflorescence (cm)	6.9	7.2	7.2	7.10 ± 0.02



**Figure 12:** (a, b, c) Transverse section of leaf, (a) T.S. of leaf showing the palisade, spongy parenchyma and trichome, (b) Single layered epidermal tissue showing the position of trichome and hypodermis, (c) position of vascular tissue

by Oxelman *et al.* (2004) in the *B. indica* showed the presence of only non-glandular 4-armed stellate trichomes, but in the present study showed more than one type of trichome.

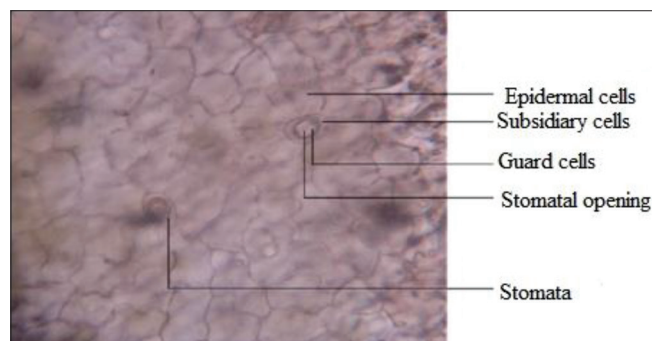
### Histo-chemical Analysis

The histo-chemical studies is an important microscopic method for the qualitative and quantitative analysis of chemical constituents including carbohydrates, calcium oxalate, protein, lipids to locate their presence in different tissues such as cortical region, vascular bundles and pith by using stains, indicators. The implication of histochemical parameters is useful for the characterization and identification

**Table 2: Histological analysis of transverse sections of the plant parts**

S. No.	Reagent	Histological analysis	Root	Stem	Leaf	Petiole
1.	Sulphuric acid (20%)	Calcium oxalate	-	-	-	-
2.	Iodine solution	Starch in cortical and pith cell	+	+	+	+
3.	Phloroglucinol+conc. HCl+alcohol	Stellar tissue	+	+	+	+

\* (+) and (-) signs indicates presence and absence of the compound, respectively.



**Figure 13:** Structure of stomata where stoma was surrounded by guard cells

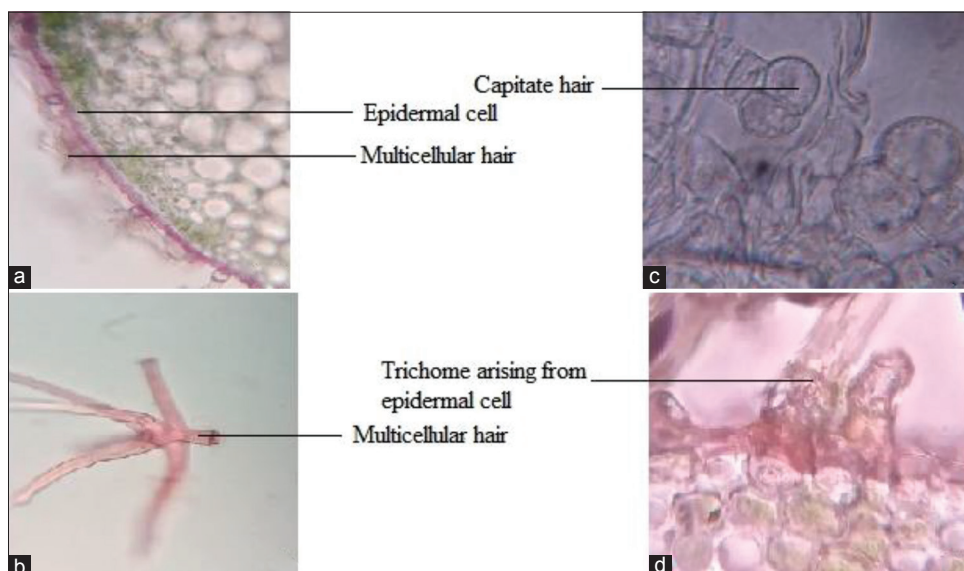
of inter and intra specific variations (Rathnakumari *et al.*, 2002); Keirman, 1999); Krishnan & Dayanandan, 2003). In the present study, transverse section of the root, stem, petiole and leaf were mounted in suitable chemical reagents to determine the presence of various chemical substances and their zone of distribution. The transverse section in different regions showed the presence of starch by staining with iodine. Starch granules were generally reported in parenchymatous cells of cortical region and in pith of stem and petiole (Table 2). The starch grain was round and oval in shape. The concentration of starch varied in different parts of the plants. The starch deposition was maximum in root as the lumen of vessels has taken dark blue colour (Figure 15). The calcium oxalate crystals were absent in all the tissues. The stellar tissues were stained pink when dipped in phloroglucinol (Figure 16).

### Appearance and Yield of Crude Extracts

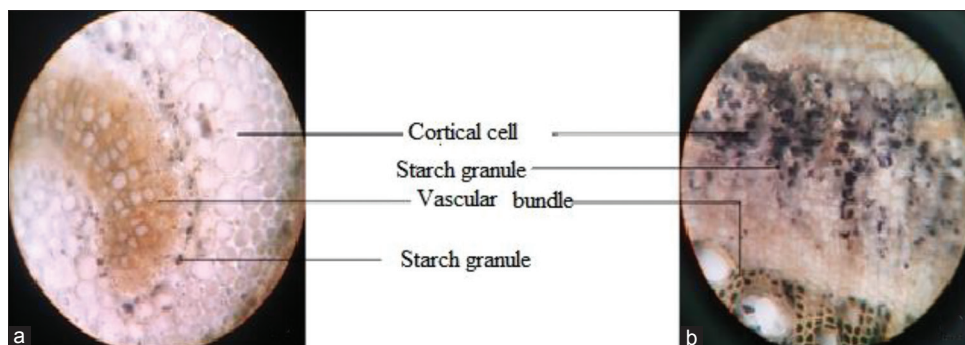
The appearance of ethanolic extract of different plant samples of *Buddleja madagascariensis* were dark green in colour. Yield of the crude extract was 5.157 g and 10.314%, respectively.

### Phytochemical Analysis

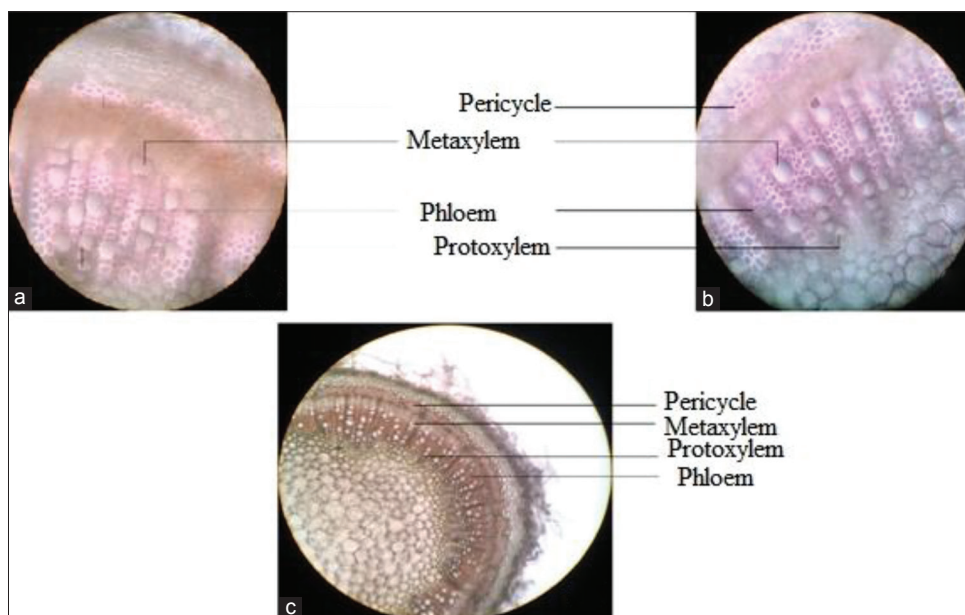
Phytoextracts or, plant derived substances have become of great interest owing to their versatile applications in modern medicines, nutraceuticals, food supplements, folk medicines etc (Ncube *et al.*, 2008). The techniques for the extraction of crude drugs include maceration, infusion, percolation, digestion of plant parts like bark, leaves, flower, roots, seeds etc *i.e.*, any part of plant may contain active components. The systematic screening of plant species with the purpose of discovering the new drugs (Tiwari *et al.*, 2011). The infusion method was used for the extraction of crude drug by using ethanol solvent. The yield of crude drug was 5.157g and 10.314%, respectively and dark green in appearance. The preliminary phytochemical



**Figure 14:** (a, b, c, d) Structure of glandular and eglandular trichome, (a) Multicellular hair arising from epidermal cell, (b) Capitate glandular hair, (c) Multicellular eglandular hair, (d) Trichome arising from epidermal cell



**Figure 15:** (a, b) Starch granules in cortical and pith region of (a) petiole and (b) root of *Buddleja madagascariensis*



**Figure 16:** (a, b, c) Stellar tissues stained by phloroglucinol

analysis of crude drug revealed the presence of carbohydrates, proteins, saponins, flavonoids, phenolic compounds and tannins, and absence of alkaloids, glycosides and free amino acids.

Traditionally the generic or, species diversity has been assessed by means of morphological, anatomical, histochemical, cytological, palynological and biochemical evidences. In the recent time, molecular characterization accomplished by various techniques including DNA fingerprinting, RAPD, AFLP, isozyme patterns were implemented for the characterization of genotypes (Peil *et al.*, 2003). Due to several disputes fashioned by molecular characterization, traditions methods like anatomical, histochemical and phytochemical evidences come in handy in recent time (Carlquist & Schneider, 2006).

## CONCLUSION

The result of the present studies on morphological, anatomical, histochemical and phytochemical characterization of plants could be used as tools to distinguish the crude drugs of *B. madagascariensis* from adulterants which are used in the preparation of traditional medicines and used as a diagnostic keys

in medicinal research. The histochemical and phytochemical studies also beneficial for the development of specific drugs from the plants. Thus, these studies will be useful in future for revealing the importance of plants, phytochemical resources for utilization in pharmaceutical industries on commercial basis in respect of the conservation of bio resources.

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