Research Article

Microscopic Evaluation of Leaves of Memecylon umbellatum Burm

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Objective. Aim of present work is to perform the microscopic evaluation and physicochemical analysis and to explore the morphology parameters of Memecylon umbellatum Burm leaves.

Methods. Fresh, dried and desiccated powdered leaf samples were studied for their morphology, microscopy, organoleptic characters, and an assortment of other WHO recommended methods for standardisation.

Results. The microscopy revealed the dorsiventral nature of the leaf. Midrib showed presence of nonlignified phloem, lignified xylem with well-defined xylem fibers, vessels, and parenchyma. Presence of Phloecentric vascular bundles surrounded by endodermis and crystal sheath. Well-defined patches of collenchyma were observed above and below the vascular bundles in the midrib area. Trichomes are mostly absent and stomata (anomocytic) were observed on both epidermal surfaces.

Conclusions. It can be concluded that the microscopic analysis and pharmacognostic parameters can serve as tool for developing standards for proper authentication, quality, and purity of Memecylon umbellatum Burm leaves.

1. Introduction

Memecylon umbellatum Burm (family: Melastomataceae) is a small evergreen shrub or tree which grows up to 8–14 m tall having young tree branches and bears numerous umbellate cymes. The plant is known as “Anjani” in Sanskrit, Anakkayavu in Malayalam, and “Ironwood tree” in English. It is distributed mostly in coastal regions of the Deccan peninsula, the eastern and southern part of India all along the Western Ghats and in the Andaman islands [1, 2]. It is also found distributed in Orissa, Assam, Sylhet, Tenasserim, Ceylon, Malay, Peninsula-Malay, and Archipelago [3]. Different extracts of Memecylon umbellatum Burm Inflorescences [4] and bark [5] have been evaluated for its antimicrobial potential. Estimation of total content of tannin [6] and seasonal variation of tannin content in different parts has also been carried out [7]. Estimation of sugars and minerals in healthy and infected parts has also been carried out [8]. Different root extract have been reported to possess antioxidant activity [9], while leaves of Memecylon umbellatum have also been evaluated for their antimicrobial activity [10]. Use of leaves was also reported in snakebite [11]. The seeds are used to cure cough and sedative [12]. The leaf powder has antidiabetic potential [13]. Leaves are used to treat eye troubles, gonorrhea, leucorrhea, wounds [14], and skin diseases [15]. It also has an antioxidant property [16]. The leaves are reported to possess antiviral activity [17–19]. Wound healing activity of ethanolic extract of the leaves has also been reported [20]. Plant contains a wide variety of phytoconstituents such as umbellactone, β-amyrin, Oleanolic acid, ursolic acid, sitosterol and organic acids [21–23].

As aforesaid, M. umbellatum has ample pharmacological activities and has the potential to cure various diseases. As this plant has special importance to humankind in their daily routine life it can be a good source of revenue. So to earn more money adulteration of this plant with various species of genus Memecylon like M. dasyanthum, M. flavescens, and M. kunstleri may take place. So it has become necessary to
set the standard parameters for proper authentication of this plant or its part. So the aim of present study is to explore the morphological and microscopic parameters of *Memecylon umbellatum* Burm leaves for its proper authentication so that it cannot be easily adulterated.

## 2. Materials and Methods

### 2.1. Materials

Plant material was located with the help of Dr. Madhukar Bachulkar sir in the region of Dajipur forest, Gaganbavada hills, and Chandoli forest area. The leaves of *Memecylon umbellatum* were collected in the month of March-April from Gaganbavada hills region, Maharashtra, India. The plant material was taxonomically identified by Dr. S. R. Yadav, Department of Botany, Shivaji University, Kolhapur, India (M.S.). The voucher herbarium specimen is deposited in the Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur.

Microscopy (T.S) and powder characters of leaf drug were done using compound microscope (Inco-Ambala), inbuilt light microscope (Metzer-Metzer Optical Instrument, Mathura), and photographs were taken using photographic microscope (Motic-Image-2003). Quantitative microscopical measurements were made using eye piece, stage micrometer (Erma-Japan), and camera lucida (Prism type—Swift-Ivis). All the reagents, solvents, and chemicals used are of A.R. grade (Merck, Loba, Qualigens) purchased from local supplier.

### 2.2. Methods

#### 2.2.1. Anatomy of Leaf

The leaf sample was studied microscopically by taking transverse section (T.S.) through midrib with small portion of lamina and thin section was double stained with hematoxylin and saffranin and observed under compound microscope and photos were taken by using photographic microscope. The powder sample was also mounted in different reagent and cellular diagnostic and diagnostic cell inclusions were observed. Quantitatively, the diameter of starch grains, length of fibers, and diameter of vessels were determined using stage and ocular micrometers while leaf constants were determined using Camera Lucida.

#### 2.2.2. Diameter of Starch Grains

Eyepiece micrometer was calibrated using stage micrometer to determine the value for one division of eyepiece micrometer (3.152 at high power and 14.87 at low power). Powder sample was gently spread over clean glass slide with the help of muslin cloth, warmed near the flame with 2–4 drops of clearing solution (chloral hydrate) for 2-3 times. Slide was stained with few drops of N/50 Iodine solution, excess stain was removed and cover slip was placed with 1-2 drops of glycerin water solution. Faint blue colored starch grains were observed and none of divisions of eyepiece micrometer occupied by individual starch grain were noted. In all group of 25 starch grains were counted and such four groups were analyzed from different slides. From the data obtained, average diameter of starch grains was determined using formula

$$D = \frac{\sum n \times d}{d} \times \text{Calibration factor}, \quad (1)$$

where $n$ = number of grains and $d$ = number of divisions.

#### 2.2.3. Length and Diameter of Fibers and Vessels

The slide was prepared with fine powder sample of leaf, cleared with clearing solution, and stained with phloroglucinol and conc. hydrochloric acid (1:1). Pink colored fibers and dark rosy pink colored vessels were selected and their length and diameter were determined in similar fashion as that of diameter of starch grains using calibrated eyepiece micrometer.

#### 2.2.4. Determination of Leaf Constants

(i) *Stomatal Number and Stomatal Index.* The upper epidermis of the leaf between midrib and lamina was peeled and transparent area was cleared with clearing solution and mounted on glass slide. The stomata and epidermal cells were traced on black sheet between 0.2 mm square using prism type camera lucida under high power (45x). The number of epidermal cells and stomata were counted as per rule from each drawn square. The experiment was repeated for five times and stomatal number was directly calculated. Stomatal index was calculated using formula

$$\text{Stomatal Index} = \frac{\text{Number of Stomata}}{\text{Number of Stomata} + \text{Epidermal cells}} \times 100. \quad (2)$$

Average values were determined and results were expressed per sq.mm.

(ii) *Vein-Islet and Vein Termination Numbers.* The leaf portion between midrib and margin was macerated in concentrated chloral hydrate solution for 24 h and decolorized with bleaching solution (5% calcium chloro-hypochlorite). The cleared lamina portion was mounted on glass slide and vein islet and vein terminations were traced on black sheet between 0.5 mm square using low power (5x). The process was repeated and values were determined per sq.mm of leaf area between midrib and margin.

(iii) *Palisade Ratio.* The leaf sample was disintegrated with caustic potash and conc. HCl by boiling on water bath, further bleached with bleaching solution, and then clarified with chloral hydrate solution. Cleared leaf surface was then mounted on glass slide. Four sets of contagious epidermal cells were traced using Camera Lucida and by lowering the objective spherical palisade cells were traced. The average numbers of palisade cells were calculated and palisade ratio was determined.

#### 2.2.5. Powder Analysis

Fine sample powder was mounted on clean glass slide and clarified with clearing solution. The powder sample was then treated with different chemical
Figure 1: Dorsal and ventral view of *Memecylon umbellatum* leaves.

Table 1: Reagents used for microscopical observations.

| S. no. | Diagnostic character | Reagent used                          |
|--------|----------------------|---------------------------------------|
| 1      | Lignified tissue     | 1:1 Phloroglucinol and conc. HCl     |
| 2      | Calcium oxalate      | Chloral hydrate/acetic acid           |
| 3      | Cystolyth            | HCl or sulphuric acid                 |
| 4      | Aleurone grains      | Picric acid                           |
| 5      | Starch grains        | N/50 Iodine solution                  |
| 6      | Fats and oils        | Sudan red III and IV                  |
| 7      | Mucilage             | Ruthenium red                         |

reagents. Stained samples were then mounted in glycerin water fluid and observed for identification of diagnostic characters. Different chemical reagents used for identification of diagnostic cellular characters and cell inclusions are as given in Table 1.

### 3. Result and Discussion

#### 3.1. Morphology

Length—leaves are 3.8–7.5 cm long,
Width—1.6–3.8 cm broad,
Shape—elliptical or ovate,
Color—dark green with polish upper and pale lower surface,
Apex—subacute or acuminate.
(see Figure 1).

#### 3.2. Microscopic Evaluation of Leaves

**3.2.1. Anatomy of Leaf (T.S.).** T.S. of leaf showed its typical dorsoventral nature. Upper and lower epidermis, lamina, mesophyll, and midrib region were observed as important diagnostic characters. Palisade tissue appeared in double layer just below upper epidermis in lamina region. Midrib shows central nonlignified phloem, lignified xylem with well-defined xylem fibers, vessels, and parenchyma. Vascular bundles are phloecentric and surrounded by endodermis and crystal sheath. Well-defined patches of collenchyma were observed above and below the vascular bundles in the midrib area. Trichomes are mostly absent and stomata (anomocytic) were observed on both epidermal surfaces. In the mesophyll region, 2-3 lignified vein lets on either side of midrib in lamina portion and loosely arranged spongy parenchyma were observed (Figures 2, 3, 5, 6, and 7).

**3.2.2. Quantitative Measurements of Diagnostic Characters.** The leaf showed diagnostic character as starch grains with average diameter ranged between 7.25–10.54–15.18 𝜇m. The diameter of xylem vessels was found to be 22–65 𝜇m and...
length of xylem fibers was observed in the range of 110–217–345 μm. The leaf surface constants such as stomatal index for upper epidermis (15.5–18.37), vein islet number (10–13/mm²), vein termination number (7–9/mm²), and palisade ratio (8–11) were found important diagnostic characters (Figure 8; Table 2).

3.2.3. Powder Characters. Powder sample of leaf showed presence of anomocytic stomata, prism and rosette type calcium oxalate crystals, aleurome and starch grains, brownish matter, upper and lower epidermis, xylem fibers, xylem vessels and palisade tissue as shown in Figures 9 and 10.

Table 2: Quantitative measurements of Memecylon umbellatum leaf.

| S. no. | Parameters studied | Proximate values |
|--------|--------------------|------------------|
| 1      | Diameter of starch grains | 7.25–10.54–15.18 μm |
| 2      | Length of fibers     | 110–345 μm       |
| 3      | Diameter of xylem vessels | 22–65 μm       |
| 4      | Stomatal index (upper epidermis) | 15.5–18.37 |
| 5      | Vein-islet number    | 13–15            |
| 6      | Vein-termination number | 7–9               |
| 7      | Palisade ratio       | 8–11              |

4. Conclusion

The detailed study of Pharmacognostic parameters of Memecylon umbellatum Burm leaves setup the standards which could be beneficial and serves as diagnostic tool for proper authentication of this medicinally important plant.

Conflict of Interests

The authors declare that they have no conflict of interests.
Figure 10: (a) Anomocytic stomata with guard cell. (b) Rosette calcium oxalate crystals and aleurone grains. (c) Prisms of calcium oxalate crystals. (d) Pericyclic fibers. (e) Epidermis and starch grains (45x). (f) Conducting strand (xylem) and brownish matter.

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