Research Article

Microscopic Evaluation of Leaves of *Memecylon umbellatum* Burm

Suresh G. Killedar,¹ Harinath N. More,² and Sameer J. Nadaf³

¹ Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Chitranagari, Kolhapur 416013, India

² Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Kolhapur 416013, India

³ Department of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Kolhapur 416013, India

Correspondence should be addressed to Suresh G. Killedar; sureshgk_64@rediffmail.com

Received 24 May 2014; Accepted 9 September 2014; Published 7 October 2014

Academic Editor: Ayman Suleiman

Copyright © 2014 Suresh G. Killedar et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 microscopical 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, Gaganavada hills, and Chandoli forest area. The leaves of *Memecylon umbellatum* were collected in the month of March-April from Gaganavada 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 safranin 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	Cystolith	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

FIGURE 2: T.S. of leaf showing midrib and lamina (4x).

FIGURE 3: T.S. of leaf showing midrib and lamina (40x).

FIGURE 4: T.S. showing upper epidermis with collenchyma (40x).

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, 4, 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

FIGURE 5: Midrib portion with lignified xylem (40x).

FIGURE 6: Part of lamina with upper epidermis and palisade tissue (40x).

FIGURE 7: Part of midrib with lower epidermis and collenchyma (40x).

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/ mm^2), vein termination number (7–9/ mm^2), 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, aleurone and starch grains, brownish matter, upper and lower epidermis, xylem fibers, xylem vessels and palisade tissue as shown in Figures 9 and 10.

FIGURE 8: Diagram showing vein islet number and vein termination number.

FIGURE 9: Epidermis with cell walls.

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.

Acknowledgment

The authors are thankful to Dr. S. R. Yadav, Department of Botany, Shivaji University, Kolhapur, India (M.S.), for their valuable guidance.

References

- [1] K. R. Kirtikar and B. D. Basu, *Indian Medicinal Plants*, Popular Prakashan, New Delhi, India, 2nd edition, 1996.
- [2] K. M. Nadkarni, *Indian Materia Medica*, Bombay Prakashan, Bombay, India, 1st edition, 1982.
- [3] H. Joshi, M. P. Gururaja, and S. Singh, "Memecylon umbellatum (Melastomataceae): a review," *International Journal of Pharmaceutical Sciences Review and Research*, vol. 11, no. 2, pp. 54–58, 2011.
- [4] S. G. Killedar and H. N. More, "Screening of antimicrobial potential and phytoconstituents for different extracts of Memecylon umbellatum Burm inflorescences," *Asian Journal of Pharmaceutical Research*, vol. 1, no. 4, pp. 114–118, 2011.
- [5] S. G. Killedar and H. N. More, "Antimicrobial potential and phytochemical screening of different bark extracts of *Memecylon umbellatum* Burm," *International Journal of Applied Microbiology*, vol. 2, no. 3, pp. 50–58, 2012.
- [6] S. G. Killedar and H. N. More, "Estimation of tannins in different parts of memecylon umbellatum burm," *Journal of Pharmacy Research*, vol. 3, no. 3, pp. 554–556, 2010.
- [7] S. G. Killedar and H. N. More, "Seasonal variation of tannin content in different parts of memecylon umbellatum burm," *Research Journal of Pharmacy and Technology*, vol. 4, no. 1, pp. 78–81, 2011.
- [8] S. G. Killedar and H. N. More, "Estimation of Sugars and minerals in healthy and infected parts of Memecylon umbellatum Burm," *International Journal of Research in Ayurveda and Pharmacy*, vol. 1, no. 2, pp. 582–585, 2010.

- [9] S. G. Killedar, H. N. More, G. Shah, and S. Gaikwad, "Phytochemical screening and in-vitro antioxidant activity of memecylon umbellatum root extracts," *World Journal of Pharmacy and Pharmaceutical Sciences*, vol. 2, no. 6, pp. 5988–5996, 2013.
- [10] S. Murugesan, A. Pannerselvam, and A. C. Tangavelou, "Phytochemical screening and antimicrobial activity of the leaves of *Memecylon umbellatum* burm. F," *Journal of Applied Pharmaceutical Science*, vol. 1, no. 1, pp. 42–45, 2011.
- [11] R. D. Kshirsagar and N. P. Singh, "Some less known ethnomedicinal uses from Mysore and Coorg districts, Karnataka state, India," *Journal of Ethnopharmacology*, vol. 75, no. 2-3, pp. 231–238, 2001.
- [12] B. Gowda, *Vanaspathi Kosha: Plant Wealth of Sringeri, Karnataka*, edited by K. N. Daivajna and Somayaji, Kalpatharu Research Academy, Bangalore, India, 2004.
- [13] M. K. Ayyanar and S. S. Ignacimuthu, "Traditional herbal medicines used for the treatment of diabetes among two major tribal groups in south Tamil Nadu, India," *Ethnobotanical Leaflets*, vol. 12, pp. 276–280, 2008.
- [14] M. L. Dhar, M. C. Dhar, B. N. Dhawan, B. N. Mehrotra, and C. Ray, "Screening of Indian plants for biological activity: part I," *Indian Journal of Experimental Biology*, vol. 6, no. 4, pp. 232–247, 1968.
- [15] Anonymous, *The Wealth of India, Publication and Information Directorate*, New Delhi; India, CSIR, 1998.
- [16] S. Karuppusamy, "Medicinal plants used by Paliyan tribes of Sirumalai hills of southern India," *Natural Product Radiance*, vol. 6, no. 5, pp. 436–442, 2007.
- [17] S. Killedar, H. More, S. Mali, S. Nadaf, S. Salunkhe, and R. Karade, "Phytochemical screening and *In vitro* antioxidant potential of *Memecylon umbellatum* Burm leaf extracts," *Journal of Drug Delivery and Therapeutics*, vol. 4, no. 2, pp. 30–35, 2014.
- [18] K. R. Kirthikar, B. D. Basu, and R. S. Anil, *Indian Medicinal Plants. Volume II*, Periodical Experts Book Agency, New Delhi, India, 1991.
- [19] B. N. Sastri, *The Wealth of India*, Publication and Information Directorate CSIR Hillside, New Delhi, India, 1962.
- [20] M. L. Dhar, M. M. Dhar, B. N. Dhawan, B. N. Mehrotra, and C. Ray, "Screening of Indian plants for biological activity: part-I," *Indian Journal of Experimental Biology*, vol. 6, no. 4, pp. 232–247, 1968.
- [21] A. Puratchikody and G. Nagalakshmi, "Wound healing activity of *Memecylon umbellatum* Burm," *Journal of Plant Sciences*, vol. 2, no. 2, pp. 179–186, 2007.
- [22] P. R. Ram and B. N. Mehrotra, *Compendium of Indian Medicinal Plants*, Drug Research Preparative: A CDRI Series, Central Drug Research Institute, Lucknow and Publications and Information Directorate, New Delhi, India, 1993.
- [23] L. V. Asolkar, K. K. Kakkar, and O. J. Chakre, *Glossary of Indian Medicinal Plants with Active Principal, Part-I. Publications and Information Directorate*, Council of Scientific and Industrial Research, New Delhi, India, 1956.