Anatomical and biochemical investigations on medicinal tree *Murraya koenigii* Spreng.

Keerti Soundappan[1, #], Richa Bajaj[2, #], Brajesh Nanda Vaidya[2], Nirmal Joshee[2, *]

[1]Houston County High School, Warner Robins, GA 31088, USA. [2]Graduate Program in Biotechnology, Agricultural Research Station, Fort Valley State University, Fort Valley, GA, 31030, USA.

Received: August 31, 2018; accepted: October 12, 2018.

Curry leaf (Curry Patta) tree is an economically important spice / medicinal tree. This investigation details morpho-anatomical features of leaf and stem and explains trichome and secretory gland structures that may be associated with medicinal properties of the plant. Transverse sections of leaf and stem clearly reveal position of oil glands (schizolysigenous glands) and other constituent cells. Oil glands are situated close to the epidermis for the release of secretion. The plant body is covered by cuticle, a uniseriate epidermis and is followed by multiple layers of cortex composed of polygonal, starch grains containing parenchymatous cells. Cortex has interspersed oil glands close to the epidermis and few cells of cortex contain calcium oxalate crystals. Inner cortex contains 2-3 layers of sclerenchymatous cells seen as a red band due to safranin staining. In the leaves, oil glands are embedded in the palisade cells near the upper epidermis and the leaf margins exhibit glands opening through a pore covered by wax. Non-glandular trichomes are present on the leaf but with higher density on mid vein. These trichomes have rough surface due to the presence of micropapillae that may function as a deterrent to the insects. Scanning electron microscopy of the trichome cross section reveals central cavity. Fluorescent staining of trichomes for the presence of secondary metabolites was positive using Diphenyl boric acid-β-ethylaminoester stain under UV light. Biochemical assays of *M. koenigii* leaf extract estimated total polyphenol and total flavonoid contents at 547 mg/g and 401 µmol/g, respectively. Same extract registered a TROLOX Equivalent Antioxidant Capacity of 211 µg/mL.

Keywords: Antioxidant; Trichome; Micromorphology.

Abbreviations: Å: Angstrom; CPD: Critical Point Dryer; DPBA: Diphenyl boric acid-β-ethylaminoester; DPX: Distyrene, Plasticizer, and Xylene; GA: Glutaraldehyde; GAE: Gallic Acid Equivalent; OsO_4: Osmium Tetraoxide; SEM: Scanning Electron Microscopy; TEAC: TROLOX Equivalent Antioxidant Capacity; TPP: Total Polyphenol; TROLOX: 6-hydroxy- 2,5,7,8- tetramethylchroman-2-carboxylic acid.

[#]Authors contributed equally.

[*]**Corresponding author:** Nirmal Joshee, Professor of Plant Science, Fort Valley State University, 1005 State University Drive, Fort Valley, GA 31030, USA. Phone: +1 478 822 7039. E-mail: josheen@fvsu.edu

Introduction

Curry patta (*Murraya koenigii* Spreng., family Rutaceae) is a traditional spice used in Indian cuisines. The name "curry patta" refers to *M. koenigii* originated from the Tamil word Kari which translates to "spiced sauce" [1]. In the early Tamil and Kannada literatures from India, the use of *M. koenigii* is described as the flavoring agent for the vegetables [2]. *Murraya koenigii* is cultivated as a crop, primarily due to the high demand for leaves as flavoring agent in various food preparations in India, Sri Lanka, Southeast Asia, Australia, Pacific Islands, and Africa [3]. Curry patta is native to India and distributed in most of South Asia.

Free radicals can cause disorders, such as atherosclerosis, central nervous system injury, and gastritis in the human body [4].

Phytochemical studies on the leaves, stem, bark, and root of this plant have shown the presence of large concentrations of alkaloids, terpenes, saponins, and phenolic compounds with high radical scavenging activity [5, 6, 7, 8]. Curry patta is a rich source of aromatic terpenes and pharmacologically important carbazole alkaloids e.g. mahanimbine, murrayanol and mahanine that are related to the antioxidant activity [6, 9]. Plant-based antioxidants block free radicals produced through oxidation, thus inhibiting chain reactions that could lead to degradation and death of cells in human body. Antioxidants are marketed as beneficial to us by protecting the skin, supporting digestion and the immune system. Antioxidant effect of curry leaf powder in chicken and goat meat products has also been reported [10, 11].

Chemical compounds are synthesized in specialized structures in plants known as trichomes, broadly divide into glandular and non-glandular types. Glandular trichomes are the synthesizing centers of many compounds [12]. Internal secretory structures include idioblasts, schizogenous cavities, secretory cells, and lysigenous cavities. Idioblasts are isolated secretory cells that usually contain distinct substances such as mucilage, tannin, oils, and calcium oxalate [13]. Schizogenous cavities are formed by the separation of cells from each other during development. These cavities are surrounded by secretory cells which fill the cavities with substances such as mucilage, oils, and resins or gums [14]. These non-metabolic compounds have important adaptive value serving mainly to protect the plant from herbivores and outside invasion from insects, attract pollinators or protect proteins and nucleic acid of the cell. These molecules produced by the secretory glands and trichomes are vital to the survival of the plant and collectively provide an adaptive edge to the plant by regulating evapotranspiration, shielding from harmful rays and deterring insects and predators [15, 16, 17]. Although *M*. *koenigii* is an important aromatic and medicinal plant, not much is known about the biosynthesis of different types of secondary metabolites at the molecular pathway level.

Recently, leaf transcriptome sequencing and analysis was carried out to understand the biosynthesis of specialized terpenoids and carbazole alkaloids in *M*. *koenigii* [18]. Thus, the level of activity of various plant chemicals is important for the evaluation of their potential health benefit to humans [19, 20]. This study was aimed at creating an understanding of micromorphology and histology of *M*. *koenigii* leaf and stem and assess antioxidant capacity of the methanolic extracts.

Materials and methods

1. Plant material.

Leaves and stem pieces of *M*. *koenigii* were collected from a two-year-old cultivated plant. The fresh plant samples were collected, weighed, measured, and prepared separately for each of the tests performed.

2. Micromorphology.

Fresh plant material was used to study surface structures under light and fluorescent microscope on leaf and stem. Another batch of plant tissue was fixed and processed to study structures in detail under a scanning electron microscope (SEM).

3. Light Microscopy.

The trichomes on the surface of fresh leaf and stem were observed under a Leica microscope MZ10F with camera DFC450C (Leica Microscope Systems, Ltd., Buffalo, IL, USA) using Leica suite v 4.4 software.

4. Fluorescent Microscopy.

In order to record the presence and secretory nature of trichomes, two leaf samples measuring one square cm were cut. These samples were submerged in Natural Product Reagent Diphenyl boric acid-β-ethylaminoester (DPBA) stain (Sigma-Aldrich, Saint Louis, MO, USA) as outlined in Heinrich et al., [21]. The hydroxyl group in

DPBA reacts with the flavonoid compound present in trichomes, which emit yellow green fluorescence [22]. The leaf samples were soaked in DPBA stain under low light for 5 min and then placed on a microscope slide, adaxial and abaxial sides facing up for observations. Two drops of DPBA were placed on the tissue, and a cover slip was placed over the samples to view under the fluorescent microscope (OLYMPUS BX-43, Olympus America, PA, USA) using DAPI filter (with excitation light at λ 386 nm; emission λ 490 nm) to capture images with camera (Olympus DP-72, Olympus America, PA, USA) and a UV source (X-cite series 120 Q, Lumen Dynamics, Ontario, Canada).

5. Scanning Electron Microscopy.

Plant samples were observed under a SEM (Hitachi S3400 NII, Hitachi High Technologies America, Inc., CA, USA). The leaf samples were excised to fit on Hitachi M4 aluminum specimen mount (Ted Pella, Inc., CA, USA) for observing abaxial and adaxial surface. Leaf and stem samples were prepared as per Vaidya et al., [23] with some modifications. The leaves were submerged in chloroform (Fisher Scientific, MA, USA) for 24 h prior to fixation. Primary fixation was done in 2% glutaraldehyde (GA) (EMS, PA, USA) for 1 h at 4°C and then washed in Sorenson's phosphate buffer saline (PBS) buffer (pH 7.2) (EMS, PA, USA) three times followed by secondary fixation in 1% osmium tetraoxide (OsO_4) (EMS, PA, USA) for 1 h at 4°C. Then the samples were washed with dH_2O three times for 15 min each. Fixed samples were then dehydrated using ascending ethanol series from 25, 50, 60, 70, 80, 95, and 100% (EMS, PA, USA) each for 15 min and in 100% ethanol three times to ensure complete dehydration. Critical Point Drying (CPD) was carried out at 35°C with CO_2 at 1,250 psi (LEICA CPD 300, LEICA, Germany). Leaf samples were placed on aluminum stubs using double-sided carbon tape. The leaf samples were sputter coated (Denton Desk V, Denton, NJ, USA) with gold for 60 s under a vacuum pressure of 0.05 torr and thickness of 50 Å.

6. Histology of stems and leaves.

For histological studies samples were fixed in Histochoice® (Amresco, OH, USA) and remaining steps (fixing, dehydration, paraffin infiltration, specimen mounting, tissue embedding, sectioning, deparaffinization, and slide preparation) were followed as per Vaidya et al., [23]. For anatomical details, sections were double stained using aqueous 1% Safranin O (Sigma Aldrich, MO, USA) for 12 h and 0.1% (w/v) Fast green (Sigma Aldrich, MO, USA) in 95% ethanol for 3-5 seconds [24]. After staining, the slides were rinsed in 100% ethanol two times to complete dehydration and remove non-specifically bound stains. Sections were cut at 6 micron thickness to reveal all internal details. Permanent slides were prepared by layering 1-2 drops of DPX mountant (Sigma Aldrich, MO, USA) and cover glass over stained and dehydrated sections, dried over night at 45°C and then observed under a microscope.

7. Trichome count and surface area calculation of leaves.

Leaf samples stained with DPBA as above were used for counting trichomes under fluorescent microscope. The fluorescing trichomes were counted on both abaxial and adaxial surfaces. The surface area of mature and immature staged *M. koenigii* leaves was calculated by placing them on a graph paper that contained unit squares measuring 5 x 5 mm^2. Five leaves each for immature and mature stage were collected and placed on the graph paper to draw their outer margins. Based on the number of full and more than 50% covered squares under the leaf on the graph paper, area was calculated, and statistical analysis carried out.

8. Biochemical assays.

(1) Preparation of methanolic leaf extract:

Protocols outlined in Vaidya et al., 2013 [20] were adopted with minor modifications for extraction and biochemical assays. Two grams of the fresh tissue (fully expanded leaves) were submerged in liquid nitrogen in a chilled mortar to facilitate grinding and minimize degradation of phytochemicals. The homogenized leaf

powder was suspended in 50 mL of HPLC grade methanol (Burdick and Jackson, MI, USA) and left overnight (18 h at 28.5°C) in the dark on an orbital shaker at 150 RPM (Benchmark Mini Incu-Shaker, NJ, USA). The suspension was then transferred to 50 mL Falcon tubes (BD, NJ, USA) and centrifuged at 13,500 RPM (Eppendorf centrifuge 5810R, NH, USA) at 25°C for 40 min. The supernatant was collected, and the remaining pellet was extracted again with 25 mL methanol for 2 h. After the second extraction, the two extracts were combined, and the pellets were discarded. The combined extract was filtered through a double layer of Whatman filter paper No. 2 (GE Healthcare Life Sciences, NJ, USA) and stored in air tight 50 mL Falcon tubes at 4°C in the dark.

(2) Assays:

Total polyphenol (TPP) content was determined by the Folin-Ciocalteu reagent method [25] as modified by Yi and Wetzstein [19], and further modified by Vaidya et al., [20]. The flavonoid content was measured using aluminum chloride colorimetric method [26] as standardized by Vaidya et al., [20]. Average flavonoid content was calculated from three different repetitions for the same extract using the Gallic Acid standard curve. Rosemary (Rosmarinus officinalis) leaf extract, a plant with high antioxidant capacity, prepared in identical fashion was used as a standard to facilitate comparison [19]. TROLOX equivalent antioxidant capacity (TEAC) of a sample was calculated based on the inhibition of radical cation absorption exerted by the standard TROLOX solution (6-hydroxy-2, 5, 7, 8-tetramethy-chroman-2-carboxylic acid) (Sigma-Aldrich, MO, USA) [27]. The TEAC assay was carried out as per the protocols optimized in our lab [20].

9. Statistical analysis.

All data are presented as means ± SE for at least three replications of each sample. Statistical analysis was based on one-way analysis of variance (ANOVA) with results at $p \leq 0.05$ level and means were separated using Tukey's post-hoc mean separation test.

Results and discussion

1. Micromorphology

(1) Light Microscopy:

Trichome distribution on the leaf was restricted to the mid-vein and margin predominantly but a few scattered on the lamina are also visible (Figures 1 A, L and 3 A). For immature leaves, abaxial and adaxial surfaces had same density of trichomes whereas mature leaves exhibited higher density of trichomes on the abaxial surface than on the adaxial surface. Only one type of long, slender non-glandular trichomes were observed on the leaf and stem samples (Figure 1, J - K).

(2) Fluorescent Microscopy:

The trichomes fluoresced bright yellow when stained with DPBA, suggesting presence of secondary metabolites (Figure 1 L and 3 A). High concentration of trichomes on leaf mid rib and margin can be seen on Figure 3A.

(3) Scanning Electron Microscopy:

Epidermal cell layer exhibits the presence of cuticle and apparently covered by wax forming chimney like pattern (Figure 1 A-B) around openings of schizolysigenous glands (Figure 2 A-D). A thick cuticle layer on the surface of leaves with numerous stomata on the abaxial surface were observed (Figure 1 F, M). Most of the non-glandular trichomes were condensed near the mid -vein section of abaxial surface (Figure 1 C, E-F). Each trichome exhibits presence of small protuberances known as micropapillae throughout on the surface and hollow cavity in the middle (Figure 1 H-I). The adaxial leaf surface is dotted with bumps present on the entire lamina (Figure 1 A-B). These structures represent central pore surrounded by multiple layers of wax on the lamina of the leaf (Figure 1 D). Stem contains trichomes in two distinct stripes alternating with blank space (Figure 1 G and K). The trichomes found are epidermal outgrowths which play an important role in plant defence especially with respect to phytophagous insects,

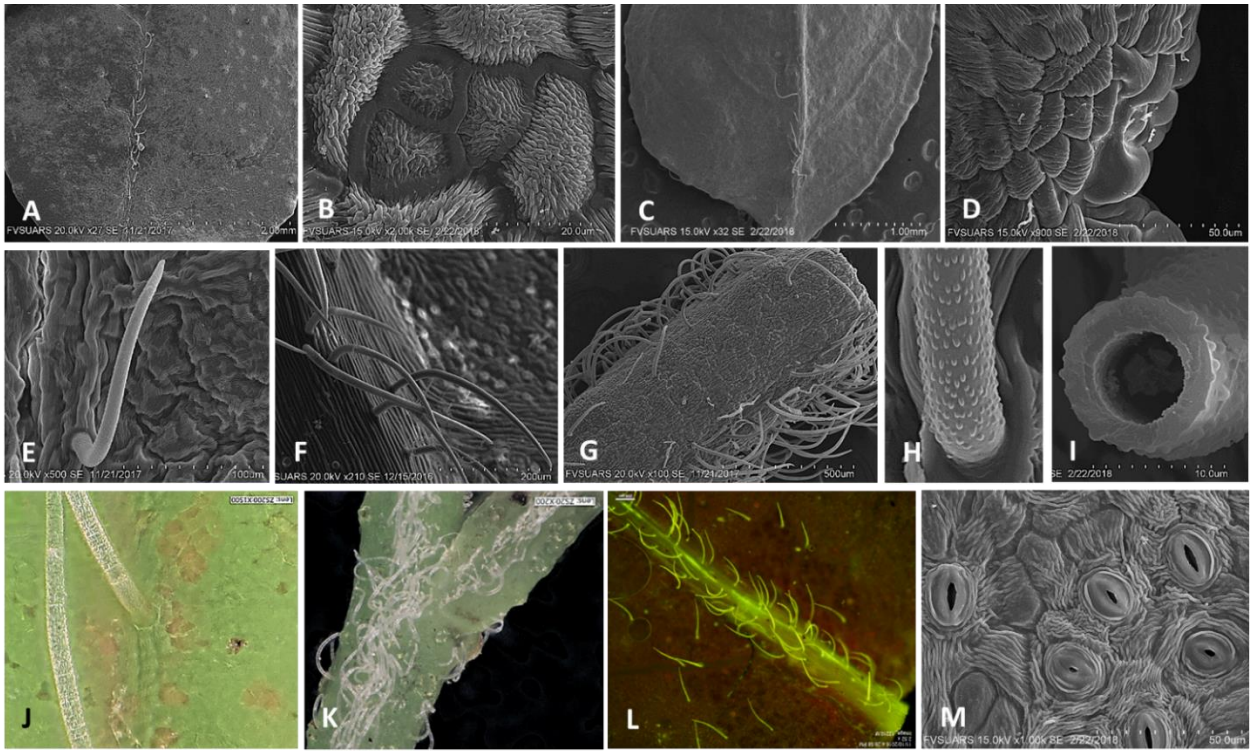

Figure 1. Micromorphology of *M. koenigii* leaf and stem. A-I and M are SEM images, J and K are light microscopy results, and L represents fluorescent imaging of trichomes on the abaxial surface of the leaf. **A:** Adaxial surface of the leaf exhibiting non-glandular trichomes confined to mid vein. **B:** Magnified view of a bump present on the lamina. Central pore surrounded by multiple layers of wax. **C:** Abaxial surface of the leaf and trichome arrangement. **D:** A pore at the margin of the leaf. **E-F:** Magnified view of a single trichome and their arrangement on the mid vein of the abaxial leaf surface. **G:** Trichome distribution on the stem exhibits bald and populated streaks. **H-I:** Magnified view of trichome presents micropapillae on the surface and hollow cavity in the middle. **J-K:** Light microscopic images exhibit trichome distribution on the leaf and stem. **L:** In the presence of fluorescent stain DPBA, trichome indicate presence of secondary metabolites. **M:** Partial removal of wax by dipping leaf in chloroform for a minute to reveal stomata.

avoiding insect feeding and oviposition responses and the nutrition of larvae [12].

2. Histology of stems and leaves

Leaves and stem sections stained with Safranin O and Fast green provided a basic understanding of the structure.

(1) Stem anatomy:

Cross section of a stem exhibits uniseriate epidermis covered with cuticle followed by many layers of cortical region (Figure 2 A). Outer three to four cortical layer cells reveal presence of starch. Thin walled parenchymatous pith is present in the center and between cortex and pith vascular bundles are arranged. A section of stem at higher magnification reveals the presence of schizolysigenous oil glands (black arrows) and calcium oxalate crystals (white arrows) in the cortex region (Figure 2 B). The schizolysigenous oil glands are present close to the upper epidermis and many suspended cells are visible in the cavity of these glands (Figure 2 C and D).

(2) Leaf anatomy:

Leaves on both surfaces and stem section possess uniseriate epidermis covered externally by a thick layer of cuticle. Internal structures such as secretory cells and lysigenous cavities were found (Figure 2). Leaf margin is serrated and contains large oil gland.

On the basis of SEM and anatomical studies, it is clear that schizolysigenous glands are present close to the surface in stem and near upper

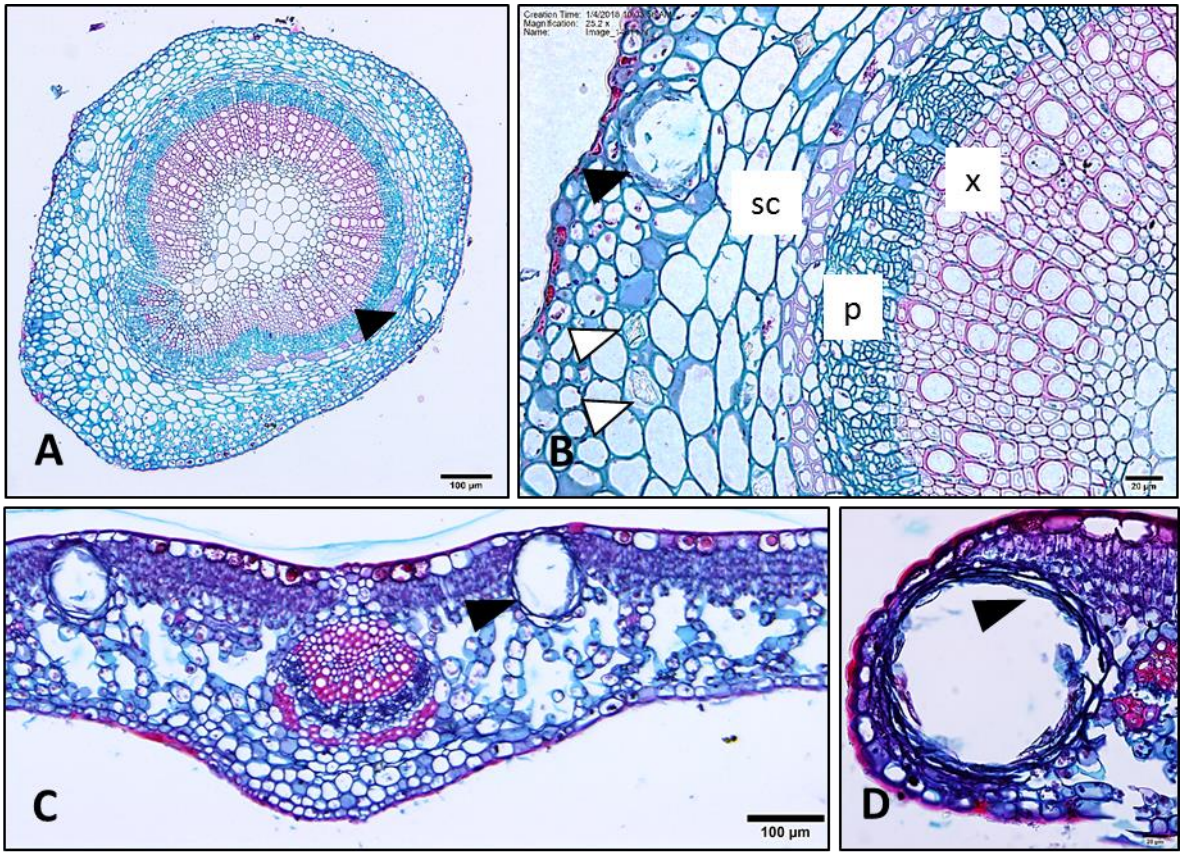

Figure 2. Histology of *M. koenigii* stem and leaf. Permanent slides were prepared, and Fast green and Safranin O staining were used to reveal internal structures. **A:** Cross section of stem showing single layered epidermis covered with cuticle followed by cortical region. Thin walled parenchymatous pith is present in the center and between cortex and pith vascular bundles are arranged. **B:** A section of stem at higher magnification to show schizolysigenous oil glands (black arrows) and calcium oxalate crystals (white arrows) in the cortical region. **C-D:** General and magnified anatomy of a leaf. Schizolysigenous oil glands are present close to the upper epidermis. Leaf margin contains large oil gland. (**Sc:** sclerenchymatous layer, **p:** phloem, **x:** xylem)

Table 1. Biochemical assays to study secondary metabolite profile of M. koenigii and antioxidant capacity.

Murraya koenigii Antioxidant Potential		
#	Assays	Average
1	Total Polyphenol Content	547.46 ± 11.56 mg/g GAE
2	Flavonoid Content	210.64 ± 4.80 µg/mL
3	TROLOX Antioxidant Capacity measurement	400.88 ± 79.61 µmol/g

epidermis in leaves. Leaf margins contain this gland which opens out through a pore (Figure 1D and 2D).

3. Surface Area calculation of leaves

The average cut off length for immature and mature leaves was 1.50 cm and 2.75 cm, respectively. Five leaves each were selected to calculated surface area. On an average, immature leaf area was 115 mm^2 and for the mature leaf the surface area was 200 mm^2.

4. Biochemical assays

The TPP content registered an average of 547.46 mg/g Gallic Acid Equivalent (GAE). A preliminary study conducted previously showed the TPP

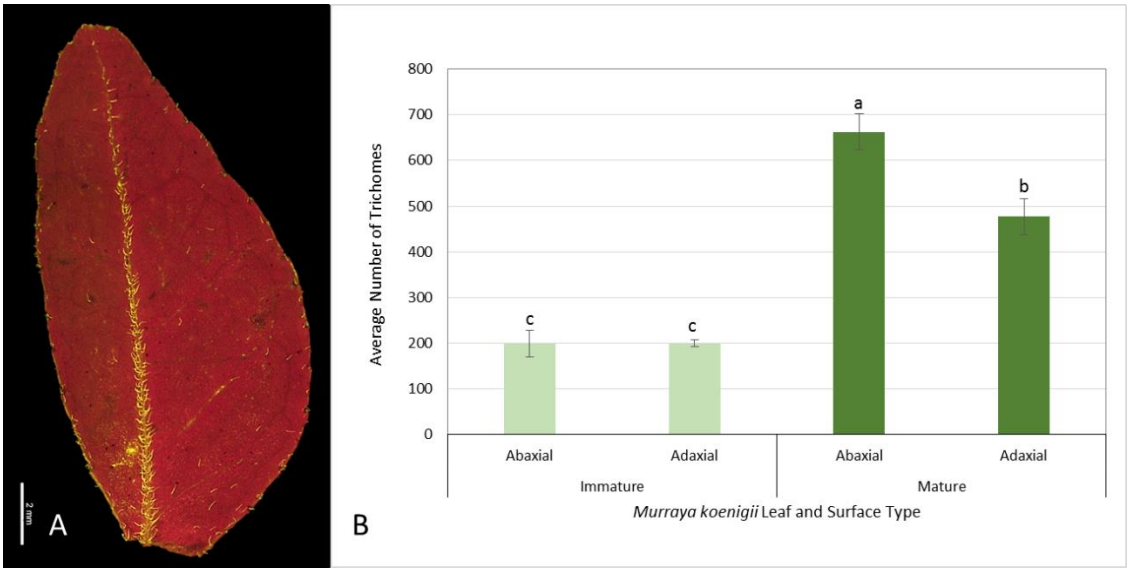

Figure 3. DPBA fluorescent staining of trichomes to facilitate counting of trichomes at two developmental stages of *M. koenigii leaf*.

content lower for Rosemary (443.23 mg/g) which was also used as an internal standard. The TEAC assay measurement results for the leaf extracts on average was 400.88 µmol/g. The total Flavonoid content in the extract registered an average of 210.64 µg/mL (Table 1).

Polyphenol supplements are found in several medications [28]. Polyphenols are known to play a role in reducing oxidative stress and preventing degenerative diseases such as cardiovascular disease and cancers [29]. Similarly, flavonoids have been found to play a role in regulation of cholesterol levels [30, 31]. Flavonoids regulate the concentration of low-density lipoproteins (LDL) and high-density lipoproteins (HDL). LDL are detrimental to our circulatory system, increasing our risk of heart disease. HDL, on the other hand, carry LDL to the liver for elimination, and have anti-inflammatory effects. In this way, flavonoids can help in improving heart health.

Curry leaf extracts have shown anticancer activity on MDA-MB-231 human breast cancer cell line [32]. Leaf extract analysis revealed the presence of significant number of flavonoid compounds such as myricetin, quercetin and epicatechin. These compounds were shown to be effective in the growth inhibition of breast cancer cells. In addition, the curry leaf extracts contain minerals which are important for the maintenance of normoglycemia or normal glucose content of blood [33]. Curry leaf extracts can also be added to meat as antioxidant since they show considerable levels of total phenolics and antioxidant properties when measured in terms of free radical scavenging and reducing activity [34].

Conclusions

Plants are used for their flavors and aromas in various cuisines around the world in addition to their health benefits. This research was conducted to form an understanding of the micromorphology and histology of *M. koenigii* leaf and stem to understand the presence and location of secretory structures that may be involved in the synthesis of compounds that are beneficial to health.

The activity of medicinal plants is closely related to their secondary metabolites that are sometimes present in the essential oil of aromatic plants. The morphology, distribution

and histochemistry of the secretory structures in plant families are well documented but little information is available on the morphology and structural aspects of secretory bodies in *M. koenigii*, apart from the observation that the essential oil is produced in schizolysigenous glands. Though this tree grows predominantly in Indian subcontinent, its culinary use has introduced it to many parts of the world. Study of trichomes, secretory glands, morphology and anatomical features provide valuable insights into desirable characters needed for the breeding programs to improve an economical crop. This study clearly highlights characters that can help in developing drought tolerant cultivars on the basis of the extent of cuticle on the plant surface and presence of sclerenchymatous ring between cortex and vascular bundles to reduce evapotranspiration. Commercially desirable cultivars may be selected on the basis of number of secretory glands present in the unit area of plant tissue.

Acknowledgements

K. Soundappan would like to thank the MS Biotechnology graduate students in the Joshee Lab, Fort Valley State University (FVSU) for their assistance and guidance. N. Joshee would like to thank Agricultural Research Station, FVSU for providing financial assistance.

Reference

1. Parrota JA: Healing plants of peninsular India. CABI Pub, USA; 2001: 639.
2. Prajapati ND, Purohit SS, Sharma AK, et al.: A handbook of medicinal plants: A complete source book. Agrobios India; 2003:401.
3. Bonde SD, Nemade LS, Patel MR, et al. 2007. *Murraya koenigii* (Curry leaf): Ethnobotany, phytochemistry and pharmacology - A Review. Intl J Pharma Phytopharma Res 4(5):45-54.
4. Calabrese V, Cornelius C, Mancuso C, et al. 2008. Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity. Neurochem Res 33(12):2444-2471.
5. Sharif K, Khalid UK, Yusuf M. 2007. Chemical constituents from stem barks and roots of *Murraya koenigii* (Rutaceae). Malays J Anal Sci. 11:173-176.
6. Tachibana Y, Kikuzaki H, Hj N, et al. 2001. Antioxidative activity of carbazoles from *Murraya koenigii* leaves. J Agric Food Chem. 49(11):5589-5594. DOI: 10.1021/jf010621r.
7. Tachibana Y, Kikuzaki H, Lajis NH, et al. 2003. Comparison of antioxidative properties of carbazole alkaloids from *Murraya koenigii* leaves. J Agric Food Chem. 51:6461-6467.
8. Jain M, Gilhotra R, Singh RP, et al. 2017. Curry leaf (*Murraya koenigii*): A spice with medicinal property. MOJ Biol Med. 2(3):00050.
9. Ningappa MB, Dinesha R, Srinivas L. 2008. Antioxidant and free radical scavenging activities of polyphenol enriched curry leaf (*Murraya koenigii* L.) extracts. Food Chem. 106:720-728.
10. Biswas AK, Kondaiah N, Anjaneyulu ASR. 2006. Effect of spice mix and curry leaf powder (*Murraya koenigii*) on the quality of meat and precooked chicken patties during refrigeration storage. J Food Sci Technol. 43:438-441.
11. Das AK, Rajkumar V, Dwivedi DK. 2011. Antioxidant effect of curry leaf (*Murraya koenigii*) powder on quality of ground and cooked goat meat. Int Food Res J. 18:559-565.
12. Bisio A. 1999. Glandular hairs and secreted material. In Salvia blepharophylla Brandegee Ex Epling grown in Italy. Ann Bot. 83(4):441-452. doi:10.1006/anbo.1998.0838.
13. Jarald EE, Sheeja E, Parial S, et al. 2008. Morphoanatomy of stems of *Murraya koenigii* Spreng. J Biol Sci. 8(3):654-658.
14. Curtis JD, Lersten NR. 1990. Internal secretory structures in *Hypericum* (Clusiaceae): *H. perforatum* L. and *H. balearicum* L. New Phytol. 114:571-580.
15. Thomas V. 1991. Structural, functional and phylogenetic aspects of the colleter. Ann Bot. 68(4): 287-305.
16. Duke SO. 1994. Glandular trichomes, a focal point of chemical and structural interactions. Int J Plant Sci. 154:107-118.
17. Fahn A. 2000. Structure and function of secretory cells. Adv Bot Res. 31:37-75.
18. Meena S, Sarma RK, Dwivedi V, et al. 2017. Transcriptomic insight into terpenoid and carbazole alkaloid biosynthesis, and functional characterization of two terpene synthases in curry tree (*Murraya koenigii*). Sci Rep. 7:44126. DOI: 10.1038/srep44126.
19. Yi W, Wetzstein H. 2010. Biochemical, biological and histological evaluation of some culinary and medicinal herbs grown under greenhouse and field conditions. J Sci Food Agric. 90:1063-1070.
20. Vaidya BN, Brearley TA, Joshee N. 2013. Antioxidant capacity of fresh and dry leaf extracts of sixteen Scutellaria species. J Med Active Plants. 2(3):42-49.
21. Heinrich G, Pfeifhofer H, Stanbentheiner E, et al. 2002. Glandular hairs of *Sigesbeckia jorullensis* Kunth (*Asteraceae*): Morphology, histochemistry and composition of essential oil. Ann Bot. 89:459-469.
22. Wollenweber E: Flavones and flavonols. In The flavonoids: advances in research. Edited by Harborne JB, Mabry TJ. London: Chapman and Hall; 1982.189-259.
23. Vaidya BN, Jackson CL, Perry ZD, et al. 2016. *Agrobacterium*-mediated transformation of thin cell layer explants of

Scutellaria ocmulgee Small: A rare plant with anti-tumor properties. Plant Cell Tiss Org. 127(1):57-69.

24. Johansen DA: Plant Microtechnique. McGraw-Hill Book Co., New York; 1940:523.
25. Lowry OH, Rosebrough NJ, Farr AL, et al. 1951. Protein measurement with the folin phenol reagent. J Biol Chem. 193:265-275.
26. Chang C, Ming-Hua Y, Hwe-Mei W, et al. 2002. Estimationof total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 10:178-182.
27. Davies MJ, Fornit LG, Willson RL. 1988. Vitamin E analogue Trolox C E.s.r. and pulse radiolysis studies of free-radical reactions. Biochem J. 255:513-522.
28. Middleton E, Kandaswami C, Theoharides TC. 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 52:673-751.
29. Dixon R, Ferreira D. 2002 Molecules of interest: genistein. Phytochem. 60: 205-211.
30. Panche AN, Diwan AD, Chandra SR. 2016. Flavonoids: an overview. J Nutri Sci. 5:1-15.
31. Ferreyra MLF, Rius SP, Casati P. 2012. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Frontiers in Plant Sci. 3:1-15. doi: 10.3389/fpls.2012.00222.
32. Ghasemzadeh A, Jaafar HZ, Rahmat A, et al. 2014. Evaluation of bioactive compounds, pharmaceutical quality, and anticancer activity of curry leaf (*Murraya koenigii* L.). Evidence-based Comp Alternative Med.
33. Singh S, More PK, Mohan SM. 2014. Curry leaves (*Murraya koenigii* Linn. Spreng.) - a miracle plant. Indian J Sci Res. 4(1):46-52.
34. Devatkal SK, Thorat PR, Manjunatha M, et al. 2012. Comparative antioxidant effect of aqueous extracts of curry leaves, fenugreek leaves and butylated hydroxytoluene in raw chicken patties. J Food Sci Tech. 49(6):781-785.