Journal of Food and Agriculture Research
Vol. 1, No. 1, 2021, pp. 1-14
© ARF India. All Right Reserved
URL: www.arfjournals.com

Micromorphological Studies in an Anxiolytic Medicinal Plant, *Scutellaria lateriflora* L.

SAMANTHA H SHERMAN[1], BRAJESH N VAIDYA[2] AND NIRMAL JOSHEE[1*]
[1]*Graduate Program in Biotechnology, Agricultural Research Station, Fort Valley State University, Fort Valley, Georgia 31030, USA*
[2]*GreenVenus LLC., Wimauma, Florida 33598, USA*
[*]*Corresponding author E-mail: josheen@fvsu.edu*

Abstract: *Scutellaria* species have been used in traditional and local medicine systems for many centuries in various parts of the world. About 400 species of the *Scutellaria* genus have been reported so far, mostly from northern hemisphere. Traditional application of *Scutellaria* species has been in practice for the treatment of inflammation, infections, jaundice, high blood pressure, and tumors in China, Japan, Korea, India, Nepal, and among South and North American countries. Flavonoids wogonin, baicalein, and baicalin extracted from *Scutellaria lateriflora* have exhibited anti-tumor properties in biomedical studies. Medicinal plants with commercial value are usually associated with indiscriminate over-collection, adulteration, and conservation issues. Over-collection may lead to biomass adulteration and the study of micromorphology may aid as an important tool for the identification of adulterants. Trichome diversity and morphology, stomatal arrangement, presence/absence of cuticle, and palisade cell ratio are important tools to assist taxonomy and in identifying adulterants. Various microscopic techniques have been used to develop a micromorphological catalog and its utility in identifying *S. lateriflora* samples.

Keywords: Adulteration, Herbal medicine, Microscopy, Trichomes

Received : 23 November 2020
Revised : 8 December 2020
Accepted : 22 December 2020
Published : 30 June 2021

TO CITE THIS ARTICLE:

Samantha H Sherman, Brajesh N. Vaidya and Nirmal Joshee. 2021. Micromorphological Studies in an Anxiolytic Medicinal Plant, *Scutellaria lateriflora* L. *Journal of Food and Agriculture Research,* 1: 1, pp. 1-14

1. Introduction

Life saving drugs originating from plants include berberine (psoriasis), caffeine (stimulant), camptothecin, capsaicin (rheumatic pains), codeine (antitussive), digoxin, diosgenin, ephedrine (stimulant), morphine (pain killer), taxol etc. (Karuppusamy, 2009; Lahlou, 2013; Vanisree *et al.* 2004). The global market

value of medicinal plant products exceeds $100 billion per annum (Sofowora *et al.* 2013). The genus *Scutellaria* is a member of the mint family (Lamiaceae) and an important source of phytochemicals. American skullcap (*Scutellaria lateriflora*) is one of the approximately 400 members of the genus *Scutellaria* (Paton, 1990). Recently, a few *Scutellaria* species were recorded from the Himalayan region and their phytochemical profile and medicinal applications were discussed (Dahal *et al.* 2021). *Scutellaria* plants are upright herbaceous perennial, rarely woody, that can grow between 10-60 cm tall. Stems are quadrangular and hollow, glabrous to sparsely hairy. The leaves are opposite, simple, and ovate. *Scutellaria lateriflora* commonly grows in riparian habitats which includes stream banks, marshes, and wet meadows. American skullcap has been used by the native Indians for medicinal usage frequently (Table 1). The plant was used by the Cherokee Indians to treat breast pain, expel afterbirth and promote menstruation (Moerman 1998, 2009). The Iroquois also used *S. lateriflora* roots in a concoction that was used to prevent smallpox and treat throat ailments (Upton, 2009). Several studies have been carried out to determine the chemical composition of *S. lateriflora* tissues to study its anxiolytic and antioxidant potential (Awad *et al.* 2003; Bergeron *et al.* 2005; Brock *et al.* 2010; Lohani *et al.* 2013; Sarris, 2007; Vaidya *et al.* 2013; Zhang *et al.* 2009). The anxiolytic properties of American skullcap are attributed to γ-aminobutyric acid (Bergeron *et al.* 2005). Biomedical studies exhibit anti-tumor properties of flavonoids wogonin, baicalein, and baicalin extracted from *S. lateriflora* and other species (Parajuli *et al.* 2009, 2011; Patel *et al.* 2013). Transgenic hairy root cultures initiated from *S. lateriflora tissues* exhibited potential for the production of bioactive secondary metabolites in response to elicitors (Wilczanska-Barska *et al.* 2012; Marsh *et al.* 2014). A thorough scrutiny of literature reports over 295 phytochemicals isolated from 35 *Scutellaria* species so far and a systematic research of large, naturally occurring germplasm could be of great medical value (Shang *et al.* 2010). Currently, American skullcap is one of the most commonly cultivated of all Skullcap species for the herbal products market and is commonly prepared as a tea. Other products are available in the form of extracts, tinctures, capsules, and granules. To meet the demand for American skullcap herb, it is commercially grown in Australia and New Zealand (Wills and Stuart, 2004). An increase in the dry matter was observed in field conditions when grown in partial shade, with application of fertilizer and manure (Similean *et al.* 2012). In greenhouse conditions, plants responded by increasing aerial parts and biomass yield to the application of nitrogen, phosphorus, and potassium fertilizer (Shiwakoti *et al.* 2015). Phosphorus applications showed the greatest effect on dry matter

and flavonoid yield. A few micropropagation studies have been conducted to establish efficient protocols for *S. lateriflora* to meet with its increased demand for herbal industry (Cole *et al.* 2007, 2009, Kawka *et al.* 2017, 2020; Tascan *et al.* 2010). Skullcap is commercially available in the form of liquid extracts, capsules, tablets, tea, and dried powder (Upton, 2009). Tinctures of skullcap are typically extracted using a mixed ethanol: water solvent that usually have a 40-60% ethanol range which produces maximum flavonoid content of about 70% (Wills and Stuart, 2004).

American skullcap has been associated erroneously with hepatotoxicity in the past due to the adulteration with germander (*Teucrium canadensis* and *Teucrium*) (Gafner and Blumenthal, 2016). Germander, which is also known as pink skullcap, is morphologically similar to American skullcap and can be misidentified even by experts. Adulteration of herbal products is an issue that is often caused by misidentification (Fennel, 2004). Study of micromorphological traits can play an important role as a taxonomic tool in identifying contaminated herbal samples (Shahin *et al.* 2019; Song *et al.* 2020; Soundappan *et al.* 2018). Microscopy is one of the most rapid, revealing, and cost-effective technologies to obtain a better understanding of plant structure and identity and purity of dried herbs and spices (Osman *et al.* 2019). Traditional medicine involves the use of plant parts in crude form, either fresh or dried. Quantitative microscopy can assist in scoring vein-islet number, vein termination number, stomatal number, stomatal index, and palisade ratio for identification of crude biomass. Vein islets and vein termination points are characteristics which are unique to all plants. These features can be used as a taxonomic fingerprint to distinguish herbal products from their adulterants and help industry meet good manufacturing practices. The palisade ratio is the average number of palisade cells that occur beneath an epidermal cell. The palisade ratio of many plants remains constant regardless of geographical location. This ratio has reliable taxonomic and pharmacognostic applications (Simon, 2018). We present investigations to develop a micromorphological catalog for medicinally and commercially important herb *S. lateriflora*.

2. Materials and Methods

Leaves of greenhouse grown *S. lateriflora* plant and a commercial sample of organic *S. lateriflora* tea (Starwest Botanicals®, Sacramento CA, USA) were used as representative samples to document micromorphological characters (Figure 1 A, D). Structures considered for the comparison and observed were palisade ratio, types of trichomes, stomata and their location, vein islets and vein

termination. Trichomes were also stained with a fluorescent dye to differentiate secretory and non-secretory trichomes.

2.1. Clearing and staining of plant material: Leaf samples were collected from greenhouse grown plants and dried, coarsely powdered commercial tea. Leaf morphology was studied in acetic acid: alcohol (glacial acetic acid 10 ml : 90 ml of 95% ethanol) fixed material. Plant material was fixed and cleared at 37 °C for 24h and then transferred to 70% ethanol for storage at 4 °C. Fixed plant material was washed 3-4 times in distilled water and then stained overnight with a few drops of 0.1% aqueous safranin 'O'. Samples were destained in water to remove excess stain, mounted on a slide and were observed for micromorphological features under Olympus BX-43 microscope, Olympus America, PA, USA, fitted with DP-72 camera (Soundappan *et al.* 2018).

2.2. Micromorphological analysis: Vein islet term is used to indicate the smallest unit of photosynthetic tissue encircled by the ultimate divisions of the conducting strands of leaves. It is the number of vein islets per mm^2 and is calculated from four contiguous square millimeters in the central part of the lamina, between the midrib and margin (Figure 2). Vein islet and termination number was recorded by taking pictures of cleared, safranin stained leaves under light microscope. Pictures were printed and six, one mm^2 grids were created (Figure 2 A). The number of vein islets and vein termination points in each grid was recorded for three leaves using six grids per leaf. To obtain palisade ratio, images were taken at the same position and the focus was adjusted to take one picture of the palisade cells and one picture of the epidermal cells (Figure 2 B-D). Pictures were printed and the images were superimposed upon one another. Images of three different leaves and ten epidermal cells per leaf were counted.

2.3. Light and fluorescent microscopy. Plant samples were processed as per Soundappan *et al.* (2018) using natural product reagent prepared with 5% (v/v) aqueous solution of aluminum chloride (Thermo Fisher Scientific®) and 0.05% diphenyl boric acid-β-ethylamino ester (DPBA) stain (Sigma-Aldrich, MO, USA) in 10% methanol (Burdick and Jackson, Muckegon, MI) for fluorescent microscopy (Heinrich *et al.* 2002). Leaf samples were soaked in DPBA solution in dark for 5-20 min, dabbed gently on the filter paper and observed under a fluorescent microscope (Olympus BX-43 fitted with Olympus DP-72 camera; Olympus America, PA, USA with ultraviolet (UV) light source from X-Cite series 120 Q; Lumen Dynamics, MA, USA) and was used to capture images.

2.4. Scanning electron microscopy. Plant material was fixed in 2% glutaraldehyde (Electron Microscopy Sciences, PA, USA) for two hours at 4 °C, and then secondary fixation was carried out in 1% osmium tetroxide for 30 min (Electron Microscopy Sciences, PA, USA). Ascending ethanol series

was used to dehydrate samples. Critical point drying (Samdri model 780-A, Tousimis, Rockville, MD, USA) and sputter coating with gold (Denton vacuum Desk V; Denton, NJ, USA) was followed. All scanning work was conducted at the Center for Ultrastructural Research, University of Georgia, Athens, USA. Gold coated samples were viewed under scanning electron microscope (Zeiss 1450EP, Zeiss SMT, Inc., Peabody, MA) at 4 KV.

3. Statistical Analysis

Data were analyzed using Analysis of variance (ANOVA) procedures of the Statistical Analysis System (Version 9.4, SAS Institute Inc., Cary, NC). Treatment means were analyzed using Tukey's post hoc mean separation test using SAS software with the least significance difference among 18 different data sets at $P < 0.05$ level for vein islet and vein termination number and 30 different data sets for palisade ratio.

4. Results and Discussion

Healthy, disease free, potted plants of *S. lateriflora* were used to sample leaves during vegetative phase for micromorphological studies. Plants start flowering in 3-4 months and flowers are 6-8 mm long, perfect, bilabiate, pedicellate, and the corolla is blue (Figure 1 A, B).

4.1. Micromorphological Observations

Palisade ratio: Palisade ratio was determined by taking pictures of safranin stained leaves (Figure 2 B-D). There was no morphological difference in the epidermal cells or in the palisade cells when the two types of cells were analyzed to derive palisade ratio. No statistically significant differences were seen in the palisade ratio between research plant material and commercial sample (Table 2). Palisade ratio provides general subjective information about the characteristics of a plant and can be used to distinguish some closely related species from each other e.g., *Agathosma* spp. (Raman *et al.* 2015). It is interesting to note that the palisade ratio is not applicable to analyze monocot leaves as differentiation within the mesophyll is not consistent (Mukherjee 2002).

Stomata morphology: Microscopic analysis of *S. lateriflora* samples revealed that stomata are mostly confined to abaxial surface (Figures 3B and 4C) confirming hypostomatic leaf. Very few stomata are present on the adaxial leaf surface. The stomata were anomocytic (Figure 3B), as the subsidiary cells surrounding stoma are not different from other epidermal cells in size and

Figure 1: Current research on *Scutellaria lateriflora*. A. Flowering experimental plants in the greenhouse. B. Close up of flowers to show floral morphology. C. Experimental organic tea sample

Table 1: Medicinal use of various skullcap species by native Indians in north America

	Scutellaria species	*Used by*	*Application*
1.	*S. galericulata*	Delaware, Oklahoma, Ojibwa	Laxative, Heart medicine
2.	*S. elliptica* / *S. incana*	Cherokee	Abortifacient and Antidiarrheal, Breast treatment, Gynecological a Kidney aid
3.	*S. lateriflora*	Cherokee, Iroquois	Abortifacient and Antidiarrheal, Emetic and Gynecological aid, Kidney and Throat aid
4.	*S. parvula*	Meskwaki	Antidiarrheal

Table 2: Micromorphological measurements of *S. lateriflora* leaves

Plant material (S. lateriflora)	*Number of Vein islets*	*Vein termination points*	*Epidermal cell and Palisade ratio*
Greenhouse plant	5.33 ± 0.28[a]	2.61 ± 0.40[b]	4.27 ± 0.22[a]
Commercial Tea	5.39 ± 0.25[a]	4.06 ± 0.40[a]	4.53 ± 0.22[a]

* Significant differences among means in a column by Tukey's test, $P < 0.05$. Data sets sharing the same letter have no significant differences.

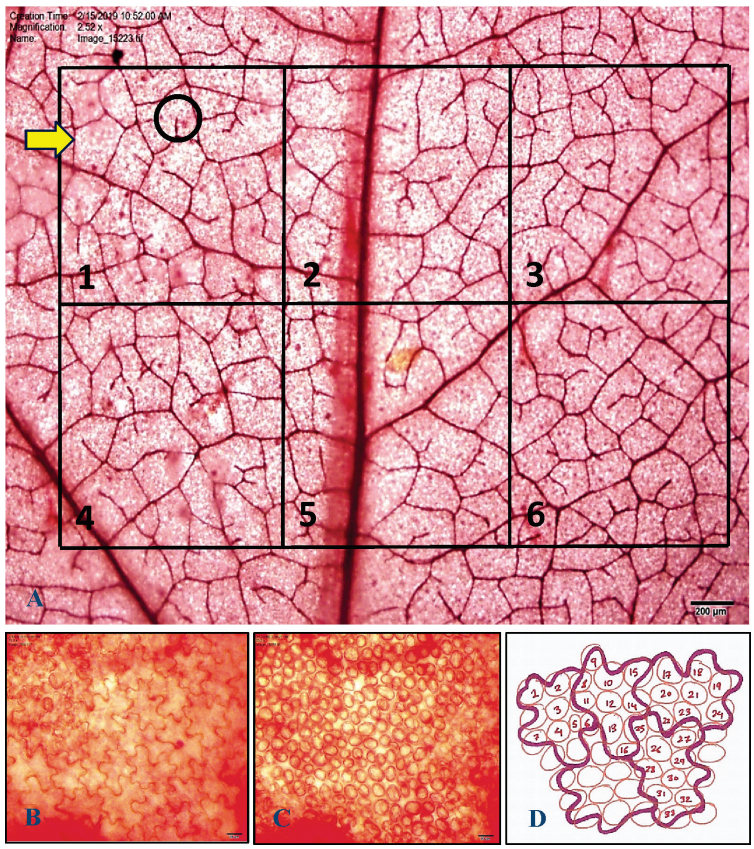

Figure 2: Micromorphological investigation in the cleared leaves of *S. lateriflora*. A. Microscopic image of *Scutellaria* leaf after clearing and staining with safranin. The yellow arrow points the area of vein islet. The black circle exhibits the vein termination point. B-D. Process series employed for the calculation of palisade ratio. B. After clearing, and staining of leaves, adaxial epidermal cells in *Scutellaria* species. C. Stained palisade cells in mesophyll layer under the epidermis. D. Palisade ratio counting process.

shape. Stoma appears to be embedded in epidermal cells and in tested samples their number ranged from three to six. These observations are in line with earlier research on the stomata of *S. lateriflora* (Cantino, 1990). No difference was observed in the type of stomata when research plant material and commercial tea sample were compared.

Vein islets and vein termination points: The vein or veinlet termination is the ultimate free termination of a vein or branch of a vein (Trease and Evans, 1966). There was no statistically significant difference in the number of vein islets in a unit area (per mm^2) of leaf when greenhouse grown plant and the commercial material were compared (Table 2), though there was difference in the number of vein termination points. This could be due to the quality of the commercial samples since they were dried and ground during processing causing distortion in tissue arrangements. This has been reported in another micromorphological study on powdered *Curcuma longa* (Jayeola, 2009). The size of the leaf samples from commercial material made it difficult to tell what area(s) of the leaf was analyzed. Another variable could be due to the age and maturity level of the commercial material. Since the material was dried and ground, distinguishing between mature and immature leaf samples proved to be a challenge. Minor venation patterns of Calycanthaceae showed that there is variability in the shape and size of aeriole and type of vein endings (Nicely, 1965). Differences in the vein termination points were observed in *Acer* that depend on the area of the leaf analyzed (Banerji and Das, 1972). This has been further supported by a study detailing the minor venation of 150 Euphorbiaceae (Sehgal and Paliwal, 1974) and 15 species of *Salix* where the minor venation was not useful in species identification (Singh *et al.* 1976). This was also supported by a study on another genus from Lamiaceae when out of the nine *Salvia* species only two could be identified on the basis of venation (Gupta and Bhambie, 1979).

Light, fluorescent and scanning electron microscopy of trichomes: Non-glandular falcate trichomes (one -, two -, and three celled) were seen on the midrib, veins, lamina, and along the margin (Figure 3 C-F). Lamina has non-glandular trichomes that are usually 1-3 celled (Figure 4A) and covered with micropapillae (Figures 3 C and 4 C,D). The non-glandular trichomes have sharp, pointed tips with basal plate comprising of 4-5 cells. There were very few capitate glandular trichomes present on the leaf surface and usually with a very short one- to two cell stalk (Figure 3A). The American skullcap is comprised of mostly non-glandular trichomes, denser on the adaxial surface of the leaf. No differences were of observed in trichome morphology of greenhouse plant material and

commercial tea leaves. Most prevalent trichomes in the leaves of *S. lateriflora* were peltate type and they were more on the abaxial surface (Figure 4).

Non glandular trichomes usually exhibited very little pale yellow or no fluorescence. Glandular capitate trichomes exhibited a green fluorescence whereas peltate trichomes showed a yellow fluorescence clearly demarcating four cells, staining large nuclei (Figure 3 I-K). Scanning electron microscopy of the leaves and flowers revealed a variety of trichomes on the plant surface (Figure 4). Non- glandular and glandular (capitate and peltate) trichomes were seen on the abaxial and adaxial surface of the leaves of *Scutellaria lateriflora* (Figure 4).

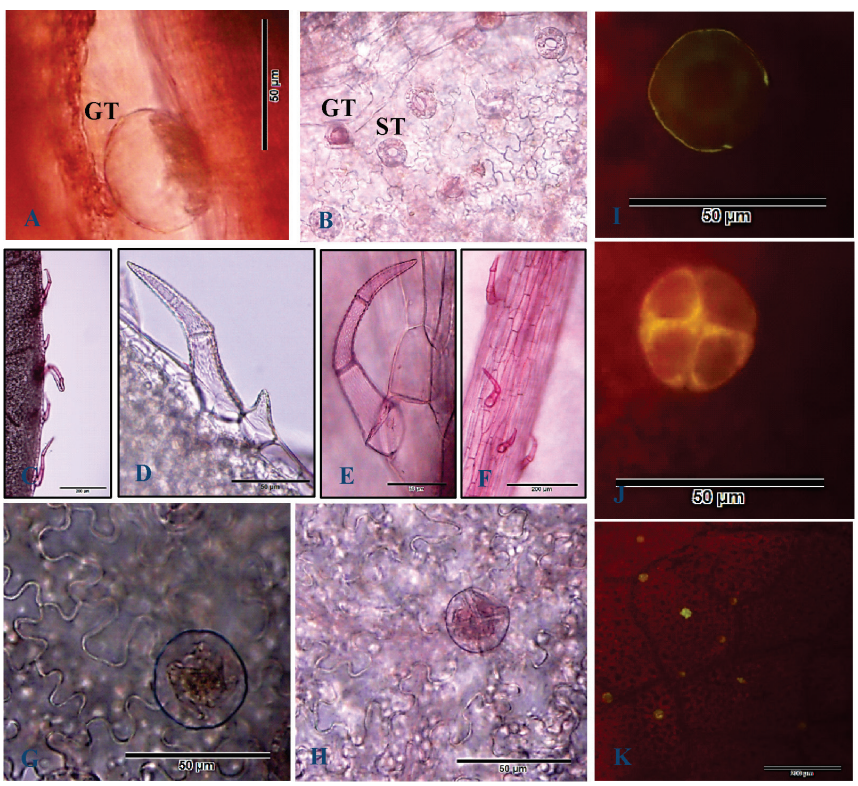

Figure 3: Micromorphological features detected in the greenhouse plant and commercial tea sample. A. Glandular trichome with a short stalk, B. Stomata present on the abaxial surface of the leaf, C. 1-4 cell long non-glandular trichomes on the leaf margin, D. One and two celled non-glandular trichomes at leaf margin, E-F. Three to four cells long non-glandular trichomes on the veins, G-H. Glandular and 4-celled peltate trichomes, I-K. DPBA staining and fluorescent detection of capitate and peltate trichomes. GT- glandular trichomes, ST- stomata

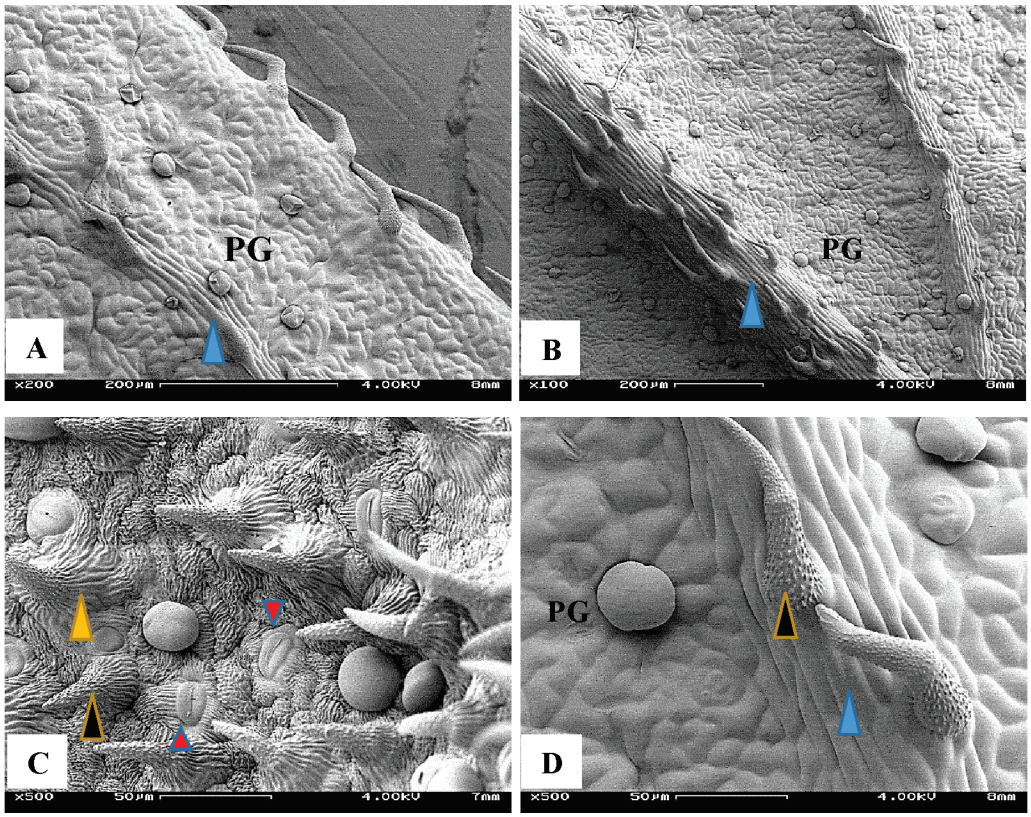

Figure 4: Scanning electron microscopy of *Scutellaria lateriflora* leaf. A. Adaxial leaf surface with non-glandular falcate and button-shaped peltate trichomes. B. Abaxial surface of leaf with long, needle like non-glandular trichomes on midrib and veins and peltate trichomes on lamina. C. A close-up of non-glandular and peltate trichomes with closed stomata. On the leaf surface cuticle is also visible. D. A close-up of non-glandular and peltate trichomes on the abaxial surface of the leaf. Arrows: Black- micropapillae on non-glandular trichome, Blue- vein, Red- stomata, Yellow- cuticle layer; PG- peltate gland

Many plant species exhibit consistent measurements and morphological characteristics that can allow for the differentiation of closely related species as was seen in case of three species of *Erythroxylum* growing in Thailand (Katib and Rungsihirunrat, 2020).

6. Future Prospects and Conclusion

Scutellaria baicalensis and *S. lateriflora* are the two species which have been used most extensively in herbal formulations. At times plant material is wild crafted, ending up with adulterated samples. A comparison of two leaf

samples of *S. lateriflora* revealed that there were no differences in trichome morphology, number of vein islets, and palisade ratio proving authenticity of the commercial sample. Microscopy is a useful preliminary tool that can help rapidly distinguish one plant from another. The demand for increased quality control should be addressed using all available methods. Microscopy is not always useful as the sole method of identification but this technique can be used in association with other analytical methods. Depending on the taxonomist and system followed, there are 360-400 *Scutellaria* species. Micromorphological features can be of great help to assign correct taxonomic position when used with other tools.

References

Awad R, Arnason JT, Trudeau V, Bergeron C, Budzinski JW, Foster BC, & Merali Z. 2003. Phytochemical and biological analysis of skullcap (*Scutellaria lateriflora* L.): a medicinal plant with anxiolytic properties. Phytomedicine, 10, 640–649.

Banerji ML & Das S. 1972. Minor venation pattern in the Indian Acers. Advances in Plant morphology. YS Murty et al. (ed.), Sarita Prakashan, Meerut, Pp. 51-57.

Bergeron C, Gafner S, Clausen E, & Carrier DJ. 2005. Comparison of the chemical composition of extracts from *Scutellaria lateriflora* using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or 70 % ethanol extraction. Journal of Agriculture and Food Chemistry, 53, 3076- 3080.

Brock C, Whitehouse J, Tewfik I, & Towell T. 2010. American skullcap (*Scutellaria lateriflor*a): an ancient remedy for today's anxiety? British Journal of Wellbeing, 1(4), 25-30.

Cantino PD. 1990. The phylogenetic significance of stomata and trichomes in the Labiatae and Verbenaceae. Journal of Arnold Arboretum, 71, 323-370.

Cole IB, Saxena PK, & Murch SJ. 2007. Medicinal biotechnology in the genus *Scutellaria*. In vitro Cell Development-Plant, 43, 318-327.

Cole IB, Farooq FT, & Murch SJ. 2009. Protocols for establishment of an *in vitro* collection of medicinal plants in the genus *Scutellaria*. In: Protocols for in vitro cultures and secondary metabolite analysis of aromatic and medicinal plants. Methods in Molecular Biology, Vol 547, 155-165. DOI: 10.1007/978-1-60327-287-2_13

Dahal N, Mahat R, Thapa A, Kunwar R, Bussmann RW, Paniagua-Zambrana NY, Turi MA, & Hussain W. 2021. *Scutellaria barbata* D. Don *Scutellaria discolor* Colebr. *Scutellaria orientalis* L. *Scutellaria prostrata* Jacq. ex Benth. Lamiaceae. In: Kunwar R., Sher H., Bussmann R.W. (eds.), Ethnobotany of the Himalayas. Ethnobotany of Mountain Regions. Springer, Cham. https://doi.org/10.1007/978-3-030-45597-2_222-1

Fennel CW, Lindsey KL, McGaw SG, Sparg GI, Setafford EE, Elgorashi OM, Grace K, & van Staden J. 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology, 94(2-3), 205-217.

Gafner S & Blumenthal M. 2016. Botanical adulterants bulletin on adulteration of skullcap. Botanical Adulterants Program. Pp. 1-5.

Gupta ML & Bhambie S. 1979. Studies in Lamiaceae III: The taxonomic value of leaf architecture in *Salvia* L. Proceedings of Indian National Science Academy, 45(6), 589-595.

Heinrich G, Pfeifhofer HW, Stabentheiner E, & Sawidis T. 2002. Glandular hairs of *Sigesbeckia jorullensis* Kunth (Asteraceae): morphology, histochemistry and composition of essential oil. Annals of Botany, 89:459–469.

Jayeola AA. 2009. Micromorphological study of plant fragments in some powdered medicinal plants. Journal of Medicinal Plants Research, 3(5), 438-442.

Karuppusamy S. 2009. A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell cultures. Journal of Medicinal Plant Research, 3(13), 1222-1239.

Katib S & Rungsihirunrat K. 2020. Macroscopic-microscopic characteristics and AFLP fingerprint for identification of *Erythroxylum novogranatense*, *E. cambodianum* and *E. cuneatum* endemic to Thailand. International Journal of Research in Pharmaceutical Sciences, 11(4), 6144-6154.

Kawka B, Kwiecien I, & Ekiert H. 2017. Influence on culture medium composition and light conditions on the accumulation of bioactive compounds in shoot cultures of *Scutellaria lateriflora* L. (American skullcap) grown *in vitro*. Applied Biochemistry and Biotechnology, 1834(4), 1414-1425.

Kawka B, Kwiecien I, & Ekeirt H. 2020. Endogeneous production of specific flavonoids and verbascoside in agar agitated microshoot cultures of *Scutellaria lateriflora* L. and biotransformational potential. Plant Cell Tissue and Organ Culture, 142, 471-482.

Lahlou M. 2013. The success of natural products in drug discovery. Pharmacology and Pharmacy, 4, 17-31.

Lohani M, Ahuji M, Buabeid MA, Schwartz D, Shannon D, Suppiramaniam V, Kemppainen B, & Dhanasekaran M. 2013. Antioxidative and DNA protecting effects if flavonoids-rich *Scutellaria lateriflora*. Natural Products Communications, 8(10), 1415-1418.

Marsh Z, Yang T, Nopo-Olazabal L, Wu S, Ingle T, Joshee N, & Medina-Bolivar F. 2014. Effect of light, methyl jasmonate, and cyclodextrin on production of phenolic compounds in hairy root cultures of *Scutellaria lateriflora*. Phytochemistry, 107, 50-60.

Moerman DE. 1998. Native American Ethnobotany. Timber Press, USA.

Moerman DE. 2009. Native American Medicinal Plants: An Ethnobotanical Dictionary. Timber Press, USA, 450 p.

Mukherjee P. 2002. Quality control of herbal drugs. New Delhi. Business Horizons, 816 p.

Nicely KA. 1965. A monographic study of the Calycantheacea. Castanea, 30, 38-81.

Osman AG, Raman V, Haider S, Ali Z, & Chittiboyina AG. 2019. Overview of analytical

tools for the identification of adulterants in commonly traded herbs and spices. Journal of AOAC International, 102, 1-10.

Parajuli P, Joshee N, Rimando A, Mittal S, & Yadav AK. 2009. In vitro anti-tumor mechanisms of various Scutellaria extracts and constituent flavonoids. Planta Medica, 75, 41–48.

Parajuli P, Joshee N, Chinni SR, Rimando AM, Mittal S, Sethi S, & Yadav AK. 2011. Delayed growth of glioma by *Scutellaria* flavonoids involve inhibition of Akt, GSK-3 and NF-kB signaling. Journal of Neurooncology, 101, 15–24.

Patel PS, Joshee N, Rimando AM, & Parajuli P. 2013. Anti-cancer scopes and associated mechanisms of *Scutellaria* extract and flavonoid wogonin. Cancer Immunology and Immunotherapy, 9, 34–42

Paton A. 1990. A global taxonomic investigation of *Scutellaria*. Kew Bull 45(3), 399–450.

Raman V, Sagi S, Galal AM, Avula B, Viljoen A, & Khan IA. 2015. Adulteration in commercial buchu dietary supplements: Analyses of commercial and authentic buchu samples and comparative studies of *Agathosma betulina* and *Agathosma crenulata* by microscopy and HPTLC. South African Journal of Botany, 100, 122-131.

Sarris J. 2007. Herbal medicines in the treatment of psychiatric disorders: a systematic review. Phytotherapy Research, 21(8), 703-716.

Sehgal L & Paliwal GS. 1974. Studies on the leaf anatomy of Euphorbia II veination patterns. Botanical Journal of Linnean Society, 68, 173-208.

Shahin L, Phaal SS, Vaidya BN, Brown JE, & Joshee N. 2019. *Aronia* (Chokeberry): an underutilized, highly nutraceutical plant. Journal of Medicinally Active Plants, 8(4), 46-63.

Shang X, He X, He X, Li M, Zhang R, Fan P, Zhang Q, & Jia Z. 2010. The genus *Scutellaria* an ethnopharmacological and phytochemical review. Journal of Ethnopharmacology, 128(2), 279 –313.

Similien A, Shannon DA, Wood CW, van Santen E, Joshee N, & Foshee WG. 2012. Partial shade, irrigation, and added nutrients maximize dry matter yield of American skullcap (*Scutellaria lateriflora*). HortScience, 47(12), 1705-1709.

Shiwakoti S, Shannon DA, Wood CW, Joshee N, Ramando A, Lawrence KS, & Kemppainen B. 2015. Nitrogen, phosphorus, and potassium effects on biomass yield and flavonoid content of American skullcap (*Scutellaria lateriflora*). Journal of Plant Nutrition, 39(9), 1240-1249.

Simon TK. 2018. Systematic significance of palisade ratio in pharmacognostic applications. IOSR Journal of Pharmacy and Biological Sciences, 13(2), 1-8.

Singh VD, Jain K, & Sharma M. 1976. Leaf architecture in *Salix*. Journal of Indian Botanical Society, 55, 140-150.

Sofowora A, Ogunbondede E, & Onayade A. 2013. The role and place of medicinal plants in the strategies for disease prevention. African Journal of Traditional Complementary and Alternative Medicine, 10(5), 210-229.

Song J-H, Yang S, & Choi G. 2020. Taxonomic implications of leaf micromorphology using microscopic analysis: A tool for identification and authentication of Korean piperales. *Plants*, *9*(5), 566. https://doi.org/10.3390/plants9050566

Soundappan K, Bajaj R, Vaidya BN, & Joshee N. 2018. Anatomical and biochemical investigations on medicinal tree *Murraya koenigii* Spreng. Journal of Biotechology Research, 9, 69-77.

Tascan A, Adelberg J, Tascan M, Rimando AM, Joshee N, & Yadav AK. 2010. Hyperhydricity and flavonoid content of *Scutellaria* species *in vitro* on polyester-supported liquid culture system. HortScience, 45(11), 1723-1728.

Upton R. 2009. Skullcap aerial parts *Scutellaria lateriflora* L. standards of analysis, quality control and therapeutics. R. Upton (ed.) American Herbal Pharmacopeia and Therapeutic Compendium®, Scotts Valley CA. p. 1-17.

Vaidya BN, Brearley TA, & Joshee N. 2013. Antioxidant capacity of fresh and dry leaf extracts of sixteen *Scutellaria* species. Journal of Medicinally Active Plants, 2(3), 42-49.

Vanisree M, Lee CY, Lo SF, Nalawade SM, Lin CY, & Tsay HS. 2004. Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. Botanical Bulletin Academia Sinica Taipei, 45(1), 1-22.

Wilczanska-Barska A, Krolicka A, Glod D, Majdan M, Kawaik A, & Krauze-Baranowska M. 2012. Enhanced accumulation of secondary metabolites in hairy root cultures of *Scutellaria lateriflora* following elicitation. Biotechnology Letters, 34(90), 1757-1763.

Wills RBH & Stuart DL. 2004. Generation of High -Quality Australian Skullcap Products. RIRDC Publication No 04/020. pp. 1-26.

Zhang Z, Lian XY, Li S, & Stringer JL. 2009. Characterization of chemical ingredients and anticonvulsant activity of American skullcap (*Scutellaria lateriflora*). Phytomedicine, 16, 485- 493.