Baez-Parra et al., Afr J Tradit Complement Altern Med., (2018) 15 (1): 110-116
https://doi.org/10.21010/ajtcam.v15i1.11

LEAF MORPHOLOGY AND ANATOMY OF VARIETIES OF *TURNERA DIFFUSA* VAR. *DIFFUSA* AND *TURNERA DIFFUSA* VAR. *APHRODISIACA* (WARD) URB

Karla Marina Baez-Parra[1], Lilia Alcaraz-Melendez[2], Apolinar Santamaria-Miranda[3], Jose Basilio Heredia[1], Josefina Leon-Felix[1], Maria Dolores Muy-Rangel[1], Miguel Angel Angulo-Escalante[1*]

[1]Centro de Investigación en Alimentación y Desarrollo, A.C. Unidad Culiacán. Km. 5.5 Carr. Culiacán-Eldorado, Col. Campo El Diez, C.P 80110, Culiacán, Sinaloa, México. [2]Centro de Investigaciones Biológicas del Noroeste, S.C. Av. Instituto Politécnico Nacional 195, Playa Palo de Santa Rita Sur, La Paz, C.P. 23096, Baja California Sur, México. [3]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional del Instituto Politécnico Nacional, Unidad Sinaloa. Bulevar Juan de Dios Bátiz Paredes #250, Colonia San Joachin. Guasave, Sinaloa, México.

***Corresponding Author Email:** mangulo@ciad.mx

Article History

Received: May. 15, 2017

Revised Received: Jul, 5. 2017

Accepted: Jul. 10, 2017

Published Online: Dec. 29, 2017.

Abstract

Background: Mexican damiana (*Turnera diffusa* Willd. Ex. Schult), specifically damiana of California has a high demand in the international market for its popularity as aphrodisiac, but its marketing has been affected by the adulteration of its products due to a lack of authentication mechanisms and limited information on the differences between the two currently known varieties. The aim of this study was to establish a leaf standard monograph with scientific bases of the varieties *aphrodisiaca* and *diffusa* of *Turnera diffusa* Willd. Ex. Schult, cultivated at the same agronomic and environmental conditions.
Material and Methods: Damiana leaves were collected from a cultivar located in the municipality of Culiacan, Sinaloa, Mexico in 2016. The pharmacognostic studies were carried out in terms of macroscopic and both optical and electronic microscopic characteristics of leaves of the two varieties for their distinction.
Results: The leaves of *diffusa* variety were twice as large as those of the *aphrodisiaca* variety, whereas papillose glandular trichomes were observed with greater density in the *aphrodisiaca* variety than those on *diffusa* leaves where unicellular trichomes were the ones observed in greater density. The leaves of both varieties are dorsiventral and hypostomatic with paracytic stomata.
Conclusion: The obtained qualitative and quantitative leaf standards provide reference information for the proper identification and monograph preparation of the *aphrodisiaca* and *diffusa* varieties of *Turnera diffusa*. Pharmacognostic characteristics such as the type of stomata, trichomes, and leaf identify the species, whereas characteristics as leaf size and trichome density differ between varieties.

Keywords: Turnera diffusa, var. diffusa, var. aphrodisiaca, medicinal plant, trichomes, microscopy.

Introduction

Turnera diffusa Willd. Ex. Schult, (Passifloraceae) usually known as damiana (Szewczyk and Zidorn, 2014) is a deciduous shrub that measures from 0.3 to 2 m height; it grows in arid and semi-arid regions of Western India, South America, Mexico, and the United States of America (Alcaraz-Meléndez et al., 2007; Zhao et al., 2007). It has alternate leaves that are ovate-lanceolate, pubescent, one to 2.5 cm long and 0.4 to 1 cm wide and light green-gray to greenish yellow color with a serrated margin dentate and venation pinnate more prominent on the underside; its smell is pleasant and aromatic (Alcaraz-Melendez et al., 2007; FEUM, 2013).

The flowers are small and yellow from eight to 12 mm long with five yellowish petals, which grow in the axils of the upper leaves. The fruit is a small capsule that measures about 5 mm and releases two to four seeds when ripening (Arbo, 2000; Zhao et al., 2007; FEUM, 2013).

Two varieties of *Turnera diffusa* have been reported by Arbo (2000), the var. *diffusa* with very variable, herbaceous, curly, fragrant, and often discolored leaves with whitish hairs, persistent after leaf fall; the var. *aphrodisiaca* also has colorless or discolored leaves sometimes glabrous and may present glandular capillary-sessile hair or with hairs on veins.

Damiana leaves have been used in traditional medicine as an aphrodisiac (FEUM, 2013; Szewczyk and Zidorn, 2014; Estrada-Reyes et al., 2016), diuretic, nerve tonic, urinary tract infections, frigidity, vaginal discharge, painful menstruation, and menopause problems (Chevallier, 1996; FEUM, 2013). The current importance of damiana in industrialized countries is not only as a medicinal plant but also as a drug for the treatment of sexual impotence where it is used in conjunction with other stimulants (Kumar et al., 2006; Szewczyk and Zidorn, 2014).

In Mexico damiana is mostly found in the state of Baja California Sur (*Turnera diffusa* Willd. ex Schult. var. *aphrodisiaca* Ward Urb.); the variety is consumed nationally and internationally and known as damiana of California. Because of its commercial importance, government programs have been established to encourage cultivation and avoid deforestation, as the wild plant is used for commercial purposes. Damiana of California marketing has been affected by the adulteration of its products, including adulteration by plants that do not belong to the genus *Turnera*, a situation that is becoming more common due to the lack of material for authentication as var. *aphrodisiaca* or for other varieties of *Turnera diffusa* that are also used (Gamez et al., 2010; Martinez de la Torre, 2013).

Despite the popular use of this medicinal plant, no conclusive morpho-anatomical analysis of the leaves has been performed with the aim of assisting in the differentiation of the *T. diffusa* var. *aphrodisiaca* to provide further information about the *T. diffusa* species. Therefore, this study aimed to establish a leaf standard monograph of the varieties *aphrodisiaca* and *diffusa* of *Turnera diffusa* Willd. Ex. Schult, cultivated under the same agronomic and environmental conditions.

Material and Methods

Plant collect and identification

Material of *Turnera diffusa* Willd ex Schult. var. *aphrodisiaca* Ward Urb. and *Turnera diffusa* Willd. ex Schult. var. *diffusa* Ward Urb. was collected from Imala, Culiacan, Sinaloa, Mexico (24° 51' 35" N; 107° 13' 1" W; 92 amsl) at the flowering and fruiting stages from October 2015 to March 2016. The varieties were identified by Professor Adrian Beltran from the School of Biology at the Universidad Autónoma de Sinaloa in Culiacan, Sinaloa, Mexico, and by Sergio Real-Cosio from Centro de Investigaciones Biológicas del Noroeste (CIBNOR). The specimens with accession numbers HCIB30319 for *T. diffusa* var. *diffusa* and HCIB300069 for *T. diffusa* var. *aphrodisiaca* were deposited in CIBNOR herbarium.

Leaf morphology

The morphological analyses including shape and size of leaves were carried out with a stereomicroscope Leica S8AP0; the photos were taken with a Leica MC120 HD (Leica Microsystems Inc. Buffalo Grove, Illinois, USA) integrated camera and documented in LAS V4.5 software; additionally size measurements of leaves were performed using a Surtek digital calibrator (Micro Precision Calibration, Inc. Grass Valley, California, USA). Color measurement was performed with a CM-700d spectrophotometer (Konica Minolta Sensing Americas, Inc., Ramsey, New Jersey, USA).

Stomata and trichome scanning electron microscopy

For the stomata and trichome analysis, five plants were selected at random from each variety, using five leaves *per* plant. The leaves were mixed and divided into two sections and gradually dehydrated with ethanol at 20, 40, 60, 80, and 100% in each solution for 30 min. The samples were placed into a critical drying chamber (Samdri-PVT-3D®, Maryland, USA), and alcohol was substituted by CO_2. The leaves were placed on aluminum sheets coated with gold (Denton Vacuum Desk II®, South Carolina, USA) and examined with a Scanning Electron Microscope at 15 mm distance and 15.00 kV of voltage (Hitachi S-30000N; Chiyoda-ku, Tokyo, Japan).

Optical microscopy of damiana leaves

The anatomical studies of damiana leaves were carried out using the technique described by other authors (Covarrubias-Carrillo et al., 2002; Osuna-Enciso et al., 2008), which consisted of fixing the leaves in FAA solution (10% formaldehyde at 37%, 50% alcohol at 96°, 5% glacial acetic acid and 35% water) and dehydrated with ethanol 70%, 80%, 96%, 100% ethanol-xylol 100% - 100%, 100% xylol and paraffin (in each solution for two hours). A centrifugal tissue processor STP 120 Thermo Fisher Scientific (Waltham; Massachusetts, USA) was used to dry out the tissues. A HistoStar Embedding

Workstation was used for tissue inclusion in paraffin; then, paraffin-embedded leaves were cut to five µm in thickness with a rotary microtome HM electronic 340E (Thermo Fisher Scientific, Waltham, Massachusetts USA). Finally, the sections were stained with safranin and fast green and observed with a compound microscope Zeiss Imager A2; photographs were taken with a camera Axiocam ERcSs integrated and documented in ZEN software lite 2012 (Carl Zeiss Microscopy GmbH, Jena, Germany). All of the reagents were analytical grade.

Statistical analysis

The ANOVA analysis was performed in the Minitab 17 statistical program; and for comparison of the mean, Tukey's test was used with 95% confidence.

Results

Morphological characteristics

Plants of the variety *diffusa* showed vertical growth (Figure 1A) with fewer secondary branches, zero to one branches with 25-cm stems and 42 leaves while the growth of the variety *aphrodisiaca* was semi-vertical with more than ten times more branches (Figure 1B), 14 secondary branches (average) with 254 leaves. The leaves of both varieties were alternate, ovate-lanceolate (29.86 ± 0.449 mm long and 11.31±0.944 mm wide for var. *diffusa*, 14.42 ± 0.388 mm long and 4.43 ± 0.253mm wide for var. *aphrodisiaca*) and green, with significant differences in chromaticity and luminosity between varieties (Table 1).

Figure 1: *Turnera diffusa* plants, A: *Turnera diffusa* var. *diffusa*, B: *Turnera diffusa* var. *aphrodisiaca*, both cultivated under similar environmental conditions in Imala, Sinaloa, Mexico.

Table 1: Color parameters of *Turnera diffusa* varieties. Values with different letters are significantly different ($p \leq 0.05$); ± standard deviation, n = 15. Different letter means significant difference

	Var. diffusa	***Var. aphrodisiaca***
°Hue	113.70a ± 0.29	113.29a ± 1.67
Luminosity	47.28b ± 0.17	48.51a ± 0.53
Chromaticity	9.04b ± 0.33	11.63a ± 0.86

Microscopic characteristics

In var. *diffusa* leaves, pubescence was observed on the adaxial and abaxial surfaces (Figure 2A, C); pubescence consisted of unicellular trichomes; in addition to these trichomes, var. *diffusa* plants showed papillose glandular trichomes (Figure 3B) on abaxial surface. On the other hand, in var. *aphrodisiaca*, unicellular hair trichomes were observed on both leaf surfaces along the midrib but with less density than those of the *diffusa* variety. In addition, papillose glandular trichomes were observed with greater density (Figure 3A) than var. *diffusa* (Figure 2B, D). Glandular trichome quantification revealed an average of 20.89 trichomes/mm on var. *diffusa* in comparison with 48.00 trichomes/mm on var. *aphrodisiaca*.

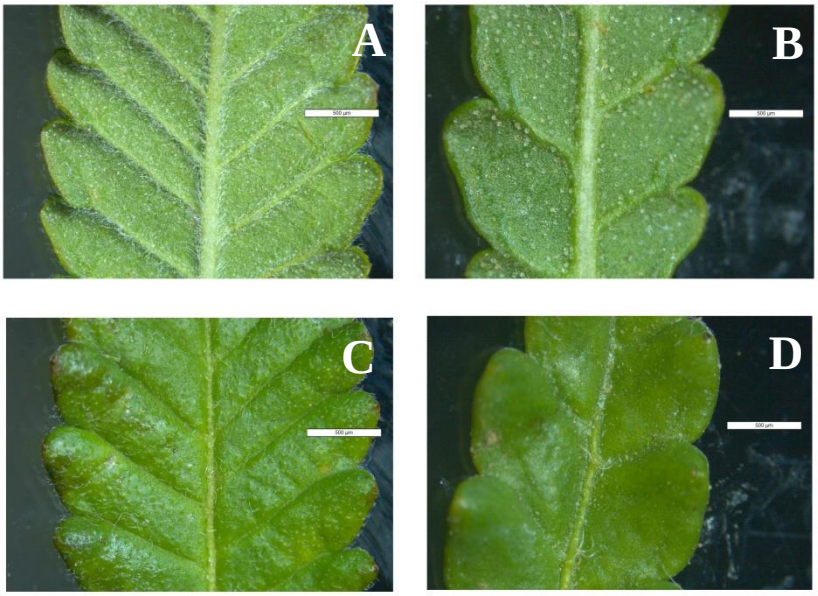

Figure 2: *Turnera diffusa* leaf micrographs with trichome presence. A: abaxial leaf surface var. *diffusa*, B: abaxial leaf surface var. *aphrodisiaca*, C: adaxial leaf surface var. *diffusa*, D: adaxial leaf surface var. *aphrodisiaca*.

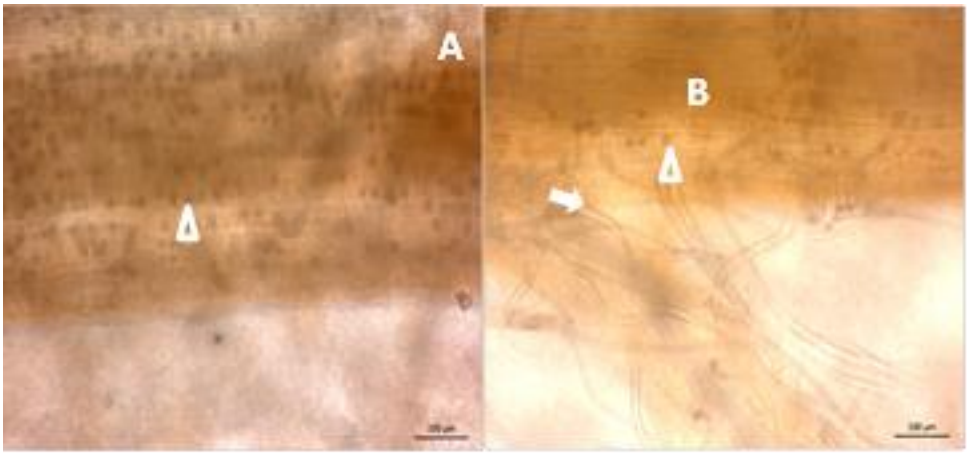

Figure 3: Discolored leaf micrographs shown unicellular (arrow) and glandular papillose trichomes (triangle) on the midrib. A: *Turnera diffusa* var. *aphrodisiaca* papillose glandular trichomes, B: var. *diffusa* unicellular trichomes and glandular papillose trichomes.

In the leaf epidermal analysis, both varieties were hypostomatic; because stomata were observed only on the abaxial surface, which were paracytic also for both varieties. Figure 4 shows adaxial surface without stomata (Figure 4A, C) for both varieties and types of stomata on the abaxial surface (Figure 5B, D); two subsidiary cells were observed next to the guard cells, characteristic of paracytic stomata.

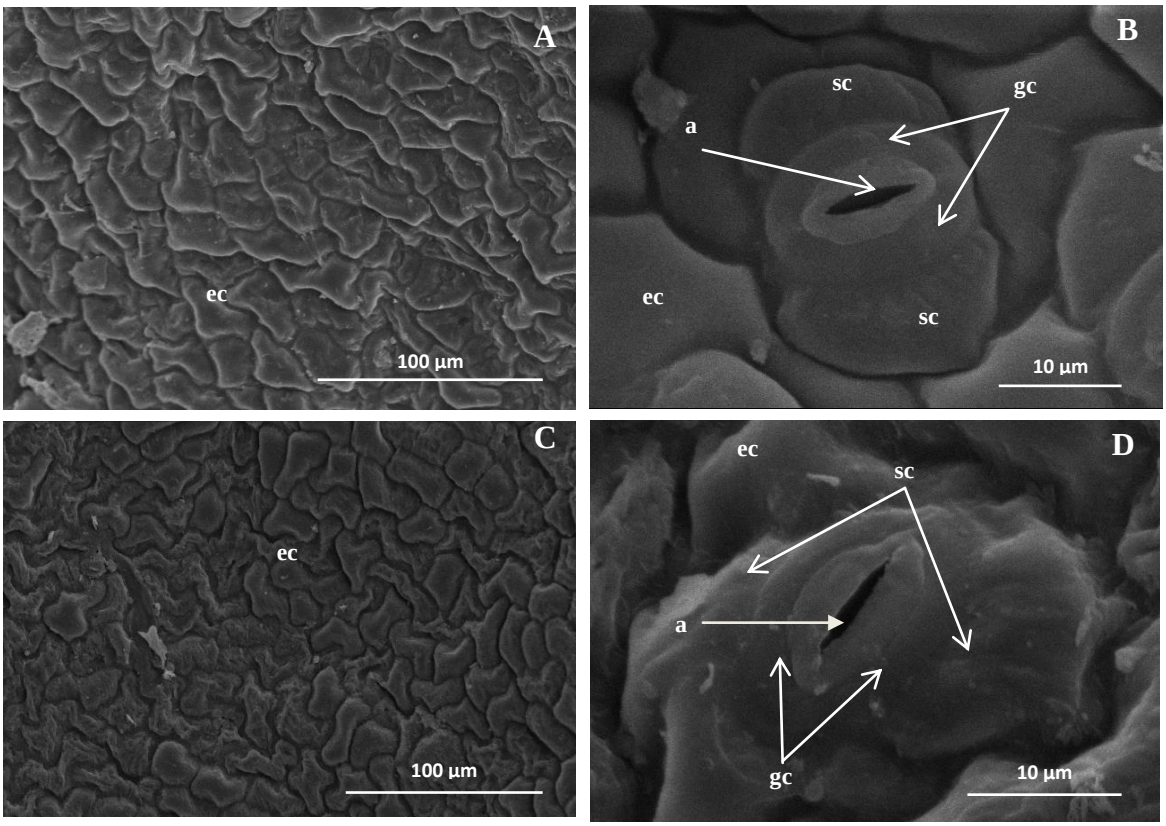

Figure 4: Scanning Electron Microscopy (SEM) analysis of damiana leaves. The images show abaxial and adaxial surface epidermis of *Turnera diffusa*, A: var. *diffusa* adaxial surface, B: var. *diffusa* stomata on the abaxial surface, C: var. *aphrodisiaca* adaxial surface, D: var. *aphrodisiaca* stomata on the abaxial surface. (ec: epidermal cell, sc: subsidiary cell, gc: guard cell, a: aperture).

Figure 5 shows the electronic micrographs of the abaxial and adaxial surface epidermis of *T. diffusa* var. *diffusa*, abaxial surface (5A, B, E) presenting unicellular and glandular papillose trichomes, (5C, D) while the adaxial surface showed only unicellular trichomes.

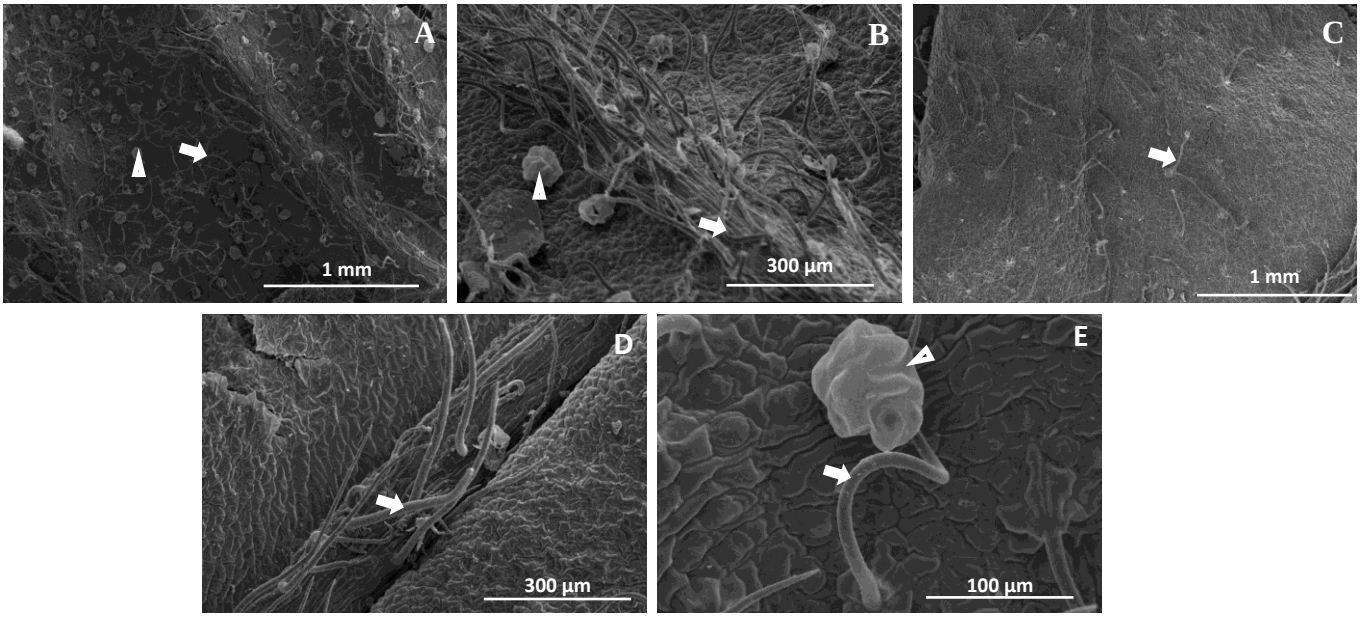

Figure 5: Scanning Electron Microscopy (SEM) analysis of *Turnera diffusa* var. *diffusa* trichomes. The images show abaxial and adaxial surface epidermis; A, B: show unicellular (arrow) and glandular papillose (triangle) trichomes on the abaxial surface; C, D: unicellular hair and glandular papillose trichomes on the adaxial surface; E: unicellular and glandular papillose trichomes.

In the same manner, the surface analysis of *T. diffusa* var. *aphrodisiaca* was performed. The adaxial surface showed glandular papillose trichomes (5A, B) and unicellular hair trichomes on the mimbrid; on the abaxial surface only unicellular trichomes were observed.

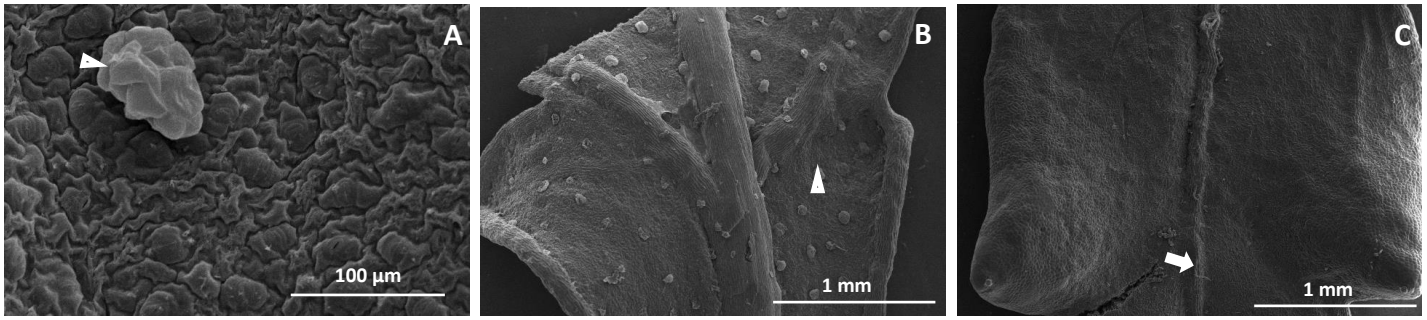

Figure 6: Scanning Electron Microscopy (SEM) analysis of *Turnera diffusa* var. *aphrodisiaca* trichomes. The images show abaxial and adaxial surface epidermis; A, B: adaxial surface show glandular papillose trichomes (triangle); C: show unicellular trichomes (arrow) only on the midrib on the abaxial surface.

In a transverse section of leaves a line of tubular cells was observed; then the mesophyll consisting of a layer of palisade cells, followed by the spongy parenchyma. The arc-shaped vascular bundle was composed of xylem on the lower side and phloem on the upper side; according to this observation, the leaves of both varieties are dorsiventral (Figure 7A, B).

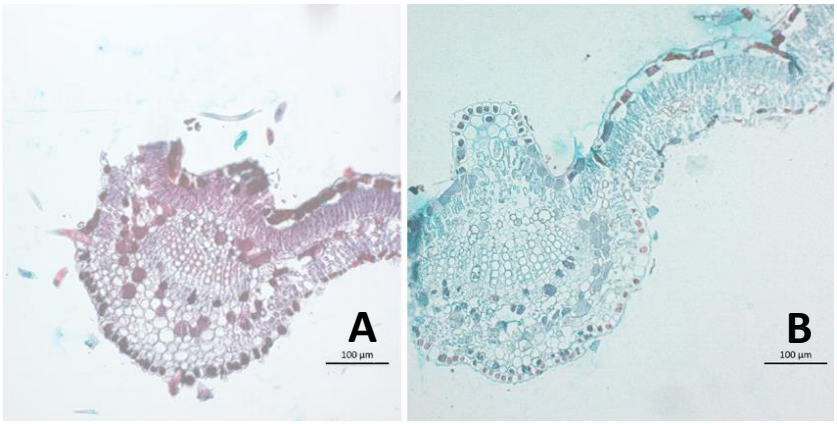

Figure 7: Micrographs of a transverse section of *Turnera diffusa* leaf. A: var. *diffusa*, B: var. *aphrodisiaca*.

Discussion

According to the World Health Organization (WHO, 2002; 2013) and the Herbal Pharmacopoeia of the Mexican United States (FEUM, 2013), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity, which must take place before any other study. Pharmacognostic studies provide an added value for the authentication of medicinal plants. For the *Turnera diffusa* varieties studied here, one of the most important parameters was the presence of trichomes, which were found to be unicellular and glandular papillose; the unicellular trichomes refract light from sunlight, thus protecting the plant from heat to avoid dehydration, which may also explain the color results, as shown in *var. aphrodisiaca* with higher luminosity and chromaticity compared with *var. diffusa* that showed a higher density of unicellular hair trichomes. On the other hand, papillose glandular trichomes secrete essential oils; thus, these trichomes are more commercially important (Alcaraz-Melendez et al., 2007). Other studies on medicinal plants, as oregano, have differentiated commercially important varieties through their trichomes; in addition, the types of trichomes have been related to the production, storage, and secretion of essential oil, showing that the species that contained a greater density of glandular trichomes contained a greater quantity of essential oil (Glas et al., 2012; Shafiee-Hajiabad et al., 2014). In var. *aphrodisiaca*, a higher density of glandular trichomes was observed (48.00 *versus* 20.89); however, a comparison of oil quantity and quality is needed to make this assertion (Moreno, 1984). The transverse sections of leaves did not show differences between varieties; both leaves were dorsiventral (Kumar et al., 2006) although it is a diagnostic characteristic for the pharmacognosy analysis in the identification of plants. Also both varieties were hypostomatic with paracytic

stomata, agreeing with the Herbal Pharmacopoeia of the Mexico (FEUM, 2013), which has reported that both paracytic and anomocytic stomata can be found in damiana; nevertheless, a different kind of stomata were observed on the abaxial surface of *Turnera diffusa* from India, which were anomocytic (Kumar et al., 2006). According to Franks and Farquhar (2007) stomata morphology diversity translates into a considerable functional diversity, mainly in function of environmental conditions.

Conclusion

This study constitutes the first step in identifying the *Turnera diffusa* varieties growing in Mexico. The results extend the information on leaf characteristics of the varieties *aphrodisiaca* and *diffusa* and define the differences between the two; pharmacognostic characteristics, such as the type of stomata, trichomes, and leaf identify the species, whereas characteristics such as leaf size and trichome density differ between varieties. These results will assist in the identification and standardization of *Turnera diffusa*, which guarantee the quality of its materials.

Acknowledgments

The authors want to thank the Laboratory of Phytopathology and the Laboratorio Nacional para la Investigación en Inocuidad Alimentaria (LANIIA), Culiacan, México for the use of their facilities; to CIBNOR staff Ariel Cruz and Federico Soto for support in SEM photos; to Diana Fischer for editorial services in English.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Alcaraz-Melendez, L., Real-Cosío, S., Suchy, V., Svajdlenka, E., 2007. Differences in essential oil production and leaf structure in pheno-types of damiana (*Turnera diffusa* willd. J. Plant Biol. 50, 378-382.
2. Arbo, M.M., 2000. Estudios sistematicos en *Turnera (Turneraceae)* 11. Series Annulares, *Capitatae*, *Microphyllae* y *Papilliferae*. Bonplandia. 10 (1-4), 1-82.
3. Chevallier, A., 1996. The Encyclopedia of Medicinal Plants. London: Dorling Kindersley.
4. Covarrubias-Carrillo, U., Cardenas-Soriano, E., Nieto-Angel, D., Gutierrez-Alonso, J.G., 2002. Histopatologia de frutos de papaya (*Carica papaya* L.) infectados por *Colletotrichum gloeosporioides* Penz. Rev. Mex. Fitopatol. 20 (001), 88-93.
5. Estrada-Reyes, R., Ferreyra-Cruz, O.A., Jimenez-Rubio, G., Hernandez-Hernandez, O.T., Martínez-Mota, L., 2016. Prosexual Effect of *Chrysactinia mexicana* A. Gray (Asteraceae), False Damiana, in a Model of Male Sexual Behavior. BioMed Res. Int., 2016 (ID 2987917) Farmacopea Herbolaria Mexicana FEUM, 2a Edicion, 2013, ISBN: 978-607-460-402-3, 91-92.
6. Franks, P.J., Farquhar, D., 2007. The Mechanical Diversity of Stomata and It Significance in Gas-Exchange Control[OA]. Plant Physiol., 142, 78-87.
7. Gamez, A.E., Ivanova, A., Martinez de la Torre, J.A., 2010. La comercializacion mundial de damiana y los pequeños productores de Baja California Sur. Comercio Exterior. 60, 3.
8. Glas, J.J., Schimmel, B.C.J., Alba, J.M., Escobar-Bravo, R., Schuurink, R.C., Kant, M. R. (2012). Plant Glandular Trichomes as Targets for Breeding or Engineering of Resistance to Herbivores. Int. J. Mol. Sci. 13(12), 17077–17103.
9. Kumar, S., Taneja, R., Sharm, A., 2006. Pharmacognostic Standardization of *Turnera aphrodisiaca* Ward. J. Med. Food. 9(2), 254–260.
10. Martínez de la Torre, J.A., 2013. Economic-Financial Assessment of a Business Plant for Dried Damiana. Revista Mexicana de Ciencia Forestal. 4, 16.
11. Moreno, N., 1984. Glosario botanico ilustrado. Instituto Nacional de Investigaciones Sobre Recursos Bioticos. Mexico.
12. Organización Mundial de la Salud, 2013. Estrategia de la OMS sobre medicina tradicional 2014-2023. WHO library. Geneva, Switzerland. www.who.int.
13. Osuna Enciso, T., Valenzuela Reyes, G., Muy Rangel, M.D, Gardea Bejar, A.A, Villarreal Romero, M., 2008. Sex Expression and Flower Anatomy of Litchi (*Litchi chinensis* Sonn.). Rev. Fitotec. Mex. 31 (1), 51–56.
14. Szewczyk, K., Zidorn, C., 2014. Ethnobotany, phytochemistry, and bioactivity of the genus Turnera (Passifloraceae) with a focus on damiana *Turnera diffusa*. J. Ethnopharmacol. 152(3), 424–43.
15. Shafiee-Hajiabad, M., Hardt, M., Honermeier, B., 2014. Comparative investigation about the trichome morphology of Common oregano (*Origanum vulgare* L. subsp. vulgare) and Greek oregano (*Origanum vulgare* L. subsp. hirtum). JARMAP e1-e9.
16. World Health Organization, 2002. Quality control methods for medicinal plants. WHO library. Geneva, Switzerland. 28-31.
17. Zhao, J., Pawar, R.S., Ali, Z., Khan, A.I., 2007. Phytochemical investigation of *Turnera diffusa*. J. Nat. Prod. 70(2), 289-292.