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MORPHOLOGICAL AND ANATOMICAL INVESTIGATION OF *ALOYSIA CITRODORA* PALAU - NEW MEDICINAL PLANT INTRODUCED IN ROMANIA

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Abstract

Lemon verbena - Aloysia citrodora Palau (Verbenaceae) is an aromati plant used for the medicinal properties of itsleaves and essential oils. The species is native to Argentina, Paraguay, Brazil, Uruguay, Chile, Bolivia and Peru. It is cultivated and commercialised as an aromatic plant for its lemon-like scent of its leaves and flowers. The dried plant and its extracts are valuable for medicinal preparations, in the perfumery industry and as an ingredient for the gourmet cuisine.It is often used in phytotherapiessuch as a digestive and diuretic, antispasmodic, carminative and sedative, antimicrobial and local analgesic. In 2015 Aloysia citrodora Palau was studied at theUniversity of Agronomic Sciences and Veterinary Medicine of Bucharest. The study targeted the stems and leaves and it shows the presence of glandular and non-glandular trichomeson all of its aerial parts. Morphological and anatomical analyses of the leaves and stems were made with the plant material cultivated in our University at the Research Center for Studies of Food Quality and Agricultural Products Greenhouse.

Key words*: Aloysia citrodora, morphological, leaf structure.*

INTRODUCTION

Aloysia citrodora, commonly called lemon verbena is a native plant - to Argentina and Chile. *Aloysia citrodora* Palau is synonymous with *Verbena triphylla* L'Hér., *Aloysia triphylla* L'Hér*., Lippia citriodora* Kunth*,* and *Lippia triphylla* (L'Hér.) Kuntze (Erikson, 2006). It is commonly known as "lemon verbena", "cedrón", "cidron", "hierba Luisa" or "verveineodorante", according to the country. The genus *Aloysia* Ortega ex Jussieu belongs to the *Verbenaceae*. This genus comprises about 30 species, distributed on the American continent from the south of the United States down to Chile and Argentina. Spanish explorers brought this plant to Spain in the 17th century at which point it was named after Princess Louisa of Parma, genus name honors Maria Louisa, princess of Parma and wife of King Carlos IV of Spain. Lemon verbena isan evergreen plant in tropical or warm winter locations, but deciduous in areas where freezing temperatures occur. In colder climates it is grown in containers and overwintered indoors, a period in which the plant is leafless and requires low amounts of water. In spring, the plant recovers with leaves. *Aloysia citrodora* has been a popular garden plant in warm southern and western parts of the U.S. The plant has a gentle sedative action and areputation for soothing abdominal discomfort, has amild tonic effect upon the nervous system and helps to depression（Gattuso et al., 2008).

The leaves of lemon verbena confer a refreshing lemon flavor to the teas and infusions isused for digestive problems (as flatulence, indigestion and acidity). The leaves and flowers are used for culinary purposes (fruit salads and jams, teas, desserts), cosmetics, for potpourris and as herbal medicines (colds, fevers, dyspepsia and diarrhea). In addition, the essential oil of lemon verbena has anti-bacterial and anti-fungal properties (Hanna et al., 2011), while the essential oils, tea and tinctures proved an antibacterial and antioxidant activity (Cowan 1999; Valentão et al., 2002; Sartoratto et al., 2004; Pereira and Meireles, 2007). Traditionally, lemon verbena is used as folk

remedy in treatments of spasms, cold and fever (Carnat et al., 1999), insomnia and anxiety (Van Hellemon, 1986; Newal et al., 1996; Wanmacher et al., 1990 a, 1990 b) and as source of analgesic, antiinflammatory and/or antipyretic remedies (Pascual et al., 2001). It is also often included in phytomedicines such as digestive and diuretic (Duke, 1985; Torrent Martí, 1985), antispasmodic (Torrent Martí, 1985), carminative and sedative (Alonso Paz et al., 1992; Mors et al., 2000), antimicrobial and local analgesic (Hieronymus, 1882; Dellacasa and Bandoni, 2003). Used alone or in combination with other herbs, the leaves make an excellent herbaltea. The essential oil is used in aromatherapy, extensively used in perfumery, especially in making eau de toilette and eau de cologne. The plants repels midges, flies and other insects (Benzi et al., 2009). The essential oil is an effective insecticide in 1-2% concentration. *Aloysia citrodora* is codified by the Argentine Pharmacopeia (1978), Francaise Pharmacopeia (1996), Argentine Alimentary Codex (1969-2007) and European Pharmacopoeia (2007). It is included in the FDA's GRAS list, i.e. the list of food additives which are Generally Regarded As Safe (Newall et al., 1996).

MATERIALS AND METHODS

Plant material

The plant material is a species and not a cultivar. The plant material to start the study was bought from plant-shop.ro as a 15 cm plant (initially in a jiffy peat pellet) in a 9 cm pot.

In the spring of 2015 Paduraru Jorj began the study of multiplying *Aloysia citrodora*. The activity took place in the Hortinvest Greenhouses and in Domnesti village (44° 24′ 1.5″ N, 25° 54′ 55.1″ E) Ilfov, 14 km of Bucharest. The crop was fertilised with BioHumusSol.

Being the first experimental study of having *Aloysia citrodora* in Romania's climate, the study began with 200 plants in the field. Considering the results of testing the planting distances of *Aloysia citrodora* in other temperate climates, such as in the experimental fields in Zalec, Slovenia, 1997, it was chosen the 40/40 cm as planting distance. At planting distances in the field of 40 x 40 cm, the average yield was 1050-1150 kg of dry leaves per hectare.

The fresh material, leaves and stems, whichwas used for the macroscopic and micro-morphologic studies, was collected in 2017, in the summer period, from the greenhouse of the Research Center for Studies of Food Quality and Agricultural Products, located in Bucharest, Romania.

The cross sections intended to illustrate the morphological and anatomical aspect were made on the same material, in Botanics laboratory. The sections were clarified with chloral-hydrate for 24 hours, and stained with Alaun-Carmine and Iodine Green suitable to optical microscopy techniques. Observations and images of the anatomical structures in stem and leaves were made with the optical microscope Leica DM1000 LED, Camera video Leica DFC295 and the Stereomicroscop Leica S8 APO, belonging to the Laboratory of microscopy and plant anatomy of the same research center.

RESULTS AND DISCUSSIONS

Macroscopical characteristics. The lemon verbena is a woody shrub, with lanceolate green leaves, with strong aroma and lemon-taste. The analysis of the foliar architecture shows simple pinnate, leaves, 5-10 cm long, lanceolate, with short petioles, in whorls of three. The color of lamina is pale green, white to pale lilac flowers, usually assembled in terminal racemose inflorescences.

Microscopical characteristics. The blades of the leaves exhibit anomocytic stomata on the abaxial side (Figure 1) and: two types of trichomes present on both sides of the leaf surfaces, non-glandular and glandular trichomes (Figure 2, Figure 4). The dorsiventral mesophyll, epidermic abaxial cells present striate cuticule around of the stomata. Metcalfe and Chalk (1972) mentioned for the genus *Aloysia* the occurrence of anomocyticstomata. Mesophyll is dorsiventral (Figure 3), with a 2-3 layer of palisade parenchyma and spongy parenchyma cells, this tissue is located next to the abaxial epidermis, the midrib consists of a collateral vascular bundle and the lower epidermis is unstratified and presents stomata. Transverse sections of the leaf measuring 10.297 to 12.318 μm and palisade parenchyma measuring 3.610 to 4.404 μm (Table 1).

Table 1. Leaf and palisade parenchyma thicknesses

Thick of leaf (µm)	Palisade parenchyma (µm)
12.065	3.770
12.206	4.304
11.306	4.292
10.638	3.859
11.306	3,398
12.318	4.404
11.430	3.705
10,297	3.610
10.915	4.224
10.407	3.871

Figure 1. Abaxial epidermis with anomocytic stomata and glandular capitata trichomes

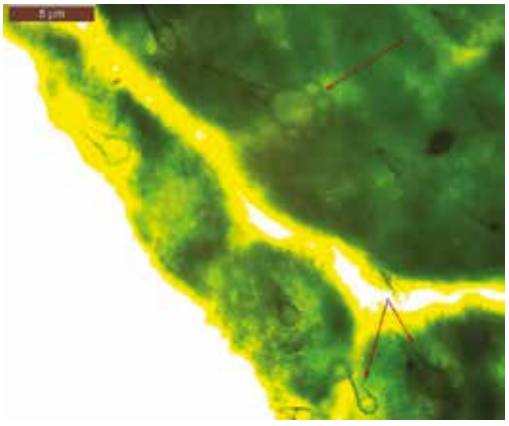

Figure 2. Adaxial epidermis with non-glandular trichomes

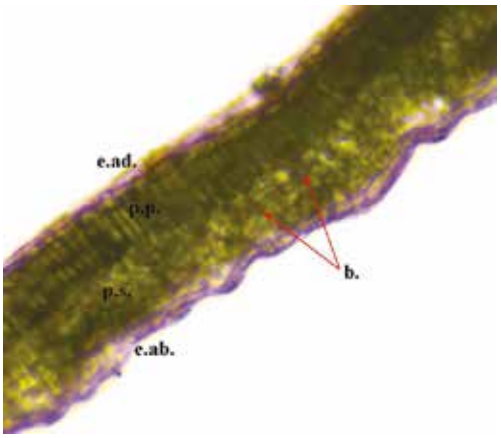

Figure 3. Leaf (cross section): e.ad. - adaxial epidermis; p.p. - palisade parenchyma; b. - colateral vascular bundle; p.s. - spongy parenchima; e.ab - abaxial epidermis

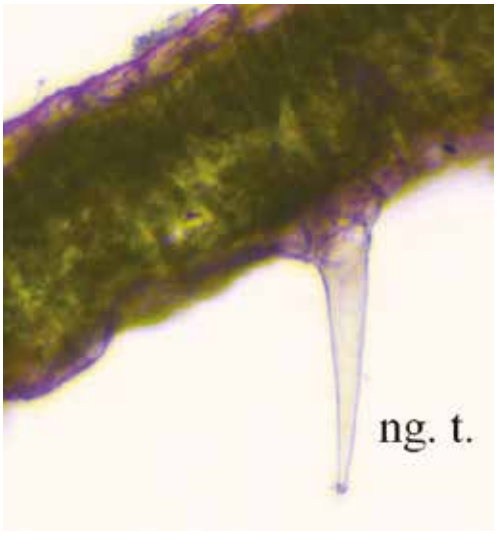

Figure 4. Detail of abaxial epidermis with non-glandular trichomes

Stem anatomy: the stem, in incipient secondary growth, presents epidermis, angular collenchyma and the vascular bundles which are open collaterally; transverse sections show a circular margin with 6 ribs, the epidermis is unstratified, with stomata.

At the level of the ribs and in a subepidermal position, 3-4 layers of angular collenchyma can be observed and cork usually appears near the phloem. Vascular bundles are open collaterally (Figures 5 and 6).

Nonglandular and glandular trichomes were observed in the unistratified epidermis as described for the leaf.

For the *Verbenaceae* family several descriptions of trichomes exist (Cantino, 1990; Yashodhara et al., 2001). Specifically for *A. citrodora*, Casadoro and Rascio (1982) in their ultrastructural study of its leaf trichomes, reported the presence of three types of glandular and one typeof non-glandular trichomes.

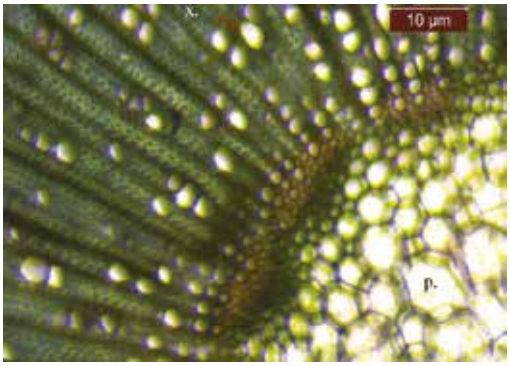

Figure 5. Stem (cross section):
x. - xylem; p. - parenchyma

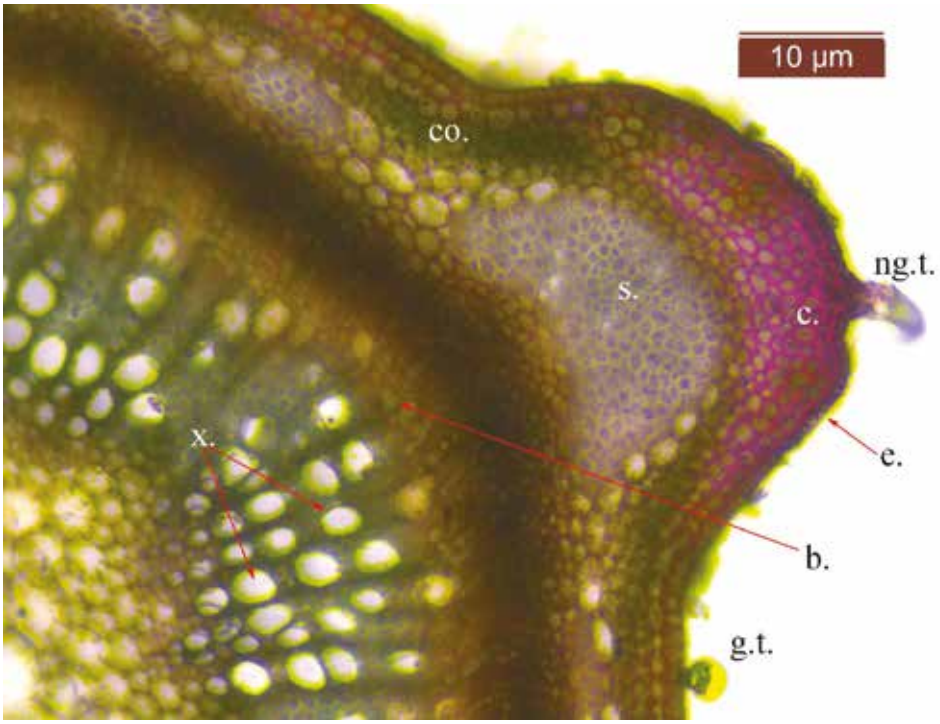

Figure 6. Stem (cross section): e. – epidermis; ng.t. - non-glandular trichome; g.t. – glandular trichome; co. - cork; c - colenchyma; s. – sclerenchyma; b. – bundle; x. - xylem

OBSERVATIONS IN THE FIELD. REVIEW

The species does well in Romania mainly as an annual crop. It showed that it reaches it's biological potential, producing leaves above the the parameters mentioned in the morphology of the species (20.0 cm^2): 10/2.5cm, 21.75 cm^2 leaf.

For mechanized harvesting, it can be planted in wide strips. The recommended plant material is vigorous rooted cuttings in a perlite and peat mix substrate, using rooting hormones from parent plants grown in the greenhouse.

The species can be cultivated successfully in Romania as an annual crop, where it has demonstrated that it reaches its biological vegetative potential, producing leaves beyond the parameters of the morphology of the species. It also blooms, but sporadically.

On the first harvest at Domnesti, after harvesting plant material on August 1st 2015, 45 days after planting, the plants continued to grow by giving vigorous shoots that grew equally fast. This fact, corroborated with the data from the literature, shows that two crops per year can be carried out, manually or mechanically cutting all leaf shoots from the entire cultivated area, in the temperate zone with scented verbena cultivated as annual.

It shows horticultural interest for taking into commercial, possibly ecological crops, a statement based on the fact that in the Domnesti field the disease attack was zero and pests only by chance and without reaching an economic threshold of harm.

The species yields higher yield when fertilized with organic fertilizer.

50 of 2 years of age plants were left outside over winter as bushes cut to 20 cm over the ground level, covered with nothing but soil as winter protection and they survived winter well 3 years in a row. However the winters were atypical and this shows that the species even hardy at only about minus 10 Celsius degrees, sometimes it can pass winters outside just well even in temperate climates depending overall conditions.

CONCLUSIONS

The following macroscopic and micro morphological parameters were established after the analysis of the studied plant material cultivated in our University.

From the morphological point of view there were determined: simple, entire, lanceolate, petiolate, pinnate leaves. The stem presented many ribs.

After this study it can be mentioned the following anatomical characteristics of the leaves: adaxial epidermis with anticlinal thin walls and strains, abaxial epidermis with anticlinal thin and sinuous walls, anomocytic stomata, non glandular and glandular trichomes, hypostomatic dorsiventral mesophyll. The above described elements should be useful for corect botanical identification of *A. citrodora* species.

REFERENCES

Alonso Paz E., Bassagoda M.J., Ferreira F., 1992. Uso Racional de las Plantas Medicinales. Ed. Fin de Siglo, Montevideo, Uruguay, 42-43.

Argyropoulou C., Argyropoulou C., Akoumianaki-Ioannidou A., Christodoulakis N.S., Fasseas C., 2010. Leaf anatomy and histochemistry of *Lippia citriodora* (*Verbenaceae*), Australian Journal of Botany, 58, 398-409.

Argyropoulou C., Daferera D., Tarantilis P.A., Fasseas C., Polyssiou M., 2007. Chemical composition of the essential oil from leaves of *Lippia citriodora* H.B.K. (*Verbenaceae*) at two developmental stages. Biochemical Systematics and Ecology 35, 831-837.

Benzi V.S., Murray A.P., Ferrero A.A., 2009. Insecticidal and insect-repellenta ctivities of essential oils from *Verbenaceae* and *Anacardiaceae* against *Rhizopertha dominica*. Natural Product Communications 4, 1287–1290.

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

Carnat A., Carnat A.P., Fraisse D., Lamaison J.L.,1999. The aromatic and poly-phenolic composition of lemon verbena tea. Fitoterapia 70, 44-49.

Casadoro G., Rascio N., 1982. Glands of *Lippia triphylla* (L'Her.) O. Kuntze.Cytobios 35, 85-94.

Código Alimentario Argentino (1969-2007): Ed. De la Canal y Asoc, Buenos Aires, Argentina.

Cowan M.M.,1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12, 564-582.

Dellacasa E., Bandoni AL., 2003. *Hierba luisa. Aloysia citriodora* Palau.Revista de Fitoterapia. 3 (1): 19-25.

Duarte M.C.T., Figueira G.M., Sartoratto A., Rehder V.L.G., Delarmelina C., 2005. Anti-Candida activity of Brazilian medicinal plants. Journal of Ethnopharmacology 97 (2), 305-311.

Duke J.A., 1985. Handbook of Medicinal Herbs. Ed. CRC Press, Florida, Estados Unidos, 33.

Duke S.O., Canel C., Rimando A.M., Tellez M.R., Duke M.V., Paul R.N., 2000. Current and potential exploittation of plant glandular trichome productivity. Advances in Botanical Research 31, 121-151.

Duke S.O.,1994. Commentary: Glandular trichomes - a focal point of chemical and structural interactions. International Journal of Plant Sciences 155, 617-620.

European Pharmacopoeia, 2007. Supplement 5.8, Monograph: Lemon verbena leaf Strasbourg: Council of Europe.

Farmacopea Nacional Argentina, VI edición, 1978. Buenos Aires, Argentina.

FrancaisPharmacopeé X Ed.,1996, Maisonneuve, Paris, Francia.

Gattuso S., Catalina M. van Baren, Gil A., Bandoni A., Ferraro G., Gattuso M., 2008. Morpho-histological and quantitative parameters in the characterization of lemon verbena (Aloysiacitriodora Palau) from Argentina. Latinoam. Caribe Plant. Med. Aromaticas Vol. 7 (4) 190-198.

Hanaa F.M.A., Hossam S.E., Nasr F.N., 2011. Evaluation of antioxidant and anti-microbial activity of *Aloysia triphylla*. Electronic Journal of Environmental, Agricultural and Food Chemistry 10 (8), 2689-2699.

Hieronymus J., 1882. Plantas Diafóricas de la Flora Argentina. Bo. l Acad. Nac. deCiencias Córdoba, Tomo IV, 404-405.

Metcalfe C.R., Chalk L., 1972. Anatomy of Dicotyledons. Vol. II. Clarendon Press, Oxford, Inglaterra. 1030-1041.

Mors W.B., Rizzini C.T., Alvarez P., 2000. Medicinal Plants of Brazil. Reference Publications INC, Algonac, Michigan, EstadosUnidos, 30.

Newall C.A., Anderson L.A., Phillipson J.D., 1996. Herbal Medicines. A Guide for Health-care Professionals. Ed. The Pharmaceutical Press, London.

Ohno T., Kita M., Yamaoka Y., Imamura S., Yamamoto T., Mitsufuji S., Kodama T., Kashima K., Imanishi J., 2003. Antimicrobial activity of essential oils against *Helicobacter pylori*. Helicobacter 8, 207-215.

Pascual M.E., Slowing K., Carretero E., Mata D.S., Villar A., 2001. Lippia: Traditional uses, chemistry and pharmacology: a review. Journal of Ethnopharmacology 276, 201-214.

Pereira C.G., Meireles M.A.A., 2007. Evaluation of global yield, composition, antioxidant activity and cost of manufacturing of extracts from lemon verbena [*Aloysia triphylla* (L'Herit.) Britton] and mango (*Mangifera indica* L.) leaves. Journal of Food Process Engineering 30, 150-173.

Santos-Gomes P.C., Fernandes-Ferreira M., Vicente A.M.S., 2005. Compositionof the essential oils from flowers and leaves of vervain (*Aloysia triphylla* (L'Herit. Britton) grown in Portugal. Journal of Essential Oil Research. 17, 73-78.

Sartoratto A., Machado A.L.M., Delarmelina C., Figueira G.M., Duarte M.C.T., Rehder V.L.G ., 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian Journal of Microbiology 35, 275-280.

Torrent Martí M.T., 1976. Algunos aspectos farmacognósticos y farmacodinámicos de *Lippia citriodora* HBK. Rev. Real Acad. Farm. 14: 39-55.

Valentão P., Fernandes E., Carvalho F., Andrade P.B., Seabra R.M., Bastos M.D., 2002. Studies on the antioxidant activity of *Lippia citriodora* infusion: Scavenging effect on superoxide radical, hydroxyl radical and hypochlorous acid. Biological and Pharmaceutical Bulletin 25, 1324-1327.

Van Hellemont J., 1986. Compendium de Phytotherapie, Service Scientifique de l'APB, Bruxeles, Belgium, pp 234-235.

Vogel H., Silva M.L., Razmilic I., 1999. Seasonal fluctuation of essential oil con-tent in lemon verbena [*Aloysia triphylla* (L'Herit.) Britton]. Acta Horticulturae 500, 75-79.

Wannmacher L., Fuchs F.D., Paol C.L., Fillman H.S., Gianlupi A., Lubiancaneto J.F., Hassegawa C.Y., Guimares F.S., 1990 b. Plant employed in the treatment of anxiety and insomnia. Effect of infusions of *Aloysia triphylla* on experimental anxiety in normal volunteers. Fitoterapia 61 (5): 449-453.

Wannmacher L., Fuchs F.D., Paoli C.L., Fillman H.S., Gianlupi A., Lubiancaneto J.F., Hassegawa C.Y., Guimares F.S., 1990 a. Plant employed in the treatment of anxiety and Insomnia. I. An Ethnopharmacological Survey in Porto Alegre, Brazil. Fitoterapia 61 (5): 445-448.

Yashodhara K., Shanmukha Rao S.R., Subba Rao J.V., 2001. Structure, distribution and taxonomic importance of trichomes in the tribe Verbeneae (*Verbenaceae*). BeiträgezurBiologie der Pflanzen 72, 265-285.