Scientific Papers. Series B, Horticulture. Vol. LXIII, No. 2, 2019
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOME MORPHOLOGICAL AND ANATOMICAL PARTICULARITIES IN *VITEX AGNUS-CASTUS* L. SPECIE GROWN IN PROTECTED SPACE

Elena SĂVULESCU, Mihaela Ioana GEORGESCU, Vasilica LUCHIAN, Vlad POPA

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: elena_savulescu@yahoo.com

Abstract

The assessed species, Vitex agnus-castus L., was taken from Thasos, Greece and cultivated in pots for acclimatization in the botanical garden, to be studied for its medicinal and ornamental use. During the vegetation period, the morphological and anatomical observations were made. It is an aromatic shrub, with opposite leaves, palmate-composed of 3-5 (7) lanceolate leaflets, entire margins or serrate-dented, 3-7 cm long and 1.5 cm width. The transversal sections were made in the root, stem and leaf. In the root and stem, it was observed that the vascular bundle had a secondary structure. In the epidermis of young stems there are non-glandular hairs and very rarely secretory hairs. In the pith of stem there were seen the starch granules and calcium oxalate crystals. On the epidermis of rachis there are non-glandular and secretory hairs. The epidermis of the leaflets is unistratified, with the corrugated walls and covered by thin cuticle. On the both epidermis of the leaflets there were observed the non-glandular hairs. The secretory hairs were identified mainly on the lower epidermis. The type of stomata is anomocytic, identified only on the lower epidermis. The mesophyll is bifacial with palisade tissue toward upper epidermis and spongy tissue toward lower epidermis.

Key words*: Vitex agnus-castus, morphology, anatomy, secretory hairs.*

INTRODUCTION

In the first taxonomic classifications, the genus *Vitex* L. is included in the *Verbenaceae* family, with approx. 250 species of trees and shrubs (Linnaeus, 1753; Jussieu, 1806; Backer, 1965; Griffiths, 1994; Takhtajan, 2009).

In some recent classifications, the *Vitex* L. genus has been transferred to the *Lamiaceae* family following morphological, micromorphological, anatomical and phytochemical investigations on pollen, petiole, stems, fruits, between *Vitex* and *Verbenaceae* species, which have provided useful characteristics in taxonomy (Abbas et al., 2006; Jamzad et al., 2006; Chantaranothai, 2011).

It grows naturally in Mediterranean countries, southern Europe, tropical area, West and Central Asia (Hegi, 1966; Tutin et al., 1972; Davis, 1982; Donald, 1994; Griffiths, 1994; Wasson, 2003).

The name *Vitex agnus-castus* derives from the Greek word "Chastity" which means virgin, being used in popular medicine for a variety of gynecological dysfunctions for over 2000 years in ancient Greece, Egypt and Rome (Christie & Walker, 1997; Chen et al., 2010).

From the plant are uses the fruit, sometimes the leaves, from which is obtained infusion, mixture, extract for different applications.

From the chemical point of view, the fruits of *Vitex agnus-castus* contain: flavonoids, iridoid glycoside, p-hydroxybenzoic acid, alkaloids, essential oils, fatty oils and essential oils such as linoleic acid, diterpenoids and steroids (Adams, 2004; Hajdu et al., 2007; Marongiu et al., 2010; Borges et al., 2012; Toplan, 2015; Hanane et al., 2016; Hina et al., 2016; Tģn et al., 2017; Allison et al., 2018; Levchyk et al., 2018).

The fruit composition have therapeutic effects for the regulation of the menstrual cycle, for the treatment of premenstrual syndrome (PMS), gynecological disorders, support the progesterone secretion, infertility in women (Mehrangiz et al., 2012; Schellenberg et al., 2012; Mohammad et al., 2015; Berrani et al., 2018; Mina et al., 2018). In addition, it has been used to reduce the symptoms of menopause such as hot flashes, depression, and

sleep disturbances, headaches, rheumatism, corpus luteum insufficiency, disrupted lactation, acne, antimicrobial, antifungal, antiepileptic, antioxidant, antitumoral, diuretic, digestive, insect repellant, larvicidal (Doll, 2009; Sarikurkcu, 2009; Khaled, 2013; Shenghong et al., 2013; Sakhavar et al., 2013; Miguel et al., 2014; Franciele et al., 2015; Katiraee et al., 2015; Yilar et al., 2016; Abeer et al., 2017; Zinat et al., 2017; Keikha et al., 2018).

The plant also has economic importance. Aromatic leaves can be used as spices and the fruits, due to their quick taste, are used as a substitute for pepper (Hanelt et al., 2001; Novak et al., 2005). Some studies show that essential oils obtained from different parts of *Vitex agnus-castus* have acaricidal potential, being tested on *Tetranychus urticae* (Hamid et al., 2010; Roberta et al., 2016).

The anatomy of vegetative organs is reconfirmed in the literature (Metcalfe, 1963; Metcalfe & Chalk, 1983; Toma & Rugină, 1998; Schweingruber et al., 2013; Garner, 2017).

From the data analysis of literature, the Vitex species have anatomical differences (Abbas et al., 2006; Bejenaru et al., 2013).

The *Vitex agnus-castus* species being little known in Romania, the purpose of this study is to identify certain anatomical features at the plants grown in protected space that will be acclimatised for planting in the field as a medicinal and ornamental species.

MATERIALS AND METHODS

The plants of *Vitex agnus-castus* of about 50 cm high were brought from Thasos, Greece at the beginning of August 2018 and planted for acclimatization in the pots, in the greenhouse of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV).

The Bucharest is located at 44º24′49″ North latitude and 26º5′48″, East longitude, 90 m altitude and temperate-continental climate, with 585 mm/year rainfall and 10.86°C the average of the temperature.

During the vegetation period, morphological, micromorphological and anatomical observations were made. Biometric measurements were performed at the leaves, and some anatomical measurements. The average was obtained from 10 measurements. The both epidermis were exfoliated and assessed.

For anatomical analysis of cross-sections were performed in very young vegetative organs (roots, stems, leaves) which were clarified with Chloral-hydrate coloured with the Carmine - Alaunte and Iodine Green (Luchian et al., 2018).

The observations, images and measurements of the anatomical structures were made with the optical microscope Leica DM1000 LED, Camera video Leica DFC295 the Stereomicroscope Leica S8 APO, and SEM, belonging to the Laboratory of Microscopy and Plant Anatomy of the USAMV of Bucharest.

RESULTS AND DISCUSSIONS

After planting, the plants were formed new composed leaves with five, rarely three leaflets, elliptical-lanceolate, with 3-7 cm long and about 1.5 cm wide with the entire margin or finely toothed in the middle part.

Analysing the internal structure of the vegetative organs this is similar to the literature data.

Anatomy of root

The contour of the cross section is circular, with a secondary structure for the most part.

From the outside to the inside there are observed: the peridermis (suber, phellogen, phelloderm), primary cortex and the central cylinder (Figure 1).

The rhizodermis is early exfoliated and replaced on the outside by the suber generated by the felogen. The suber consists of 2-5 rows of elongated, suberified cells with the first rows being exfoliated.

The phelloderm is parenchymatous tissue, consisting of 2-4 rows of isodiametric cells with thin walls. The cortex of primary origin is reduced to a row of cells. The vascular tissues are collateral, of secondary origin, generated from the cambium and consisting of two concentric rings.

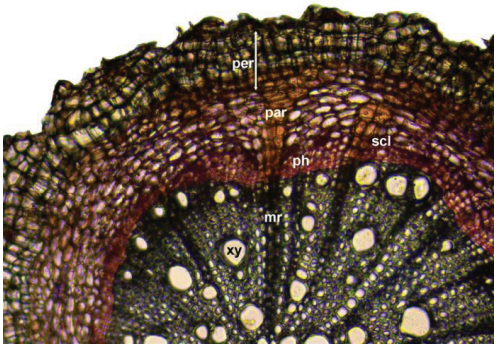

Figure 1. Cross section in the root of *Vitex agnus-castus*: per - peridermis; par - parenchyma; ph - phloem; scl - sclerenchyma; xy - xylem; mr - medullary rays

The ring of secondary phloem, located on the outside of cambium, is very thin at about 22.73 µm, consisting of sieved vessels, annexe cells and parenchyma. At the exterior of the phloem were observed small and thin fragments of sclerenchyma of primary origin, originating from pericycle.

The ring of secondary xylem is thick, approx. 210.53 µm, with a lot of libriform, strongly lignified, disorderly dispersed and metaxylem vessels with large diameter in average of 30.37 µm.

Anatomy of stem

The stem structure corresponds to the data from the speciality literature.

In the annual stems, the contour of the cross section is oval, with 4 edges, consisting in the epidermis, cortex and central cylinder (Figure 2).

The epidermis is formed by one layer of cells with about 25 µm thick and covered by a thin cuticle. In the epidermis were observed non-glandular, unicellular or multicellular hairs, with a conical to sharp peak of about 36.4 µm long.

On the epidermis of the young stems were sporadically identified the multicellular secretory hairs with 2 (3) cells, with spherical secretory cell (Figures 3 and 4).

In the literature have not been reported the secretory hairs in the epidermis of the stem (Yunus et al., 2008).

In the young stems, under the epidermis is the collenchymatous tissue more developed on the

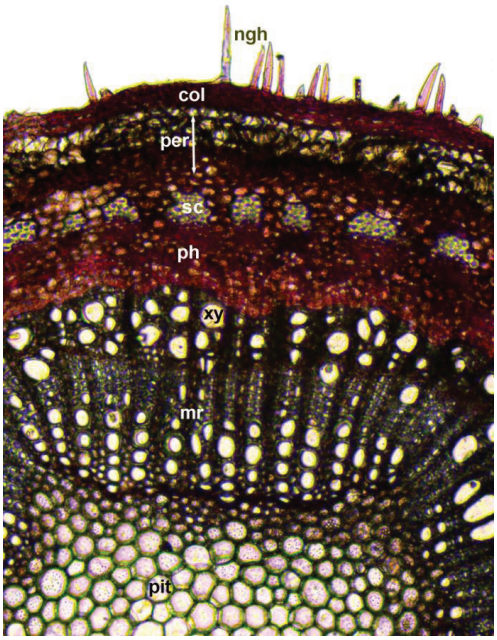

Figure 2. Cross section in the stem of *Vitex agnus-castus*: ngh – non glandular hairs; col – collenchyma; per – peridermis; ph – phloem; sc – sclerenchyma; xy – xylem; mr – medullary rays; pit – pith

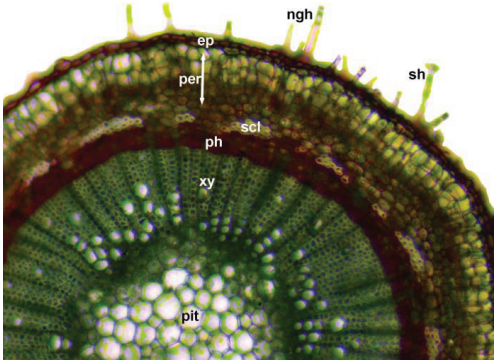

Figure 3. Cross section in the stem of *Vitex agnus-castus*: ep – epidermis; ngh – non glandular hairs; sh – secretory hairs; per – peridermis; ph – phloem; scl – sclerenchyma; xy – xylem; pit – pith

edges followed by the suber, phellogen and phelloderma.

The primary cortex is reduced to 1-2 rows of cells.

In some stems there were observed also the lenticels (Figure 5).

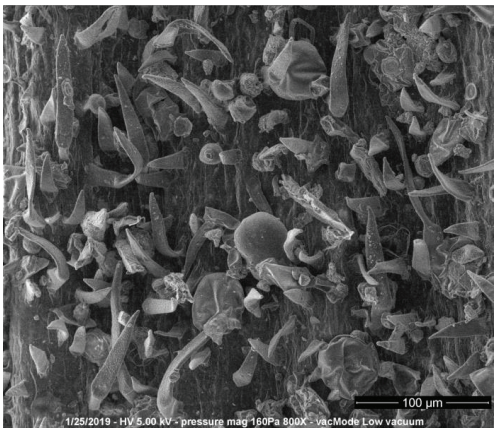

Figure 4. Micromorphological analysis of the stem (SEM 800X)

In the mature stem, the epidermis is replaced by the suber generated by the phellogen, consisting on 2 (3) rows of elongated cells.
In the central cylinder, the vascular tissues are collateral, consisting of two concentric rings, generated by the cambium.
At the outside of cambium is a thin ring of phloem and inside of it is a thick ring of the xylem.
At the base of the secondary xylem there was observed a thin primary xylem from place to place.
The ring of secondary phloem is very thin at about 52.70 µm, consisting of sieved vessels, annexe cells and thin layer of parenchyma. At the exterior of the phloem were observed fragments of sclerenchyma.
The ring of secondary xylem with a thickness of 181.17 µm with irregularly dispersed vessels in the basic libriform is separated by the medullary rays. The metaxylem vessels have an average diameter of 30.80 µm.
The pith is a well-developed parenchymal tissue in which have been observed starch granules and prismatic calcium oxalate crystals (Figure 6).

Anatomy of leaf rachis
The shape of leaf rachis is semicircular in cross section in accordance with the data from the literature (Figure 7).
The structure of the rachis consists of the epidermis, collenchyma and fundamental parenchyma with vascular tissues.

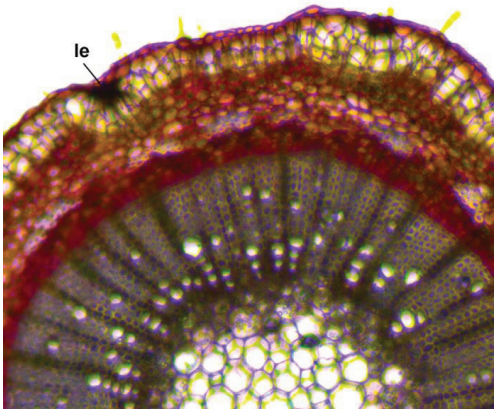

Figure 5. Cross section in the stem of *Vitex agnus-castus*: le – lenticels

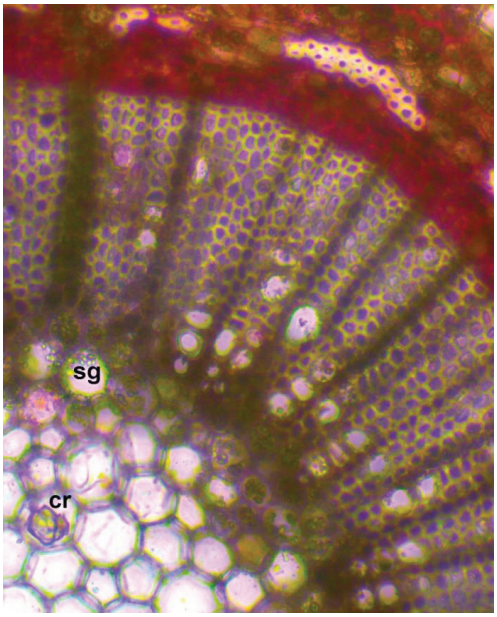

Figure 6. Cross section in the stem of *Vitex agnus-castus*: cr - calcium oxalate crystal; sg - starch granules

The epidermis is represented by the single layer of isodiametric cells of approximate 25 µm and covered by thin wax layer.
From place to place, on the epidermis there are observed the typically multicellular non glandular hairs with an average length of about 80 µm and also secretory multicellular hairs 2 (3) cells of approx. 20 µm long with the spherical secretory cell.

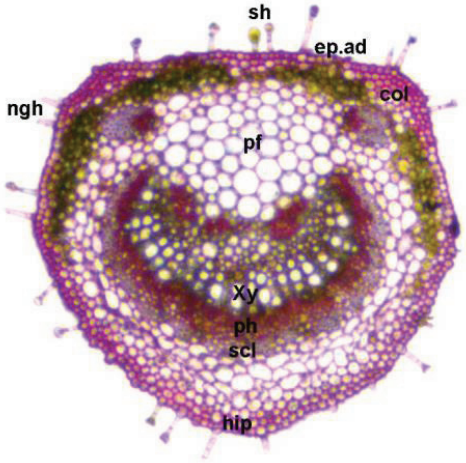

Figure 7. Cross section in the leaf rachis of *Vitex agnus-castus*: ep.ad – adaxial epidermis; hip – hypoderma; ngh – non glandular hairs; sh – secretory hairs; col – collenchyma; pf – fundamental parenchyma; ph – phloem; xy – xylem; scl – sclerenchyma

Under the epidermis there is one layer of collenchymatous cells but it is multi layered on the adaxial corners of the rachis.

Into the fundamental parenchyma it was observed one big central vascular bundle and two minor vascular bundles in the adaxial corners.

The vascular bundles are collateral with a primary structure, delimited by sclerenchyma tissue.

The phloem is thin, consisting of sieved vessels, annexe cells and little parenchyma. The xylem has vessels arranged in ray strings, separated by cellulosic parenchyma.

Anatomy of the leaflets

The epidermis, seen from the front is made up of cells with strongly corrugated walls (Figure 8).

The leaflets are hypostomatic of anomocytic type and annexe cells similar to epidermal cells, reconfirmed by the data from the literature (Figures 9 and 10).

In the cross-section of the leaflet, the midrib is strongly prominent on the underside with a unistratified epidermis and a thin cuticle. On the epidermis of midrib are observed non-glandular hairs and very rarely the secretory hairs (Figure 11 A).

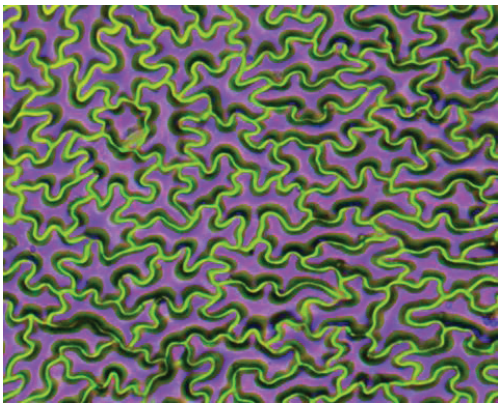

Figure 8. Analysis of upper epidermis (Ob. 10X)

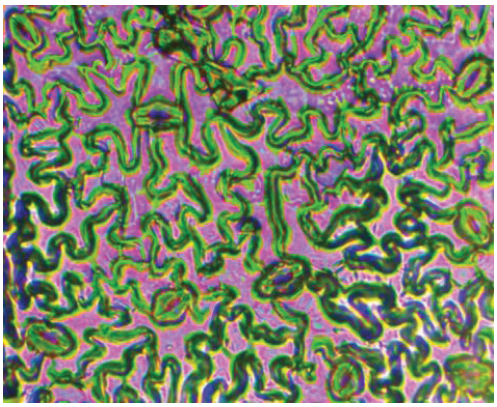

Figure 9. Analysis of lower epidermis (Ob. 20X)

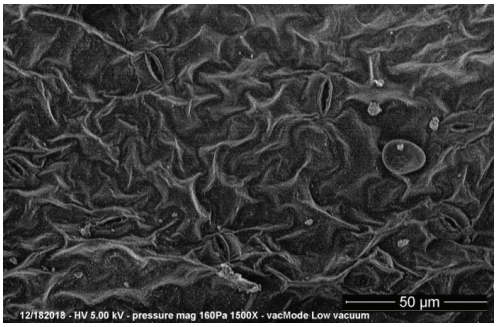

Figure 10. Micromorphological analysis of the lower epidermis (SEM 1500X)

In the fundamental parenchyma of the median rib there is a collateral vascular bundle, similar to the one in the rachis.

Both leaflets epidermis are unistratified with isodiametric cells and covered by a thin cuticle.

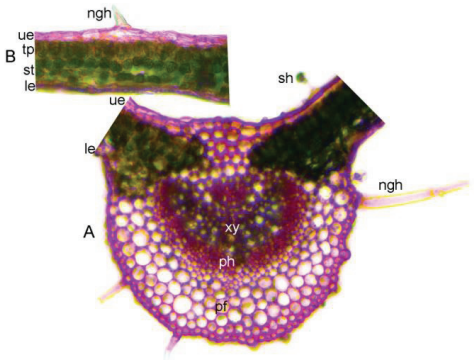

Figure 11. Cross section in the leaflet of *Vitex agnus-castus*: A. midrib; B. leaflets; ue – upper epidermis; le – lower epidermis; sh – secretory hairs; ngh – non glandular hairs; pf – fundamental parenchyma; ph – phloem; xy – xylem; tp – palisade tissue; st – spongy tissue

The upper epidermis has cells of about 11.80 µm larger than the lower epidermis which is about 7.12 µm.

In both epidermises there we observed non-glandular hairs, usually unicellular, with a conic-sharp tip.

The secretory hairs are particularly present in the lower epidermis along the ribs and this is also mentioned in some speciality papers (Mamoucha et al., 2016).

The mesophyll is bifacial type with palisade tissue toward upper epidermis and spongy tissue toward the lower epidermis (Figure 11 B).

Palisade tissue consists of a single layer of slightly elongated cells, rich in chloroplasts, without inter cellular spaces and with approx. 16.59 µm thickness.

The spongy tissue consists of two rows of isodiametric cells with a lower content in chloroplasts and intercellular spaces of approx. 29.65 µm thickness.

In the mesophyll there were observed the Calcium oxalate crystals and vascular bundles of collateral type were present.

In the studies of Yunus et al., published on 2008 year, there were not identified the secretory hairs on the epidermis of the leaflets and under the upper epidermis it was identified a layer of hypodermis.

CONCLUSIONS

After anatomically analysing the structure of young roots, stems and leaves of *Vitex agnus-castus* species were identified some characteristics:

The structure of the root is mainly secondary.

On the epidermis of the stem there were identified non-glandular hairs and rarely the secretory hairs.

In some stems there were observed the lenticels.

The leaflets are hypostomatic with anomocytic type of stomata.

In both epidermis of the leaflets there were present the non-glandular hairs but also secretory hairs, especially in the lower epidermis, along of the ribs.

The mesophyll of leaflet is bifacial type with only one cell layer of palisade parenchyma toward upper epidermis and two cells layer of spongy parenchyma toward the lower epidermis.

REFERENCES

Abbas Azimi, R., Jamzad, Z., Sefidkon, F., Bakhshi-Khaniki Gh. (2006). The potential value of phytochemical and micromorphological characters in taxonomic treatment of genus *Vitex* L. (Lamiaceae). *Journ. Bot.* 12 (1), 15-35.

Abeer, Y. Ibrahim, Samah, A. El-Newary, Eman, R. Youness, Amr, M.M. Ibrahim, Walaa, A. El Kashak. (2017). Protective and therapeutic effect of *Vitex agnus-castus* against prostate cancer in rat. *Journal of Applied Pharmaceutical Science*, Vol. 7 (12), 133-143.

Adams, R.P. (2004). *Identification of essential oil components by gas chromatography/mass spectroscopy*. Carol Stream, Illinois, USA: Allured Publishing Corporation.

Aissaoui, H., Algabr, M., Mezhoud, S., Mekkiou, R., Boumaza, O., Seghiri, R., Benayache S., Benayache, F. (2016). Chemical constituents of Vitex agnus-castus (Verbenaceae). *Der Pharma Chemica*, 8(1), 491-494.

Allahtavakoli, M., Honari, N., Pourabolli, I., Arababadi, M.K., Ghafarian, H., Roohbakhsh, A., Esmaeili Nadimi, A., Shamsizadeh, A. (2015). *Vitex agnus-castus* Extract Improves Learning and Memory and Increases the Transcription of Estrogen Receptor α in Hippocam - pus of Ovariectomized Rats. *Basic Clin Neurosci,* 6(3), 185–192.

Allison, M. Heskes, Tamil, C.M. Sundram, Berin A. Boughton, Niels B. Jensen, Nikolaj L. Hansen, Christoph Crocoll, Federico Cozzi, Simon Rasmussen, Britta Hamberger, Bjorn Hamberger, Dan Staerk, Birger L. Møller and Irini Paterak. 2018.

Biosynthesis of bioactive diterpenoids in the medicinal plant *Vitex agnus-castus*. *The Plant Journal*, 93, 943–958.

Backer, C.A., Bakhuizen van den Brink, R.C. (1965). Verbenaceae. *Flora of Java*. Vol. 2:594-614. (N.V.P. Noordhoff: Groningen).

Bejenaru, L.E., Bejenaru, C., Mogoşanu, G.D., Oancea, C.N., (2013). Histo-anatomical study of the annual off spring and leaf's limb of *Vitex negundo* L. (Verbenaceae) species, Scientific Conferences with International Participation. *Annals of the University of Craiova – Agriculture, Montanology, Cadastre Series* XLIII (1), 40–46.

Berrani, A., Lrhorfi, L. Aicha, Larbi, Ouahidi, My, Z., Meryem, L., Ould A., Boufellous, Moncef, Bengueddour, R., 2018. Ethnobotanical study of two medicinal plants: *Vitex agnus castus* and *Anabasis aretioïdes*. *Journal of Advances in in Biology*. Volume 9(4), 2017-2026.

Betül, T., Cemal, K., Emre. S., (2017). Evaluation of Chemical Composition of *Vitex agnus-castus* (Verbenaceae) Fruits Essential Oils Grown in Aydın/Turkey. *Turkish Journal of Life Sciences* 2(2), 171-174.

Borges A. R., Aires J. R. A., Higino T. M. M., Graças M., Medeiros F., Citó A. M. G., Lopes J. A. D and Figueiredo R. C. B. Q. (2012). Trypanocidal and cytotoxic activities of essential oils from medicinal plants of Northeast of Brazil. *Experimental Parasitology*, 132, 123-128.

Chantaranothai, P., (2011), *Trop. Nat. Hist*. 11 (2), 91–118.

Chen, SN, Friesen, BJ, Webster, D, Nikolic, D, Breemen, van RB, Wang, ZJ, Fong, HHS, Farnsworth, NR, Pauli, GF. (2010), *Fitoterapia*, 82, 528–533.

Christie, S., Walker, A. (1997). *Vitex agnus-castus*; review of its traditional and modern therapeutic use, Current use from a survey of practitioners. Eur. *I Herb. Med.* 3, 29-45.

Davis, P.H. (1982). *Flora of Turkey and the East Aegean Islands.* Vol. **7**. Edinburgh Univ. Press, Edinburgh.

Dogan, Y. Ay G., Kozuharova, E. (2008). A study on the anatomical characteristics of *Vitex agnus-castus* (Verbenaceae). *Phytologia Balcanica,* 14 (1), 97–101.

Doll, M., (2009). The Premenstrual syndrome (PMS): effectiveness of *Vitex agnus-castus*. Med. *Monatsschr Pharm.,* 32(5), 186-191.

Donald, JB, (1994). *Vitex agnus-castus* Clinical Monograph, Herbal Research Review, Quarterly Review of Natural Medicine.

Garner, C. (2017), *Hallmark Features of Stem Anatomy in the Family Lamiaceae*, University of South Carolina, Scholar Commons, 30.

Ghanbari, Z., Foroughifar, T., Zebardast, J., Salimi, S., Tootoonchi, S., Shabihkhani, S., Mokhtari, M.G., Forooghifar, T. (2017). The Effect of Vitagnus on Menopausal Symptoms. *J Obstet Gynecol Cancer Res*. 2(2), e10195.

Griffiths, M., 1994. *Index of Garden Plants*, Royal Horticultural Society, 1209.

Hajdu, Z., Hohmann, J., Forgo, P., Martinek, T., Deruarics, T., Zupko, M., Falkay, I., Cossuta, G., Mathe D.I. (2007). Diterpenoids and flavonoids from the fruits of *Vitex agnus-castus* and antioxidant activity of the fruit extracts and their constituents. *Phytotherapy Research* 21, 391-394.

Hamid, A.A., Usman, L.A., Adebayo, S.A., Zubair, M.F., Elaigwu, S.E., (2010). Chemical Constituents of Leaf Essential Oil of North-central Nigerian Grown *Vitex agnus-castus*. *Advances in Environmental Biology*, 4(2), 250-253.

Hanelt, P., Kilian, R., Kilian, W., (2001). *Mansfeld's Encyclopedia of Agricultural and Horticultural Crops (Except ornamentals).* Institut Fur Pflanzengenetik Und Kulturpflanzenforschung Gatersleben (COR), Springer, Berlin.

Hegi, G. (1966). *Illustrierte Flora von Mittel-Europa. Band* 5(3). Carl Hanser Verlag, Munchen.

Jamzad, R.Z., Sefidkon, F., Bakhshi-Khaniki, Gh. (2006). The potential value of phytochemical and micromorphological characters in taxonomic treatment of genus *Vitex* L. (Lamiaceae). *Iran. Journ. Bot.* 12 (1), 15-35.

Jussieu, A.L., (1806). Sur la Famine des Plantes Verbenacees Ann. Mus. *Hisi Nat. Paris*. 7, 63-77.

Katiraee, F, Mahmoudi, R , Tahapour, K, Hamidian, G, Emami, S.J. (2015). Biological properties of Vitex agnus-castus essential oil (Phytochemical component, antioxidant and antifungal activity). *Biotechnology and Health Sciences* 2:e26797, 196.

Keikha, N., Shafaghat, M., Mousavia, SM, Moudi, M., Keshavarzi, F. (2018). Antifungal effects of ethanolic and aqueous extracts of *Vitex agnus-castus* against vaginal isolates of *C*andida albicans. *Current Medical Mycology* (CMM**)** 4(1), 1-5.

Levchyk N., Levon V., (2018). Cold Resistance of Plant Species of the Genus Vitex l. Introduced in M.M. Gryshko National Botanic Garden of NAS of Ukraine. *International Journal of Secondary Metabolite***,** Vol. 5, No. 3, 186–199.

Li, S., Qiu, S., Yao, P., Sun, P., Fong, H., Zhang, H.S, (2013). *Compounds from the Fruits of the Popular European Medicinal Plant Vitex agnus-castus in Chemoprevention* via NADP (H): Quinone Oxidoreductase Type 1 Induction. Evidence-Based Complementary and Alternative Medicine Volume p. 7.

Linnaeus, C., (1753). *Didynamia Angiospermia. Species Plantarum*. Edn 1, vol. 2:602-609 (Laurentius Salvius: Stockholm).

Luchian, V., Săvulescu, E., Păduraru, J., Popa, V., Lagunovschi-Luchian, V., (2018). Morphological and anatomical investigation of *Aloysia citrodora* Palau – new medicinal plant introduced in Romania. *Scientific papers. Series B. Horticulture*, Volume LXII, 643-648.

Mamoucha, S., Rahul, J., Christodoulakis, N. Anatomical and Histochemical Investigation of the Leaf of Vitex agnus-castus L. 2016. *International Journal of Medical and Health Sciences,* Vol. 10, No, 7, 1885.

Marongiu, B., Piras, A., Porcedda, S., Falconieri, D., Goncalves, M.J., Salgueiro, L., Maxia, A., Lai, R. (2010). Extraction, separation and isolation of volatiles from *Vitex agnus-castus* L. (Verbenaceae)

wild species of Sardinia, Italy, by supercritical CO2. *Nat Prod Res Part A: Structure and Synthesis* 24, 569-579.

Melih, Y., Yusuf, B., Abdurrahman, O. (2016), Chemical Composition and Antifungal Effects of Vitex agnus – castus L. and Myrtus communis L. *Not Bot Horti Agrobo*, 44(2), 466-471.

Metcalfe, C.R. (1963). Comparative Anatomy as a Modern Botanical Discipline. IN: *Adv. Bot. Res*. (ad. R.D. Preston), Academic Press, London, Vol. I, 101-147.

Metcalfe, C.R., Chalk, L. (1983), *Anatomy of the dicotyledones*. 2nd. Vol. II, Clarendon press. Oxford.

Miguel. M., Bouchmaaa. N., Aazza. S., Gaamoussi. F. Lyoussi. B. (2014). Antioxidant, anti-inflammatory and anti-acetylcholinesterase activities of eleven extracts of Moroccan plants. *Fresen Environ Bull* 23, 1375-1388.

Moreno, F.N., Campos-Shimada, L.B., da Costa, S.C., Garcia, R.F., Cecchini, A.L., Marçal, M.R., Natali, de Souza Vitoriano, A., Ishii-Iwamoto, E.L., Salgueiro-Pagadigorria, C.L. (2015). *Vitex agnus-castus* L. (Verbenaceae) Improves the Liver Lipid Metabolism and Redox State of Ovariectomized Rats. *Evidence-Based Complementary and Alternative Medicine*, 1-14.

Neves, R.C.S., Da Camara, C.A.G. (2016). Chemical composition and acaricidal activity of the essential oils from *Vitex agnus-castus* L. (Verbenaceae) and selected monoterpenes. *Annals of the Brazilian Academy of Sciences, Epub aug 04*.

Niroumand, M.C., Heydarpour, F., Farzaei, M.H. (2018). Pharmacological and therapeutic effects of *Vitex agnus-castus* L., *Pharmacognosy Plant review*. Vol. 12, 103-114.

Novak, J., Draxler, L., Gohler, I., Franz, C.M. (2005). Essential oil composition of *Vitex agnus-castus*, comparison of accessions and different plant organs. *Flavour and Fragrance Journal*, 20, 186–192.

Rashed, K. (2013). Antioxidant activity of different extracts of *Vitex agnus-castus* (L.) and phytochemical profile. *Research in Pharmacy* 3(6), 01-05.

Sakhavar, N., Teimoory, B., Razavi, M., Mirteimoori, M., Arbabisarjou, A., Ghaljeh, M. (2013). The effect of vitagnus on treatment of hot flash in menopause. *Life Sci J.* 10(1), 628–32. doi: 10.13140/2.1.2877. 4405.

Sarikurkcu, C., Arisoy, K. Tepe, B. Cakir, A. Abali, G., Ebru Mete E. (2009). Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitex agnus-castus* L. fruits from Turkey. *Food and Chemical Toxicology,* 47, 2479-2483.

Schellenberg, R., Zimmermann, C., Drewe, J., Hoexter, G., Zahner C. (2012). Dose-dependent efficacy of the *Vitex agnus-castus* extract Ze 440 in patients suffering from premenstrual syndrome, *Phytomedicine*, vol. 19, no. 14, 1325–1331.

Schweingruber, F.H,, Börner A., Schulze E-D., (2013). *Atlas of Stem anatomy in Herbs, Shrubs and Trees*, vol 2, 288-292.

Takhtajan, A. (2009). *Flovering plants* (Second Edition). Springer science+Business Media V.P., 871.

Toma C., Rugină R. (1998). *Anatomia plantelor medicinale. Atlas*. Editura Academiei Române, Bucureşti, 172-181.

Toplan, G.G, Kürkçüoğlu, M, Baser, KHC, Sarıyar, G. (2015). Composition of the essential oils from samples of *Vitex agnus-castus* L. growing in Turkey. *Journal of Essential Oil Research*, 27(4), 337-342.

Tutin, T.G., Heiwood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., et al. (1972). *Flora Europaea*. Cambridge, UK: University Press.

Wasson, E. (2003). *The complete encyclopedia of trees and shrubs descriptions, cultivation requirements pruning and planting*. San Diego, United States, Thunder Bay Press.

Zahid, H., Rizwani, G.H., Ishaqe, S. (2016). Phytopharmacological Review on *Vitex agnus-castus*: A Potential Medicinal Plant. *Chinese Herbal Medicines*, 8(1), 24-29.

Zamani, M., Neghab, N., Torabian, S. (2012). Therapeutic Effect of *Vitex agnu-castus* in Patients with Premenstrual Syndrome. *Acta medica Iranica*, Vol. 50, No. 2, 101-106.