Scientific Papers. Series B, Horticulture. Vol. LXV, No. 2, 2021
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOME ASPECTS REGARDING THE MORPHO-ANATOMY AND ANTIOXIDANT POTENTIAL OF THE MEDICINAL PLANT *EUCOMMIA ULMOIDES* OLIV.

Vasilica LUCHIAN[1], Mihaela Ioana GEORGESCU[1], Elena SĂVULESCU[1], Minodora GUTUE[1], Mariana TOMA [1, 2], Aurora DOBRIN[3], Vlad POPA[3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Institute of Research - Development for Processing and Marketing of Horticultural Products - "Horting", 5N Drumul Gilăului, District 4, Bucharest, Romania
[3]Research Center for Studies of Food Quality and Agricultural Products, USAMV of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author's email: vasi_botanica@yahoo.com

Abstract

Eucommia ulmoides Oliv. - Gutta-percha, Hardy rubber tree, known as Du Zhong in China, is commonly used in Chinese herbalism, being considered to be one of the 50 fundamental herbs. E. ulmoides *is native to China and has been widely cultivated in central and south-eastern regions of China and other countries worldwide. The studied individuals have been cultivated in the Bucharest, at the University of Agronomic Sciences and Veterinary Medicine's Botanical Garden which maintained this species for over 40 years. Different studies have been carried out with regard to leaf morphology, as well as the anatomy of the stem, leaf and petiole. Morphological analyses show that leaves vary in length between 12 and 15 cm, in width between 5.2 and 8 cm, and the petiole length measures 1.5-2 cm. The leaves of Eucommia ulmoides Oliv. feature an epidermis (upper and lower) covered by cuticle. Their mesophyll is bifacial. Ethanolic extracts from the fresh leaves and fruits were prepared in order to determine the total phenolic, flavonoid and associated antioxidant activity. The results showed that leaves had higher total polyphenol and flavonid content than fruits and fruits had higher antioxidant activity than leaves. The findings of this study suggest that Eucommia ulmoides can be also used as a dietary source of phenolic compounds with antioxidant potential.*

Key words: *Eucommia ulmoides, leaf anatomy, stem anatomy, polyphenol, trichomes.*

INTRODUCTION

Eucommia ulmoides (*E.u.*) (Gutta - Percha, The Hardy Rubber Tree) is a small dioecious tree, the only species of genus *Eucommia* (Family *Eucommiaceae*), being native to China. It has been widely cultivated in central and south-eastern regions of China and other countries worldwide (Li & Du, 2001). Gutta-percha, known as Du Zhong in China, is commonly used in Chinese herbalism, where it is considered one of the 50 fundamental herbs (Duke & Ayensu, 1985). *Eucommia* is a primitive angiosperm and fossils reportedly show that a few species were distributed worldwide up to the late around the world about 6 million years ago. Fossils of other *Eucommia* species have been found in 10 -to 35-million-year-old brown coal deposits in central Europe and widely in North America (Call & Dilcher, 1997). Some fossil specimens assigned to the living *Eucommia ulmoides* were found from the Miocene and Pliocene in Germany, Poland and Romania (Guo Shuang - Xing, 2000). The pharmacological properties and efficacy of *E. ulmoides* have been well documented in ancient Chinese medicinal books such as Shennong's *Classic of Materia Medica* and *Compendium of Materia Medica* (Bamba et al. 2002, 2010). Some 112 compounds have been isolated from *E. ulmoides*, including lignans, iridoids, phenolics, steroids and other compounds. Delicious tea formula made from *E. ulmoides* leaves was reported to reduce fattiness and enhance energy metabolism (Hussain et al., 2016). The leaf of *E. ulmoides* has been found to be a rich source of aminoacids, vitamins, minerals and flavonoids, such as quercetin, rutin and geniposidic acid (Chen et al., 2004; Takamura et al., 2007). The leaves of *E. ulmoides* have been reported to enhance bones' strength and body muscles, thus leading to longevity and promoting fertility in humans. Flavonoids are important compounds which are common in nature and are considered as secondary metabolites. Analyses of *E. ulmoides* isolated a total of 7 flavonoids (Cheng et al., 2000).

The effect of *Eucommia ulmoides* substances is antibacterial (against *Acinetobacter baumannii* and *Staphylococcus aureus*), antifungal (against *Aspergillus fumigatus*), antiviral and anti-inflammatory (Nakano, 1997; Lv et al., 2008; Kim et al., 2009; Tsai et al., 2010; Zang et al., 2013; Peng et al., 2014; Kwon, 2016). Two antifungal peptides from the bark of *E. ulmoides* inhibited 8 pathogenic fungi from cotton, wheat, potato, tomato, and tobacco, including *Phytophthora infestans*, *Ascochyta lycopersici*, *Verticillium dahlia*, *Gibberella zeae*, *Alternaria nicotianae*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Colletotrichum gossypii* (Huang et al., 2002). In the Chinese traditional medicine, *Eucommia* is considered a major herbal tonic for cardiac patients (Luo et al., 2010; Greenway et al., 2011). Antioxidant compounds from the *Eucommia* plant reduced the level of free radicals (Cai et al., 2004) and improved the disease condition caused by oxidative stress (Akinmoladun et al., 2010). The *Eucommia* cortex extract can be used in the control of osteoporosis. Therefore, the mentioned extract can be established as a therapeutic agent under conditions of osteoporosis (Ha et al., 2003). Antioxidant properties of the *Eucommia* leaf extract were also reported to contribute positively to the promotion of bone growth by improving cell integrity during oxidative stress (Lin et al., 2011). Previous studies have shown that *E. ulmoides* has also properties that help the human body fight against obesity and the antimetabolic syndrome (Hirata et al., 2011; Kobayashi et al., 2012; Dai et al., 2013). Stem bark extract of *E. ulmoides* showed higher protection activity against memory dysfunctions (Kuon et al., 2010; 2013). It has also been shown that the activities of sex hormones in the body are optimized with the application of *E. ulmoides* (Ong & Tan, 2007). *Eucommia* also increases the level of other antioxidant enzymes in the blood to neutralize free radicals (Park et al., 2006). Aucubin, Geniposidic contained in *E. ulmoides* may be therapeutic candidates for non-alcoholic fatty liver disease (Lee et al., 2014). β-carotene may also be relevant to the anti-cancer effects of *Eucommia*. The leaf and bark of *E. ulmoides* have been widely used in traditional Chinese medicine for the treatment of hypertension (Deyama et al., 2001; Tagawa et al., 2005). The leaves are also used as the basic ingredient of Tochu tea (Du zhong tea) and the plant is cultivated in Japan. Numerous studies have been conducted to reveal the medicinal properties of the species (Duke & Ayensu, 1985; Yeung Him-Che, 1985; Li Dong et al., 1986; Hong et al., 1987; Chevallier, 1996; Yen & Hsieh, 1998; Stuart, 1998; Zang et al., 2007; Choi et al., 2008; Zhou et al. 2009; Zang et al., 2009; Horii et al., 2010; Jin et al., 2010; Luo et al., 2010; Jiang et al., 2011; Kwon et al., 2010, 2013; Li et al., 2016; Kim et al., 2012; Fujikawa et al., 2012; Zang et al., 2012; Zang et al., 2014; Guo et al., 2015; Li et al., 2016; Hussain et al., 2016; Do et al., 2018). *E. ulmoides* has excellent resistance to insect and disease problems. Its general morphology, systematics, anatomy, pollen, chemical elements, growth, development and habitat are studied for *E. ulmoides* (Tippo, 1940; Metcalfe and Chalk, 1957; Metcalfe, 1967; Erdtman G., 1969; Zhang Hong-Da et al., 1979; Cronquist, 1981, 1988; Li et al., 1981; Zavada & Dilcher, 1986; Nakazawa et al., 2013; Zhang Yu-Iong et a1., 1988; Zhang Zhi-Yu et al., 1990; Zhang Kang-Jan, 1990; Cheng Jun-Qing et al., 1992; Watson and Dallwitz, 1992; Yan, 1999; Yu Pang et al., 2008). In Romania, to date no studies have been conducted regarding the morphology, anatomy and biochemistry of this species.

MATERIALS AND METHODS

The material of *Eucommia ulmoides* Oliv. (female plants) originated from the field of the Botanical Garden of the University of Agronomical Sciences and Veterinary Medicine in Bucharest, Romania. The species exhibits good adaptation here, having been cultivated for more than four decades. It showed immunity to various pests. The material used in this study was sectioned by hand using razor blades to obtain semipermanent and permanent slides for microscopic studies. Fresh leaves, stems and petioles were collected for anatomical study in the year 2019. Thereafter, the sections were cleansed with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine (Georgescu et al., 2015). Analyses and observations of the obtained cross-sections were performed at the Center for the Study of Food and Agricultural Products Quality at USAMV - Bucharest. Photos were taken and measurements were made using the Leica DM1000 LED, the Leica DFC295 Video Camera and the Leica S8 APO Stereo Microscope, Novex Holland, Optika Microscope, as well as a Sony photocamera. Photos were taken using a light microscope with different magnifications. The physicochemical analysis were carried out in the laboratories of the Research Center for Studies of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest. The extracts were prepared using 50% (v/v) ethanol, after the pharmacopoeia method. The total phenolic content was determined using Dobrin et al. (2018) method, employing the Folin-Ciocalteu reagent and expressed as mg gallic acid equivalents per grames of fresh material (mg GAE/g FW). Calibration curve of the gallic acid had the folowing concentrations: 0, 5, 10, 20, 30, 40, 50, 60, 70 and 80 μg/ml. The $AlCl_3$ modified assay after Dobrin et al. (2018) was used for quantifying the total flavonoid content of the ethanolic plant extracts. The standard catechine solutions for the calibration curve were 0, 0.01, 0.05, 0.10, 0.20, 0.30, and 0.40 mg/ml. The total flavonoid content was expressed as mg catechin equivalents/g fresh weight (Xu et al., 2010). The radical scavenging activity (RSA) assay was made after Dobrin et al., 2018. The results were expressed in DPPH inhibition percentage. All the solvents used were of the analytical grade. All the absorbances were measured using a Specord 210 Plus UV/VIS spectrophotometer. All the samples were analyzed in triplicates.

RESULTS AND DISCUSSIONS

Stem anatomy

The stem has a circular shape (Figures 1 and 2).

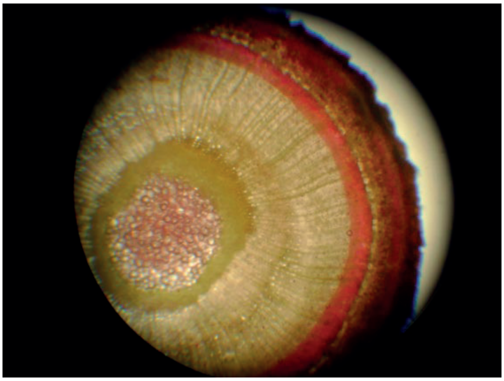

Figure 1. Stem anatomy - cross section

Cross-sections performed in young stem branches reveal an outer epidermis consisting of a polygonal cells layer, covered by a smooth cuticular layer.

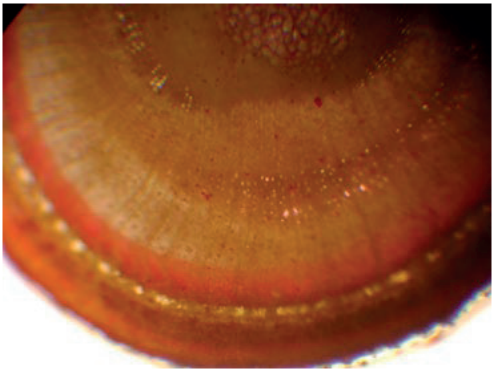

Figure 2. Stem anatomy: cross section details

Under the epidermis there is the primary cortex, made up of 8-12 layers of cells (see Figure 3).

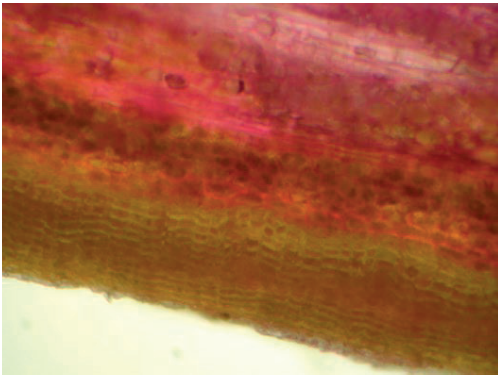

Figure 3. Stem cross-section: details of the outer region

More specifically, the primary cortex is made up of collenchyma tissue, phellogen consisting of 1 or 2 oblong cells, phelloderm, perycicle with loose ring of fibers and parenchymatous cells, xylem and phloem, as continuous cylinders traversed by narrow rays, and heterogeneous pith cells in the middle (see Figure 4).

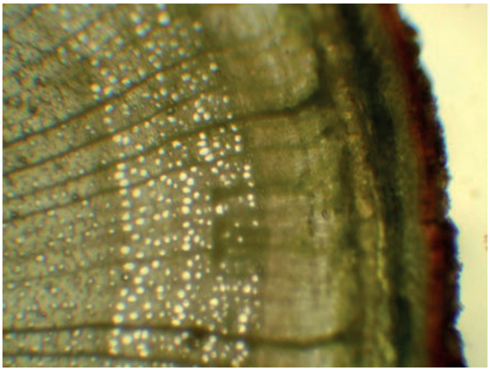

Figure 4. Stem cross-section: phloem, xylem and rays

Between the phelloderm and phloem there are several layers of cells and fibre. The phloem rays are made up of 1 to 3 cell columns. There are articulated laticifers present in the phloem and cortex, as well as scattered latex-cells in some of the other tissues. The wood is of a diffuse-porous type with distinct growth rings as shown in the cross section in Figure 5.

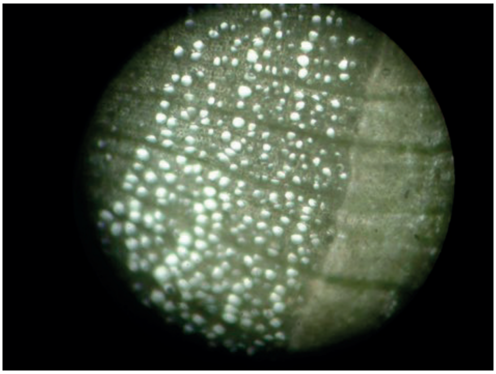

Figure 5. Diffuse-porous wood

There are some solitary vessels with a circular to angular contour. The xylem rays are uni and biseriate. Laticiferous cells are present in the primary cortex, phloem and pith (Metcalfe & Chalk, 1957). The xylem has small vessels (the average tangential diameter measures 9.43-23.28 mm), nearly all are solitary, in shape of half ring, porous type, with spiral thickening, simple perforation plate (Figure 6), ovate and elliptic, lateral wall with bordered pits and spiral thickenings, intervascular pitting uncommon owing to the solitariness of the vessels; small, usually opposite, scalariform perforation plate (Figure 7).

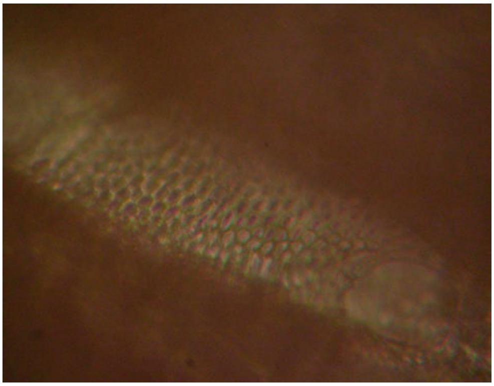

Figure 6. Cross section: simple perforation plate

The rays are almost homocellular or slightly heterocellular.

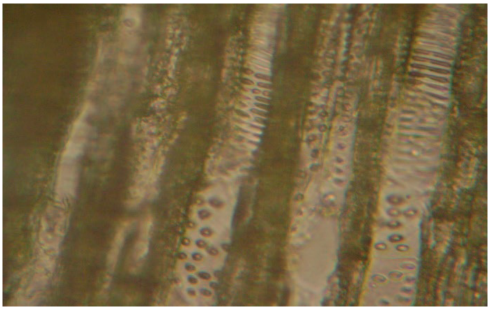

Figure 7. Cross section: simple and scalariform perforation plate

Leaf morphology

Leaves are simple, alternate (Figure 8), deciduous, serrate, astipulate; the blade has an elliptical, ovate or oblong shape, varying in length between 12 and 15 cm and in width between 5.2 and 8 cm; the length of the petiole measures 1.5-2 cm; hairs are simple, unicellular; the edge is simple-serrate and pinnate venation.

Figure 8. Leaf

If a leaf is torn across, strands of latex exuded from leaf veins solidify into rubber and hold the two parts of the leaf together (Figure 9).

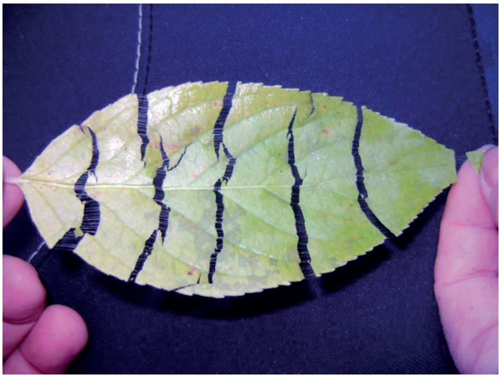

Figure 9. Leaf with rubber

Leaf anatomy

The leaves of *E. ulmoides* Oliv. feature an epidermis (upper and lower) covered by cuticle. Their mesophyll is bifacial; it consists of a pallisade tissue (63.32 µm), just beneath the upper epidermis, whereas there are more chloroplasts and a spongy tissue (87.10 µm) toward the lower epidermis. The dorsi-ventral blade is 174.27-130 µm thick; the upper and lower epidermis is somewhat irregular in shape. The upper epidermal layer consists of compact cells (11.70 µm with cuticle), while the lower epidermis consists of one layer of cells (7.28 µm), with stomata and no glandular trichomes,unicellular The palisade tissue has two cell layers. The cells of the secondary layer are shorter, irregular, columnar. The spongy tissue consists of 5-6 cell layers, with intercellular spaces (Figures 10 and 11).

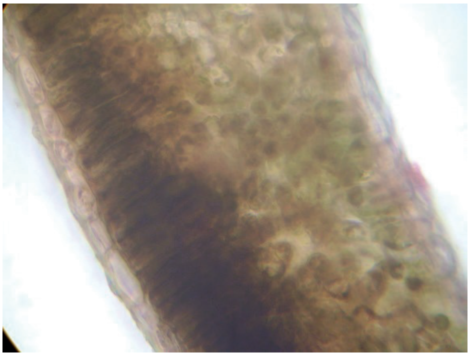

Figure 10. Leaf anatomy: lamina

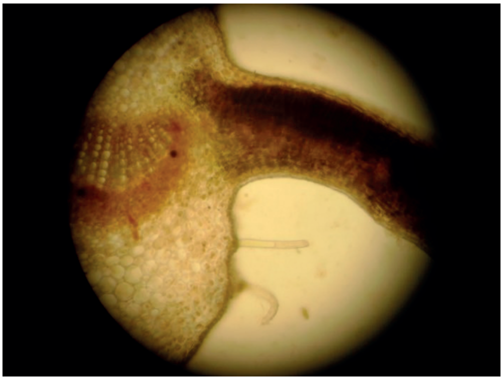

Figure 11. Leaf anatomy: lamina with midrib

The vascular bundle in the middle vein has an arc shape, with several layers of parenchyma cells on the exterior enclosing them. There are scattered rubber cells, 2-4 layers of collenchyma cells under the middle vein/ midrib epidermis (Figure 12).

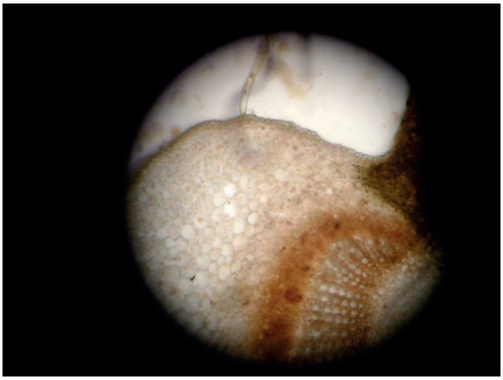

Figure 12. Midrib

The leaf epidermis was scanned by through electron microscopy (SEM) (Figures 13, 14, 15, and 16).

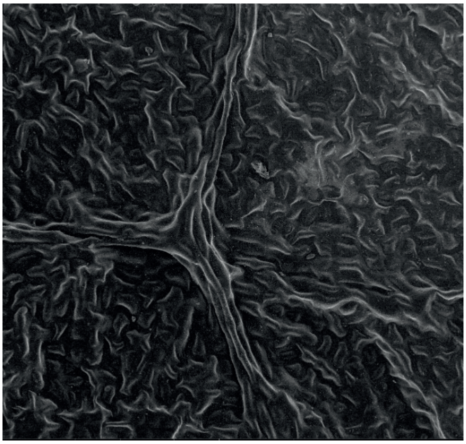

Figure 13. Upper epidermis (SEM)

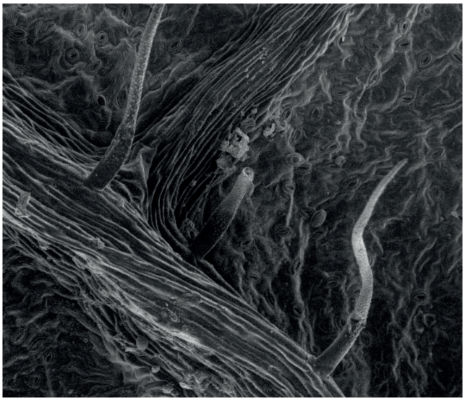

Figure 14. Lower epidermis: stomata, no glandular trichomes

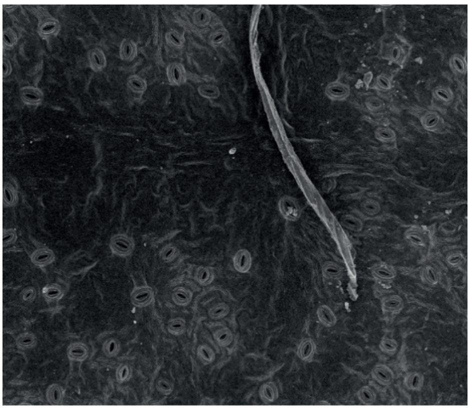

Figure 15. Lower epidermis: stomata, no glandular trichomes

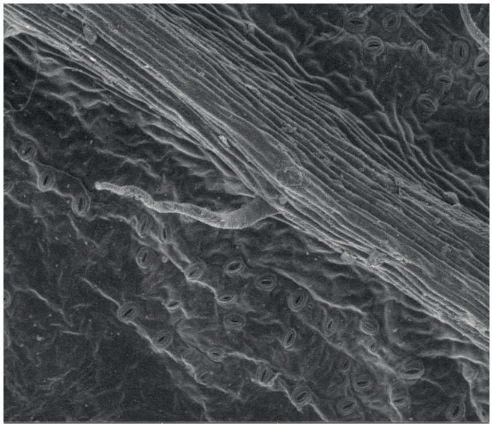

Figure 16. Lower epidermis: stomata, no glandular trichomes

Petiole anatomy

The petiole is thick, crescent-shaped, with 1-layer cuticled epidermis; the sclerenchyma, consisting of 4-6 layers, is present on the lower surface of epidermis, cortex parenchyma inside, the cells are large, 6-7 layer cells thickness. The vascular bundle of midrib has an arc shape (Figure 17).

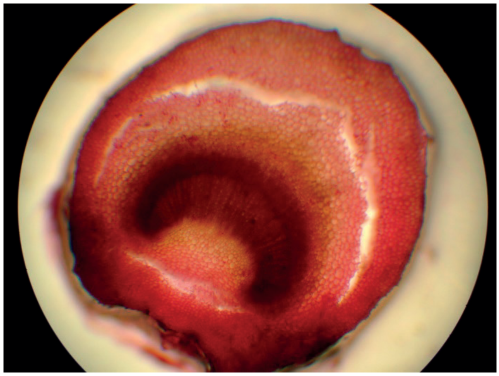

Figure 17. Petiole anatomy

Phenolic, flavonoids and antioxidant activity

It was found that the total phenolic content was higher in leaves (1398.65 mg GAE/g FW) than in fruit (803.22 mg GAE/g FW).

Total flavonoid content was found also in a very hight content in leaves (10,24 mg CE/g FW) than in fruit (5.33 mg CE/g FW).

The scavenging efect on DPPH radical of *E. ulmoides* leaves extract (61.40 %) was lower than in fruits (69.79%). This results are in accordance with the studies of Xu et al., 2018, Wang et al., 2012, and Xu et al., 2018, who found DPPH scavenging activity of 65.9% in leaves extracts and Wang et al., 2012, who found that DPPH scavenging activity of *E. ulmoides* varied between 56.32% and 90.37%.

CONCLUSIONS

The morphological and anatomical studies that we carried out on stem and leaf cross-sections, as well as on leaf surface sections, demonstrate for the first time in Romania the anatomy of organs of *E. ulmoides* Oliv. specimens growing in our country.

The microscopic observations performed on leaves, petioles and stems of *Eucommia ulmoides* are very significant and useful, representing relevant information to the specialists of systematic botany and to the taxonomists.

These preliminary results suggest that *E. ulmoides* can be an ideal candidat for capitalizing its potential in pharmaceutical, food and biomedical industries.

REFERENCES

Akinmoladun, A.C., Obuotor, E.M. Farombi, E.O. (2010). Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants, *Journal of Medicinal Food*, vol. 13, no. 2: 444–451.

Bamba, T., Fukusaki, E., Nakazawa, Y., Kobayashi, A. (2002). In situ chemical analyses of trans-polyisoprene by histochemical staining and fouRier transform infrared microspectroscopy in a rubber-producing plant, *Eucommia ulmoides* Oliver. Planta. 215. pp. 934–939.

Bamba, T., Murayoshi, M., Gyoksen, K., Nakazawa, Y., Okumoto, H., Katto, H., Fukusaki, E., Kobayashi, A. (2010). Contribution of mevalonate and methylerythritol phosphate pathways to polyisoprenoid biosynthesis in the rubber-producing plant *Eucommia ulmoides* Oliver. Z. Für. Nat. C J. Biosci. 65:363–372.

Cai, Y., Luo, Q., Sun, M., Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, vol. 74, no. 17: 2157–2184.

Call, V. B., Dilcher, D. L. (1997). The fossil record of *Eucommia* (Eucommiaceae) in North America. *American Journal of Botany* 84(6): 798–814.

Chen, Z., He, J., Tang, D., Shi, Q., Qunxue, L. (2004). Nutrient distribution and uptake characteristics in Fucommia ulmoides Oliv. plant. *Journal of Northwest Forestry College*. 19(3):15–17.

Cheng Jun-Qing, Yang Jla-Ju & Liu Pang (1992). Chinese timber anatomy: 220-221. Chinese Forestry Press, Beijing.

Cheng, J., Zhao, Y. Y., Cui, Y. X., and Cheng ,T. M. (2000). Studies on flavonoids from leave of Eucommia *ulmoides* Oliv," *Zhongguo Zhong Yao Za Zhi*, vol. 25, no. 5:284–286.

Chevallier, A. (1996) *The Encyclopedia of Medicinal Plants* Dorling Kindersley. London 1996 ISBN 9-780751-303148.

Choi, M.S., Jung, U.J., Kim, H.J. Do, G.M. Jeon, S.M., et al. (2008) Du-zhong (Eucommia ulmoides Oliver) leaf extract mediates hypolipidemic action in hamsters fed a high-fat diet. *Am J Chin Med* 36:81–93.

Cronquist, A. (1981). An integrated system of classification of flowering plants. Columbia University Press, New York:1262.

Cronquist, A. (1988). The evolution and classification of flowering plants. 2nd Ed. The New York Bot. Gard., pp.555.

Deyama, T., Nishibe, S., and Y. Nakazawa (2001). Constituents and pharmacological effects of Eucommia and Siberian ginseng. Acta Pharmacol Sin. 22:1057-1070.

Do, M.H.; Hur, J.; Choi, J.; Kim, M.; Kim, M.J.; Kim, Y.; Ha, S.K. (2018). *Eucommia ulmoides* Ameliorates Glucotoxicity by Suppressing Advanced Glycation End-Products in Diabetic Mice Kidney. *Nutrients* 2018, *10*, 265. https://doi.org/10.3390/nu10030265.

Dobrin, A., Popa V.I., Potor, C.D., Georgescu, M.I. (2018). Phytochemical research on aerial parts of Raphanus raphanistrum subsp. landra (Moretti ex DC.) Bonnier & Layens. Scientific Papers-series A-Agronomy. Jan 1;61:458-63. 2018.

Duke, J. A. , Ayensu, E. S.(1985). *Medicinal Plants of China* Reference Publications, Inc. 1985 ISBN 0-917256-20-4.

Erdtman, G., (1969). Handbook of palynology. Morphology-Taxonomy Ecology. An introduction to the study of pollen grain and spores, Munksgard, Copenhagen, pp. 486.

Fujikawa, T., Hirata, T., Hosoo, S., Nakajima, K., Wada, A., Yurugi, Y., Soya, H., Matsui, T., Yamaguchi, A., Ogata, M., & Nishibe, S. (2012). Asperuloside stimulates metabolic function in rats across several organs under high-fat diet conditions, acting like the major ingredient of Eucommia leaves with anti-obesity activity. *Journal of nutritional science*, *1*, e10. https://doi.org/10.1017/jns.2012.12

Georgescu, M.I., Săvulescu, E., Dobrescu, E., Mușat, M. (2015). Seseli gigantissimum Ciocârlan-anatomy of leaves. Scientific Papers-Series B, Horticulture, Vol. 59, 347-349.

Greenway, F., Liu, Z., Yu, Y., Gupta, A. (2011). A clinical trial testing the safety and efficacy of a standardized *Eucommia ulmoides* oliver bark extract to treat hypertension. *Alternative Medicinee Review*. 16(4):338–347.

Guo Shuang-Xing, (2000). Evolution, palaeobiogeography and palaeoecology of Eucommiaceae, Palaeobotanist 49:65-83, 0031- 0174.

Guo, H., Shi, F., Li, M.J., Liu, Q., Yu, B. 2015). Neuroprotective effects of *Eucommia ulmoides* Oliv. and its bioactive constituent work via ameliorating the ubiquitin-proteasome system. BMC Complement. Altern. Med.15:151. doi: 10.1186/s12906-015-0675-7.

Ha, H., Ho, J., Shin, S., Kim, H., Koo, S., *et al.* (2003). Effects of Eucommiae Cortex on osteoblast-like cell proliferation and osteoclast inhibition. *Arch Pharm Res* 26, no.11: 929-936.

Hirata, T., Kobayashi, T., Wada, A., Ueda, T., Fujikawa, T., et al. (2011). Anti-obesity compounds in green leaves of Eucommia ulmoides. *Bioorganic & Medicinal Chemistry Letters*. 21(6):1786–1791.

Hong, N. D., Rho, Y. S., Won, D. H., Kim, N. J., Cho, B. S. (1987). Studies on the anti-diabetic activity of *Eucommia ulmoides* Oliver. *Korean J. Pharmacogn.* 18, 91–100.

Horii, Y., Tanida, M., Shen, J., Hirata, T., Kawamura, N., et al. (2010). Effects of Eucommia leaf extracts on autonomic nerves, body temperature, lipolysis, food intake, and body weight. *Neurosci Lett* 479:181-186.

Huang, R.H., Xiang, Y., Liu, X.Z., Zhang, Y., Hu, Z., Wang, D.C. (2002). Two novel antifungal peptides distinct with a five-disulfide motif from the bark of *Eucommia ulmoides* Oliv. *FEBS Lett*. 521(1-3):87-90.

Hussain, T., Tan, B., Liu, G., Oladele, O. A., Rahu, N., Tossou, M. C., et al. (2016). Health-promoting properties of *Eucommia ulmoides*: a review. *Evid. Based Complement. Alternat. Med.* :5202908. doi: 10.1155/2016/5202908.

Jiang, L., Wang, Z., Zhu, H.W., Di, H.Y., Li, H., et al. (2011). Beneficial effect of Eucommia polysaccharides on systemic lupus erythematosus-like syndrome induced by Campylobacter jejuni in BALB/c mice. *Inflammation* 34:402-411.

Jin, X., Amitani, K., Zamami, Y., Takatori. S., Hobara, N., Kawamura, N., Hirata T., Wada, A., Kitamura, Y., Kawasaki, H. (2010). Ameliorative effect of Eucommia ulmoides Oliv. leaves extract (ELE) on insulin resistance and abnormal perivascular innervation in fructose-drinking rats. *J Ethnopharmacol* 128: 672-678.

Kim, B. H., Park, K. S., Chang, I.M. (2009). Elucidation of anti-inflammatory potencies of *Eucommia ulmoides* bark and *Plantago asiatica* seeds. *Journal of Medicinal Food*. 12(4):764–769.

Kim, M.C., Kim, D.S., Kim, S.J., et al.(2012). "Eucommiae cortex inhibits TNF-*α* and IL-6 through the suppression of caspase-1 in lipopolysaccharide-stimulated mouse peritoneal macrophages," *The American Journal of Chinese Medicine*, vol. 40, no. 1: 135–149.

Kobayashi, Y., Hiroi, T., Araki, M., Hirokawa, T., Miyazawa, M., et al. (2012). Facilitative effects of Eucommia ulmoides on fatty acid oxidation in hypertriglyceridaemic rats. *J Sci Food Agric* 92: 358–365.,

Kwon SH, Ma SX, Hwang JY, Ko YH, Seo JY, Lee BR, Lee SY, Jang CG.(2016). The Anti-Inflammatory Activity of Eucommia ulmoides Oliv. Bark. Involves NF-κB Suppression and Nrf2-Dependent HO-1 Induction in BV-2 Microglial Cells. Biomol Ther (Seoul). 2016 May 1;24(3):268–82.

Kwon, S.H., Lee, H-K., Kim, J.A., et al. (2010). Neuroprotective effects of *Eucommia ulmoides* Oliv. Bark on amyloid beta$_{25-35}$-induced learning and memory impairments in mice. *Neuroscience Letters*. 2011;487(1):123–127

Kwon, S.H., Ma, S.X., Joo, H.J., Lee, S.Y., Jang, C.G. (2013). Inhibitory Effects of *Eucommia ulmoides* Oliv. Bark on Scopolamine-Induced Learning and Memory Deficits in Mice. Biomol. Ther. 21:462–469.

Lee, G. H., Lee, M. R., Lee, H. Y., Kim, S. H., Kim, H. K., Kim, H. R., et al. (2014). *Eucommia ulmoides* cortex, geniposide and aucubin regulate lipotoxicity through the inhibition of lysosomal BAX. *PloS One* 9, e88017. doi:10.1371/journal.pone.0088017.

Li Dong, Wang Long. Chen Jia-ming & Xu Jun-wen, (1986). Studies on chemical constituents of Duzhong (the bark of Eucommia ulmoides (Oliv). Acta Botanica Sinica 28: 528–532.

Li, X., Tang Z., Fei, D., Liu, Y., Zhang, M., et al. (2016). Evaluation of the sedative and hypnotic effects of astragalin isolated from Eucommia ulmoides leaves in mice. *Nat Prod Res* 26: 1–5.

Li, Z.L., Cui, K.M., Yu, C.S., Chang, X.L. (1981). Anatomical studies of regeneration after ringing of Eucomma ulmoides, Acta Botanica Sinica, vol. 23: 6–13.

Lin, J., Fan, Y. J., Mehl, C., Zhu, J. J., Chen, H., Jin, L. Y., Xu, J. H., & Wang, H. M. (2011). Eucommia ulmoides Oliv. antagonizes H2O2-induced rat osteoblastic MC3T3-E1 apoptosis by inhibiting expressions of caspases 3, 6, 7, and 9. *Journal of Zhejiang University. Science. B*, *12*(1), 47–54.

Luo, L.F., Wu, W.H., Zhou, Y., J., Yan, J., Yang, G.P., Ouyang, D.S. (2010). Antihypertensive effect of *Eucommia ulmoides* Oliv. extracts in spontaneously hypertensive rats," *Journal of Ethnopharmacology*, vol. 129, no. 2: 238–243.

Lv, L., Sun, Y. R., Xu, W., Liu, S. W., Rao, J. J., Wu, S. G. (2008). Isolation and characterization of the anti-HIV active component from *Eucommia ulmoides* . *Zhong Yao Cai*. 2008;31(6):.847–850.

Metcalfe, C.R .& Chalk, L, (1957). Anatomy or the Dicotyledons. Oxford. Vol. I.:31–34.

Metcalfe, C.R. Distribution of latex in the plant kingdom. *Econ Bot* **21,** 115–127 (1967). https://doi.org/10.1007/BF02897859.

Nakano M., Nakashima H., Itoh Y. (1997). Anti-human immunodeficiency virus activity of oligosaccharides from rooibos tea (*Aspalathus linearis*) extracts in vitro. *Leukemia*. 11(supplement 3):128–130.

Nakazawa Y., Takeda T., Suzuki N., Hayashi T., Harada Y., Bamba T., Kobayashi A. (2013) Histochemical study of trans-polyisoprene accumulation by spectral confocal laser scanning microscopy and a specific dye showing fluorescence solvatochromism in the rubber-producing plant, *Eucommia ulmoides* Oliver. *Planta*, 238(3):549-60. doi: 10.1007/s00425-013-1912-2. Epub 2013 Jun 18. PMID: 23775438.

Ong, V.Y., Tan, B.K. (2007). Novel phytoandrogens and lipidic augmenters from Eucommia ulmoides. *BMC Complement Altern Med* 7: 3.

Park, S.A., Choi, M.S., Jung, U.J., Kim, M.J., Kim, D.J, et al. (2006) *Eucommia ulmoides* Oliver leaf extract increases endogenous antioxidant activity in type 2 diabetic mice. *J Med Food* 9: 474-479.

Peng, W., Ge, S., Li, D., Mo, B., Daochun, Q., Okoshi, M.(2014) Report: Molecular basis of antibacterial activities in extracts of *Eucommia ulmoides* wood. Pak. *J. Pharm. Sci*., 4;27: 2133–2138.

Stuart. Rev. G**. A.** *Chinese Materia Medica.* Taipei. Southern Materials Centre, *Medicinal Plants in the Republic of Korea*, World Health Organisation, Manila 1998, ISBN 92 9061 120

Tagawa, C., Nakazawa, Y., Tagashira, E., Ueda, T., Yamaguchi, Y., et al. (2005). Effect of Eucommia Leaf (*Eucommia ulmoides* Oliver; Du-Zhong yge) Extract on Blood Pressure (2), *Nat Med*, 59: 117–120.

Takamura C., Hirata T., Ueda T., et al.(2007). Iridoids from the green leaves of *Eucommia ulmoides* . *Journal of Natural Products*. 2007;70(8).

Tippo, O.. (1940). The comparative anatomy of the secondary xylem and the phylogeny of the Eucommiaceae, *American Journal of Botany*, 27, 9:832-838.

Tsai, T.H., Tsai, T.H., Wu, W.H., Tseng, J.T.P., Tsai, P.J.. (2010). *In vitro* antimicrobial and anti-inflammatory effects of herbs against *Propionibacterium acnes*," *Food Chemistry*, vol. 119, no. 3: 964–968.

Wang J-H., Liu D-M., Liu Y-L., Li C-L., Cheng Y-Y., Li Y. (2012). Antioxidant Capacity and Polyphenolic Content of *Eucommia ulmoides*, Advanced Materials Research Vols. 396-398 (2012) pp 1349-1352, doi:10.4028/www.scientific.net/AMR.396-398.1349.

Watson, L., and Dallwitz, M.J. (1992) onwards. The families of flowering plants: descriptions, illustrations, identification, and information retrieval. Version: 11th February 2021. delta-intkey.com.

Xu Z., Tang M., Li Y., Liu F., Li X., Dai R. (2010) Antioxidant Properties of Du-zhong (Eucommia ulmoides Oliv.) Extracts and Their Effects on Color Stability and Lipid Oxidation of Raw Pork Patties, *Journal of Agricultural and Food Chemistry*, 58(12), 7289-7296, DOI: 10.1021/jf100304t;

Xu, J., Hou, H., Hu, J. et al. (2018). Optimized microwave extraction, characterization and antioxidant capacity of biological polysaccharides from *Eucommia ulmoides* Oliver leaf. Sci Rep 8, 6561 (2018). https://doi.org/10.1038/s41598-018-24957-0;

Yan, R.F.(1999). The Characteristics of the chain structure of Eucommia ulmoides Gum and Its Material Engineering. Proceeding of the first international symposium on Eucomma ulmoides. "China Forestry Publishing House publisher" : 102–110.

Yen, G.C., Hsieh, C.L. (1998). Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) toward various lipid peroxidation models *in vitro* . *Journal of Agricultural and Food Chemistry*. 46(10): 3952–3957.

Yeung Him-Che. (1985**).** *Handbook of Chinese Herbs and Formulas.* Institute of Chinese Medicine, Los Angeles 1985.

Yu Pang, Jing Zhang, Jing Cao, Shen-Yi Yin, Xin-Qiang He, Ke-Ming Cui,(2008) Phloem transdifferentiation from immature xylem cells during bark regeneration after girdling in *Eucommia ulmoides* Oliv, *Journal of Experimental Botany*, Volume 59, Issue 6:1341–1351.

Zavada MS & Dilcher DL (1986). Comparative pollen morphology and its relationship to phylogeny of pollen in the Hamamelidae. *Annales Missouri Botanical Garden*, 73: 348–381

Zhang Hong-Da & Vall Su-Zhu (1979). Eucommiaceae, Flora Reipublicae Popularis Sinicae. Tom. 35: 116-118. Science, Press. Beijing.

Zhang Kang-Jan (1990). Eucommia, 118 Chinese Forestry, Press Beijing.

Zhang Zhi-Yu, Lu An-men, Pan Kdi- Yu & Wen Je (1990). Anatomy embryology and systematic relation of Eucommiaceae. *Acta Phytotaxonomica Sinica*, 28: 430–441.

Zhang, L., Ravipati, A.S., Koyyalamudi, S.R,. Chul Jeong, S.C., Reddy, N. John Bartlett, J., Smith, P.T. Cruz M., Monteiro, M.C., Ángeles, Melguizo, A., Jiménez, E., Vicente, F. (2013). Anti-fungal and anti-bacterial activities of ethanol extracts of selected traditional Chinese medicinal herbs. *Asian Pacific Journal of Tropical Medicine*, vol. 6, no. 9: 673–681.

Zhang, Q., Su, Y.Q., Yang, F.X., Peng, J.N., Li, X.H., Sun, R.C. (2007). Antioxidative activity of water extracts from leaf, male flower, raw cortex and fruit of *Eucommia ulmoides* Oliv. *Forest Products Journal*. 57(12). 74–78.

Zhang Yu-Long. Wang Fu-Hsiung & Chien Nan-Leng (1988). A study on Pollen morphology of *Eucommia ulmoides* Oliver, *Acta Phytotaxonomica Sinica*, 26: 267-370.

Zhang, R., Liu, Z.G., Li, C, Hu, S.J., Liu, L., et al. (2009). Du-Zhong (*Eucommia ulmoides* Oliv.) cortex extract prevent OVX-induced osteoporosis in rats. *Bone* 45. 553-559.

Zhang, R., Pan, Y.L., Hu, S.J., Kong, X.H., Juan, W., Mei, Q.B. (2014) Effects of total lignans from *Eucommia ulmoides* barks prevent bone loss *in vivo* and *in vitro*. *J. Ethnopharmacol*. 112: 104–112.

Zhang, W., Fujikawa, T., Mizuno, K., Ishida, T., Ooi, K., et al. (2012). A Eucommia leaf extract (ELE) prevents OVX-induced osteoporosis and obesity in rats. *Am J Chin Med*, 40: 735–752.

Zhou, Y., Liang, M., Li, W., Li, K., Li, P., et al. (2009) Protective effects of Eucommia ulmoides Oliv. bark and leaf on amyloid β-induced cytotoxicity. *Environ Toxicol Pharmacol*, 28: 342–349.

World Conservation Monitoring Centre (1998). "Eucommia ulmoides".

The IUCN Red List of Threatened Species. IUCN. ***1998****: e.T31280A9614313. doi:10.2305/IUCN.UK.1998.RLTS.T31280A9614313. en. Retrieved 9 January 2018.*