Contribuţii Botanice – 2023, LVIII: 19-26 DOI: 10.24193/Contrib.Bot.58.2
Grădina Botanică "Alexandru Borza"
Cluj-Napoca

SECRETORY STRUCTURES IN *ARTEMISIA ABROTANUM* L. (ASTERACEAE)

Mircea TĂMAŞ[1], Lucian BARBU-TUDORAN[2,3], Ana COSTE[4]
[1] Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Haţieganu", 12 I. Creangă st.,
400010 Cluj-Napoca, Romania
[2] Electron Microscopy Center, Faculty of Biology and Geology, Babeş-Bolyai University,
5-7 Clinicilor st., **400006 Cluj-Napoca, Romania**
[3] Electron Microscopy Integrated Lab., Nat. Inst. for R&D of Isotopic and Molecular Technologies,
67-103 Donath st., **400293 Cluj-Napoca, Romania**
[4] Institute of Biological Research Cluj-Napoca, branch of NIRDBS Bucharest, 48 Republicii st.,
400015 Cluj-Napoca, România
email: ana.coste@icbcluj.ro

Abstract: The genus *Artemisia* includes a large number of aromatic plants that produce secondary metabolites with numerous applications in the health, cosmetics, food and pest management sectors. However, relatively little is known of the dedicated organs where many of these metabolites are synthesized and accumulate. In this context, the aim of this paper was to identify the essential oil-secreting tissue of *Artemisia abrotanum* L. (Asteraceae) popularly known as southern wormwood. The morphology of its secretory tissue was studied using optical microscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). For the first time, secretory structures composed of stalked bicellular glands have been identified in the leaf sinuses on the abaxial side of the leaf blades. These secretory structures have not been described before in vascular plant taxa indigenous (native or naturalized) to Romania, but were reported in other Asteraceae species worldwide.

Keywords: *Artemisia abrotanum*, secretory tissues, essential oil, scanning and transmission electron microscopy.

Introduction

Artemisia abrotanum L. (Asteraceae), known worldwide as southern wormwood or in Romanian folk medicine as "God's wood", is a bush with a tall stem that can grow up to 30-100 cm tall. It has pinnately divided leaves with linear, narrow lobes and small, globular, stalked nodding flower-heads. It is a species of South-West Asian and South European origin, grown in gardens as an aromatic and ornamental plant [26]. Little interest, however, has been paid to study the botanical, chemical, and pharmacological importance of this species, although it was considered to be "the most widespread aromatic plant in peasant gardens" and was frequently used in religious ceremonies and rituals [5]. Chemically, *A. abrotanum* leaves contain essential oils, tannins, bitter substances, flavonoids, coumarins, polyphenolic carboxylic acids and choline [8; 14]. A more in-depth study of the polyphenolic compounds in *Abrotani herba* was conducted by Băiceanu et al. [1]. In terms of pharmacological properties and phytotherapeutic indications, the plant is known to have cholagogue, hepatoprotective, emmenagogue and vermifuge effects

[12-14]. Recent studies on the biological activity of extracts from the above-ground parts of the plant and/or derived essential oils have provided evidence of other possible applications related to their antibacterial, antifungal, antioxidant, anticancer and antiallergic properties [15; 21], as well as biocontrol agents in pest management [29] or as a source of valuable cosmetic ingredients [9]. Additionally, a recent review shows the great potential of *Artemisia* plants in the food and dietary supplement industry [33].

Although *A. abrotanum* is considered an aromatic medicinal plant, the essential oils and the secretory structures responsible for their synthesis have been less studied. Studies on the chemical essential oil profile in fresh (0.475%) and dried (0.4-1.4%) *A. abrotanum* plants have been reported by Gildemeister and Hoffman [10], with a maximum content registered before the flowering stage. Carbonyl (45.6%) and alcoholic functional groups (18%) were identified as components of the essential oils [10]. In Romania, the first research on *A. abrotanum* essential oil was conducted by Radu et al. [25], who investigated the essential oil content and its accumulation dynamics during different vegetative stages. They reported a 1.05-2.10% content of essential oils in dry leaves, with a maximum content at the begining of the flowering stage and identified eucalyptol as the main component of the essential oil through TLC (Thin Layer Chromatography). Later, Tibori et al. [31] also confirmed by GC (Gas Chromatography) that eucalyptol (1,8-cineole) is the most important oil component (38%) in *A. abrotanum*. However, Burzo et al. [6] reported only 17.59% eucalyptol in *A. abrotanum*, possibly due to differences in the local climatic and environmental conditions under which the plants grew. Other studies were focused on the enrichment of *A. abrotanum* essential oil with eucalyptol through fractional distillation [2; 3]. Another survey reported 0.24 ml/100 g vp essential oil content in *Abrotani herba* originating from experimental crops grown at SCDPMA – Fundulea [11]. Investigations on the essential oil's antimicrobial and antifungal properties were also reported [30], while other studies targeted the introduction of this medicinal plant into experimental cultivation systems [24; 27].

The morphology and density of secretory structures are considered taxonomic trademarks for the identification of *Artemisia* species and the certification of medicinal plants [19]. Moreover, considering the importance of specific biosynthesis and/or accumulation sites identification, it is necessary to investigate the vegetative organs of species with therapeutic potential. Therefore, our study provides a more comprehensive microscopic identification and additional evidence for the correct identification of the secretory structures responsible for essential oil production in *A. abrotanum*.

Material and Methods

Fresh samples of leaves of *A. abrotanum* were collected in August 2022 from plants cultivated in Cluj-Napoca. The voucher specimen was identified and deposited in the herbarium of "Alexandru Borza" Botanical Garden from Cluj-Napoca under number CL 672119.

For optical microscopy (OM) investigations, freshly collected leaves were cut into small pieces of 0.5-1 cm and stained with Sudan Red [34], then rinsed with water and included in glycerine as a mounting agent of the specimen on the microscope slide. The stained fragments were analysed with an Olympus BX51 light microscope at 10x, 20x, and 40x magnifications. Secretory structures with specific red coloration (essential oils) were searched for on the surface of the analysed fragments.

For semi-thin sections, leaf segments were embedded, after fixation, in epoxy resin. The resin blocks were sectioned using the Leica UC 6 ultramicrotome (Leica Microsystems, Germany). The thinner sections (250-400 nm) were collected using a glass capillary and mounted on standard glass slides. The slides were heated until the water has evaporated and the sections have adhered to the supporting slide. Epoxy-embedded specimens were stained with epoxy tissue staining solution containing toluidine blue and basic fuchsin [28]. The glass slide was gently heated until a yellow-green ring began to form around the stain drop. Evaporation was halted by rinsing with distilled water. The slides with semi-thin sections were dried and examined using the Olympus BX 51 light microscope at 10x, 20x, 40x magnifications and by immersion at 100x magnification.

Transmission electron microscopy (TEM) analysis was performed according to Leopold et al. [18]. For this survey, the leaves were prefixed in 2.7% glutaraldehyde (Sigma-Aldrich-Sigma Chemical Co. Ltd., St Louis MO, USA) for 60 minutes, then washed with saline phosphate buffer (PBS) four times. Following the second fixation (1.5 hours) with osmium tetroxide, the samples were washed four times with PBS. After 24 hours of dehydration in increased acetone concentrations, the samples were embedded in Epon 812 resin [20], kept for 72 hours at 60^{o}C for polymerization, then sectioned and double stained with Pb (lead) citrate and uranyl acetate and examined under the transmission electron microscope (Jeol JEM 1010, Tokyo, Japan)

The protocol for scanning electron microscopy (SEM) was previously described by Ciorîță et al. [7]. For SEM examination, leaves were prefixed immediately after dissection by immersion in 2.7% glutaraldehyde solution in 0.1 M PBS, pH 7.2. After 1 hour, the glutaraldehyde solution was removed and the samples were rinsed four times with 0.1 M PBS. Afterwards, the leaves were cut in half and then dehydrated by placing them in increased acetone concentrations, as follows: 30 minutes in 30% acetone in water, 30 minutes in 50% acetone, and 30 minutes in 70% acetone, 1 hour in 80% acetone, 1 hour in 90% acetone, and 4 hours in 100% acetone, changing the acetone every hour. Subsequently, the samples were placed in hexamethyldisilazane:acetone solution (1:2, 1:1, 2:1, 1:0, each for 1 hour). All steps were performed at 4^{o}C. The samples were coated with a 9 nm layer of gold using a turbomolecular pump for sputtering - Quorum Q 150T ES (Quorum Technologies London UK) and examined with a HITACHI SU8230 SEM apparatus (Hitachi, Tokyo Japan). All reagents were purchased from Sigma Aldrich (Merck, Bucharest, Romania).

Results and Discussions

SEM analysis (Fig. 1A-F) revealed both glandular and non-glandular hairs (trichomes) on the leaf surface. Non-glandular trichomes were evenly distributed on the upper surface of the leaves, while arranged in two rows in longitudinal grooves on the abaxial leaf surface (Fig. 1A-D). Non-glandular protector hairs are dense, filamentous, curly, interwoven and whitish (Fig. 1 A-E). On the lower surface of the leaf, among these non-glandular trichomes, sunken elliptical glands with bilobed external appearance are observed (Fig. 1B-E). These glands are about 60 x 30 µm in size, slightly constricted in the middle and are more frequent in the grooves from the lower side of the leaves. They present a rigid and opaque cell wall, turgid and fragile (Fig. 1D).

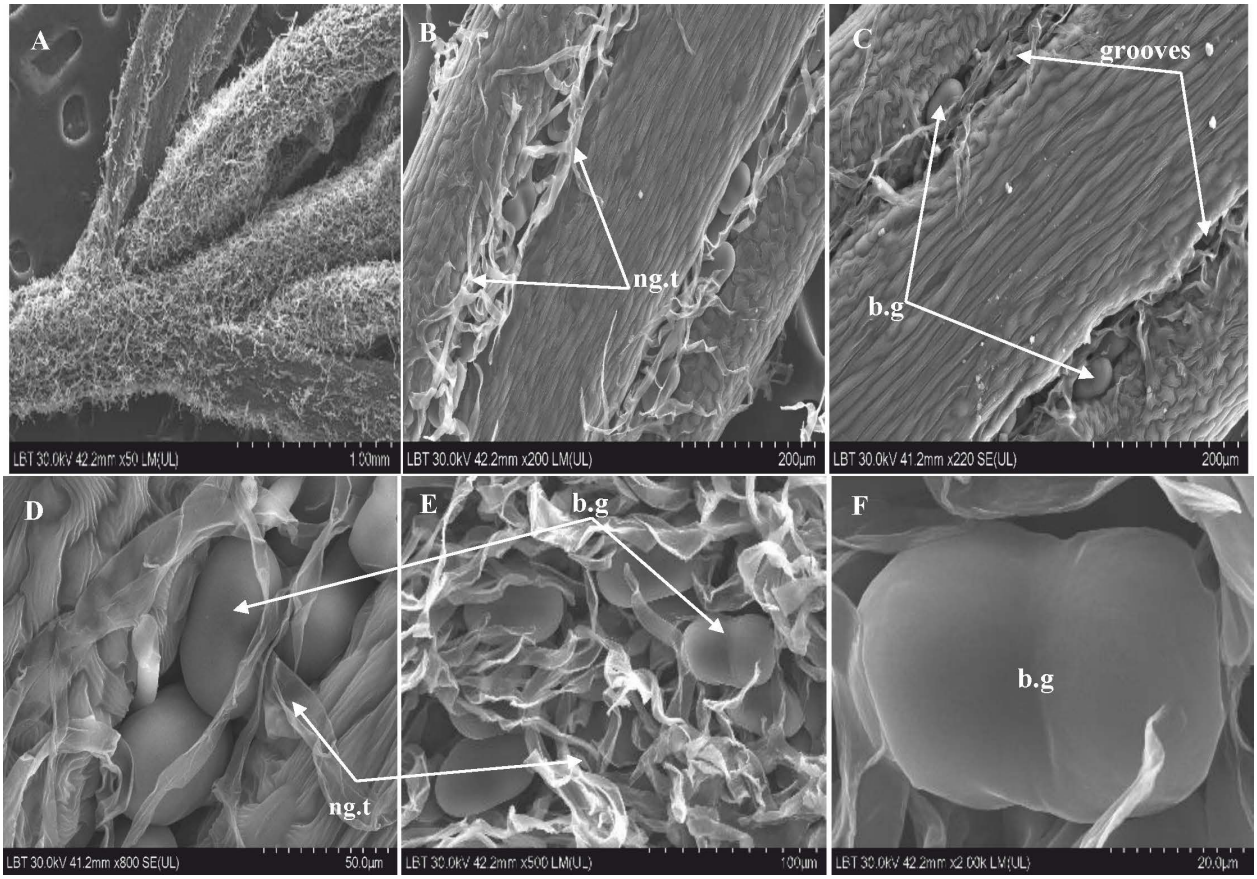

Fig. 1: Leaf surface structure under SEM in *Artemisia abrotanum.* A: Branched pubescent stems, Scale bar = 100 mm; B-C: Abaxial side with two grooves filled with filamentous non-glandular trichomes (ng.t) and mature bicellular secretory glands (b.g) with bilobed external appearance, Scale bar = 200 µm; D-F: Abaxial side with non-glandular trichomes (ng.t) and bicellular mature secretory glands (b.g). Scale bar = 100 µm, 50 µm, 20 µm.

The longitudinal and transverse semi-thin cross-sections of the leaves revealed a circular central part flanked by two lateral circular lobes and delimited by two upper and lower grooves. The central part of the leaf is composed of several layers of continuous palisade cells surrounding the midvein and revealing a few air lacunae (Fig. 2). Semi-thin cross-sections also revealed secretory stalked structures (glandular hairs) in the groove area on the abaxial side of the leaves. These glandular hairs present a small, thin pedicel which seems easily detachable during histological processing.

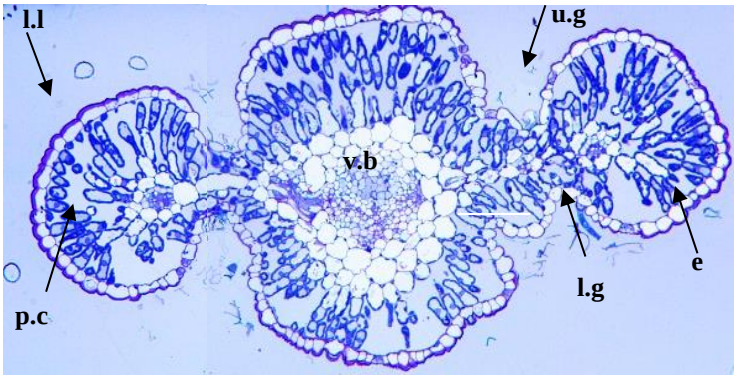

Fig. 2: Semi-thin cross-sections of the leaves: l.l - lateral lobe; u.g – upper groove; l.g – lower groove; e - epidermis; v.b – midvein; p.c – palisade cells

Transmission electron microscopy (TEM) technique revealed bicellular glandular hairs consisting of a basal stalked cell, and a more elongated terminal secretory cell, with a small constriction between the cells and a thickened cell wall (Fig. 3A-E). This might explain the resistance to penetration and why the dye reagent Sudan III was unable to cross the cell wall and enter the secretory cells. Inside these cells, TEM sections revealed numerous essential oil droplets (oil-bodies with light content) (Fig. 3B-C). These secretory structures seem to be in an early developmental stage and are sunken in the epidermal tissue. Over time, the two secretory cells grow and become approximately equal in length (Fig. 3E-F). Glandular hairs present a thin, short-stalked basaloid cell (Fig. 3A-B), which we suppose to be is easily detachable during processing. The fragility of this stalk might explain a possible ready detachment of these glandular secretory structures which then appear loosely distributed between the epidermal cells in the grooves found on the abaxial side of the leaves. Higher magnification images revealed essential oil droplets located in the cytoplasm and a large central vacuole (Fig. 3F).

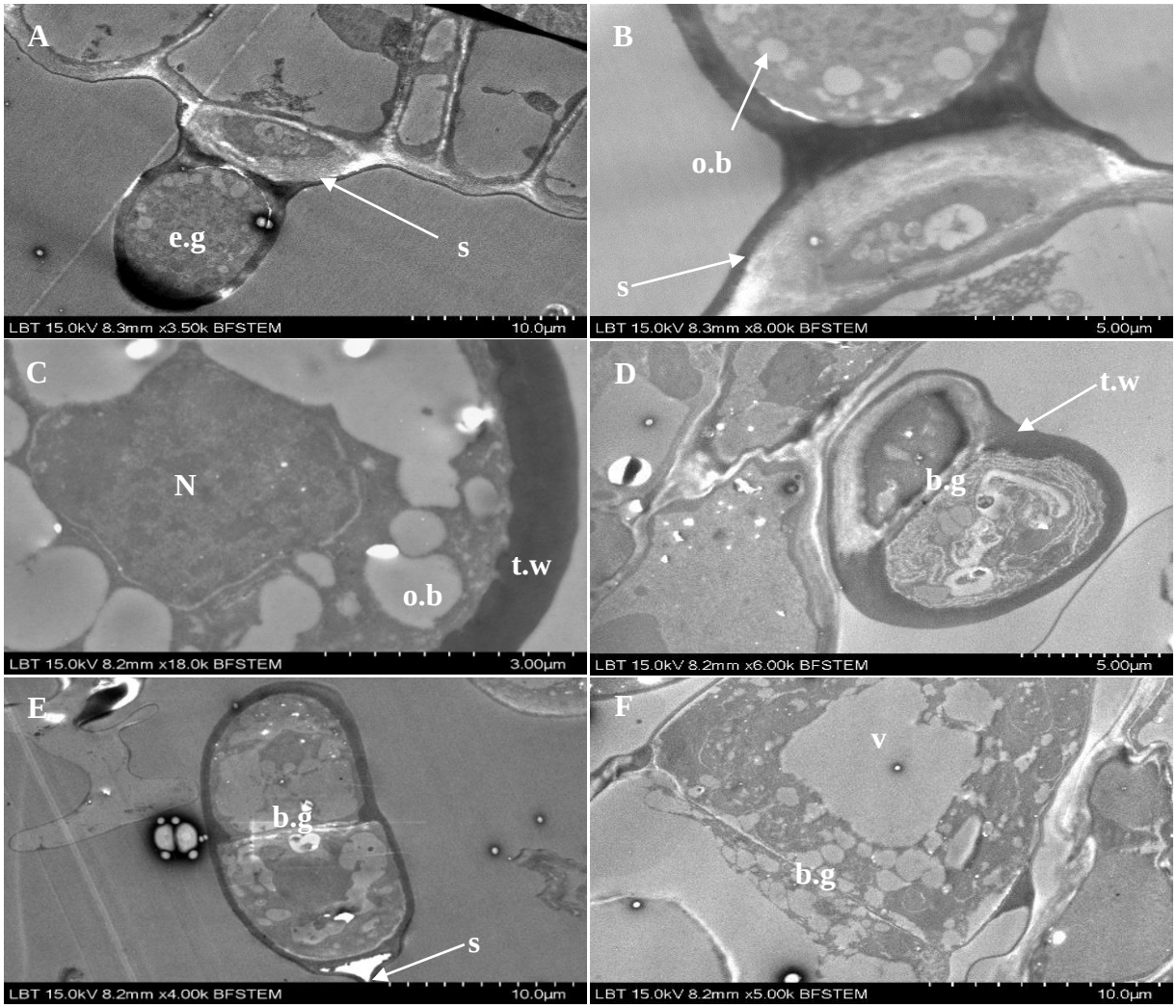

Fig. 3: TEM of secretory glands of *Artemisia abrotanum*. A: Stalked secretory gland at early developmental stage - one-cell stage, Scale bar = 10 µm; B-C: Secretory gland with thickened cell walls an essential oil drops at early developmental stage - one-cell stage, Scale bar = 5 µm, 3 µm; D: Two-cell stage – unequal cells, Scale bar = 5 µm.; E-F: Two-cell stage – equal cells with large vacuoles filled with essential oils (oil-bodies with light content), Scale bar = 10 µm; e.g – early stage secretory gland; s – stalk (pedicel); o.b – oil bodies; N = nucleus; t.w – thickened secretory cell wall; b.g - bicellular secretory gland; V – vacuole.

Most *Artemisia* species produce essential oils in the secretory hairs found on the surface of aerial organs but also through the secretory ducts in the parenchyma tissue [16]. We note that such secretory structures of essential oils have not been described/reported in any Romanian autocthonous [native or naturalized?] vascular plant species, but they are known and reported in other *Artemisia* species [19; 23] or in some other Asteraceae species [4; 22].

A. abrotanum leaf histoanatomy was previously described based on optical microscopy by Toma and Rugină [32]. The authors reported rare tetracellular glandular secretory hairs on the petioles. However, these structures cannot justify the high essential oil content reported in *A. abrotanum* and their accumulation in the leaves. Other researchers described branched stems with penate sectated leaves, pubescent on the lower side with unicellular and pluricellular tectorial hairs in *Abrotani herba* [11]. Yet, none of these studies have revealed the lower grooves filled with loosely distributed secretory glands described in the present paper. In other related species, such as *Artemisia absinthium* (wormwood), the authors reported ellipsoidal, slightly constricted secretory cells [4], while glandular hairs have been described in other Compositae species [22]. Histo-anatomical studies of vegetative organs have also been carried out in other *Artemisia* species: *A. absinthium, A. annua, A. vulgaris* [4; 17; 22].

Conclusions

Essential oil secretory structures of *A. abrotanum* were studied by optical microscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Semi-thin cross-sections revealed a circular central part flanked by two lateral circular lobes, delimited by two upper and lower grooves. The leaflets were covered by elongated, dense non-glandular protector hairs on both sides. On the abaxial side of the leaves, in the grooved area, among these hairs, stalked bicellular glands with opaque cuticle were detected. In the assimilatory parenchyma tissue (chlorenchyma), elongated palisade cells were observed beneath and perpendicular to the epidermis. Mature secretory glands revealed a large central vacuole surrounded by a dense layer of cytoplasm with numerous essential oil droplets. This is the first report to provide additional evidence for the correct identification of the secretory structures responsible for the large amounts of essential oils reported in *A. abrotanum*. Such structures have both taxonomic and medicinal importance.

Funding: This work was funded through the Core Project BIORESGREEN, subproject BioClimpact, project no. 7N/03.01.2023, project code 23020401 and Projects for Excellence Financing in RDI, project 2PFE/2021.

REFERENCES

1. Băiceanu, E., Vlase, L., Băiceanu, A., Nanes, M., Rusu, D., Crișan, G., 2015, New polyphenols identified in *Artemisia abrotani herba* extract, *Molecules,* **20** (6): 11063-75. DOI: 10.3390/molecules200611063.
2. Bâtiu, I., 1995, *Contribuții la tehnologia de separare și valorificare superioară a uleiurilor volatile*, PhD thesis, Univ. „Babeș-Bolyai" Cluj-Napoca.
3. Bâtiu, I., Mărculescu, A., Faur, M., Tămaș, M., 1990, Cercetări privind înnobilarea în eucaliptol a unui ulei volatil indigen, în vol. „*Realizări și perspective în cercetarea biochimică românească*" Cluj-Napoca, Acad. Rom. Fil. Cluj-Napoca: 38-45.

4. Bisset, N.G., Wichtl, M., 1994, Herbal Drug and Phytopharmaceuticals, Medpharm Sci. Publ., Stuttgart, CRC Press, Boca Raton: 91-95.
5. Borza, A., 1925, Flora grădinilor ţărăneşti române II. Plante de podoabă, de leac, de farmece şi credinţe, *Bul. Info. Grăd. Bot. Muz. Bot. Univ. Cluj*, **5** (3-4): 49-74. In: Cristea, V., (ed.), 2014, Plantele vasculare: Diversitate, Sistematică, Ecologie și Importanță, Presa Univ. Clujeană.
6. Burzo, I., Ciocârlan, V., Delian, E., Dobrescu, A., Bădulescu, L., 2008, Researches regarding the essential oil composition of some *Artemisia* L. species, *Analele Ştiinţifice ale Al.I. Cuza Iaşi*, Tomul 54, fasc.2, seria II a, *Biologie vegetală*: 86-91.
7. Ciorîță, A., Tripon, S.C., Mircea, I.G., Podar, D., Barbu-Tudoran, L., Mircea, C., Pârvu, M., 2021, The morphological and anatomical traits of the leaf in representative *Vinca* species observed on indoor- and outdoor-grown plants, *Plants*, **10** (4): 622. https://doi.org/10.3390/plants10040622.
8. Ekiert, H., Knut, E., Świątkowska, J., Klin, P., Rzepiela, A., Tomczyk, M., Szopa, 2021, A. *Artemisia abrotanum* L. *(*Southern Wormwood*)* - History, Current Knowledge on the Chemistry, Biological Activity, Traditional Use and Possible New Pharmaceutical and Cosmetological Applications, *Molecules*, **26** (9): 2503. DOI: 10.3390/molecules26092503
9. European Commission Cosing CosIng-Cosmetic Database. Available online:
10. https://ec.europa.eu/growth/tools-databases/ cosing/ (accessed on 5 July 2023).
11. Gildemeister, E., Hoffman, F.R., 1961, Die ¨Aetherischen ¨Ole Bd. VII, Akademie Verlag, Berlin: 478.
12. Gîrd, C.E., Flutur, R., Sandu R., 2006, Comparative pharmacognostic study of *Artemisia absinthium* L., *Artemisia abrotanum* L. and *Artemisia dracunculus* L., *Farmacia*, **54** (1): 26-34.
13. Grigorescu, E., Lazăr, M.I., Stănescu, U., Ciulei, I., 2001, *Index fitoterapeutic*, Edit. Cantes Iași: 61.
14. Hiller, K., Melzig, M.F., 2010, *Lexicon der Arzneipflanzen und Drogen*, 2 Aufl., Spektrum Akad. Verl. Heidelberg: 59.
15. Hoppe, H.A., 1975, *Drogen Kunde*, Bd.I, 8 Aufl., Walter de Gruyter, Berlin, New York: 122.
16. Hrytsyk, R.A., Kutsyk, R.V., Yurchyshyn, O.I., Struk, O.A., Kireev, I.V., Grytsyk, A.R., 2021, The investigation of antimicrobial and antifungal activity of some *Artemisia* L. species, *Pharmacia*, **68** (1): 93-100. https://doi.org/10.3897/pharmacia.68.e47521.
17. Ivănescu, B., Burlec, A.F., Crivoi, F., Roşu, C., Corciovă, A., 2021, Secondary metabolites from *Artemisia* genus as biopesticides and innovative nano-based application strategies, *Molecules*, **26** (10): 3061. https://doi.org/10.3390/molecules26103061.
18. Ivănescu, B., Miron, A., Lungu, C., 2015, Histo-anatomy of vegetative organs of some *Artemisia* species, *Rev. Med. Chir. Med. Nat*., Iaşi, **119** (3): 917-924.
19. Leopold, L.F., Coman, C., Clapa, D., Oprea, I., Toma, A., Iancu, Ș.D., Barbu-Tudoran, L., Suciu, M., Ciorîță, A., Cadiș, A.I., Mureșan, L.E., Perhaița, I.M., Copolovici, L., Copolovici, D.M., Copaciu, F., Leopold, N., Vodnar, D.C., Coman, V., 2022, The effect of 100-200 nm ZnO and TiO2 nanoparticles on the in vitro-grown soybean plants, *Colloids Surf B: Biointerfaces*, **216**: 112536. https://doi.org/10.1016/j.colsurfb.2022.112536.
20. Liu, Z., Li, X., Zhao, Y., Jin, Y., Zhou, J., Huang, L., Zha, L., & Yuan, Y., 2022*,* Microscopic characterization of five *Artemisia* crude herbs using light microscopy, scanning electron microscopy, and microscopic quantitative analysis, *Microscopy Research and Technique*, **85** (7): 2428-2437. DOI: 10.1002/jemt.24098.
21. Luft, J.H., 1961, Improvements in epoxy resin embedding methods, *J. Biophys. Biochem. Cytol.*, **9** (2): 409-14. DOI: 10.1083/jcb.9.2.409.
22. Melzig, M.F., 2019, *Artemisia abrotanum* L., *Eberraute. Z. Phyther.,* **40** (06): 283-288. DOI: 10.1055/a-1015-5223.
23. Metcalfe, C.R., Chalk, L., 1957*, Anatomy of the Dicotyledons*, Oxford at the Clarendon Press: 557.
24. Minarchenko, V., Karpiuk, U., Kovalska, N., Tymchenko, I., Dvirna, T., Cholak, I., 2023, Diagnostic micromorphological features of leaf surface of some *Artemisia* species (Asteraceae), *Hacquetia*, **22** (1): 131-141. https://ojs.zrc-sazu.si/hacquetia/article/view/10691.
25. Muntean, L.S., Tămaș, M., Muntean, S., Muntean, L., Duda, M., Vârban, D., Florian, S., 2016, *Tratat de plante medicinale cultivate și spontane*, ed.II, Edit. Risoprint Cluj-Napoca: 571-574.
26. Radu, A., Tămaș, M., Băncilă, E., 1973, Cercetări asupra uleiului volatil din *Artemisia abrotanum* L. identificarea eucaliptolului, *Farmacia*, **21** (7): 417-423.

27. Săvulescu, T., Ghişa, E., Grinţescu, I., Guşuleac, M., Morariu, I., Nyarady, E.I., Prodan, I., 1964, *Flora R.P.R.*, Ed. Academiei Române, vol. IX, Bucureşti: 460.
28. Scoreico, E., Stoianov, R., 1993, Cercetări privind stabilirea modului de înmulțire la *Artemisia abrotanum* L., *Herba Romanica*, **12**: 33-38.
29. Spurlock, B.O., Skinner, M.S., Kattine, A.A., 1996, A simple rapid method for staining epoxyembedded specimens for light microscopy with the polychromatic stain Paragon-1301, *Amer. J. Clin. Path*., **46**: 252-258. DOI: 10.1093/ajcp/46.2.252.
30. Tabanca, N., Demirci, B., Blythe, E.K., Bernier, U.R., Ali, A., Wedge, D.E., Khan, I.A., 2011, Composition of *Artemisia abrotanum* and *A. pontica* essential oils and their repellent activity against, *Aedes aegypti*, 59th International Congress and Annual Meeting of the Society-for-Medicinal-Plant-and-Natural-Product-Research, Antalya, Turkey.
31. Tămaș, M., Bergner, E., Radu, A., Kevorkian, F., 1975, Cercetări privind acțiunea biologică a unor uleiuri volatile, *Clujul Medical*, **48** (2): 265-269.
32. Tibori, G., Tămaș, M., Bucur, H., 1981, Analiza uleiului volatil de *Artemisia abrotanum* L., „*Orientări în fitoterapie*" USSM Alba: 177-179.
33. Toma, C., Rugină, R., 1998, *Anatomia plantelor medicinale*, Edit. Acad. Rom., Bucureşti: 93-95.
34. Trendafilova, A., Moujir, L.M., Sousa, P.M.C., Seca, A.M.L., 2021, Research advances on health effects of edible *Artemisia* species and some sesquiterpene lactones constituents, *Foods*, **10** (1): 65. https://doi.org/10.3390/foods10010065.
35. Verzar-Petri, G., 1979, Drogatlasz (Drogok mikroszkopos vizsgalata), *Medicina Könyvkiado*, Budapest: 28.

ȚESUTUL SECRETOR DIN FRUNZELE DE *ARTEMISIA ABROTANUM* L. (ASTERACEAE)

(Rezumat)

Genul *Artemisia* include o mare varietate de plante aromatice care produc metaboliți secundari cu potenţial de utilizare în industria farmaceutică, cosmetică, alimentară și a combaterii dăunătorilor. Cu toate acestea, se cunosc relativ puține aspecte despre organele specializate în care se sintetizează și se acumulează acești metaboliți. În acest context, scopul acestui articol a fost de a identifica țesuturile secretoare producătoare de ulei volatil la specia *Artemisia abrotanum* L. (Asteraceae), cunoscută și sub numele de Lemnul Domnului. Morfologia țesutului său secretor a fost studiată utilizând tehnicile microscopiei optice, a microscopiei electronice de transmisie (TEM) și electronice de baleiaj (SEM). Sunt descrise, pentru prima dată, structuri secretoare formate din peri glandulari bicelulari, pedicelați, dispuși pe partea inferioară în șanțurile laciniilor foliare. Aceste glande secretoare nu au mai fost descrise la plantele vasculare din România, dar sunt cunoscute și menționate în literatura de specialitate internaţională la alte specii de Asteraceae.

Received: 25.10.2023; Accepted: 26.11.2023