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ANATOMICAL AND BIOCHEMICAL RESEARCH ON THE SPECIES *ARTEMISIA ABSINTHIUM* L. (ASTERACEAE) FROM THE SOUTHERN PART OF ROMANIA

Monica Luminița BADEA, Vasilica LUCHIAN, Liliana BĂDULESCU, Elena DELIAN, Elena SĂVULESCU

Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: monica.badea@horticultura-bucuresti.ro

Abstract

Artemisia absinthium L. (Wormwood) is a medicinal plant known for its antibacterial, antioxidant, anti-inflammatory, antifungal, antimalarial, and choleretic properties. The essential oil, extracted through hydrodistillation, was analyzed using GC-MS. The main chemical compounds identified in the essential oil were linalyl isovalerate (16.65%), sabinene (8.90%), myrcene (8.85%), geranyl isobutyrate (6.97%), and β-cymene (6.92%) in the stems; sabinene (23.67%), myrcene (20.53%), β-cymene (9.74%), and α-phellandrene (9.23%) in the leaves; and chrysanthenone (5.48%), sabinene (11.18%), beta-pinene (15.53%), and linalool (17.97%) in the flowers. Anatomical analysis was performed on cross-sections of the stems and leaf lamina. Transverse sections of the stems and leaves were examined under an optical microscope. The A. absinthium stems and leaves are covered with dense T-shaped glandular and non-glandular trichomes, on both sides, the most vigorous being those on the stem and petioles.

Key words: *Artemisia, essential oil, chromatography, glandular trichomes.*

INTRODUCTION

Artemisia absinthium L. (Wormwood) is a medicinal and aromatic bitter herb (Nguyen & Németh, 2016), belonging to the Asteraceae family, the Artemisia genus, including over 500 species (Hayat et al., 2009; Ivănescu et al., 2021; Tojić & Rančić, 2023). It is a perennial plant native to the temperate regions of Asia, Europe, North Africa, Canada and the United States (Bhat et al., 2019). Extracts from these plants contain several classes of secondary metabolites such as: flavonoids, glycosides, coumarins, polyacetylenes, sterols and terpenoids (Tan et al. 1998). The secondary metabolites of Artemisia species are used in the health field but also in food and cosmetics (Ivănescu et al., 2021).

The main secondary metabolites that are responsible for the aromatic odor are found in the leaves and flowers (Bordean et al., 2020). Studies have shown that *Artemisia absinthium* L. plants have antimicrobial, anti-inflammatory, antioxidant, antifungal, antibacterial, antimalarial, anti-tumour choleretic and gastrointestinal effects (Bora & Sharma, 2010; Ebrahimzadeh et al, 2010; Hussain et al., 2017; Goud & Swamy, 2015; Bhat et al., 2019; Batiha et al., 2020; Szopa et al., 2020; Malla et al., 2023). Also, the chemical compounds present in the volatile oil from Artemisia plants show parasiticidal, insecticidal and phytotoxic properties, being recommended as possible biological control agents against plant pests (Ivănescu et al., 2021).

This species is well known for its use in the production of the absinthe drink, being restricted in many countries due to neurotoxicity (Sharifi-Rad et al., 2022). The species of this genus show great therapeutic potential being used against various health problems around the world (Hussain, 2020).

The anatomical elements of species are very important in the classification and correct identification of plants (Hussain et al., 2019; Hussain, 2020).

Studies related to the anatomy of this species were conducted by Konowalik & Kreitschitz (2012), Ivanescu et al., (2015), Ivashchenko et al. (2017) and Janaćković et al. (2019).

The objective of this study is to highlight the importance of *Artemisia absinthium* L. by analyzing the chemical composition of the essential oil extracted from the stems, leaves, and flowers, as well as analyzing the plants from an anatomical perspective.

MATERIALS AND METHODS

Biological material

For the anatomical analysis, mature stems and leaves (leaf blade and petiole) of the species *Artemisia absinthium* L. were used. Regarding the essential oil analysis, it was extracted from fresh plants (stems, leaves, flowers).

The plants were collected from the southern part of Romania, Teleorman County.

Anatomical characterization

The microscopic examination was performed on transverse sections obtained from leaves and stems, analyzing fresh preparations treated with chloral hydrate and stained with carmine alum and methyl green (Luchian et al., 2018), according to the classical method.

The study was carried out in the Botany Laboratory – Faculty of Horticulture Bucharest. The photos have been take using an Optika optical microscope with different objectives, and a Motorola digital camera.

Volatile oil analysis

The essential oil was extracted and analysed within the Faculty of Horticulture Bucharest. Some herbal parts (stems, leaves and flowers) were subjected to hydro distillation for 3h in a fresh condition, using a Clevenger apparatus. The components were separated and identified using an Agilent gas chromatograph equipped with a quadrupole mass spectrometer detector.

A DB-5 capillary column (25 m in length, 0.25 mm inner diameter, and 0.25 µm film thickness) was used, with helium as the carrier gas.

The oven temperature was initially set at 60°C and gradually increased to 280°C at a rate of 4°C per minute (Burzo et al., 2008). The identification of essential compounds was performed using the NIST spectral database, with confirmation based on comparison of Kovats retention indices.

RESULTS AND DISSCUTIONS

The composition of the volatile oil

From the analysis of the volatile oil in the *Artemisia absinthium* L. species, a higher number of chemical compounds was observed in the flowers (40) compared to the stems and leaves which had the same number (30). The chemical composition of the essential oil varied according to the organ, as follows: in the stems, the chemical compounds present in greater quantity were: linalyl isovalerate (16.65%), sabinene (8.90%), myrcene (8.85%), geranyl isobutyrate (6.97 %), β-cymene (6.92%), as shown in Figure 1, followed smaller amounts of isopropyl methyl hexenyl acetate (4.45%), neryl butyrate (2.57%), linalol (2.39%), α-phellandrene (2.36%), ocimene (2.14%), α-curcumene (1.83%), camphene (1.68%), nerolidol (1.05%) and terpinen-4-ol (1.01%). Other constituents had a value below 1% (cis β ocimene, eucalyptol, γ terpinene, myrtenol, lavandulol, nerol, hexenyl methyl butyrate, neryl acetate, hexyl methyl butenoate, terpinyl acetate, β elemene, geranyl propionate, isoaromodendron epoxide, α- bisabolol and cedren 13-ol).

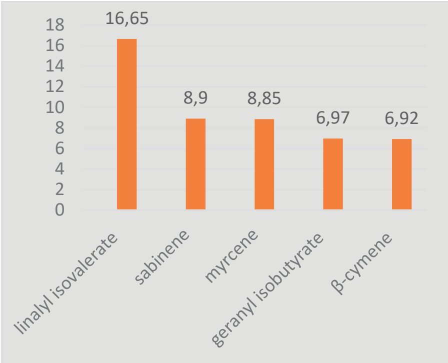


Figure 1. Major chemical compounds of stems, %

In the leaves, the chemical compounds had a greater weight: sabinene (23.67%), myrcene (20.53%), α-phellandrene (9.23 %) and β-cymene (9.74%), as shown in Figure 2, followed smaller amounts of lianlol (4.75%), eucalyptol (2.04%), γ terpinene (1.29%) and myrtenol (1.39 %).

Other constituents had a value below 1% (methyl octene, α-thujene, α-pinene, α-terpinene, cis β ocimene, isopropyl methyl cyclohexen 1-ol, α-terpinolene, hexyl isobutyrate, linalol oxide, lavandulol, dimethyl octadiene diol, α-terpineol, isopulegol, nerol, beta-caryophylene, β-farnesene, β-himachalene, β-selinene, tetra methyl dehydronaphthalene, selinen-4-ol and cis-farnesol).

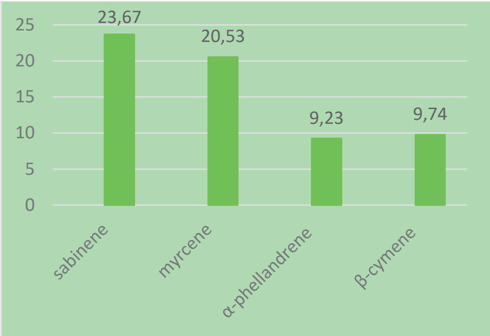


Figure 2. Major chemical compounds of leaves, %

In the essential oil extracted from the flowers, the chemical compounds were in greater quantity: chrysanthenone (5.48%), sabinene (11.18%), β-pinene (15.53%), linalol (17.97%), as shown in Figure 3, followed smaller amounts of tetramethyl-1, 2-dehydronaphalene (4.80%), geranyl butyrate (3.47%), β-himachalene (2.99%), terpinene-4-ol (2.87%), α-phellandrene (2.48 %), selinen 4-ol (2.11%) , linalyl isovalerate (2.31%), r-muurolene (1.58%), γ-terpinene (1.43%), β-cymene (1.26%), β-phellandrene (1.12%) and nuciferol (1.04 %).

Other constituents had a value below 1% (α-thujene, α-pinene, camphene, octanol, α-terpinene, β-thujene, trans-β-ocimene, α-terpinolene, cis p-terpineol, lavandulol, cis-β-ocimene, nerol, neryl acetate, geranyl isobutyrate, β-caryophyllene, β farnesene, β selinene, nerolidol, epiglobulol, caryophyllene oxide, chamazulene, methyl propenyl tetrahydronaphthalene 2-ol and cis-lanceol).

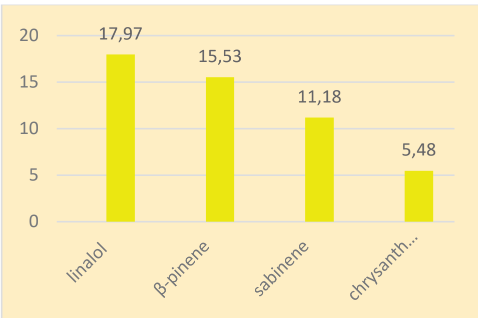


Figure 3. Major chemical compounds of flowers, %

Sabinene was present in greater quantity in the composition of the essential oil extracted from leaves (23.67%), followed by flowers (11.18%) and stems (8.90%). Regarding the chemical compound β-cymene, a larger amount was observed in the stems and leaves (6.92-9.23%), and a much smaller amount in the flowers (1.26%).

Linalol was present in greater quantity in the essential oil extracted from the flowers (17.97%), and in the volatile oil extracted ace from the stems and leaves was in smaller quantity (2.39-4.75%).

α-Phelandrene was identified in a larger amount in the composition of the essential oil extracted from the leaves (9.23%), compared to the amount obtained from the volatile oil extracted from the stems and flowers, where the values were approximately equal (2.36-2.48%).

Myrcene was present in greater quantity in the essential oil extracted from the stems and leaves (8.85-20.53%), being absent in the volatile oil extracted from the flowers.

The presence in greater quantity of substances specific to each vegetative organ should be noted. Thus, in the case of the essential oil extracted from the stems, the presence of esters was highlighted: linalyl isovalerate (16.65%), geranyl isobutyrate (6.97%) and isopropyl methyl hexenyl acetate (4.45%), and in the case of the essential oil extract from flowers of monoterpenes: β-pinene (15.53%) and chrysanthenone (5.48%).

Regarding the chemical compounds with a smaller weight, it was observed that there were chemical compounds common to the analyzed organs, such as: α-thujene, α-pinene, α-terpinene, α-terpinolene, α-terpineol, β-caryophyllene, β-selinene, selinen 4-ol (leaves and flowers), eucalyptol and myrtenol in stems and leaves, cis-β-ocimene, lavandulol, terpinene 4-ol and nerol in stems, leaves and flowers. Research by Lopez-Lutz et al., 2008, showed that the volatile oil from *Artemisia absinthium* L. plants from western Canada had a high content of trans-thujone (10.1%), myrcene (10.8%) and trans-sabinyl acetate (26.4%).

The analysis of the volatile oil carried out by Burzo, 2015 on *Artemisia absinthium* L. plants from Romania showed that the majority chemical compounds were the following: β-pinene (41.65%), myrtenyl acetate (12.77%), sabinene (6.68%), α-phellandrene (7.02%) and β-thujone (4.60 %). A high content of sabinene and myrcene (9.2% - 38.9%) were characteristic of samples from Hungary, Moldova and Scotland (Orav et al., 2006).

The essential oil of *Artemisia absinthium* L. from Lithuania was rich in trans-sabinyl acetate and thujones (Judzentiene et al., 2009).

According to Sharopov et al., 2012, the major chemical compounds present in the essential oil obtained from *Artemisia absinthium* L. plants from three different locations in central Tajikistan were: cis-chrysanthenyl acetate, myrcene, germacrene D, dihydrochamazulene isomer, linalool, linalool acetate and α-phellandrene.

According to the research carried out by Nguyen et al., 2018, a higher content of essential oil was observed in the flowers than in the leaves, regardless of the phenophase (0.31mL/100 g, after flowering stage to 0.91mL/100 g, flowering stage in trans sabinil acetate.

The essential oil content is also influenced by environmental factors, such as light and temperature; the ratio of cis-chrysanthenyl acetate grew from 8.0% to 13.8% (in the "cold chamber" and "warm chamber") with the Spanish plants (Nguyen et al., 2018).

Anatomical characterization

Stem anatomy

Cross-sections have been performed at the internode; fresh slides thereof have been observed under the microscope.

The stem is pubescent, greenish-grey, with a circular contour especially toward the base, where some areas are evidently ridged.

The following areas can be noticed looking from the exterior to the interior of the cross-section: the epidermis, made up of one layer of isodiametric, oval, cuticle-covered cells. It presents dense, simple, T-shaped tector trichomes, bicapitate glandular trichomes and, here and there, stomata (Figure 4 a, b, c).

The cortex is composed of a 7-12 layered collenchyma and a 3-5 layered intercostal parenchyma.

The endodermis is monolayered.

The vascular bundles are collateral, separated from the parenchymatous cells, with 2-12 layers of strongly lignified schlerenchyma above the phloem.

The phloem has small, irregularly-shaped elements. The xylem is made up of 3-8 parallel rows of xylem elements, each row comprising 2-7 vessels.

The central cylinder is sclerified. Small secretory canals can be observed within the cortex. The central part of the cross-section is occupied by the pith, well-developed, formed of parenchyma cells with intercellular spaces. Moreover, the pith also contains small secretory canals (Figure 5 a, b, c, d). Inside the cortex and pith there are secretory canals for essential oils (Figure 6 a, b, c, d,; 6.1. a, b, c).

The longitudinal sections through the stem reveal spiral and punctiform vessels as well as fibers (Figure 7 a, b, c).

The stem epidermis has polygonal, elongated cells, tector and glandular trichomes (Figure 8 a, b, c; 8.1 a, b, c).

The lignified stem presents secondary structure elements, a periderm with overlapping cells, xylem and secondary phloem generated by the cambium.

Anatomy research for this species has also been carried out by Hussain et al., 2019, while Liu et al., 2022, conclude that the morphology and density of the glandular and tector trichomes are highly significant for the diagnosis of *Artemisia* species.

Petiole anatomy

The petiole cross-section has irregular edges and presents the following elements: a monolayered epidermis covered by a thin cuticle, with non-glandular and glandular trichomes resembling those present on the stem and leaves, a parenchymatic tissue including collenchyma, schlerenchyma, secretory canals, and small, medium, and large collateral conducting bundles, made up of xylem and phloem (Figure 9 a, b, c, Figure 10 a, b, c).

The petiole epidermis has polygonal, elongated cells, simple and T-shaped tector trichomes, as well as glandular, bicapitate trichomes (Figure 11 a, b, c).

Leaf blade anatomy

The leaf is equifacial, tomentous on its lower side, with both the upper and lower epidermis having a thin cuticle, with simple and T-shaped non-glandular trichomes, as well as large, silvery-silky glandular trichomes, epidermic cells having their anticlinal walls with a wavy contour, both for the upper (Figure 12 a, b, c, d, e, f) and the lower (Figure 13 a, b, c, d) epidermis.

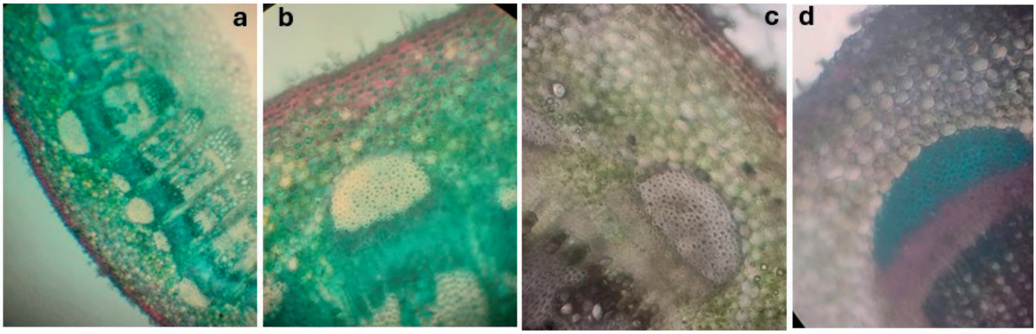


Figure 4. Transversal section of the stem

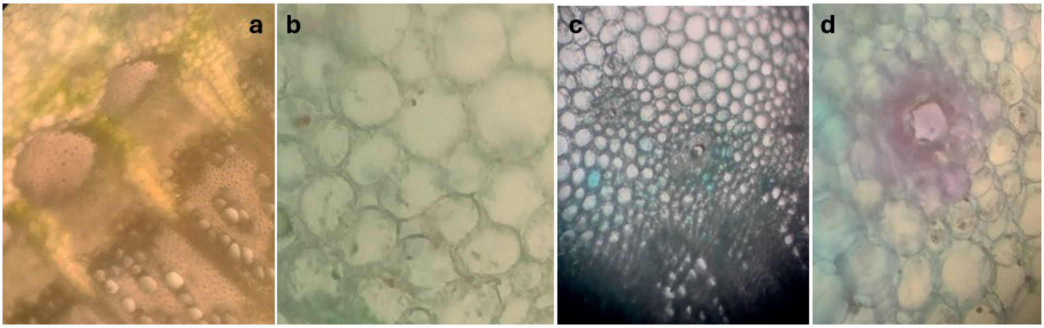


Figure 5. a - Stem - cortex with conductive bundles, b - pith, c, d - secretory canals in the pith - overall view and detail

Figura 6. a, b, c, d -Secretory canals in the cortex - overall view

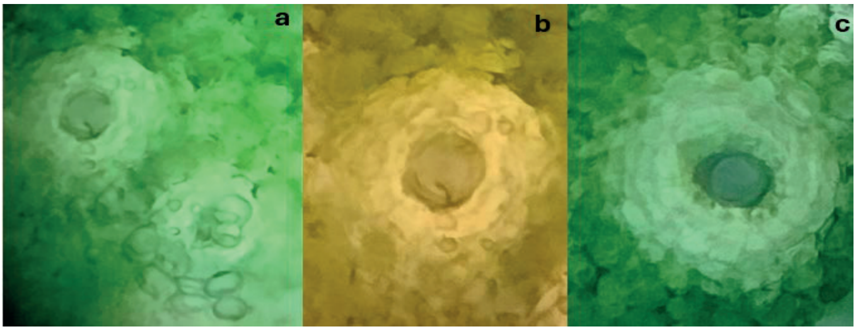


Figure 6.1. a, b, c - Secretory canals in the cortex - b, c - detail

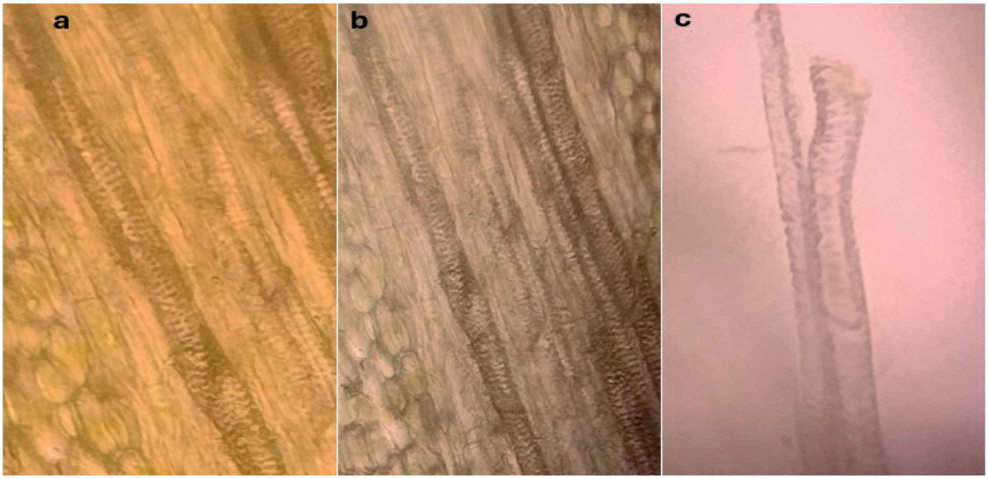


Figure 7. a, b, c, - a,b - Longitudinal section in the stem - punctate conductive vessels and fibers - overall view, c - detailed punctiform vessels

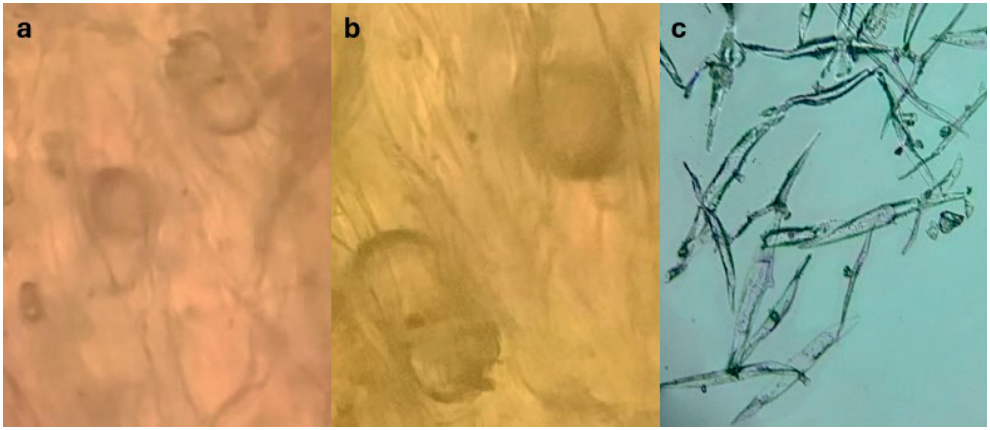


Figure 8. a, b, c - Stem epidermis with tector and glandular trichomes - overall view and detail, c - non-glandular hairs - overall view

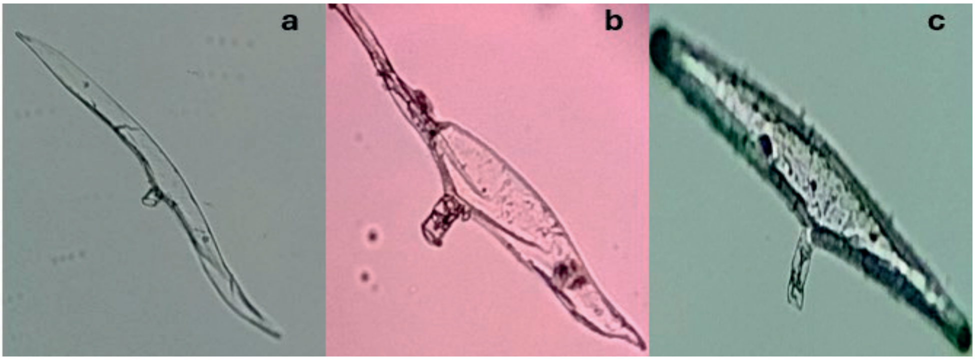


Figure 8.1. a, b, c - Stem epidermis with tector trichomes - overall view and detail, non-glandular hairs - T-shape detail

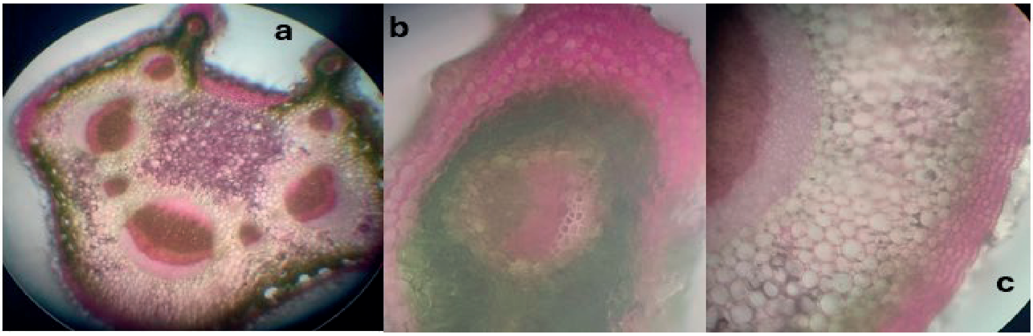


Figure 9. a, b, c - a - Petiole - overall view, b - section margin with conductive bundle,
c - detail of the outer zone

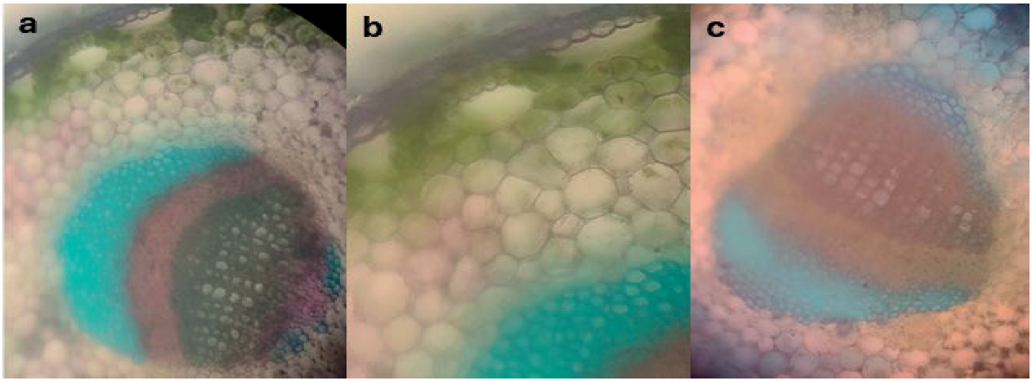


Figure 10. a, b, c - a, b - Petiole with secretory canals and, c - conductive bundle

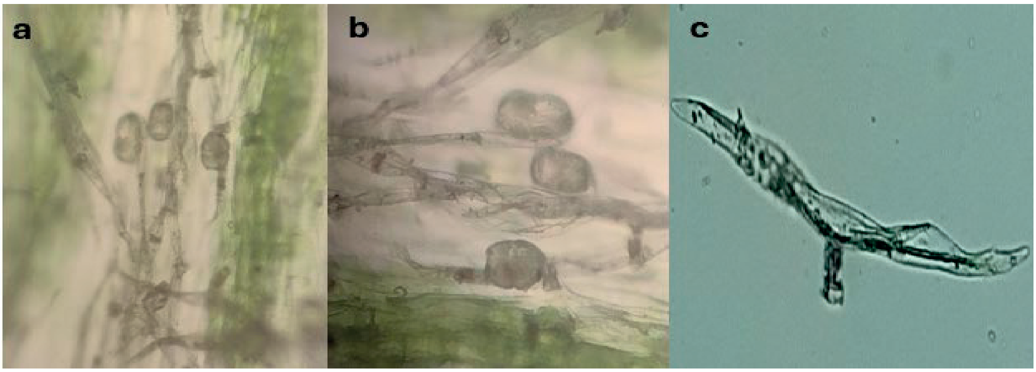


Figure 11. a, b, c - a- Petiole epidermis with tector and glandular trichomes - overall view, b - detail,
c - non-glandular trichome in the shape of the letter T - detail

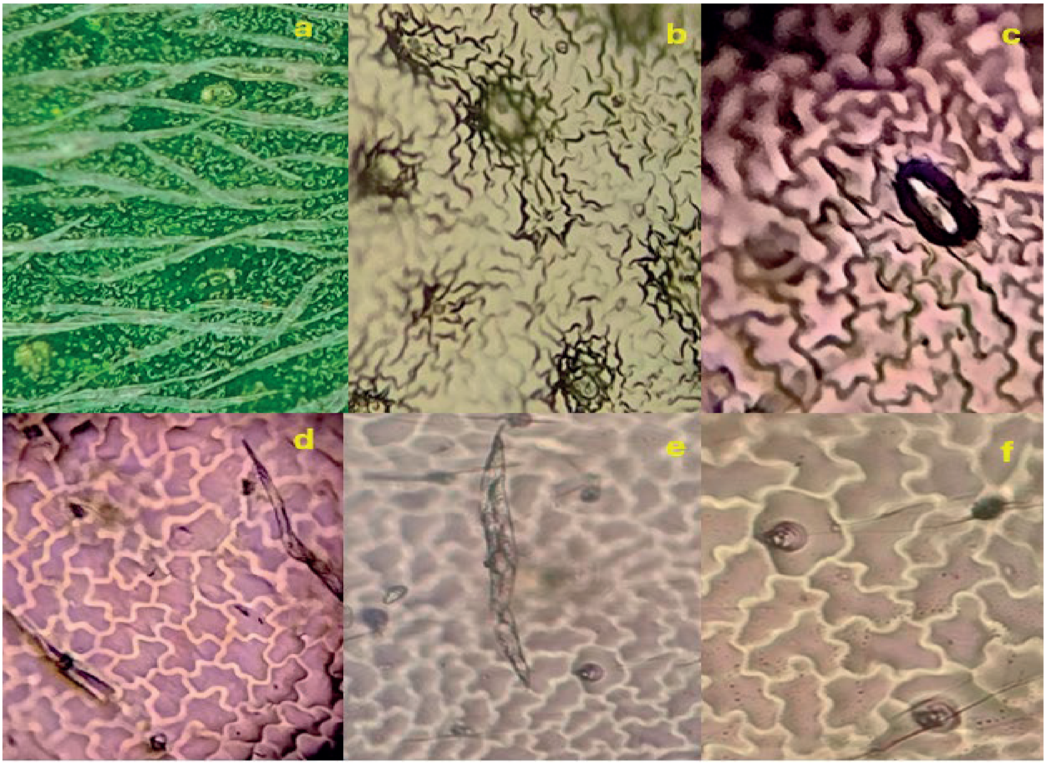


Figure 12. a, b, c, d, e, f -a-Upper epidermis with T-shaped trichomes and glandular trichomes - overall view, b - upper epidermis with glandular trichomes - overall view, c - upper epidermis with glandular trichomes - detail, d, e, f - upper epidermis with T-shaped and simple trichomes

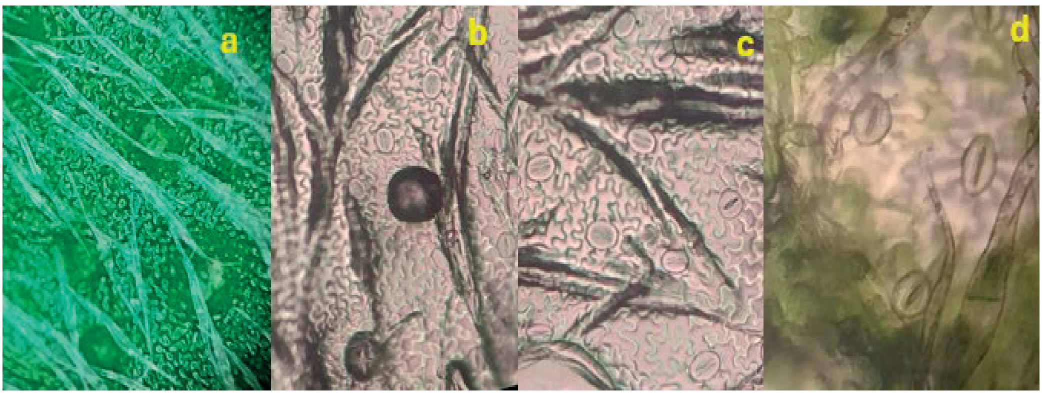


Figure 13. a, b, c, d - Lower epidermis with simple trichomes, T - shaped trichomes, and glandular trichomes, b - lower epidermis with stomata, T - shaped trichomes, simple trichomes, and glandular trichomes, c, d - lower epidermis with stomata, T - shaped trichomes, and simple trichomes

The upper and lower epidermis are both monolayered, with non-glandular simple, pointed or T-shaped trichomes, as well as large, bicapitate, prominent glandular trichomes. Stomata are found only on the abaxial epidermis.
Under the epidermis with T-shaped trichomes (Figure 14 a, b) there is the mesophyll differentiated into a palisadic and a lacunar tissue.
The mono-or bilayered palisadic tissue is positioned adaxially and abaxially, being made up of columnar cells rich in chloroplasts, small-sized lacunar tissue, and collateral conducting bundles in the area of the main and secondary ridges; the xylem is located toward the upper epidermis, with the phloem toward the lower epidermis (Figure 15 a, b, c, Figure 16 a, b, c).
There were many studies indicating that volatile substances of *Artemisia* species are produced and released through glandular trichomes (Zhou et al., 2020). Stem and leaf anatomy research for *Artemisia* species has also been carried out by Osmanlıoğlu et al., (2023) Toma & Rugină (1988).

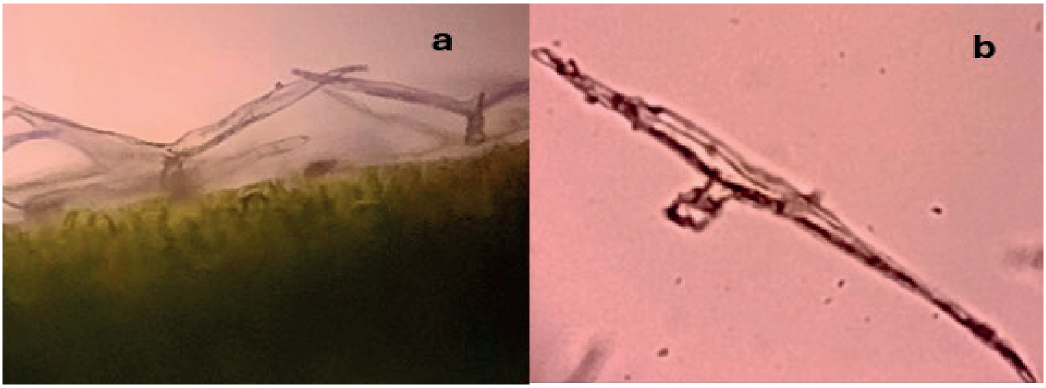


Figure 14. a, b - a - Transverse section in the lamina with epidermis containing T-shaped trichomes - overall view, b - detail of trichome hairs

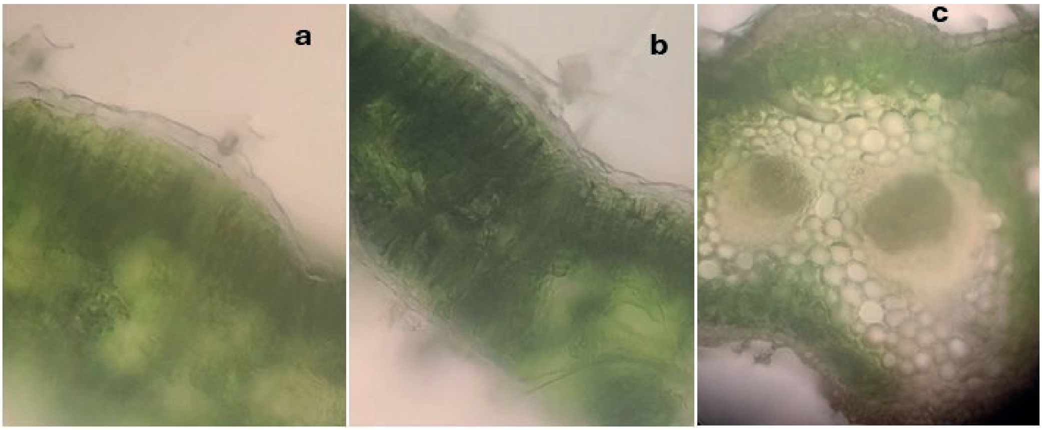
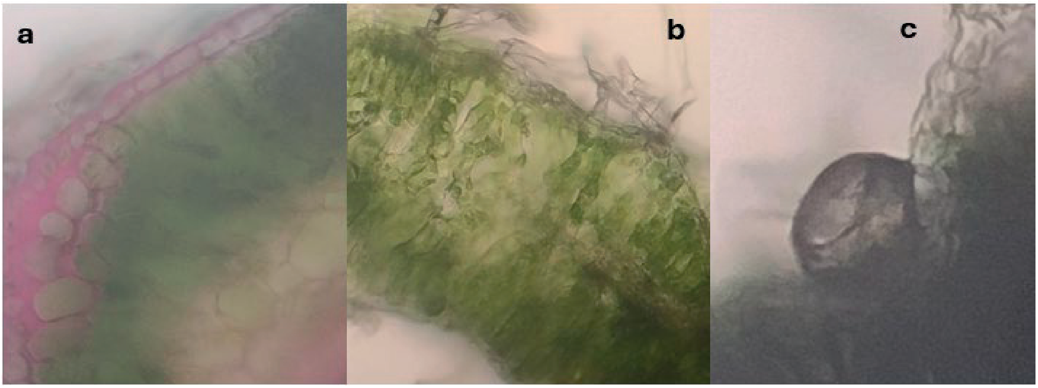


Figure 15. a, b, c - Transverse section in the lamina



Figurae 16. a, b, c - Transverse section in the lamina. a - epidermis, b - mesophyll with palisade tissue beneath both epidermis, c - epidermis with glandular trichomes

CONCLUSIONS

The *Artemisia absinthium* L. species presents a high therapeutic potential with beneficial effects for multiple conditions. The chemical compounds present in greater quantity in *Artemisia absinthium* L. species were: linalyl isovalerate, sabinene, myrcene, geranyl isobutyrate, β-cymene in stems, sabinene, myrcene, α-phellandrene, β-cymene in the leaves and sabinene, beta-pinene, linalol, chrysanthenone in the flowers.

The amount and composition of the essential oil, according to the results obtained, and the existing data in specialized writings, vary depending on the parts of the plant (stems, leaves, flowers), phenophase, ecotype, temperature and light.

The anatomy elements looked at in this paper are very important for plant systematics and taxonomy, facilitating a correct diagnosis of the species.

The species *Artemisia absinthium* L. (Asteraceae) has distinctive traits, such as the cuticle showing a sinuous structure and the presence of simple and T-shaped non-glandular trichomes, as well as large and bicapitate glandular trichomes.

Knowledge of anatomy elements is also important due to the accumulation and production of secondary metabolites in the glandular trichomes.

The *A. absinthium* stems and leaves are covered with dense T-shaped glandular and non-glandular trichomes, on both sides, the most vigorous being those on the stem and petioles.

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