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Comparative study of the micromorphological and anatomical characteristics of two *Artemisia* species

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SUMMARY

The genus *Artemisia* has attracted great interest from plant researchers because of the chemical composition and biological diversity of its species. The aim of this study was to investigate general anatomical features of vegetative organs of two species of the genus *Artemisia* (*A. vulgaris* and *A. absinthium*) that could be of taxonomic value and potentialy relevant to the production of secondary metabolites. In this study, transverse sections of leaves, stems and roots were prepared and microslides were observed under the light microscope. The main anatomical difference between these two species is reflected in the presence of trichomes. T-shaped non-glandular trichomes are present on both sides of the leaf epidermis, and on the stem surfaces in both species. However, a much lower glandular trichomes was observed in *A. vulgaris* than in *A. absinthium*, which may indicate a lower production of secondary metabolites, especially volatiles.
***Keywords*:** anatomy, *Artemisia vulgaris*, *Artemisia absinthium*, trichomes.

INTRODUCTION

The genus *Artemisia* L. belongs to the family *Asteraceae* and is characterized by a large number of herbs and shrubs (about 500) (Vallès and McArthur, 2000). Most *Artemisia* species are perennial, while annual and biennial species are rare (Vallès et al., 2003). Species of the

genus *Artemisia* are mainly distributed in the temperate zones of the northern hemisphere, while a limited number of taxa occur in the southern hemisphere (Oberprieler et al., 2009). They are found in Asia, Europe, North America, South Africa, and North Africa (Bremer, 1994; Torrel et al., 1999). Central Asia is considered to be the center of diversity of *Artemisia*, as it is characterized by the largest number of different species of this genus (McArthur and Plummer, 1978; Wang, 2004). The number of species occurring in Europe is estimated at 57 (Tutin et al., 1976), and the presence of 9 species of this genus has beed reported in the former Yugoslavia: *A. vulgaris, A. absinthium, A. annua, A. pontica, A. petrosa, A. lobelii, A. maritima, A. campestris,* and *A. scoparia* (Josifović, 1975). The association *Arctio-Artemisietum vulgaris* is also very common in ruderal habitats in the Balkans and is characterized by a rich floristic composition with 123 species (Stanković-Kalezić et al., 2009).

Many members of the genus *Artemisia* are ruderal and invasive weed species that also occur in agroecosystems where they can affect agricultural production. In addition, some species (e.g., *A. annua*, *A. absinthium*, *A. vulgaris*) are highly valued for their richness in secondary metabolites and have been used in traditional medicine in Europe and Asia since ancient times (Proksch, 1992). Many extracts obtained from *Artemisia* sp. are used to treat anxiety, epilepsy, depression, insomnia, irritability, psychoneuroses, and stress (Walter et al., 2003). Also, these species have a wide range of other biological activities such as antimalarial, antiseptic, antibacterial, anticancer, hepatoprotective, antirheumatic, etc. (Walter et al., 2003; Terra et al., 2007; Hussain et al., 2017). Another important role of secondary metabolites of *Artemisia* species is their allelopathic activity. The allelopathic activity of *A. vulgaris* (Barney et al., 2005; Pannacci et al., 2020), *A. absinthium* (Kapoor et al., 2019; Jiang et al., 2021), *A. frigida* (Wang et al., 2022), *A. scoparia* (Kaur et al., 2010), *A. selengensis* (Shi et al., 2021) and many other species has been confirmed. The allelopathic potential of the secondary metabolites synthesized by *Artemisia* species has led to increased interest in research on their morphological, anatomical, and phytochemical properties.

The genus *Artemisia* includes numerous species characterized by polymorphism. Phenotypic plasticity is the source of numerous morphological and anatomical variations that can promote adaptive mechanisms (Valladares et al., 2007). *Artemisia* species are widespread and occur in a variety of habitats, but most of them are native to arid regions (Bremer and Humphries, 1993; Polyakov, 1995). For this reason, many species have evolved adaptive mechanisms that often have xeromorphic traits (Metcalfe and Chalk, 1979). One of the most striking characteristics of xerophilous plants is dense pubescence that allows plants to adapt to dry conditions by limiting transpiration, rejecting strong radiation, or even promoting water uptake (Lyshede, 1979). In addition, trichomes may be involved in the control of water loss and temperature regulation (Johnson, 1975). Morphological variability in the genus *Artemisia* is a well-known phenomenon and occurs at different levels of organization (Torrell et al., 1999; Kreitschitz, 2003). The most variable feature at the species level of this genus is leaf morphology. The shape of leaves often vary along the stem of an individual, but there are also differences between individuals of the same species (Kreitschitz, 2003). Numerous researchers have also studied the anatomical characteristics (especially of the leaves) of *Artemisia* species

(Rabie et al., 2006; Noorbakhsh et al., 2008; El-Sahhar et al., 2011; Konowalik and Kreitschitz, 2012; Hussain et al., 2019; Janaćković et al., 2019; Liu et al., 2022). Characteristics of the leaf epidermis such as size and shape of epidermal cells, distribution and orientation of stomata, size of guard cells, number of subsidiary cells, type of glandular or non-glandular trichomes are important for species identification (Dickison, 2000). Micromorphological characters, including trichomes, are an important taxonomic feature for plant identification at the genus and species level (Hayat et al., 2009; Gavrilović et al., 2018). In addition to its importance in taxonomy, the presence of trichomes, especially glandular trichomes, is closely associated with the production of secondary metabolites, since trichomes are the major source and site of their synthesis (Slone and Kelsey, 1985). Many studies have reported that volatiles in *Artemisia* species are synthesized and released by glandular trichomes (Zhou et al., 2020). At the same time, it was found that the morphology and density of trichomes can be used to identify species within the genus (Yang et al., 2020). In the analysis of 24 different species of the genus *Artemisia*, eight different types of trichomes were found (Hayat et al., 2009). In another study, four types of glandular trichomes (capitate, peltate, pluricellular and thin neck trichomes) and six types of non-glandular trichomes (conical type, stinging hair trichomes, abuncate, unicellular calavate, unicellular filiform, unicellular tector) were described (Hussain et al., 2019). Knowledge of the anatomical characteristics of weed leaves is also important with regard to their control. Previous studies have shown that differences in leaf anatomical structure influence weed sensitivity and response to herbicide applications (Vranješ et al., 2017; Božić et al., 2019). Božić et al. (2020) also suggest that the high trichome density in tolerant compared to sensitive sunflower hybrids prevents herbicides from reaching the surface of the epidermis, resulting in their lower uptake.

The taxonomic position of plants is very important, but previous studies on the genus *Artemisia* indicate that the morphological similarity of the species causes problems in their identification (Hayat et al., 2009). In a recent classification, the genus *Artemisia* was divided into six large groups, considered at the section level: *Absinthium* DC., *Artemisia* L., *Dracunculus* Besser, *Pacifica*, *Seriphidium* Besser, and *Tridantatae* (Rydb.) McArthur (Hussain, 2020). The species *A. vulgaris* is classified in section *Artemisia* and *A. absinthium* in *Absinthium* (Nazar and Mahmood, 2011).

A. vulgaris and *A. absinthium* are broad-leaved perennial weeds that form rhizomes that contribute to their rapid vegetative reproduction thanks to their high regenerative capacity. *A. vulgaris* occurs in Serbia in ruderal areas, roadsides, waste areas, and tends to spread in crops as well. In North America, it has the status of an invasive weed species (Weston et al., 2005), and it is also considered as a cosmopolitan weed (Vallès and McArthur, 2001). The stem of *A. vulgaris* is straight, angular, woody over time, and generally grows to a height of 70 to 150 cm, but can also reach over 200 cm in some parts of the world. The herb forms a branched main root and many thinner, fibrous lateral roots that form a dense underground network that can penetrate to a depth of 7-18 cm in soil (Barney and DiTommaso, 2003; Weston et al., 2005). *A. vulgaris* is characterised by having 5-10 cm long pinatissect or bipinatissectas leaves, with lanceolate or oblong segments. The flower heads are numerous, 3-4 mm long, 2-3

mm wide, and arranged in racemes panicles. The flower receptacle is glabrous. The flowers in the centre are hermaphrodite, on the edge they are female. All flowers are yellowish or reddish brown. *A. absinthium* is a straight, branched herb, 60-120 cm tall, with woody and strongly branched rhizomes. The herb is covered with white trichomes that give it a silvery appearance. The leaves on the ground are stalked and three-lobed, while the leaves on the steam are short-stalked or stalkless, 1-2-pinnately lobed, oblong-lanceolate and pointed. The flower heads are 3-4 mm in diameter and form panicle inflorescences. The flower receptacle is hairy. The flowers are yellow and are all fertile. In the centre, the flowers are hermaphrodite, while on the edge of the head they are female. *A. absinthium* is clearly distinguished from *A. vulgaris* by the presence of silvery-silky hairs on both sides of the leaf (Alex, 1992). In addition to knowledge of morphological characteristics, the familiarity with anatomical features is also very important, not only for species identification, but also for a better understanding of the production and accumulation of secondary metabolites. Therefore, the aim of this study is to complete the previous knowledge of these two species (*A. vulgaris* and *A. absinthium*) by examining the anatomical characteristics of their vegetative organs.

MATERIAL AND METHODS

Plant material of *A. vulgaris* and *A. absinthium* was collected in June 2023 during the pre-flowering period at a ruderal habitat near Belgrade (N 44°43'58.72"; E 20°25'11.56"). Plant material included parts of the root, leaves in the middle of the stem and apical parts of the stem (under the inflorescence). Samples were sectioned fresh or kept in 50% ethanol until processing.

The method described by Ruzin (1999) was used to prepare permanent microslides. This paraffin method was applied for preparing cross-sections (8-10 μm thick) of roots, leaves and stems. The sections were double stained with Safranin O (1%, w/v, 50% ethanol) and Alcian blue (1% w/v, aqueous) and then mounted with Canada balsam. For temporary slides, handmade cross sections of fresh plant material were prepared using sharp razor blades, followed by staining in Toluidine blue (0,05%, w/v, aqueous) (O'Brien et al., 1964), or phloroglucinol-HCl to detect lignified cell walls (Jensen, 1962). Leaf epidermal prints were made according to the procedure described by Wolf (1950). Paraffin and hand sections were combined to obtain better slides for accurate observations and analyses. The permanent microslides are stored at the Department of agrobotany, University of Belgrade, Faculty of Agriculture. The prepared slides were observed with a Leica DM2000 light microscope equipped with a Leica DFC320 digital camera and Leica IM 1000 imaging software. Images of the surface of vegetative organs of *A. vulgaris* and *A. absinthium* and micromorphological features were observed by Nikon SMZ18 stereomicroscopes.

RESULTS AND DISCUSSION

Anatomical characteristics of the leaf. The differences between *A. vulgaris* and A. *absinthium* leaves were observed at both anatomical and morphological level (Figure 1 and 2). Common characteristics of the cross sections of the leaf of both species are an elongated linear shape and the epidermis is single-layered on both sides of the leaf. The epidermal cells have an irregular, polygonal shape with wavy walls (Figure 5a,c,d). In *A. vulgaris* leaves, the palisade tissue is located on the adaxial side and the sponge tissue on the abaxial side (Figure 3b), while in *A. absinthium* the palisade tissue is located under both adaxial and abaxial epidermis (Figure 4b,d). Abiri et al. (2018) found that the single-layer palisade tissue is present under both epidermal layers of *A. vulgaris,* which can probably be attributed to the influence of various environmental factors. In both species, vascular bundles in leaves are collateral, with the xylem oriented toward the adaxial surface and the phloem toward the abaxial surface, especially well developed within midrib (Figure 3a and 4a). Leaf epidermal prints are shown in figure 5. Due to the presence of a large number of T-shaped nonglandular trichomes on the abaxial side of the leaf of *A. vulgaris*, stomata and epidermal cells are difficult to notice by this method (Figure 5b). The stomatal type is anomocytic in both species and more numerous on abaxial side (Figure 5b, d). Hussain et al. (2019) observed four different types of stomata in the genus *Artemisia* (anomocytic, anomotetracytic, anisocytic and diacytic). Srilakshmi and Naidu (2014) also observed anomocytic type of stomata in *A. vulgaris*. T-shaped, non-glandular trichomes are present on the surface of both sides of the epidermis in both species, but less often on the

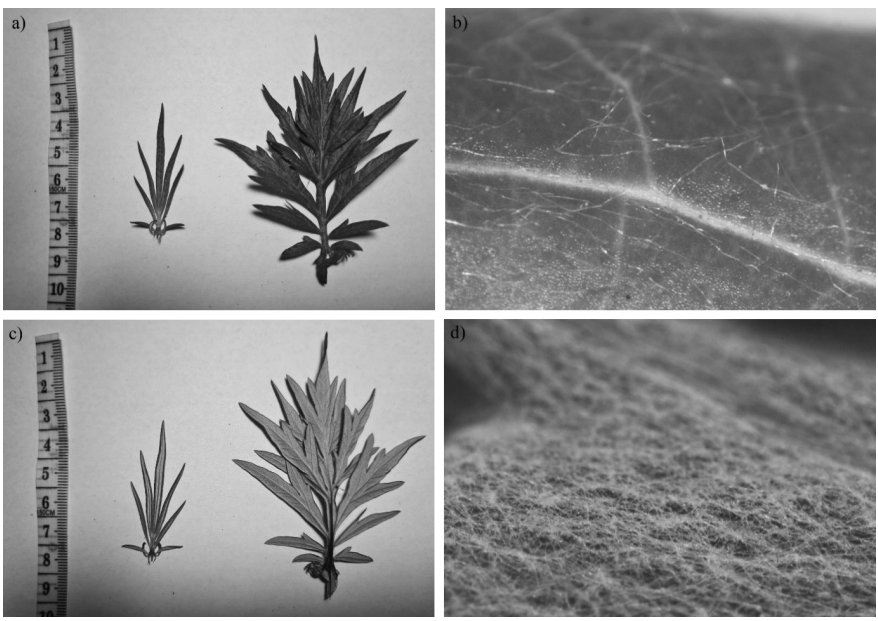

Figure 1. Leaves of *A. vulgaris*: adaxial side (a, b); abaxial side (c, d)
Slika 1. Listovi *A. vulgaris*: lice lista (a, b); naličje lista (c, d)

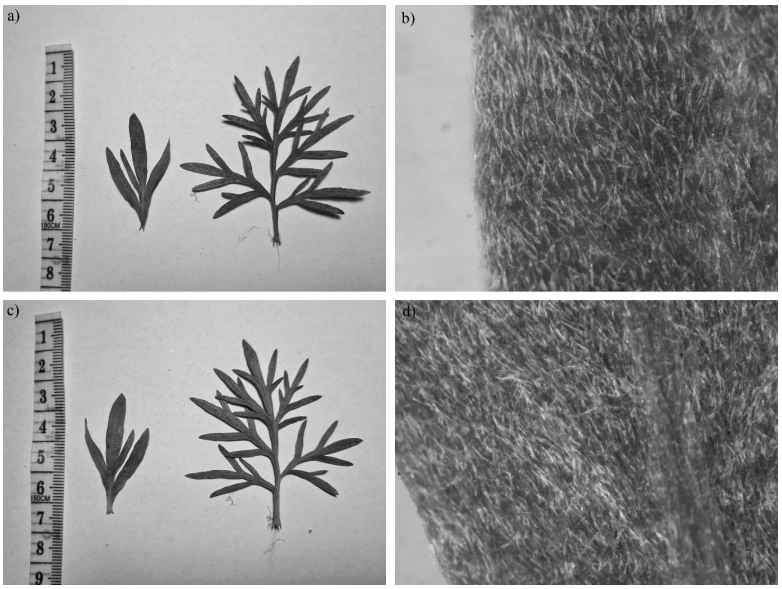

Figure 2. Leaves of *A. absinthium*: adaxial side (a, b); abaxial side (c, d)
Slika 2. Listovi *A. absinthium*: lice lista (a, b); naličje lista (c, d)

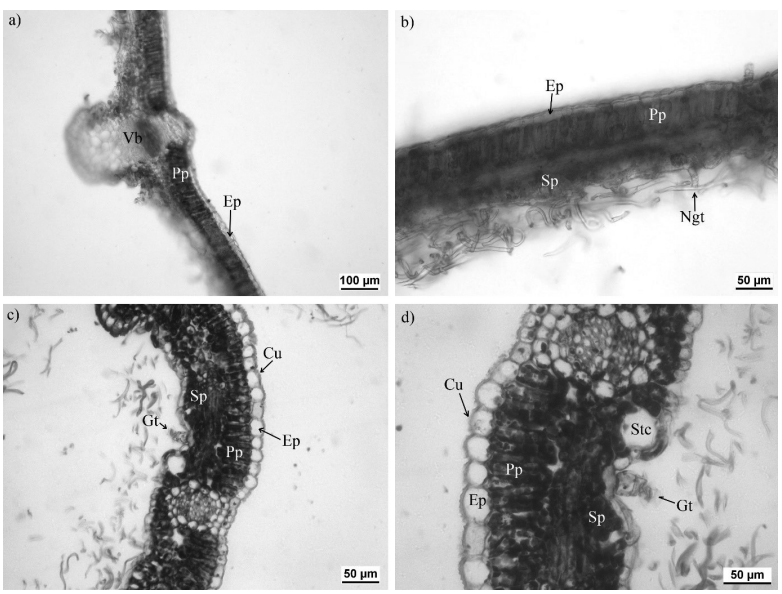

Figure 3. Leaf transverse section of *A. vulgaris*. Abbreviations: Cu = cuticule; Ep = epidermis; Gt = glandular trichome; Ngt = non-glandular trichome; Pp = palisade parenchyma; Sp = spongy parenchyma; Stc = stomatal crypt; Vb = vascular bundle
Slika 3. Poprečni presek lista *A. vulgaris*. Skraćenice: Cu = kutikula; Ep = epidermis; Gt = žlezdane dlake; Ngt = nežlezdane dlake; Pp = palisadni parenhim; Sp = sunđerasti parenhim; Stc = stomina šupljina; Vb = provodni snop

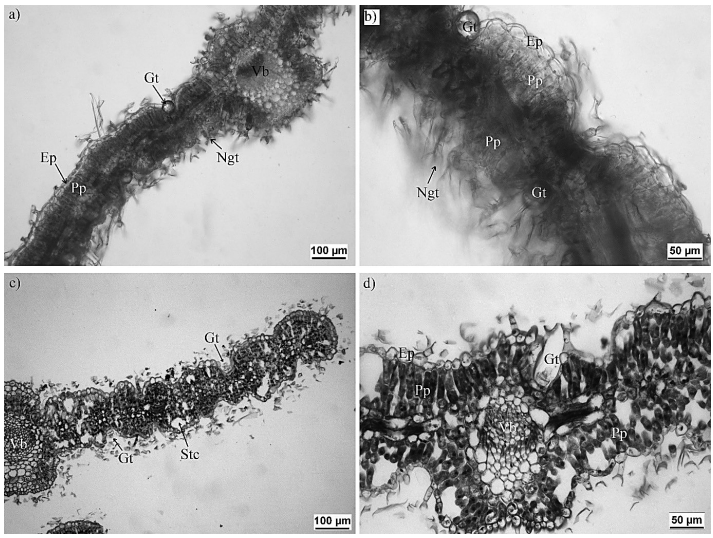

Figure 4. Leaf transverse section *A. absinthium*. Abbreviations: Cu = cuticule; Ep = epidermis; Gt = glandular trichome; Ngt = non-glandular trichome; Pp = palisade parenchyma; Sp = spongy parenchyma; Stc = stomatal crypt; Vb = vascular bundle

Slika 4. Poprečni presek lista *A. absinthium*. Skraćenice: Cu = kutikula; Ep = epidermis; Gt = žlezdane dlake; Ngt = nežlezdane dlake; Pp = palisadni parenhim; Sp = sunđerasti parenhim; Stc = stomina šupljina; Vb = provodni snop

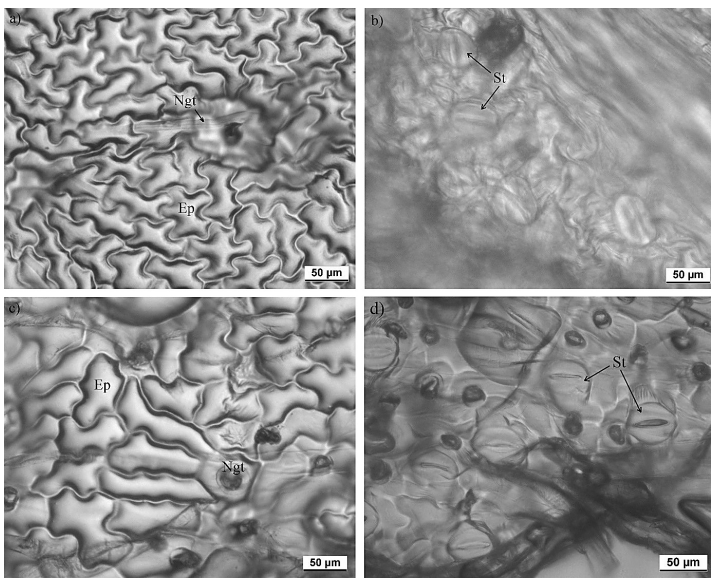

Figure 5. Leaf epidermal prints of the adaxial side of *A. vulgaris* (a) and *A. absinthium* (c) and abaxial side of *A. vulgaris* (b) and *A. absinthium (*d). Abbreviations: Ep = epidermis; Ngt = non-gladular trichomes; St = stoma

Slika 5. Epidermalni otisci lica lista *A. vulgaris* (a) i *A. absinthium* (c) i naličja lista *A. vulgaris* (b) i *A. absinthium* (d). Skraćenice: Ep = epidermis; Ngt = nežlezdane dlake; St= stoma

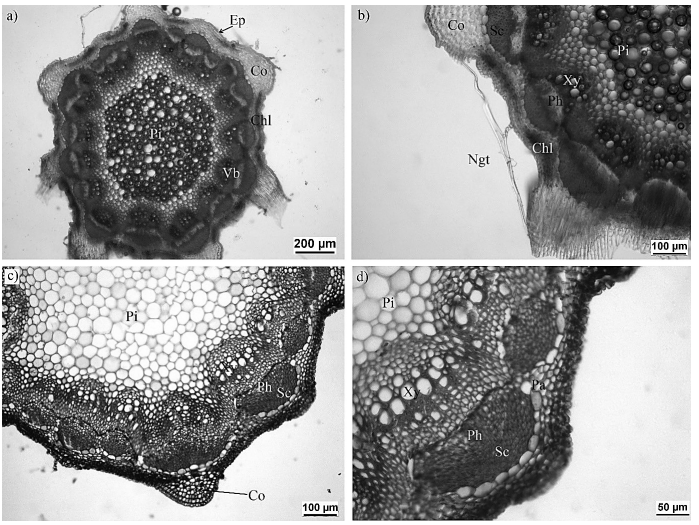

Figure 6. Transverse section of the stem of *A. vulgaris.* Abbreviations: Chl = chlorenchyma; Co = collenchyma; Ep = epidermis; Gt = glandular trichomes; Ngt = non-glandular trichomes; Pa = parenchyma; Ph = phloem; Pi = pith; Sc = sclerenchyma; Vb = vascular bundle; Xy = xylem

Slika 6. Poprečni presek stabla *A. vulgaris.* Skraćenice: Chl = hlorenhim; Co = kolenhim; Ep = epidermis; Gt = žlezdane dlake; Ngt = nežlezdane dlake; Pa = parenhim; Ph = floem; Pi = srž; Sc = sklerenhim; Vb = provodni snop; Xy = ksilem

upper epidermis in *A. vulgaris* (Figure 1b). On a temporary slide, glandular trichomes of the capitate type are found on the adaxial and abaxial sides of *A. absinthium* (Figure 4b, c), while Konowalik and Kreitschits (2012) suggested that glandular trichomes occurred sparsely on both leaf surfaces. Glandular trichomes are found in *A. vulgaris* only on the abaxial side, but they are much less numerous than in *A. absinthium.* (Figure 3c, d). Capitate glands and T-shaped non-glandular trichomes are the most common trichome type in *A. vulgaris* and *A. absinthium* (Hayat et al., 2009). Based on the results obtained from the observation of 5 *Artemisia* species (*A. annua, A. argyi, A. absinthium, A. leucophylla* and *A. lavandulaefolia*), Liu et al. (2022) claimed that microscopic characters such as differences in morphology and density of glandular and non-glandular trichomes may be crucial for distinguishing species within the *Artemisia* genus. In addition, Tang et al. (2014) demonstrated that the density of glandular trichome and the amount and type of volatile compounds synthesized were positively correlated in three species of the genus *Artemisia* (*A. annua*, *A. argyi*, and *A. hedinii*). The species *A. annua* had the highest density of glandular trichomes as well as the highest amount of volatile compounds, which then decreased proportionally in other species (*A. annua* > *A. argyi* > *A. hedinii*).

Anatomical characteristics of the stem. The stem surface is strongly ribbed and angular in both *Artemisia* species (Figure 6; Figure 7), especialy in the upper part of the plant. The same shape of the stem has been described by many authors (El-Sahhar et al., 2011; Konowalik

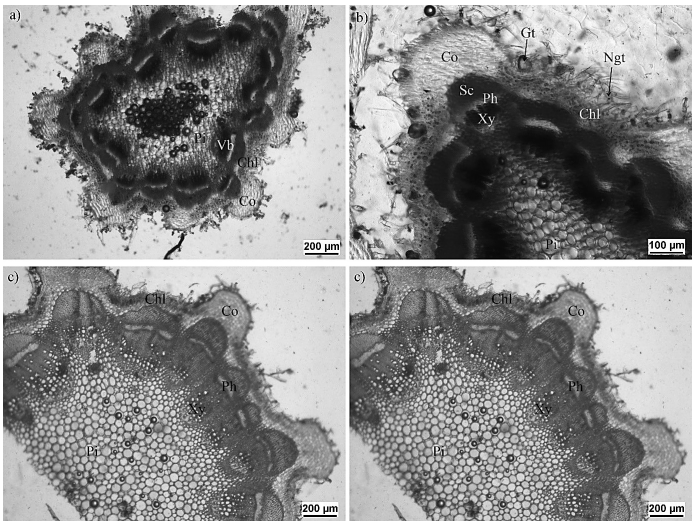

Figure 7. Transverse section of the stem of *A. absinthium*. Abbreviations: Chl = chlorenchyma; Co = collenchyma; Ep = epidermis; Gt = glandular trichomes; Ngt = non-glandular trichomes; Pa = parenchyma; Ph = phloem; Pi = pith; Sc = sclerenchyma; Vb = vascular bundle; Xy = xylem

Slika 7. Poprečni presek stabla *A. absinthium*. Skraćenice: Chl = hlorenhim; Co = kolenhim; Ep = epidermis; Gt = žlezdane dlake; Ngt = nežlezdane dlake; Pa = parenhim; Ph = floem; Pi = srž; Sc = sklerenhim; Vb = provodni snop; Xy = ksilem

and Kreitschitz, 2012; Abiri et al., 2018). Moreover, El-Sahhar et al. (2011) observed that the main stem has an almost cylindrical outline at its basal part. The same feature is observed in young stems with increasing age (Janaćković et al., 2019). The ribs on the stem are mostly composed of mechanical tissue, collenchyma (Figure 6c and 7c). Chlorenchyma is positioned between the ribs, under the epidermis (Figure 6b and 7b). The vascular bundles are arranged in a ring and the stele consists of about 20 collateral bundles in both species (Figure 6a and 7a). Well-lignified sclerenchyma tissue surrounds each vascular bundle and each bundle contains well-defined phloem and xylem (Figure 6d and 7d). The pith occupies the largest part of the cross section and consists of polygonal parenchyma cells with intercellular spaces (Figure 6c and 7c). In some studies, small secretory canals were found in the pith of *A. absinthium* (Konowalik and Kreitschitz, 2012; Janaćković et al., 2019), which can be considered as a specific characteristic of this species. A single layered epidermis occurs on the surface, which is covered with T-shaped non-glandular trichomes (Figure 6b). The main difference between *A.vulgaris* and *A. absinthium* is the presence of glandular trichomes, which are very abundant on the surface of *A. absinthium* (Figure 7b), whereas we observed none in A. *vulgaris*.

Anatomical characteristics of the root. *A. vulgaris* and *A. absinthium* are rhizomatous perennial weeds that also develop adventitious roots. The roots of these two species have very similar anatomical characteristics. The transverse sections of the root at the primary growth stage are shown in figure 8. The transverse section of the root is circular in outline (Figure 8a,

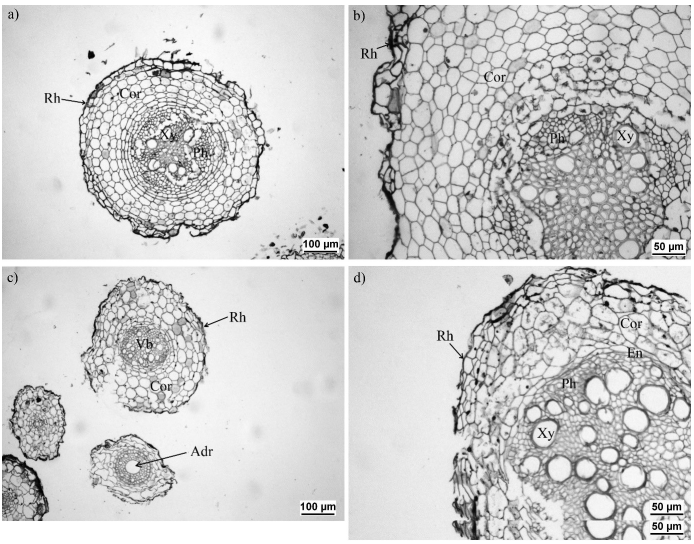

Figure 8. Transverse section of the root of *A. vulgaris* (a, b) and *A. absinthium* (c, d). Abbreviations: Adr = adventitous root; Cor = cortex; En = endodermis; Ph = phloem; Rh = rhizodermis; Xy= xylem
Slika 8. Poprečni presek korena *A. vulgaris* (a, b) i *A. absinthium* (c, d). Skraćenice: Adr = adventivni koren; Cor = korteks; En = endodermis; Ph = floem; Rh = rizodermis; Xy = ksilem

c). There is a single layer of rhizodermis on the surface. Under the rhizodermis is the cortex with several layers of polygonal parenchyma cells with small intercellular spaces (Figure 8b, d). The thickness of the cortex varies and depends on the age of the root. The innermost layer of cells of the cortex forms the endodermis surounding the vascular cylinder in the most central part of the root. The vascular cylinder is composed of xylem and phloem, arranged in one single radial vascular bundle. The root cortex of many *Artemisia* species contains parenchyma secretory canals, but in our study those canals were probably not noticed. The rhizome of *A. vulgaris* has a similar anatomical structure to the adventitous root, but the rhizome cortex is composed of more layers of parenchyma cells (10-12) and the pith consistutes about 70% of the whole transverse section area (El-Sahhar et al., 2011).

CONCLUSION

The most noticeable differences in the anatomical structure of *A. vulgaris* and *A. absinthium* were found at the leaf level. The mesophyll of *A. vulgaris* consists of spongy and palisade parenchyma, whereas palisade tissue was observed under both epidermal layers of *A. absinthium*. The main anatomical difference between these two species is reflected in the presence of trichomes. A large number of non-glandular trichomes were observed on both sides of the leaf of *A. absinthium*, whereas in *A. vulgaris* non-glandular trichomes were much less common on the adaxial side. *A. absinthium* is characterized by numerous glandular

trichomes on both sides of the leaf as well as on the stem, while in *A. vulgaris* much fewer glandular trichomes were observed only on the abaxial side of the leaf. The differences in the anatomical structure of the stem and roots are less pronounced.

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Uporedna analiza mikromorfoloških i anatomskih karakteristika dve vrste iz roda *Artemisia*

REZIME

Rod *Artemisia* privlači veliku pažnju istraživača zbog hemijskog sastava i biološke raznovrsnosti vrsta koje obuhvata. U ovom radu proučavali smo vegetativne organe dve vrste roda *Artemisia* (*A. vulgaris* i *A. absinthium*) sa ciljem da se ispitaju anatomske karakteristike koje bi mogle da imaju taksonomski značaj i koje mogu biti povezane sa proizvodnjom sekundarnih metabolita. Ova studija je obuhvatala pripremu poprečnih preseka listova, stabla i korena, kao i njihovo posmatranje pod svetlosnim mikroskopom. Glavna anatomska razlika između posmatranih vrsta ogleda se u prisustvu dlaka. Nežlezdane dlake u obliku slova T primećene su na površini stabla i u epidermisu lica i naličja lista kod obe vrste. Žlezdane dlake su prisutne u mnogo većem broju kod vrste *A. absinthium* u odnosu na vrstu *A. vulgaris*, što se može dovesti u vezu sa većom proizvodnjom sekundarnih metabolita, naročito isparljivih jedinjenja.

***Ključne reči*:** anatomija, *Artemisia vulgaris*, *Artemisia absinthium*, dlake.