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Anatomical structure and saponin content in roots of *Acanthophyllum albidum* in Uzbekistan

Ergashali Ruzimatov[1]*, Khusanboy Yuldashev[2], Tolibjon Madumarov[1], Nilufar Ibragimova[1], Mamura Sharopova[1]

[1] Andijan State University, Department of Ecology and Sustainable Development, University Str. 129, Andijan, Uzbekistan
[2] Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan, Molecular Phylogeny and Biogeography, Durmon Yuli Str. 32, Tashkent, Uzbekistan
*Corresponding author. E-mail: ruzmatovergashali@gmail.com

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Abstract

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Plant root anatomy and saponin accumulation may vary with plant age and environmental conditions. This study investigated age-related and population-level variation in the root anatomy and saponin content of *Acanthophyllum albidum* Schischk. (Caryophyllaceae), a Central Asian endemic species. We collected root samples from six localities in Uzbekistan, representing contrasting foothill and mountain habitats. Anatomical characteristics were examined using light microscopy, while saponin content was assessed gravimetrically in two- and four-year-old plants. We used thin-layer chromatography to compare the chromatographic profiles of root extracts. Anatomical observations revealed age-related differentiation of secondary vascular tissues, development of internal vascular bundles and differences in the relative proportions of conducting and mechanical elements among populations. Mean saponin content across the six localities increased from 54.17 ± 6.52 mg/g in two-year-old plants to 113.87 ± 15.70 mg/g in four-year-old plants. A paired comparison of locality-level values indicated a significant difference between the age groups (Wilcoxon signed-rank test, $W = 0$, $p = 0.031$). The lowest saponin content was recorded in Khurjun and the highest in Mindon. Thin-layer chromatography revealed a common Rf value of 0.44 across the investigated samples. However, the available data did not establish a statistically supported quantitative relationship between individual anatomical traits and saponin content. The findings highlight the importance of developmental stage and population origin in evaluating root anatomical variation and estimated saponin content in *Acanthophyllum albidum*, while indicating the need for further compound-specific phytochemical analyses.

Keywords: Caryophyllaceae, Fergana Valley, population variability, root anatomy, saponin content, secondary xylem.

INTRODUCTION

The genus *Acanthophyllum* C.A. Mey. (Caryophyllaceae) comprises 82 currently accepted species distributed predominantly across the Irano–Turanian floristic region (POWO, 2026). Central Asia represents an important region of species diversity for the genus, with 31 species reported from the region

(Li & Tojibaev, 2024). The geographical distribution of individual Central Asian species, including *Acanthophyllum gypsophiloides* Regel, has also been investigated (Jorayev et al., 2025). The major centres of diversity include northeastern Iran, southern Turkmenistan and western Afghanistan, where considerable species richness and endemism have been documented (Mahmoudi Shamsabad et al., 2012, 2019). Molecular phylogenetic investigations have revealed complex evolutionary relationships within *Acanthophyllum* and contributed to revisions of its generic boundaries and infrageneric classification (Pirani et al., 2014, 2020). Pirani & Rabeler (2017) have addressed additional nomenclatural aspects of the genus.

Beyond their taxonomic and ecological significance, *Acanthophyllum* species are important sources of bioactive secondary metabolites, particularly triterpenoid saponins (Ruzimatov et al., 2026), which have surface-active properties and potential medicinal and industrial applications (Mamadalieva et al., 2024; Rezazadeh et al., 2026). Recent studies have revealed considerable phytochemical variation among species and ecotypes. Balali et al. (2026), investigating ten *Acanthophyllum* species and 68 ecotypes, have demonstrated substantial differences in root saponin content, indicating that phytochemical characteristics vary both among species and within species. Furthermore, Mursaliyeva et al. (2026) have investigated relationships between root growth characteristics, developmental stages and saponin accumulation in wild populations of *Acanthophyllum pungens* (Bunge) Boiss., showing that saponin accumulation patterns vary during plant development. Together, these studies highlight the importance of considering both biological variation and developmental processes when evaluating saponin-producing plants.

Among the Central Asian representatives of the genus, *Acanthophyllum albidum* is of particular interest because of its restricted geographical distribution and conservation significance. The species is native to Uzbekistan and Tajikistan and is therefore endemic to Central Asia (POWO, 2026). In Uzbekistan, *Acanthophyllum albidum* is included in the national Red Data Book (Khassanov, 2019). The species occurs in foothill and mountainous habitats of the Fergana Valley and adjoining mountain areas of Uzbekistan, where populations experience contrasting environmental conditions. Habitat loss and fragmentation are identified as conservation concerns in the region. These characteristics make *Acanthophyllum albidum* an important subject for investigating population-level anatomical and phytochemical variation and for better understanding its ecological differentiation.

Although previous studies have advanced our understanding of *Acanthophyllum* taxonomy, root morphology and phytochemical diversity, important questions remain concerning the relationships between internal root anatomy and saponin accumulation. For example, Balali et al. (2026) have demonstrated phytochemical variation among species and ecotypes, whereas Mursaliyeva et al. (2026) have examined changes in root morphometry and saponin content across different developmental stages. However, these investigations did not specifically address how the differentiation of secondary vascular tissues, including xylem, mechanical tissues and parenchyma, relates to variation in root saponin content among geographically separated populations of *Acanthophyllum albidum*. Comparative anatomical observations combined with phytochemical extraction data may therefore provide additional insight into the relationships between root development, population differentiation and saponin accumulation.

To address this research gap, the present study aimed to investigate the anatomical characteristics of *Acanthophyllum albidum* roots from different populations in Uzbekistan and to evaluate their variation in relation to plant age, ecological conditions and saponin content. We hypothesised that differences in root anatomical structure, particularly in the development of conducting, mechanical and parenchymatous tissues, may be associated with variation in saponin content among populations. The study addressed three research questions: (a) How does the anatomical structure of *Acanthophyllum albidum* roots change with plant age, and what differences occur among populations inhabiting contrasting ecological environments? (b) How does root saponin content vary between two- and four-year-old plants and among the investigated populations? (c) What comparative patterns can be identified between root anatomical differentiation and saponin content across the investigated populations?

MATERIALS AND METHODS

Study species and study sites

We studied *Acanthophyllum albidum* Schischk. (Caryophyllaceae), a perennial plant species occurring in Uzbekistan. We conducted field investigations and collected plant material at six geographically separated localities representing contrasting foothill and mountain environments in Uzbekistan (Table 1). The investigated localities included Chust and Pop in Namangan Region, Chimyon and Mindon in Fergana Region, Sulukota Mountain in the Kurama Range of the Western Tien Shan, and Khurjun Mountain in the Alay mountain system. Chust, Pop, Chimyon and Mindon represented foothill habitats, whereas Sulukota and Khurjun represented mountain habitats (Table 1). The Sulukota sampling sites were situated at elevations of approximately 1200–1600 m above sea level.

For anatomical comparisons, we grouped plants from Chust and Pop into a dry foothill ecoform based on their occurrence in arid continental foothill habitats and their shared morphological and anatomical characteristics. This group was characterised by comparatively short and narrow juvenile leaves and delayed segmentation of the secondary root xylem, which occurred at approximately five to six years of age. For extraction analysis, we treated Chust and Pop as separate sampling localities.

Plant sampling and material preparation

We collected plant material from the investigated populations during field expeditions. According to the original sampling protocol, 5–8 plants were sampled from each investigated plant group, and 6–8 specimens were collected from different plant organs.

Root material intended for anatomical examination was fixed in 70% ethanol. Before sectioning, we softened the preserved roots in a 20% aqueous glycerol solution. Depending on root thickness, the material was heated in the solution and subsequently maintained at approximately 80°C for 1–3 days to facilitate section preparation. We prepared transverse, radial, longitudinal, and tangential longitudinal sections using conventional anatomical techniques. We stained temporary microscopic preparations with safranin and methylene blue to distinguish root tissue components.

Microscopic examination of root anatomy

We performed anatomical observations using a Carl Zeiss light microscope. Measurements of anatomical structures were obtained using an MOV-1.5× ocular micrometre. Anatomical illustrations were prepared using a PA-6 drawing apparatus.

We examined root anatomical structure at different developmental stages, with particular attention to the organisation of primary and secondary xylem, phloem differentiation, development of mechanical tissues, formation of internal vascular bundles, and changes in the distribution of parenchymatous elements. Comparative observations were conducted to characterise differences in root anatomical organisation among the investigated populations and plant age groups.

Gravimetric estimation of root saponin content

Air-dried root samples from two- and four-year-old *Acanthophyllum albidum* plants were used to gravimetrically estimate root saponin content by methanolic extraction. For each sample, 25 g of air-dried root material was placed in a 250 mL round-bottom flask containing 150 mL of methanol. The flask was equipped with a reflux condenser, and the

Table 1. Geographical characteristics of the investigated localities of *Acanthophyllum albidum* in Uzbekistan

Study site	Administrative affiliation	Longitude (°E)	Latitude (°N)	Altitude (m a.s.l.)	Habitat
Chust	Chust District, Namangan Region	71.22642	41.00240	855–939	Dry foothills
Pop	Pop District, Namangan Region	71.09721	40.87511	400–1200	Dry foothills
Chimyon	Fergana District, Fergana Region	71.52136	40.25589	400–1200	Foothills
Mindon	Fergana District, Fergana Region	71.55306	40.22680	400–1200	Foothills
Sulukota	Pop District, Namangan Region	70.55018	41.04584	1200–1600	Mountains
Khurjun	Fergana Region, Alay Mountain area	71.81195	39.98517	2200–2400	Mountains

mixture was heated in a water bath for 2 h. The liquid extract was separated by decantation, and the remaining plant material was extracted again with fresh methanol for another 2 h under the same conditions. The two extracts were combined and transferred into a previously weighed flask. Methanol was removed under reduced pressure, and the remaining dry residue was determined gravimetrically using an analytical balance. Saponin content was estimated gravimetrically using the following equation: saponin content (mg/g) = [mass of dry extract (g) / initial mass of air-dried root material (g)] × 1000. We also expressed the resulting values as percentages of the initial dry root mass for comparison with the original experimental data. The gravimetric measurements represent the total dry extract residue rather than the separately quantified mass of individual saponin compounds. We further examined the extracts by thin-layer chromatography, as described below.

Thin-layer chromatography

Thin-layer chromatography (TLC) was performed to examine the chromatographic characteristics of the obtained dry root extracts. For each sample, 0.013 g of dry extract was dissolved in 0.1 mL of distilled water, and equal aliquots of the prepared solutions were applied to the starting line of silica gel TLC plates. Chromatographic separation was performed in a closed chamber using a chloroform–methanol–water solvent system (65:35:8, v/v/v).

After chromatographic development, the plates were removed from the chamber and dried. We then treated the chromatograms with a 20% phosphotungstic acid solution and heated them at 120°C for 10–15 min to visualise the separated compounds. Retention factors (Rf) were calculated as the ratio of the distance travelled by each chromatographic spot to the distance travelled by the solvent front. We compared chromatographic patterns and Rf values among root extracts from the investigated populations.

Data analysis

We compared root anatomical characteristics descriptively among the investigated populations and developmental stages. We obtained anatomical measurements using an ocular micrometre. Root saponin content was estimated gravimetrically from the dry residue obtained after methanolic extraction and expressed as milligrams per gram of air-dried root material (mg/g). Mean values and standard deviations (SD) were calculated across the six sampling localities for each age group. We evaluated differences in estimated saponin content between two- and four-year-old plants using a two-sided exact Wilcoxon signed-rank test, treating the six localities as paired observations (n = 6). We performed statistical calculations using Python and SciPy 1.17.0. Because individual plant-level measurements were unavailable, we restricted statistical comparisons to locality-level saponin content values. We descriptively evaluated relationships between root anatomical characteristics and saponin content.

RESULTS

Age-related and population-level variation in root anatomy

The anatomical structure of *Acanthophyllum albidum* roots showed age-dependent changes and differences among the investigated populations in Uzbekistan. In young roots, the primary xylem generally exhibited a diarchal arrangement. In contrast, subsequent development involved the differentiation of secondary vascular tissues and changes in the distribution of conducting and mechanical elements.

In the dry-foothill ecoform (Chust and Pop), two-year-old roots were characterised by well-developed phloem and predominantly non-lignified libriform in the secondary xylem (Fig. 1a, b).

By the fourth year, the conducting elements accounted for approximately 60–70% of the xylem cross-sectional area (Fig. 1, Fig. 2).

Further development was accompanied by the formation of internal vascular bundles and increasing vascular complexity, as observed in six- and seven-year-old roots (Fig. 3).

In the Sulukota population, secondary xylem differentiation was already evident in two-year-old roots. During subsequent development, the xylem became divided into several groups, while the primary diarchal xylem gradually underwent structural changes. In contrast, the Mindon population exhibited a comparatively high proportion of libriform in

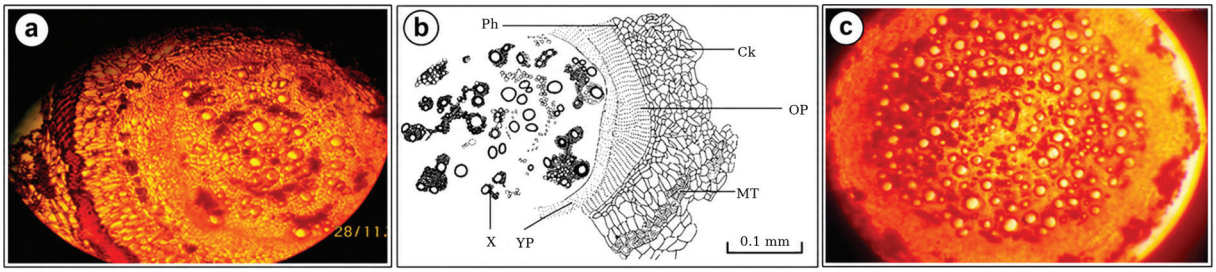


Fig. 1. Anatomical structure of *Acanthophyllum albidum* roots from the dry foothill ecoform (Chust and Pop); (a) transverse section of a two-year-old root (photomicrograph); (b) schematic representation of a two-year-old root; (c) transverse section of a four-year-old root (photomicrograph). Abbreviations: Ph – phloem; Ck – cork; OP – old phloem; YP – young phloem; MT – mechanical tissue; X – xylem. Scale bar in (b) = 0.1 mm.

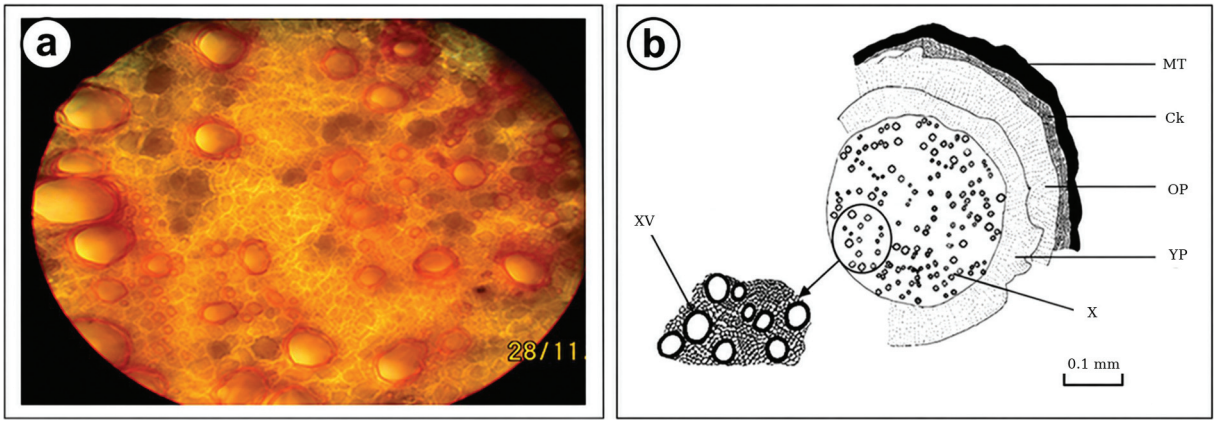


Fig. 2. Anatomical structure of a four-year-old *Acanthophyllum albidum* root from the dry-foothill ecoform (Chust and Pop); (a) transverse section (photomicrograph); (b) schematic representation of the root structure with an enlarged detail of xylem vessels. Abbreviations: MT – mechanical tissue; Ck – cork; OP – old phloem; YP – young phloem; X – xylem; XV – xylem vessels. Scale bar in (b) = 0.1 mm.

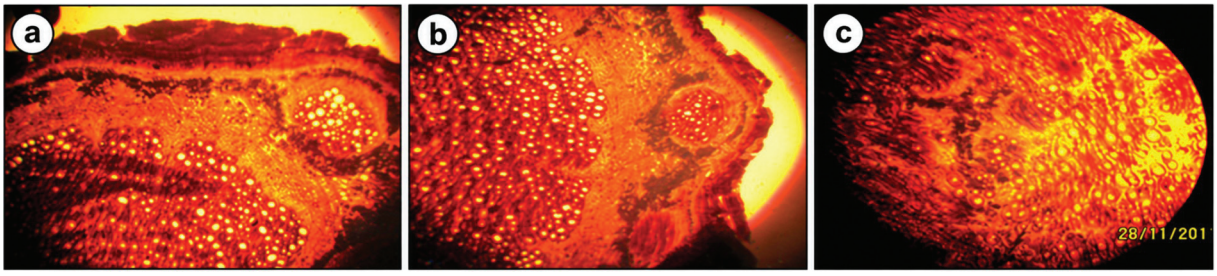


Fig. 3. Anatomical structure of *Acanthophyllum albidum* roots from the dry foothill ecoform (Chust and Pop) at later developmental stages; a – root transverse section (photomicrograph); b – six-year-old root; c – seven-year-old root.

young roots, with conducting elements occupying approximately 20–25% of the xylem area in the second year and about 30% in the fourth year.

In the Chimyon population, first-year roots retained the epidermis and cortical parenchyma, with two to three rows of phloem developing beneath the mechanical tissues. In two-year-old roots, the phloem increased from five to eight cell layers, occasionally reaching ten, and occupied about half of the root cross-sectional area. The xylem was predominantly represented by libriform cells, while occasional concentric vascular bundles were observed. By the end of the second growing season and the beginning of the third, further differentiation of the peripheral tissues became evident.

The Khurjun population was distinguished by

the relatively early development of internal vascular bundles, which appeared by the third year. Their number increased with plant age, indicating progressive reorganisation of the root vascular system.

Overall, these observations demonstrate that root anatomical differentiation in *A. albidum* depends on plant age and varies among populations, particularly in the timing of internal vascular bundle formation and the relative development of conducting and mechanical tissues.

Variation in root saponin content among populations and age groups

Root saponin content varied with plant age and sampling locality in *Acanthophyllum albidum* (Table 2). In all six investigated localities, four-year-old plants exhibited higher saponin content than two-year-old plants. Across localities, mean saponin content increased from 54.17 ± 6.52 mg/g in two-year-old plants to 113.87 ± 15.70 mg/g in four-year-old plants. A paired comparison of locality-level values using the exact Wilcoxon signed-rank test indicated a difference between the two age groups ($W = 0$, $p = 0.031$). The reported standard deviations reflect variation among sampling localities rather than among individual plants within each locality.

Geographical differences were also observed. The mountain populations of Khurjun and Sulukota exhibited lower saponin content than the foothill populations of Pop, Chust, Chimyon and Mindon. The highest saponin content was recorded in four-year-old plants from Mindon (131.6 mg/g), whereas the lowest was observed in two-year-old plants from Khurjun (42.0 mg/g). Thin-layer chromatography of root extracts from all six investigated localities revealed a common *Rf* value of 0.44. The chromatographic observations showed similar migration characteristics of the detected spots under the experimental conditions used.

Comparative patterns of root anatomical characteristics and saponin content

Comparative analysis revealed differences in root anatomical structure and saponin content among the investigated populations of *Acanthophyllum albidum*. Anatomical differences included differentiation of secondary vascular tissues, the formation of internal vascular bundles, and the relative development of conducting and mechanical elements. The Khurjun population, characterised by the relatively early formation of internal vascular bundles, exhibited the lowest saponin content in both age groups (42.0 and 85.2 mg/g in two- and four-year-old plants, respectively). In contrast, the Mindon population showed the highest saponin content (60.0 and 131.6 mg/g, respectively), accompanied by a different pattern of vascular tissue differentiation. The remaining populations showed intermediate values. These observations indicate population-level differences in anatomical development and saponin content. However, the present analysis did not establish a direct quantitative relationship between individual anatomical traits and saponin accumulation.

DISCUSSION

This study revealed age-related and population-level differences in the root anatomical structure and root saponin content of *Acanthophyllum albidum* in Uzbekistan. The observed anatomical changes included the differentiation of secondary vascular tissues, progressive development of internal vascular bundles and variation in the relative proportions of conducting and mechanical elements. These findings

Table 2. Root saponin content in *Acanthophyllum albidum* according to plant age and sampling locality (mg/g of air-dried root material)

Population	Habitat	Two-year-old plants (mg/g)	Four-year-old plants (mg/g)	*Rf*
Khurjun	Mountain	42.0	85.2	0.44
Sulukota	Mountain	52.0	109.6	0.44
Pop	Lower hill	57.0	118.4	0.44
Chust	Lower hill	56.0	118.8	0.44
Chimyon	Lower hill	58.0	119.6	0.44
Mindon	Lower hill	60.0	131.6	0.44

indicate that root structural development is not uniform among populations, although the relative contributions of plant age, environmental conditions and genetic differences remain unresolved. The developmental differences observed in *Acanthophyllum albidum* are particularly relevant to recent investigations of root growth and secondary metabolite accumulation in saponin-producing Caryophyllaceae. Mursaliyeva et al. (2026) have examined root morphometric characteristics and total saponin content in *Acanthophyllum pungens* and two *Gypsophila* species from southeastern Kazakhstan. Their findings demonstrated species-dependent relationships between root growth characteristics, developmental stages and saponin accumulation. In *Acanthophyllum pungens*, relatively high saponin accumulation coincided with increased root growth parameters toward the end of the growing season. Although our study focused on anatomical differentiation rather than seasonal root morphometry, both investigations emphasise the importance of considering plant development when evaluating the potential phytochemical productivity of underground organs.

In the present study, root saponin content was consistently higher in four-year-old plants than in two-year-old plants across all six sampling localities. The locality-level statistical comparison supports an age-associated difference in saponin content. However, the available data do not allow assessment of variation among individual plants or establishment of the independent effects of age and environmental conditions. Furthermore, the investigated age range does not indicate whether saponin content continues to increase during later developmental stages. Previous studies of saponin-bearing Caryophyllaceae have demonstrated that phytochemical accumulation may vary with ontogenetic stage (Mursaliyeva et al., 2026). Therefore, further investigations involving replicated samples and additional age groups are required. Population-level variation in saponin content is consistent with the broader phytochemical diversity reported within *Acanthophyllum*. Balali et al. (2026) have investigated ten Iranian *Acanthophyllum* species represented by 68 ecotypes and demonstrated substantial differences in phytochemical characteristics, including root saponin content. Their results indicate that phytochemical variation occurs both among species and among ecotypes, supporting the importance of examining geographically distinct populations. In our study, the comparatively lower saponin content observed in the mountain populations of Khurjun and Sulukota, relative to the lower-hill populations, suggests that growing conditions may contribute to the observed variability. Nevertheless, because environmental variables were not experimentally isolated, the differences cannot be attributed directly to altitude, temperature or growing-season duration.

The relationship between root anatomical organisation and phytochemical productivity warrants particular attention. Anatomical differentiation may affect the relative abundance of tissue types involved in transport, mechanical support and storage. However, the present observations do not establish whether specific anatomical characteristics directly influence saponin content. Recent experimental investigations have shown that *Acanthophyllum* root tissues can produce specialised metabolites under controlled culture conditions. For example, Arabjafari et al. (2026) have established hairy-root cultures of *Acanthophyllum korshinskyi*, *Acanthophyllum glandulosum* and *Acanthophyllum squarrosum* as a platform for investigating and improving saponin production. Such approaches could complement field-based anatomical studies by helping to distinguish developmental effects from environmental influences.

The phytochemical findings of the present study are based on the extraction and chromatographic examination of saponin-containing root material from *Acanthophyllum albidum*. In the original investigation, saponin content was assessed gravimetrically by determining the dry residue obtained after methanolic extraction, followed by thin-layer chromatography. The results revealed differences in gravimetrically estimated saponin content among populations and developmental stages. All six sampling localities showed a common Rf value of 0.44, indicating similar chromatographic migration characteristics under the applied experimental conditions. These findings provide comparative information on root saponin content, although individual saponin compounds were not quantified separately. More detailed chemical characterisation using advanced analytical techniques, such as LC–ESI–MS (Rezazadeh et al., 2026), would further clarify the composition of these fractions. The findings highlight the importance of considering plant developmental stage and popula-

tion origin when evaluating root anatomical characteristics and saponin content in *Acanthophyllum albidum*. The comparative observations suggested an association between root anatomical differentiation and saponin accumulation, although the strength of this relationship was not established statistically. Further investigations combining replicated quantitative anatomical measurements with compound-specific saponin analysis are needed to clarify this relationship.

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ER https://orcid.org/0009-0004-8713-3873
KY https://orcid.org/0009-0002-8249-0228
TM https://orcid.org/0009-0006-7342-8480

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