Cryo-SEM and Raman spectroscopy reveals an integrated secretory network of resin in *Juniperus phoenicea* L. leaves and cones

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Abstract: Resin secretory structures in conifers remain poorly characterised. Cryo-scanning electron microscopy (cryo-SEM) and Raman microscopy were applied to study native resin duct architecture and chemical composition in *Juniperus phoenicea* and provide a detailed structural description of the species secretory system. Samples of *J. phoenicea* leaves, cones and leaves collected from the Morrocan coastline were processed using both techniques. Multiple anatomical regions were analysed to ensure representative chemical profiling. Cryo-SEM analysis revealed that the species possesses a multi-scale integrated resin secretory network rather than isolated glandular structures previously described for Cupressaceae. The system exhibits hierarchical organization with large primary resin ducts (120—150 µm diameter) connected to numerous smaller secondary droplets (2—50 µm diameter) distributed throughout both leaf and cone tissues. Raman spectroscopy confirmed α-pinene as the dominant chemical component (characteristic peaks at 1640 cm^{-1} and 666 cm^{-1}), with additional oxidized terpenoids detected in mature cones. The spatial and cellular organisation reveal a dynamic terpene-rich defence network. Cryo-SEM ensures superior preservation of resins and secretory structure, the current methodological standard contrasting with isolated glands in other Cupressaceae

Keywords: Cryo-scanning electron microscopy, *Juniperus phoenicea*, resin secretory system, α-pinene, Raman spectroscopy, conifer anatomy

1. Introduction

Conifers possess specialized secretory structures that produce oleoresin, which serves as both a physical and chemical defence against a wide range of herbivores and pathogens. Oleoresin is composed of a complex blend of terpenoid compounds, including monoterpenes, sesquiterpenes, and diterpenes, the latter typically present as diterpene resin acids. These substances accumulate naturally within various anatomical features, such as resin cells, blisters, glands, and ducts (Fahn, 1979). Furthermore, conifers can enhance their defensive capacity by forming additional resin ducts in response to environmental stresses, both biotic and abiotic. Resin ducts are essential structural components of the plant defence system enabling the rapid mobilization of antimicrobial metabolites and the secretion of wound-healing resins (Franceschi et al., 2005; Hudgins & Franceschi, 2004). Their architecture underpins an integrated strategy against biotic and abiotic stress, combining chemical protection with mechanical resilience (Kroken et al., 2003; Nagi et al., 2000). Structurally, resin ducts and glands are characterised by an extracellular cavity surrounded by secretory epithelial cells responsible for terpene production (Kshatriya et al., 2018). Resin is a secondary metabolite produced by trees primarily as a defence mechanism and is secreted in response to injury to protect against microbes and insects (Kroken & Nagy 2012). Resin ducts serve to store and transport oleoresin, a complex terpenoid mixture (Panda 2008). Resin channels arise from cambial cell divisions and form interconnected networks. Resin ducts in gymnosperms, especially in *Pinus*, develop from the cambium or procambium through a process of schizogenous or lysigenous formation. These ducts may form a complex, interconnected system within the secondary xylem and phloem. The formation depends on the species and tissue type as the use of the cambial cell divisions is generally appropriate, especially for secondary ducts (Franschi et al., 2005). Their epithelial and sheath cells differ anatomically among species, with some forming thick, lignified walls. (Wu & Hu 1997; Franschi et al., 2005). Oleoresin consists mainly of monoterpenes and diterpenes; the volatile components evaporate while the diterpenes polymerise to seal wounds (Phillips & Croteau 1999). Stored oleoresin is released during pest attacks, forming barriers and acting as a toxin. Although these substances are metabolically expensive, their synthesis supports tree defence, possibly compromising some growth and reproduction. The challenges of separating subdermal structures make it difficult to fully understand the production of plant secretions, as conventional SEM and histological sectioning require dehydration and physical dissection that might disrupt native tissue organisation. Cryo-SEM offers an alternative approach by maintaining samples in their frozen state,

preserving native morphology. In some *Cupressaceae*, resin may accumulate and drip, especially at leaf tips or from bark wounds (Farjon 2005), but a full comparative study of resin ducts across organs and growth stages is still needed Cryo-scaning electron microscopy (cryo-SEM) keeps structures, including liquids, intact by rapid freezing samples in ultra-low temperature (Wightman 2022). This method allows for high-resolution structural analysis without the need for extensive chemical processing, especially important for volatile or delicate samples. Cryo-SEM has successfully visualised glandular trichomes and their secretory activity, confirming its effectiveness in preserving native structures. It is particularly suited for studying plant tissues sensitive to traditional fixation Anatomical studies on *Cupressaceae* have primarily focused on light microscopy-level descriptions of resin ducts in vegetative tissues. These investigations include leaf anatomical analyzes examining resin canal dimensions and tissue organisation (Bercu et al., 2010; Hamidipour et al., 2011); More recently, Della Rocca et al. (2022) conducted an ultrastructural investigation of traumatic resin duct formation in *Cupressus sempervirens* following fungal infection, revealing the involvement of plastids and endoplasmic reticulum in terpenoid biosynthesis. While morphological and structural variation at the reproductive organs level has been documented through cone scale studies (Jagel and Dörken, 2015; Dörken et al., 2017), a significant gap remains regarding resin structures within these organs themselves. The study Objectives are: i) compare resin duct morphology using cryo-SEM between *Juniperus phoenicea*'s leaf cones and stem ii) locate the resin secretory structures using Raman spectroscopy; iii) Establish a protocol for multi-modal structural-chemical analysis.

2. Methods

2.1 Plant Material and Sample Collection

Leaves and cones of *J.phoenicea* were collected from the Saidia coastal region in north-east Morocco (GPS coordinates: 35°10' N, 2°28'E, elevation 57.15m asl) during November 2024. Five healthy, mature shrubs of J. phoenicea were selected based on approximatively equal age class, and absence of visible disease or mechanical damage. From each shrub, five samples of small sections of one-year-old stems with scale-like adult leaves, three samples of 2 years cones and three samples of one-year cones were collected from the mid-crown branches with southern exposure, the samples were carefully collected to minimise the damage on the plant. The voucher reference samples are deposited at the herbarium of Mohammed Premier

University, Oujda, Morocco, under voucher number HUMPOM023. All plant material was stored in ethanol to inactivate pathogens for transport to the UK

2.2 Cryo-SEM Protocol

Sample Preparation: the plant materials were frozen intact to limit artifacts, mechanical damage, and resin loss. Samples were frozen using liquid nitrogen. Ethanol preserved tissues were carefully hand-sectioned using a sterile double-edged razor blade. Samples were mounted onto aluminium specimen stubs using a small amount of adhesive consisting of 10:1 Tissue-Tec: Colloidal graphite to ensure secure placement during subsequent freezing, good thermal contact and conductivity. Mounted specimens were rapidly plunge frozen in nitrogen slush to preserve tissue integrity for imaging in the high vacuum environment of the SEM chamber. Samples were then transferred under vacuum to the cryo-preparation chamber attached to a cryo-scanning electron microscope. Samples were subsequently sputter-coated with a gold-palladium to a thickness of approximately 6 nm. During imaging, samples were maintained at -145°C to maintain structural integrity. Cryo-SEM analysis was performed using a Zeiss EVO LS 15, equipped with a Quorum cryo-stage system. Imaging was conducted at an acceleration voltage of 6 kV, with a working distance maintained between 8-10 mm. A range of magnifications from 100x to 5,000x was used to capture both surface topography and the anatomical details of the plant tissues.

2.3 Raman Spectroscopy parameters

Raman spectral acquisition was carried out using a Renishaw InVia Raman microscope, equipped with a 785 nm diode laser, selected to minimise sample autofluorescence. The objective was sample dependant: A Leica 40x HC APO L NA 0.8 water dipping lens was used for leaf samples (leaf samples were set on the surface of 1% v/v agar and submerged in water) and a Leica 50x N Plan EPI NA 0.75 dry objective lens used for cones. The acquisition software was WiRE 4.2. The laser power at the sample surface was maintained at < 10mW to prevent thermal degradation of sensitive plant tissues and the pinhole was engaged. Each spectrum was recorded with an acquisition time of 10-40 seconds per spot, depending on signal intensity and sample type. The spectral range was set to include 400 to 1700 cm^{-1}, allowing clear identification of characteristic vibrational bands of organic compounds. Multiple spectra were collected from each anatomical region of interest to ensure reproducibility and representative chemical profiling. To aid visualisation of all major peaks,

spectra were smoothed using the WiRE software using a smooth window of 7 and polynomial order set to 2.

2.3. Light microscopy imaging

Light microscopy imaging was carried out using a Keyence VHX-7000 digital microscope to observe leaves and cones.

3. Results

3.1 Leaves

Cross-sections of *J. phoenicea* scale-like leaves revealed resin ducts located on the upper leaf surface (Fig. 1a, 1b). Resin deposits were visible on the leaf surface (Fig. 1a, arrows), while shoot cross-sections showed typical single resin ducts (RD) positioned on the upper epidermis of preserved leaves (Fig. 1b)

3.1.1 Cryo-SEM microscopy of leaves

Light microscopy can resolve large resin ducts (Fig 1a, 1b) but gives insufficient detail of morphology and the surrounding tissue. Using the cryoSEM, five cross sections were examined of the upper leaf surface regions where there was a single prominent duct. Diameters range from 150-200 μm (**Fig. 2**). The ducts contained dense visible resin accumulations that either partially or fully occupy the cavity lumen. The organization of the surrounding secretory tissues shows a distinct compartmentalization within the mesophyll, with a well-defined central cavity that might serve as primary repositories for lipophilic metabolites and characteristic droplets distributed along specific tissues margins. Resin secretion on *J. phoenicia* leaves contributes both to storage and protection as discussed by Al-Edany & Khidhir, 2022. The Cupressaceae species possess structures to optimize storage of terpenoids (Baran et al., 2023). Cryo-SEM preserves volatile compounds and reveals the presence of a well-developed secretory epithelium in *J. phoenicea* leaves. The epithelial zone is characterized by a distinct layer of specialised secretory cells. The epithelial cells are continuous and line the upper the resin cavity (**Fig. 3**). These cells are tightly packed with their long axes oriented perpendicular to the cavity surface. The epithelial cells are morphologically distinct from the surrounding mesophyll parenchyma (**Fig.3**).

Resin droplets of varying sizes were observed distributed throughout intercellular spaces, suggesting secretion and accumulation within these regions (**Fig.4**).

156 *3..1.2 Raman microscopy analysis of the external resin drop of the leaves*

157 The Raman spectrum (**Fig. 5**) of a resin droplet shows dominant peaks characteristic of α-
158 pinene, including signals at 262, 668, 772, 1044, 1373, and 1659 cm^{-1}. These peaks closely
159 match Raman data published elsewhere (Pan et al. 2016, Yang et al. 2020)

160 *3.2-Cones:*

161 The *Juniperus phoenicea* cone exhibits a highly developed, multi-scale secretory system,
162 supported by a specialised cellular architecture that facilitates abundant resin production and
163 distribution. At the structural level, the resin duct walls display a multi-layered organization
164 with clearly defined secretory epithelia consistent with observations inother *Juniperus* species
165 confirming that resin ducts are lined by several layers of secretory and sudsidiary cells
166 (Radoukova et al., 2024; Tulik & Jura-Muraweic., 2023). In the cones, resin is not restricted
167 to central cavities but is also distributed through an integrated secretory network as reported
168 in related taxa where ducts are interconnected and supported by parenchymatous tissues
169 (Guvenç et al., 2011), suggesting the presence of resin movement pathways with a well-
170 organised cellular arrangement (Cabrita., 2018).

171 The light microscopy of longitudinal cross-section of the cone highlights the differentiation of
172 tissue zones. A distinct resin secretion zone is visible, demarcated by a translucent, amber-like
173 region. Embedded within this zone are single prominent resin ducts, appearing as a well-
174 defined void surrounded by secretory tissues. (**Fig. 6a,6b)** offers a magnified view of the resin
175 duct observed in **Fig. 6**

176 *3.2.1 Cryo-SEM microscopy of cones*

177 Cryo-scanning electron microscopy of transverse sections through *Juniperus phoenicea* cones
178 revealed a complex dual-component resin secretory system consisting of both large secretory
179 cavities and numerous dispersed resin droplets (**Fig. 7**). The ultrastructural preservation
180 achieved through Cryo-preparation allowed detailed visualization of the three-dimensional
181 organization of secretory structures without the artifacts typically associated with
182 conventional chemical fixation of lipophilic compounds.

183 The resin is secreted from a complex secretory system, characterised by large primary resin
184 cavities located in the peripheral region of the cone (**Fig. 8**). This rounded cavity is

accompanied by multiple additional smaller cavities of various sizes spread throughout the cone tissue and spread mostly in the basal part of the cone, indicating a distributed rather than isolated secretory system. The architecture of this system reveals multiple resin cavities arranged across the cone, with a size gradient from primary cavities (larger) of the scale-like leaves to smaller secondary ones of the basal part, reflecting an integrated network that extends throughout the cone. There is a possibility that the resin from the small cavities will flow into the larger one facilitated with the surrounding parenchyma (**Fig.9**), which might explain the significant amount of resin observed in the large cavities.

The architecture demonstrates a prominent resin cavity (approximately 120—150 µm in diameter) observed in the peripheral cone tissue, exhibiting the characteristic smooth, rounded morphology typical of lysigenous or schizolysigenous secretory spaces. The cavity displayed well-defined boundaries with surrounding parenchymatous tissue (**Fig 8** ; **Fig. 9**), suggesting organized secretory activity rather than random tissue degradation. The empty or partially depleted appearance of the cavity might indicate active resin mobilization.

A network of resin droplets was found throughout the cone basal part, ranging in diameter from 2—50 µm. These droplets exhibited heterogeneous size distribution and non-random spatial organization, with areas of high droplet density alternating with regions of lower secretory activity. The droplets appeared both intercellular and intracellular, suggesting multiple mechanisms of resin accumulation and storage within the fruit tissue. The cone parenchyma surrounding the secretory structures consisted of large, thin-walled cells with extensive intercellular spaces, facilitating resin movement and distribution throughout the fruit (**Fig.9**)

3.2.2 Raman microscopy in a mature cone (2 years)

One-year-old cones were excluded from the study we used 2 years cones. the resin is mainly composed of peaks corresponds to oxidised terpenoids (Pan et al. 2016, Vergas Jentzsch et al 2021)

The Raman spectrum (**Fig. 10**) shows a very strong peak at 1642 cm^{-1} corresponding to α-pinene with characteristic ring breathing mode at 667 cm^{-1}. The 1374, 1440—1470 cm^{-1} peaks are found in cyclic monoterpenes like α-pinene. A cluster of low-to-mid frequency peaks (620—842 cm^{-1}) are consistent with the bicyclic ring system of α-pinene. α-pinene

oxide, verbenone and pinocarvone. The band at 1614 cm^{-1} may indicate minor phenolic content, possibly from flavonoid derivatives or oxidation artifacts (Daferera et al. 2002, Vergas Jentzsch et al 2015).

The comparison between leaf and mature cone spectra (Fig5 and Fig 10) indicates that both tissues contain α-pinene, as evidenced by similar characteristic Raman bands. The spectra are, however, not identical and suggests variations in the local chemical environment of α-pinene or the presence of other metabolites that differ between the two tissues

Discussion

Combining Cryo-SEM and Raman analyses of *J.phoenicea*

The application of Cryo-SEM has revealed a complex resin secretory architecture of *J. phoenicea*, demonstrating the importance of preservation methodology in structural studies of secretory systems. The preservation of plant structures and droplets, achieved through plunge freezing in slush nitrogen, allowed for detailed visualisation of intact resin distribution patterns and secretory cavity morphology without the dissolution or displacement artifacts typically associated with organic solvent processing (Tshilande et al., 2024). The positive identification of Raman droplets using Raman microscopy represents a significant methodological advancement, bridging the gap between anatomical and chemical analyses that has historically limited comprehensive understanding of plant secretory systems.

Integrated secretory network architecture

The current study reveals that *J. phoenicea* possesses an integrated terpene-rich resin secretory network, while earlier studies on anatomical organisation in the *Cupressaceae* have mainly documented isolated resin glands. The investigations document solitary abaxial resin glands or discreet resin ducts in *Juniperus* leaf scales (Adams, 2014; Zlatković et al., 2011; Hamidipour et al., 2011), *Cupressus*, *Thuja*, and *Platycladus species* (Hamidipour et al., 2011), where resin is sequestered in non-connected structures. In other studies, interconnected resin duct networks, where axial and radial ducts form continuous, three-dimensional systems, are described in certain *Pinaceae* genera trees (Tulik & Muraveic, 2023; Franceschi et al., 2005).

The observed size gradient from large primary cavities (120—150 µm diameter) to numerous smaller secondary droplets (2—50 µm diameter) indicates a hierarchical organization that likely facilitates both storage and rapid mobilisation of defensive compounds.

The presence of both intercellular and intracellular resin accumulation sites suggests multiple mechanisms of resin production and storage within the same tissue that all resin is made within specialised cells and then secreted in to the ducts, indicating a more complex secretory biology than the single-pathway models typically described for *Pinaceae* species (Franceschi et al., 2005; Wu & Hu, 1997). This architectural complexity may represent an evolutionary adaptation to the specific ecological pressures faced by Mediterranean conifers, where rapid response to multiple stress factors is essential for survival. The distinct, diffuse resin secretory system in *J. phoenicea*, compared to the well-defined ducts of *Pinaceae* (Krokene & Nagy, 2012), underscores the need for broader comparative studies, as these differences may reflect varying evolutionary strategies for chemical defence among conifer families, The widespread presence of resin throughout the young cone tissue suggests a protective strategy aimed at safeguarding reproductive structures—likely an adaptation to high seed predation in arid Mediterranean environments.

This study reveals that *J. phoenicea* has developed a dynamic and comprehensive chemical defence strategy, characterized by rapid resin mobilization and the gradual chemical transformation of defensive compounds, effectively protecting both vegetative and reproductive tissues against the diverse stresses of its Mediterranean habitat.

While this study provides the first detailed structural characterization of the *J. phoenicea* secretory system, several limitations should be acknowledged. The analysis was conducted on samples from a single geographical location, and regional variations in secretory architecture and chemistry remain to be explored. CryoSEM was carried out on ethanol preserved, not freshly harvested material. Although freshly harvested material retains native hydration levels, cryogenic conditions aid the preservation of tissue integrity, including any spaces and voids, during SEM imaging in a vacuum.

Future research should expand this integrated structural-chemical approach to other Cupressaceae species to determine whether the observed architectural complexity is characteristic of the family or represents a species-specific adaptation. Long-term studies

examining seasonal variations in secretory activity and chemical composition would provide valuable insights into the dynamic nature of these defence systems.

Conclusion: the current work establishes cryo-SEM and Raman microscopy as useful tools for accurate characterisation of plant secretory systems and reveals that *Juniperus phoenicea* possesses more sophisticated resin defence architecture than previously recognised. The integrated secretory network identified here represents a significant advancement in our understanding of conifer chemical defence strategies and provides a foundation for future comparative studies across the Cupressaceae family. Future research should build upon the morphological (e.g. cryoSEM) and chemical analyses presented here. We now have a clearer understanding of the spatial distribution of resin-generating and transporting structures and can use this for future work in the context of pathogen defence and comparing to other closely and more distally related species.

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References:

Adams, R.P. (2014). Junipers of the World: The genus Juniperus (4th ed.). Trafford Publishing.

Bercu, R., Broasca, L., & Popoviciu, R. (2010). Comparative anatomical study of some gymnospermae species leaves. Botanica Serbica, 34(1).

Cabrita, P. (2018). Resin flow in conifers. Journal of Theoretical Biology, 453, 48-57.

Daferera, D., Pappas, C., Tarantilis, P. A., & Polissiou, M. (2002). Quantitative analysis of α-pinene and β-myrcene in mastic gum oil using FT-Raman spectroscopy. Food Chemistry, 77(4), 511-515.

Della Rocca G, Papini A, Posarelli I, Barberini S, Tani C, Danti R and Moricca S (2022) Ultrastructure of Terpene and Polyphenol Synthesis in the Bark of Cupressus sempervirens After Seiridium cardinale Infection. Front. Microbiol. 13:886331. doi: 10.3389/fmicb.2022.886331

Dörken, V. M., Nimsch, H., & Jagel, A. (2017). Morphology, anatomy and morphogenesis of seed cones of Cupressus vietnamensis (Cupressaceae) and the taxonomic and systematic implications. Flora, 230, 47-56.

Della Rocca G, Papini A, Posarelli I, Barberini S, Tani C, Danti R and Moricca S (2022) Ultrastructure of Terpene and Polyphenol Synthesis in the Bark of Cupressus sempervirens After Seiridium cardinale Infection. Front. Microbiol. 13:886331.

Dörken, V. M., Nimsch, H., & Jagel, A. (2017). Morphology, anatomy and morphogenesis of seed cones of Cupressus vietnamensis (Cupressaceae) and the taxonomic and systematic implications. Flora, 230, 47-56.

Dörken, V. M., Ladd, P. G., & Parsons, R. F. (2023). Leaf dimorphism in Cupressus nootkatensis D. Don and Cupressus vietnamensis (Farjon & TH Nguyên) Silba (Cupressaceae) and its ecological and evolutionary significance. Trees, 37(4), 1267-1279.

Fahn A. (1979). Secretory tissues in plants. Academic Press. London UK

Farjon, A. (2005). A monograph of Cupressaceae and Sciadopitys. Richmond, Surrey: Royal Botanic Garden Kew

Franceschi, V. R., Krokene, P., Christiansen, E., & Krekling, T. (2005). Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New phytologist*, *167*(2), 353-376.

Güvenç, A., Hurkul, M. M., & Erdem, A. (2011). The leaf anatomy of naturally distributed Juniperus L.(Cupressaceae) species in Turkey. Turkish Journal of Botany, 35(3), 251-260.

Huang, B. Q., & Yeung, E. C. (2015). Chemical and physical fixation of cells and tissues: an overview. Plant microtechniques and protocols, 23-43

Hamidipour, A., Radjabian, T., Charlet, D. A., & Zarrei, M. (2011). Leaf anatomical investigation of Cupressaceae and Taxaceae in Iran. Kärntner Botanikzentrum, 18, 95-111.

Huang, B. Q., & Yeung, E. C. (2015). Chemical and physical fixation of cells and tissues: an overview. Plant microtechniques and protocols, 23-43.

Hudgins, J. W., & Franceschi, V. R. (2004). Methyl jasmonate-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. *Plant Physiology*, *135*(4), 2134-2149.

Humbel, B. M., Schwarz, H., Tranfield, E. M., & Fleck, R. A. (2019). Chemical fixation. *Biological field emission scanning electron microscopy*, *1*, 191-221.

Jagel, A., & Dörken, V. (2015). Morphology and morphogenesis of the seed cones of the Cupressaceae-part III: Callitroideae.

Krokene, P., & Nagy, N. E. (2012). Anatomical aspects of resin-based defences in pine. Pine resin: biology, chemistry and applications, 661(1431), 67-86.

Krokene, P., Nagy, N. E., & Krekling, T. (2008). Traumatic resin ducts and polyphenolic parenchyma cells in conifers. In *Induced plant resistance to herbivory* (pp. 147-169). Dordrecht: Springer Netherlands.

Lakušić, B., & Lakušić, D. (2011). Anatomy of four taxa of the genus Juniperus sect: Juniperus (Cupressaceae) from the Balkan peninsula. Botanica Serbica, 35(2), 145-156.

Nagy, N. E., Franceschi, V. R., Solheim, H., Krekling, T., & Christiansen, E. (2000). Wound-induced traumatic resin duct development in stems of Norway spruce (Pinaceae): anatomy and cytochemical traits. *American Journal of Botany*, *87*(3), 302-313.

Pan An, Cheng-Qian Yuan, Xian-Hu Liu, De-Bao Xiao, Zhi-Xun Luo, Vibrational spectroscopic identification of isoprene, pinenes and their mixture, Chinese Chemical Letters, Volume 27, Issue 4, 2016, 527-534, ISSN 1001-8417,

Phillips, M. A., & Croteau, R. B. (1999). Resin-based defenses in conifers. Trends in plant science, 4(5), 184-190.

Radoukova, T., Semerdjieva, I., & Zheljazkov, V. D. (2024). A Comparative Morphological and Anatomical Study of Juniperus communis L., J. sibirica Burgsd., and J. pygmaea K. Koch from Bulgaria. *Plants*, *13*(17), 2419.

Tshilande, N., Mammino, L., & Bilonda, M. K. (2024). The Study of Molecules and Processes in Solution: An Overview of Questions, Approaches and Applications. *Computation*, *12*(4), 78.

Tulik, M., & Muraveic, M. (2023). The morphology and anatomy of resin-secreting systems in plants. Trees, 37, 13-38.

Tulik, M., & Jura-Morawiec, J. (2023). An arrangement of secretory cells involved in the formation and storage of resin in tracheid-based secondary xylem of arborescent plants. *Frontiers in Plant Science*, *14*, 1268643.

Vargas Jentzsch, P., Sandoval Pauker, C., Zarate Pozo, P., Sinche Serra, M., Jacome Camacho, G., Rueda-Ayala, V., & Ciobotă, V. (2021). Raman spectroscopy in the detection of adulterated essential oils: The case of nonvolatile adulterants. *Journal of Raman Spectroscopy*, *52*(5), 1055-1063.

Wightman, R. (2022). An Overview of Cryo-Scanning Electron Microscopy Techniques for Plant Imaging. *Plants*, *11*(9), 1113. https://doi.org/10.3390/plants11091113

Wu, H., Hu, Zh. Comparative anatomy of resin ducts of the Pinaceae. Trees 11, 135–143 (1997). https://doi.org/10.1007/s004680050069.

Xie, J. B., Zhang, B. N., & Wang, Z. Y. (2022). Relationships between xylem transport, anatomical, and mechanical traits at organ level of two Cupressaceae species. Forests, 13(10), 1564.

Yang, H., Wang, P., & Wu, G. (2020). The Raman optical activity of pinane as compared with (-) α-and (-) β-pinene: The perspective of intramolecular enantiomerism. *Vibrational Spectroscopy*, *106*, 102988.

Yousif, I. Z., & Shahbaz, S. E. (2023). Comparative Anatomical Study of Leaves in Five Cupressaceae Gray. Taxa in Kurdistan Region-Iraq. Science Journal of University of Zakho, 11(1), 22-29.

Zlatković, B.K., Lakušić, D.V., & Lakušić, B.S. (2011). Anatomy of four taxa of the genus Juniperus sect. Juniperus (Cupressaceae) from the Balkan peninsula. Botanica Serbica, 35(2), 159-168.