

# Microscopic analysis and the histochemical staining of three species from *Asparagaceae* family: *Hosta plantaginea* (Lam.) Asch., *Ornithogalum umbellatum* L. and *Ornithogalum dubium* Houtt.

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## Introduction

The genus *Hosta* has been baffling scientists for decades with its morpho-anatomical characteristics; numerous debates among botanists have existed with the thematic of placing the genus in the right family. The species *Hosta plantaginea* (Lamarck) Ascherson is the most well-known representative of this genus, and it has been documented that it is used in Chinese traditional medicine for its therapeutic effects (Yang, He 2021). The genus *Ornithogalum* it is considered one of the largest genera of *Asparagaceae*. *Ornithogalum umbellatum* L. is a well-known species in Eastern Europe while *Ornithogalum dubium* Houtt. has its origin in South Africa.

Different studies (Langat et al. 2019, Wei et al. 2020) on species from these genera exist pointing out the fact that some of them have potential use in the medical field for different treatments. The following procedures (histochemical tests) are a starting point to identify the potential compounds and their exact location. The limited number of studies (Andrić et al. 2016, Langer et al. 2021,) is one of the key reasons for choosing these three species of *Asparagaceae*.

## Materials and methods

### Materials

Plant materials of *Hosta plantaginea* and *Ornithogalum umbellatum* were obtained from a local garden in Ploiești, Prahova, Romania, while the plant material of *Ornithogalum dubium* was purchased from a local supplier of decorative plants; all three species were in blooming season. The chemical reagents used for the staining procedures were purchased from Sigma-Aldrich. The microscope used for examination was Euromex oxion series 110-240V/50-60 Hz, with 4-100x lenses, and pictures were taken with a CEMEX 5 DC 5000 C camera.

### Double staining with iodine green and alum carmine

For this procedure cross-sections were made with a scalpel and stained with iodine green (1% aqueous solution) and alum carmine (5.5% aqueous solution). The sections were mounted in distilled water onto the slide (Dinu M., et al. 2012).

### Histochemical staining

The cross-sections obtained similarly to the previous method were stained with specific reagents for every different class of compounds: potassium dichromate 10%

aq. solution - for phenol compounds; ruthenium red 0.1% aq. solution – for mucilage identification; eosin 1% ethanol solution and picric acid 1% et. solution – nuclei and protein formations identification; Lugol solution for starch identification; Dragendorff reagent, 5% sodium nitrite and tartaric acid 5% et. solution – for alkaloid identification (Badria F.A., 2019).

## Results and discussion

### *Double staining with iodine green and alum carmine*

This procedure was used to set apart the differences between the morpho-anatomical characteristics of the two genera which might help on further studies and examinations. The cross-sections from *H. plantaginea* were made in the median part of the following organs: leaf (petiole and lamina), rhizome, and adventive roots. The tissue arrangement was representative for a monocotyledonous species but with some key distinctions: two types of lignification thickening in xylem cells (spiral and scalariform) - longitudinal section; secretory canals in root parenchyma; “U” shaped cellular wall thickening of the cells in the endoderm (roots), unifacial, asymmetric leaf structure.

For *O. umbellatum* cross-sections were made in the middle part of the leaf blade and fertile stem, while for *O. dubium* in the middle part of the adventive root, leaf blade and fertile stem. These 2 species exhibit typical morphology for monocotyledonous plants, nonetheless with special characteristics such as: unicellular trichomes on the margin of the leaf and asymmetric, unifacial leaf structure (such as *O. umbellatum*) of *O. dubium*; Caspary strip in the roots of *O. dubium*; three-layered palisade in the leaf of *O. umbellatum*. The mesophyll differs in both *Ornithogalum* species: the number of palisade layers and the size of mucilage-containing cells (bigger in *O. umbellatum*). The sclerenchyma in the scape of *O. dubium* is lignified with more cell layers, while *O. umbellatum* has a cellulose structured sclerenchyma (collenchyma) with fewer cell layers. *O. dubium* has a similar structure to *O. nutans*, although *O. nutans* does not have trichomes.

In all three species were present needle-shaped crystals (raphides), in *H. plantaginea* stiloids, and *Ornithogalum. sp.* idioblast crystals (Andric et al. 2016).

### *Histochemical staining*

*H. plantaginea* presented a significant number of brown formations after applying Dragendorff reagent, which confirms that the compounds are alkaloids, and a smaller quantity of mucilage and crystal deposits of proteins. In *O. dubium* and *O. umbellatum* mucilage was

found to be the most abundant in the tissues, a lower number of crystal deposits of proteins as well as alkaloids. All three species presented small formations of starch that were colored in dark blue after applying Lugol solution, confirming that the reserve substance of all three species is starch.

## Conclusion

On the basis of histochemical staining, it has been deduced that the three species are most abundant in alkaloids and mucilage, unevenly dispersed through organs, whereas smaller amounts of protein crystals and starch deposits are also detectable. The staining procedure for identifying phenol compounds pointed out just a small number of brown colored formations in *H. plantaginea* leaf blade. Further studies need to be pursued for a better analysis of the secondary metabolites of these three plant species to better identify the quantity and chemical structures of these compounds.

## References

- Andrić, A.M., Rat, M.M., Zorić, L.N., Luković, J. Ž., 2016. Anatomical characteristics of two *Ornithogalum L. (Hyacinthaceae)* taxa from Serbia and Hungary and their taxonomic implication. *Acta Bot. Croat.* 75 (1), 67–73. <https://doi.org/10.1515/botcro-2016-0002>.
- Badria, F.A., Walaa, S.A., 2019. Plant Histochemistry: A Versatile and Indispensable Tool in Localization of Gene Expression, Enzymes, Cytokines, Secondary Metabolites and Detection of Plants Infection and Pollution. *Acta Scientific Pharmaceutical Sciences*, 3, 7, 88-100. <https://doi.org/10.31080/ASPS.2019.03.0318>.
- Dinu, M., Ancuceanu, R., Hovanet, M.V., Anghel, A.I., Cretu, O.D., Rebegea, O.C., Oлару, T.O., 2012. Baze teoretice si practice. *Citologie. Histologie. Organografie*. 2nd edition, Ed Universitara, Bucuresti. pp.67-92.
- Langat, L., Langat, M.K., Mulholland, D.A., 2019. Homoisoflavonoids and cardenolides from cultivated *Ornithogalum* species: *Ornithogalum dubium* Houtt. and *Ornithogalum ponticum* ‘Sochi’. *South African Journal of Botany* 126, 317-321. <https://doi.org/10.1016/j.sajb.2019.04.025>.
- Langer, M., Speck, T., Speck, O., 2021. Petiole-Lamina Transition Zone: A Functionally Crucial but Often Overlooked Leaf Trait. *Plants*, 10, 774. <https://doi.org/10.3390/plants10040774>.
- Wei, R., Ma, Q., Zhong, G., He J., Sang, Z., 2020. Isolation and characterization of flavonoid derivatives with anti-prostate cancer and hepatoprotective activities from the flowers of *Hosta plantaginea* (Lam.) Aschers. *Journal of Ethnopharmacology*, 253, 112685. <https://doi.org/10.1016/j.jep.2020.112685>.
- Yang, L., He, J., 2021. Traditional uses, phytochemistry, pharmacology and toxicological aspects of the genus *Hosta (Liliaceae)*: A comprehensive review. *Journal of Ethnopharmacology* 265(30), 113323. <https://doi.org/10.1016/j.jep.2020.113323>.