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Natural Volatiles &
Essential Oils

PP-086. Essential oil composition of wild growing and *in vitro* cultivated *Isula britannica* L.
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Isula britannica L. is well known medicinal plant used as an antibacterial, carminative, diuretic remedy and for treating asthma, hepatitis and tumors (Khan et al., 2010). The presence of sesquiterpene lactones, main bioactive components of this species in *in vitro* cultivated plants was reported previously (Todorova et al., 2014). Here we compare the volatile components of *in vitro* cultivated and wild collected material. The plant was collected from a native population in Bulgaria and leaves and flowers were analyzed separately. *In vitro* culture was initiated from steam segments of the plant in 0.5 benzyl adenine supplemented medium. The essential oils were obtained by micro steam distillation-extraction. Identification and quantification were performed by GC/MS. Eighty-three components were registered as chromatographic peaks in concentration more than 0.2% at least in one of the samples. Insignificant difference in the qualitative composition of flower (F) and leaf (L) volatiles was observed. Both oils were rich in oxygenated components reaching 74.56% in L. Higher terpenoid content in comparison with other compound types was also characteristic for the analyzed samples. It should be noted that the ratio terpenoids:others was 1.48 in F and 3.97 in L. The results revealed that the sesquiterpenoids exceeded monoterpenoids. The amount of monoterpenoids in F and L was almost equal, while sesquiterpenoids in L were 20% higher than in F. β -Pinene (1.71% in F and 1.21% in L) and 1,8-cineole (2.00% in F and 1.06% in L) were the main monoterpenoids. Principal sesquiterpenoids were viridiflorol (7.17% in F and 8.20% in L) and himachalol (3.45% in F and 8.71% in L) followed by β -selinene, intermedeol, caryophyllene oxide, T-cadinol, and T-murolol. Aliphatic hydrocarbons (C₁₀-C₁₅) dominated in F (17.03%), while their content in L was 3.64%. The composition of the volatiles of *in vitro* culture was very similar to the ones from leaves (mainly sesquiterpenoids with viridiflorol and himachalol as major components). The obtained results confirmed our previous study that the *in vitro* culture system can be utilized as a biotechnological source for targeted delivery of phytopharmaceuticals, characteristic for *I. britannica*.

Keywords: *Isula britannica* L., essential oils, sesquiterpenoids, *in vitro* culture.

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PP-087. Are relations between essential oil profile and endogenous cytokinins independent of rooting in *Artemisia alba* *in vitro* model?
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Artemisia alba Turra is a fragrant shrub, characterized by a marked variability of its essential oils depending on the origin of collection of the wild growing plant. Previously we established that inhibition of rooting caused by combination of low concentration of benzyl adenine (BA) combined with different indole-3-butyric acid (IBA) supplementations significantly lowered monoterpenoid/ sesquiterpenoid ratio in the essential oils of the aerial parts of the plant *in vitro* (Danova et al., 2012), which was also related to a substantial drop of the endogenous cytokinins in these samples (Krumova et al., 2013). The observed effect was logically attributed to a drop of endogenous cytokinins biogenesis due to the inhibited rooting, as it is widely accepted that roots are the main spot of endogenous cytokinins synthesis in the plant. In the present work we further developed the *A. alba* shoot cultures model by inhibiting rooting and stimulation of callusogenesis by treatments of BA alone, as well as with different combinations of BA and IBA. Interestingly, though BA treatments completely inhibited rooting, they did not affect monoterpenoid levels to such extent, as the BA and IBA combinations. Noteworthy, while IBA decreased the ratio of irregular towards regular oxygenated monoterpenes in the oils, on the contrary, BA increased this parameter. In addition, it was established that in the BA treatments, bioactive cytokinins levels were also not as strongly reduced, comparing with non-treated control as, were the IBA and BA combinations. Little is still known on the interplay between endogenous hormonal status and the biosynthetic capacity of plants. A recent work sheds light on this problem, showing that for the five plant secondary metabolic gene clusters reported so far, the enzymes for the first committed steps all appear to have been recruited directly or indirectly from primary metabolic pathways involved in phytohormone synthesis (Chu et al., 2011). Further research is in progress for the elucidation of physiologic factors affecting terpenoid biogenesis in *A. alba* *in vitro* model system.

Keywords: *Artemisia alba* Turra, *in vitro* culture, terpenoid biogenesis, phytohormone treatment, cytokinins.

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