INFLUENCE OF ABIOTIC AND BIOTIC ELICITORS ON SESQUITERPENE LACTONE PRODUCTION IN *ARNICA MONTANA* L. *IN VITRO* SHOOT CULTURES

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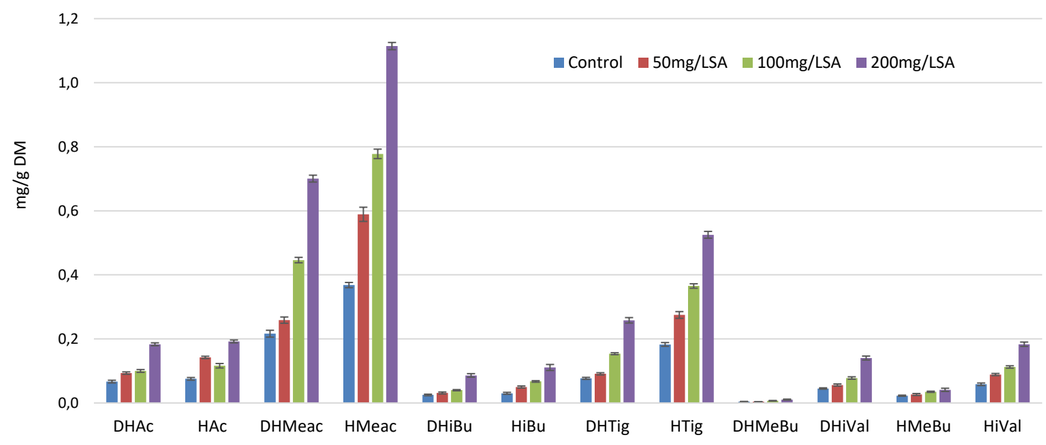
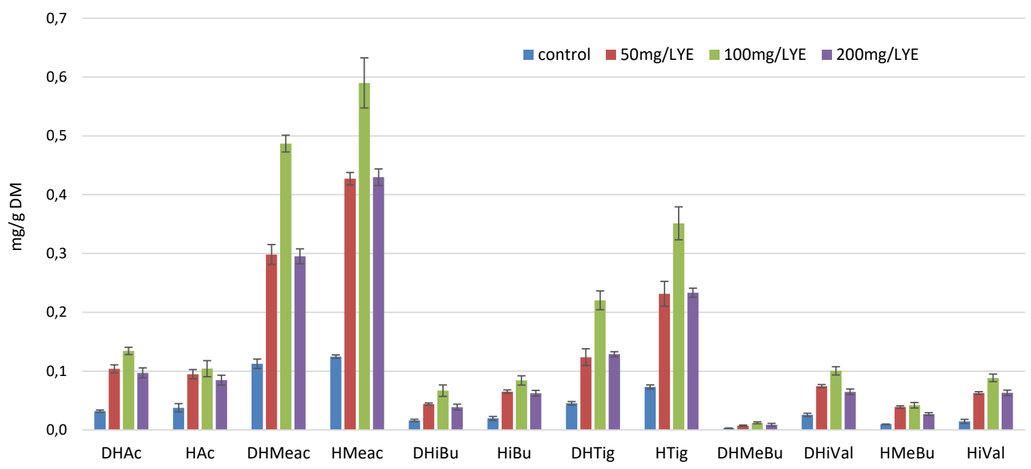
***Arnica montana* L. (Asteraceae) is an endemic plant to Europe, used for centuries in ethnomedicine and is currently applied widely in pharmacy and cosmetics due to its antiseptic, anti-inflammatory, and antioxidant properties. The biological activity of this species is due to the presence of sesquiterpene lactones of helenalin and 11,13-dihydrohlenalin type. The plant is listed as threatened in many European countries due to habitat loss and extensive harvesting for commercial purposes. *In vitro* cultivation is an attractive and ecofriendly approach for large-scale plant production, and elicitation is one of the most effective biotechnological tools for modulating, generating, and enhancing valuable plant secondary metabolites.**

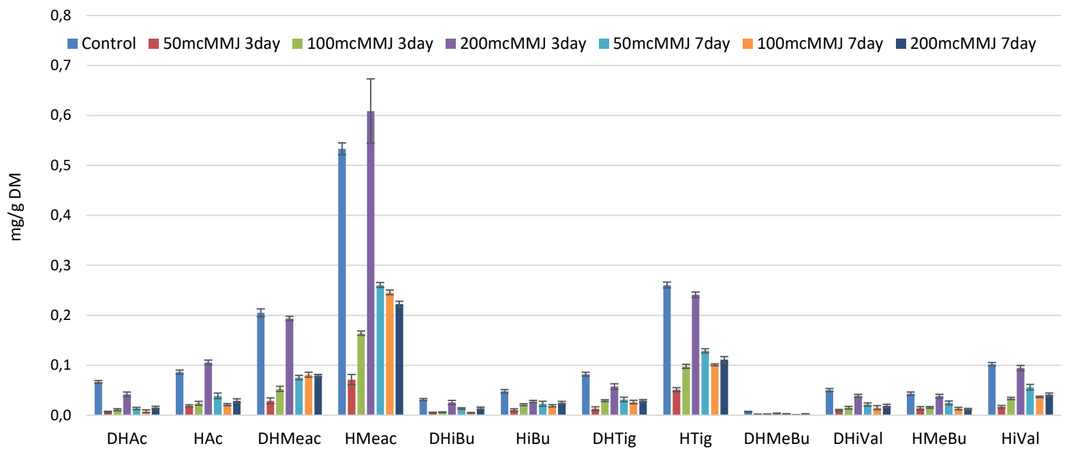
The aim of the current study was to assess the effect of abiotic (salicylic acid SA and methyl jasmonate MJ) and biotic (yeast extract YE) elicitors applied at different concentrations on sesquiterpene lactone accumulation and lactone profile of *in vitro* micropropagated *A. montana* shoots.



SL content in *in vitro* micropropagated *A. montana* shoots treated with different concentrations of salicylic acid (SA)



SL content in *in vitro* micropropagated *A. montana* shoots treated with different concentrations of yeast extract (YE)



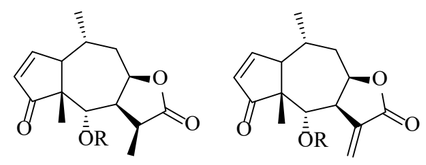
SL content in *in vitro* micropropagated *A. montana* shoots treated with different concentrations of methyl jasmonate (MJ)

Acknowledgements:The authors are thankful to project КП-06-Н76/5 (05.12.2023)., National Science Fund, Bulgaria for the financial support.

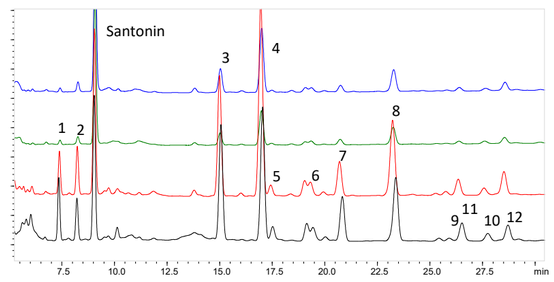
Material and methods:

Two-month-old *in vitro* shoot cultures were used as a source of stem segments for elicitor treatment studies. Control shoots were grown on Murashige and Skoog medium supplemented with 0.5 mg/l BA (MSB0.5) without elicitor. Yeast extract (50, 100, or 200 mg/l) was added to MSB0.5 before autoclaving. The stock solutions of MeJ and SA were filter-sterilized through a 0.22 µM Minisart® Syringe Filter. SA (50, 100, or 200µM) were added to a MSB0.5 medium after being autoclaved. YE and SA were medium constituents for the whole period of the cultivation (5 weeks). Methyl jasmonate was sterilized and added to the MSB0.5 medium at the end of the 4 weeks of cultivation with varying concentrations (50, 100, or 200µM). The *in vitro* shoots were collected on the 3rd and 7th days after treatment with MJ. The media were solidified with 0.6% agar.

The plant material (0.1 g) was extracted with $CHCl_3$ in ultrasonic bath for 30 min. The extract was purified by SPE (C18) and analyzed by HPLC-DAD method using santonin as internal standard.



R	Dihydrohelenalins (DH)	Helenalins (H)
Ac	DHAc (1)	HAc (2)
Meac	DHMeac (3)	HMeac (4)
iBu	DHiBu (5)	HiBu (6)
Tig	DHTig (7)	HTig (8)
MeBu	DHMeBu (9)	HMeBu (10)
iVal	DHiVal (11)	HiVal (12)

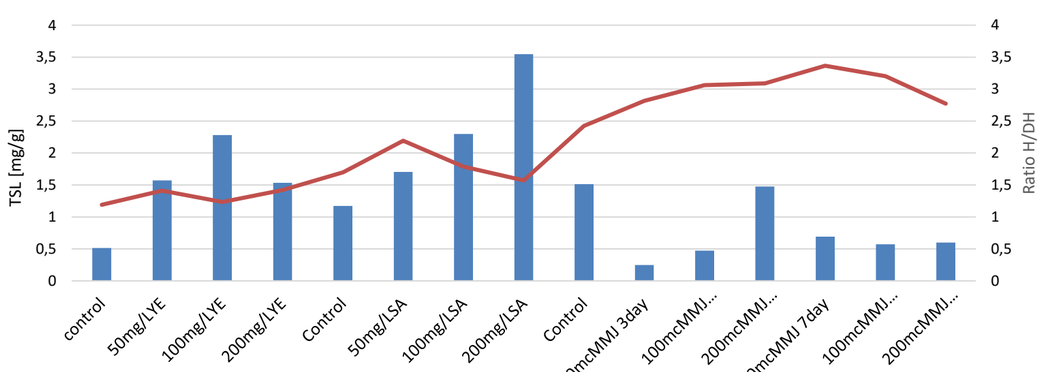


HPLC chromatograms of SL in *in vitro* micropropagated *A. montana* shoots at 225 nm

Results:

The results showed that salicylic acid and yeast extract enhanced the production of sesquiterpene lactones in the shoot culture compared to the untreated *in vitro* shoots. In this study, the elicitor methyl jasmonate was ineffective. All samples revealed the same lactone profile assessed by HPLC analysis. The maximum sesquiterpene lactone content was measured in micropropagated shoots treated with 200 µM salicylic acid. The treatment with 100 µM salicylic acid and 100 mg/l yeast extract was also effective. Helenalins were dominant in all tested *in vitro* samples. The main sesquiterpene lactones in the studied samples are methacryloyl esters of helenalin and 11α,13-dihydrohelenalin.

This is the first report to demonstrate higher production of sesquiterpene lactones in shoot cultures of *A. montana* by elicitation with salicylic acid and yeast extract.



Total SL content in *in vitro* micropropagated *A. montana* shoots and ratio H/DH