ACCUMULATION OF CAFFEOYLQUINIC ACIDS IN *ARNICA MONTANA* L. IN VITRO SHOOTS UNDER ELICITATION

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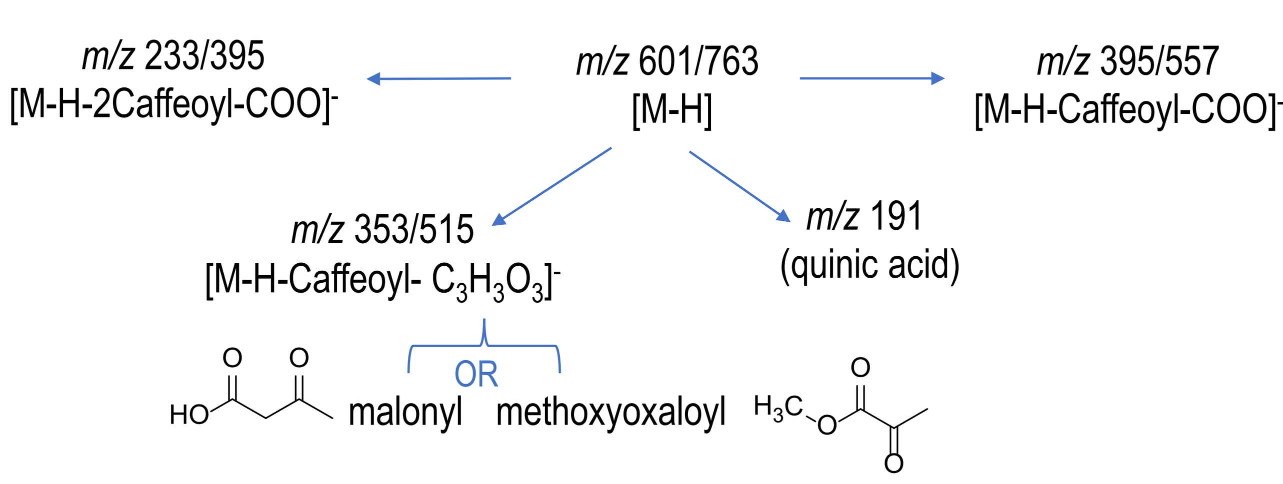
Selected *In vitro* micropropagated *A. montana* shoots

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

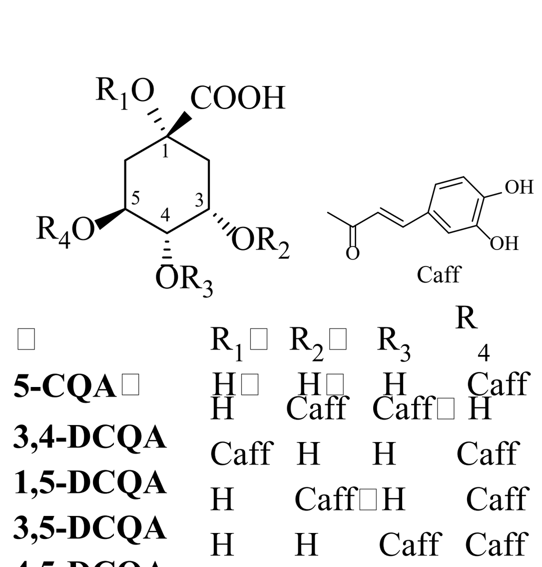
Table 1. Identification of the compounds in the methanol extract of *A. montana* shoots by UHPLC-MS/MS

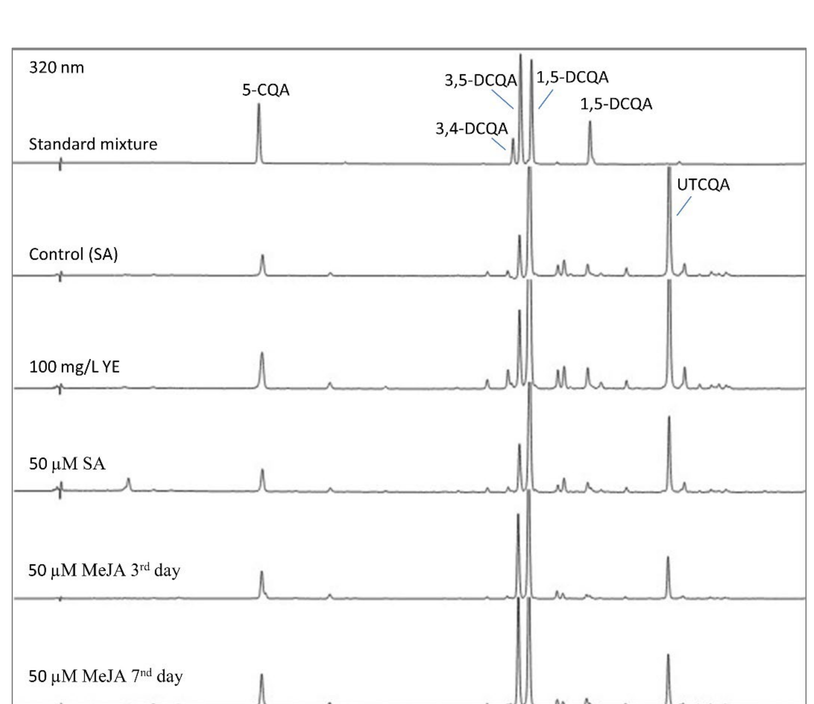
Rt, min	Compound	Molecular formula	$[M-H]^-$, m/z	MS/MS fragments
0.93	Quinic acid*	$C_7H_{11}O_6$	191.0555	**191**, 127, 85
1.00	Dihydroxybenzoic acid O-hexoside*	$C_{13}H_{15}O_9$	315.0732	315, 153, 152, 109, **108**
1.39	Hydroxy-methoxybenzoic acid O-hexoside*	$C_{14}H_{17}O_9$	329.0883	**167**, 152, 123, 108
1.43	Dihydroxybenzoic acid O- hexoside*	$C_{13}H_{15}O_9$	315.0728	315, 153, 152, 109, **108**
1.61	Syringic acid O-hexoside*	$C_{15}H_{19}O_{10}$	359.0992	359, **197**, 167, 153, 123
1.83	Neochlorogenic acid (3-CQA)**	$C_{16}H_{17}O_9$	353.0880	353, **191**, 179, 135
2.94	**Chlorogenic acid (5-CQA)****	$C_{16}H_{17}O_9$	353.0884	353, **191**, 179, 135
3.86	Caffeic acid**	$C_9H_7O_4$	179.0341	179, **135**
11.96	**3,4-Dicaffeoylquinic acid (3,4-DCQA)****	$C_{25}H_{23}O_{12}$	515.1202	353, 191, 179, **173**, 135
12.15	**1,5-Dicaffeoylquinic acid (1,5-DCQA)****	$C_{25}H_{23}O_{12}$	515.1203	353, **191**
12.19	Kaempferol 3-O-glucoside**	$C_{21}H_{19}O_{11}$	447.0938	447, 284, 255, **227**
12.53	**3,5-Dicaffeoylquinic acid (3,5-DCQA)****	$C_{25}H_{23}O_{12}$	515.1202	353, **191**, 179, 135
12.61	Methoxyoxaloyl dicaffeoylquinic acid/Malonyl dicaffeoylquinic acid***	$C_{28}H_{25}O_{15}$	601.1210	395, 353,335, **233**, 191, 179, 173, 162
12.86	Isorhamnetin hexoside*	$C_{22}H_{21}O_{12}$	477.1044	477, 315, 299, **271**, 243
13.98	**4,5-Dicaffeoylquinic acid (4,5-DCQA**) **	$C_{25}H_{23}O_{12}$	515.1201	353, **191**, 179, 135
14.77	Methoxyoxaloyl dicaffeoylquinic acid/Malonyl dicaffeoylquinic acid***	$C_{28}H_{25}O_{15}$	601.1213	395, 353, **233**, 191, 179, 173, 162
15.26	1,3,5-Tricaffeoylquinic acid (TCQA)*	$C_{34}H_{29}O_{15}$	677.1526	515, 353, **191**, 179, 161, 135
19.37	1,4,5-Tricaffeoylquinic acid (TCQA)*	$C_{34}H_{29}O_{15}$	677.1527	515, 353, 191, 179, **173**, 161, 135
19.4	3,4,5-Tricaffeoylquinic acid (TCQA)*	$C_{34}H_{29}O_{15}$	677.1529	515, 353, 191, **179**, 173, 161, 135
19.55	Methoxyoxaloyl tricaffeoylquinic acid/Malonyl tricaffeoylquinic acid(UTCQA)***	$C_{37}H_{31}O_{18}$	763.1532	557, 539, 515, **395**, 233, 191, 179, 173, 161, 135
21.26	Hispidulin*	$C_{16}H_{11}O_6$	299.0565	299, **284**
21.31	Methoxyoxaloyl tricaffeoylquinic acid/Malonyl tricaffeoylquinic acid (UTCQA)***	$C_{37}H_{31}O_{18}$	763.1533	515, **395**, 233, 191, 179, 173, 161, 135
22.37	Dihydroxy-dimethoxyflavone*	$C_{17}H_{13}O_6$	313.0721	313, 298, **283**, 255

* Tentative identification; ** Compariosn with standards; *** MS data did not allow the unambiguous identification



Six caffeoylquinic acids were chosen for monitoring the impact of elicitors by HPLC-PDA. The content of chlorogenic (5-CQA), 3,4-, 1,5-, 3,5- and 4,5-dicaffeoylquinic (DCQA) acids in *A. montana* shoots was determined from the regression equations of the corresponding calibration curves, while the quantity of the undetermined tricaffeoylquinic acid derivative (UTCQA) was calculated as mg equivalents of 1,5-DCQA per g DW.





Stacked HPLC chromatograms of CQAs in *in vitro* micropropagated *A. montana* shoots at 320 nm

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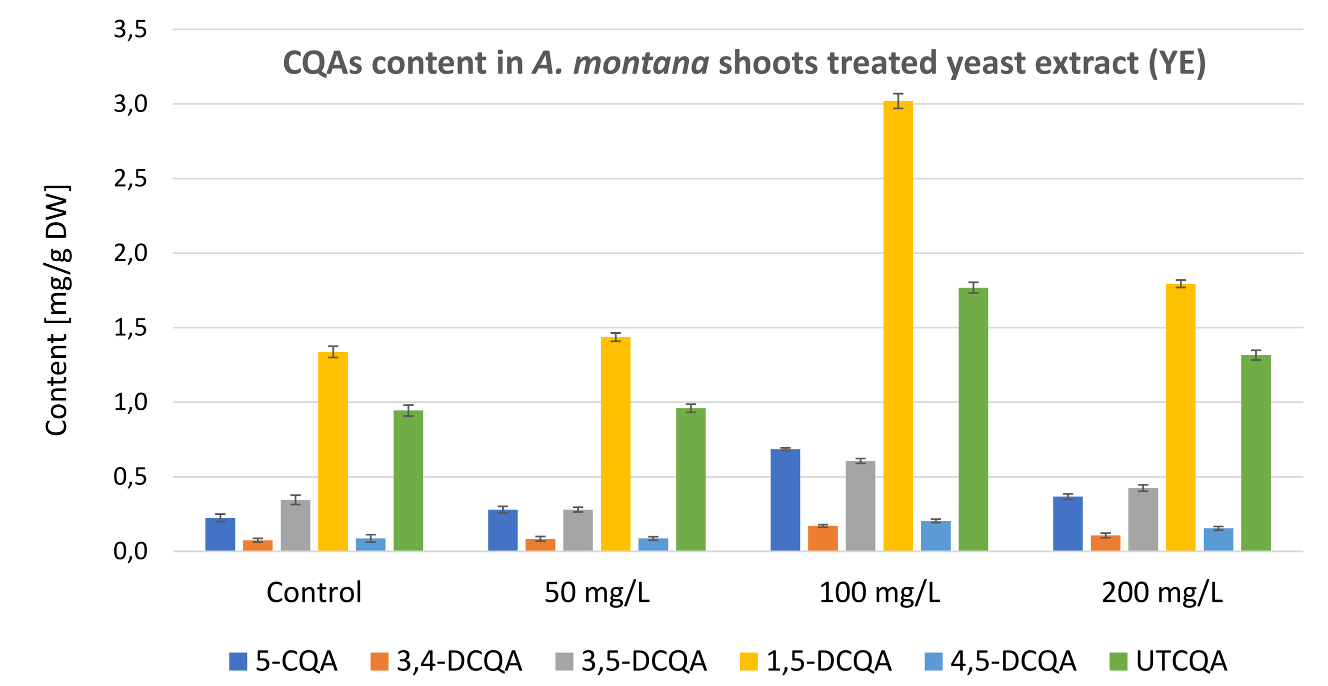
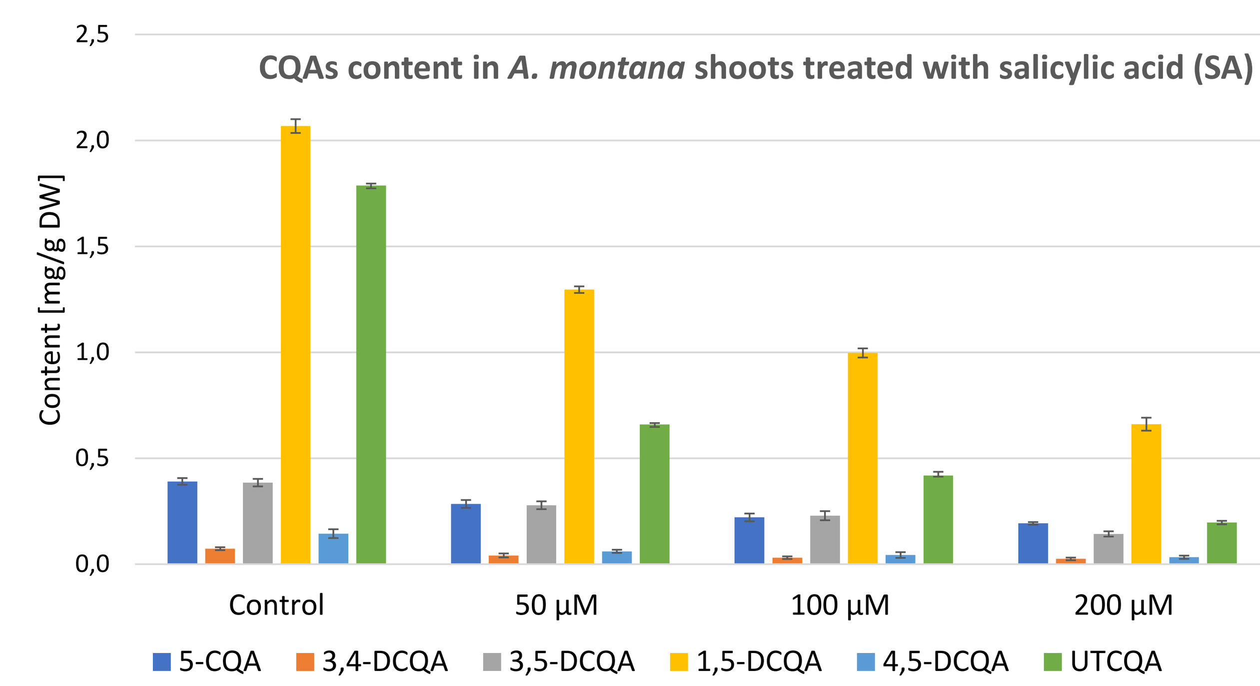
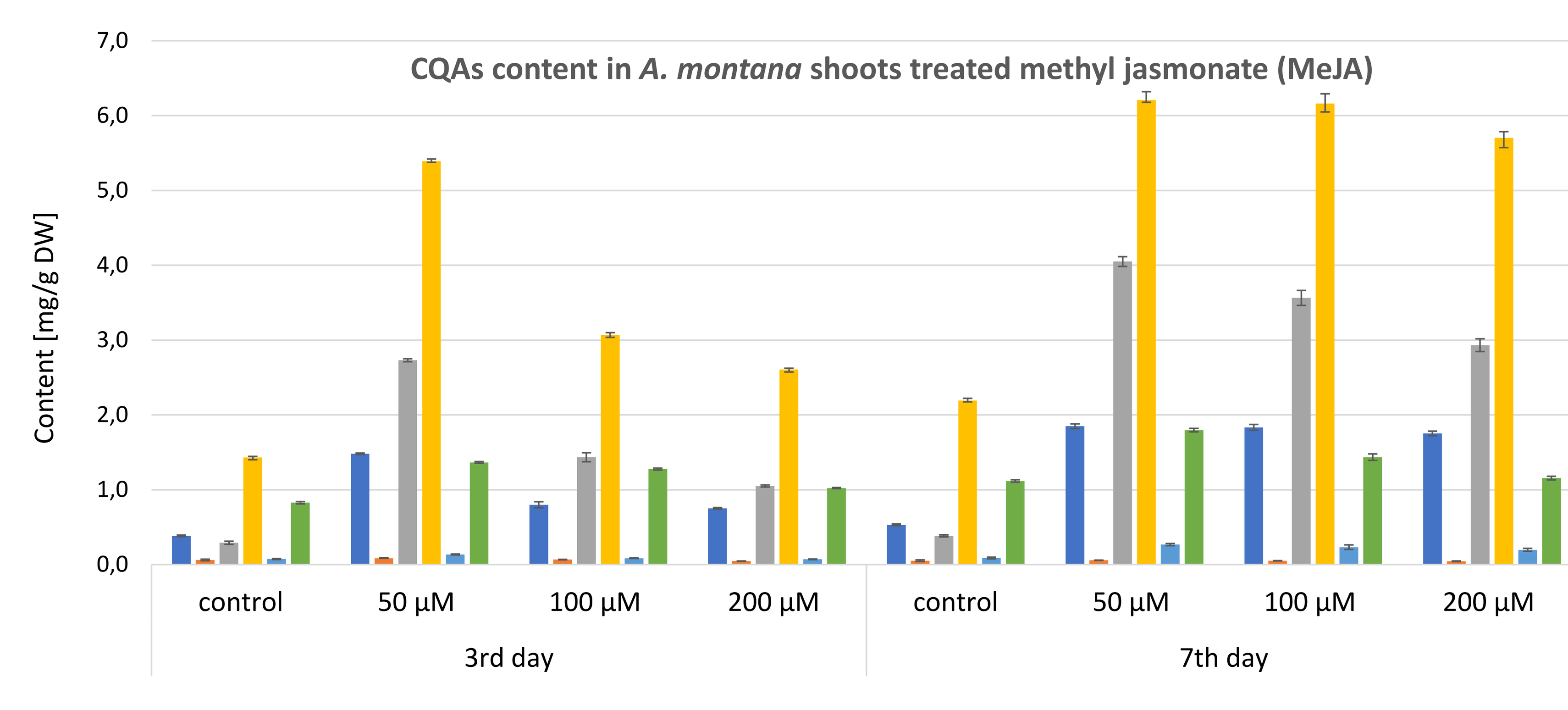
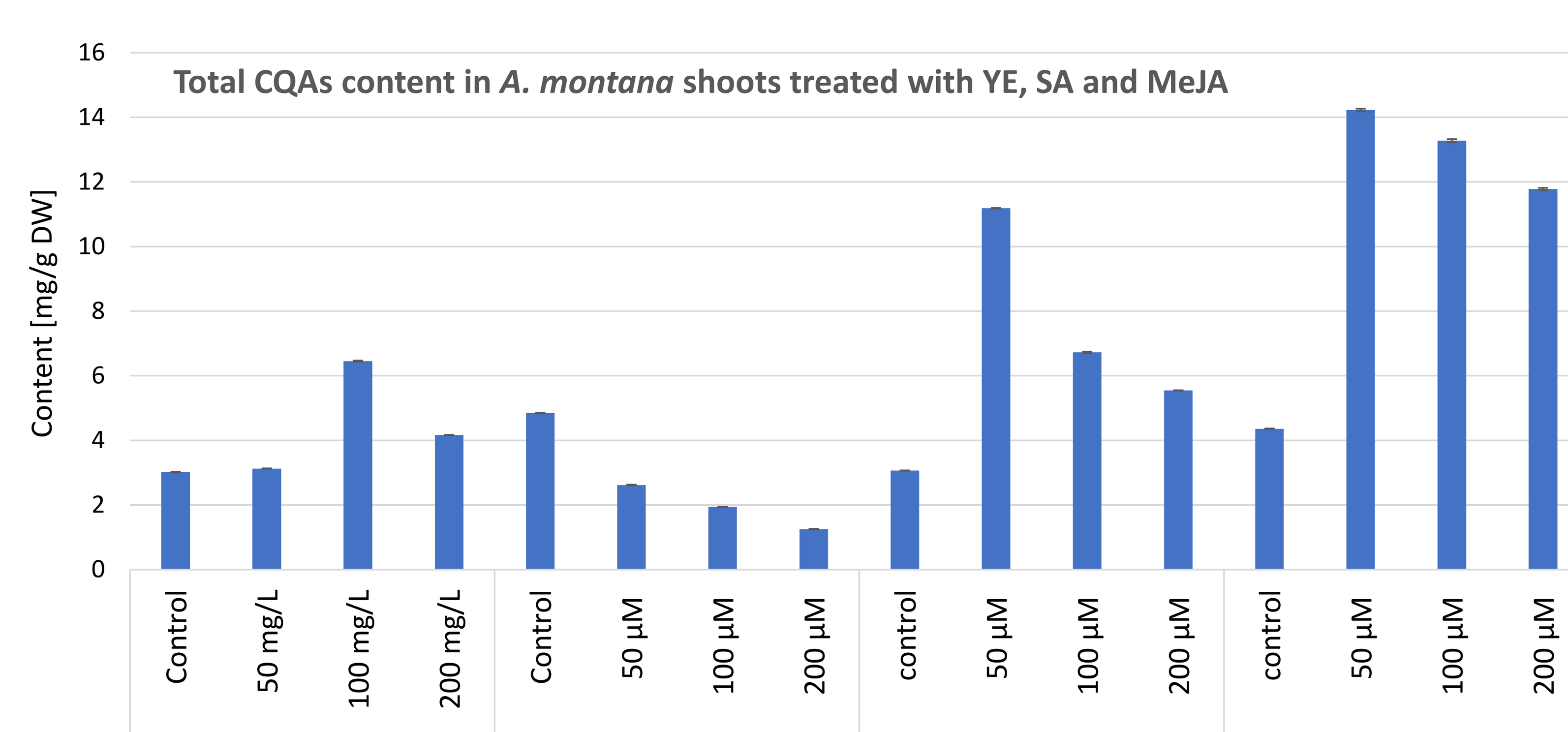
Arnica montana L. (Asteraceae) is an herbaceous perennial plant growing on northern and central European highlands. The plant is a rich source of secondary metabolites - sesquiterpene lactones, thymol derivatives, phenolic acids, flavonoids, etc. The species has a number of activities - antibacterial, antitumor, antioxidant, anti-inflammatory, antifungal, immunomodulatory, etc. The increased demand for *A. montana* herbal material highlights the need for the development of alternative methods to increase the production of biomass and bioactive compounds. Elicitation is a proven strategy for improving the secondary metabolite yields in numerous plant species.









The results showed that methyl jasmonate could effectively stimulate the caffeoylquinic acids accumulation. The highest total content of caffeoylquinic acids was measured in shoots harvested on the 7th day after treatment with 50 µM MeJA compared to control untreated shoots. The synthesis of caffeoylquinic acids was enhanced also with the application of yeast extract but inhibited under salicylic acid treatment. Our results provide insight into the increase in phenolic compounds in this highly valued medicinal plant.