Effect of elicitation on the content of triterpenoid saponins in suspension culture of soapwort *Saponaria officinalis* L.

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Introduction

Saponaria officinalis is a perennial plant from the *Caryophyllaceae* family that has long been valued for its high content of triterpenoid saponins. These amphiphilic glycosides are responsible for the plant's characteristic foaming properties and have traditionally been exploited as natural detergents, emulsifiers and foaming agents. Beyond its historical and ethnobotanical uses, soapwort has gained increasing attention in biotechnology and applied biosciences as a sustainable source of bioactive saponins. These compounds exhibit a broad spectrum of biological activities, including antimicrobial, antifungal, cytotoxic and anti-inflammatory effects, making them promising candidates for pharmaceutical, cosmetic and agricultural applications. In plant biotechnology soapwort is also studied as a model system for the production of valuable secondary metabolites in suspension cultures. *In vitro* cultures provide a controlled platform for studying metabolic pathways and for enhancing saponin biosynthesis through elicitation strategies, such as treatment with jasmonates or chitosan. Such approaches enable the optimization of metabolite yield without relying on field cultivation, supporting the development of sustainable biotechnological production systems for high value natural products.

Fig1.Morphological characteristics of *Saponaria officinalis*

Experimental

Plant material: Suspension cultures of *Saponaria officinalis* L. were established according to the procedure described for *Calendula officinalis* L. (Długosz et al. 2018). Four culture lines were initiated from the following primary explants: apical bud (B), cotyledon (C), hypocotyl (H), and embryonic root (R), which were excised from sterile 7–8-day-old seedlings. The established cultures were maintained in darkness at 24 ± 2 °C on a rotary shaker at 120 rpm. Subculturing was performed every 2 weeks.

Elicitation: For jasmonic acid (JA) elicitation, suspension cultures (7.5 g inoculum/100 mL medium in 300 mL flasks) were treated after 3 days with JA to a final concentration of 50 µM. Control cultures were supplemented with an equal volume of sterile 70% (v/v) ethanol. For chitosan (Ch) elicitation, cultures were prepared analogously; Ch was added after 6 days to a final concentration of 25 mg/L, while control cultures received sterile water (pH 4.0). All cultures were analyzed after 14 days of cultivation.

Saponin extraction: Cultures were separated into biomass and medium via filtration using a Büchner funnel under reduced pressure. The biomass, air-dried at room temperature, was crushed and extracted with boiling methanol. The medium was extracted three times with *n*-butanol. The resulting extracts were evaporated to dryness and hydrolyzed with 10% HCl in methanol. Triterpenic acids were then extracted with ethyl ether, purified by TLC, and methylated.

GC/GC-MS Quantitative analysis was performed using a Shimadzu GC-2014 gas chromatograph equipped with a ZB-1 capillary column (30 m × 0.25 mm × 0.25 µm, Phenomenex) and a flame ionization detector (FID). Separation was carried out at a column temperature of 270 °C, with injector and detector temperatures set to 290 °C. Nitrogen served as the carrier gas at a flow rate of 1.2 mL/min. Qualitative analysis was performed using an Agilent Technologies 7890A gas chromatograph coupled with a 5975C mass spectrometric detector (GC–MS). Separation was achieved on an HP-5MS UI capillary column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies). Helium was used as the carrier gas at 1 mL/min. Analyses were performed either under isothermal conditions at 280 °C or using a temperature program (160 °C for 2 min, ramped to 280 °C at 5 °C/min, and held for 44 min). Additional parameters: injector/detector 290 °C; MS transfer line 275 °C; quadrupole 150 °C; ion source 230 °C; EI at 70 eV; m/z range 33–500. Gas flows for FID were 30 mL/min for H_2 and 400 mL/min for air. Compounds were identified by comparing mass spectra with Wiley 9th Edition and NIST 2008 libraries (SW Version 2010).

Results

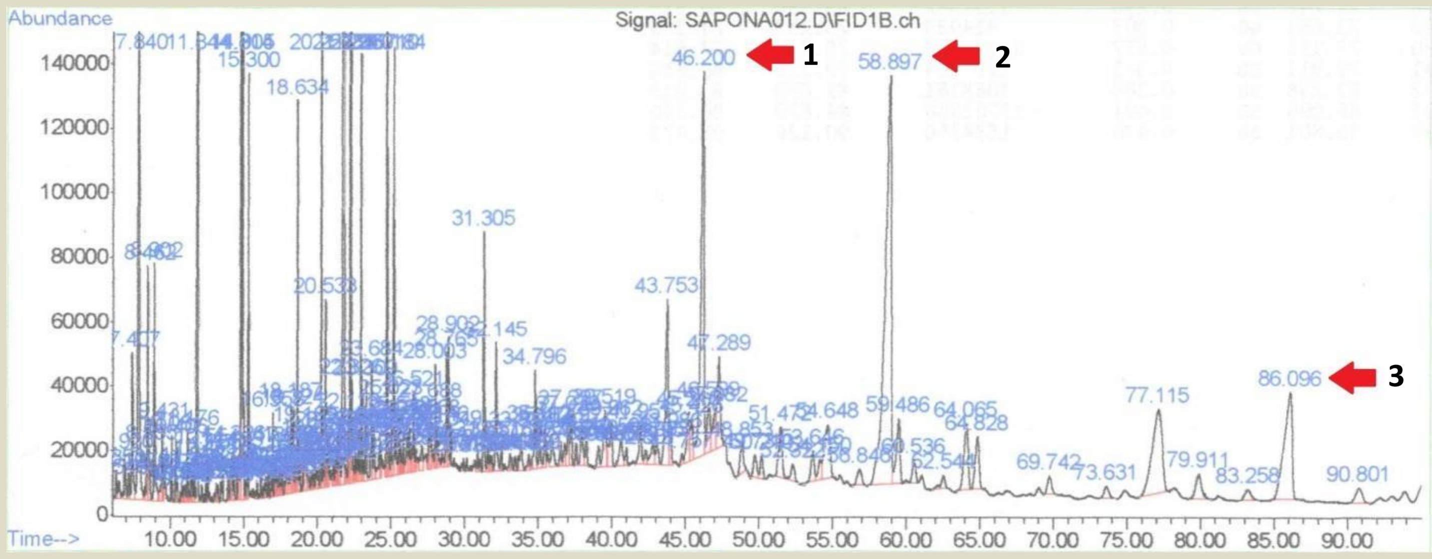


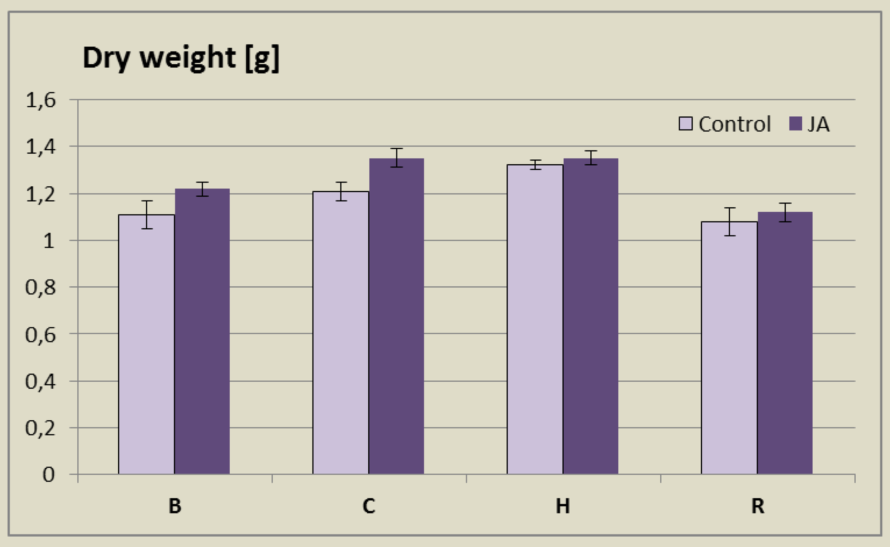
Fig 2. FID chromatogram of the TA fraction obtained from suspension culture (R) elicited with JA.

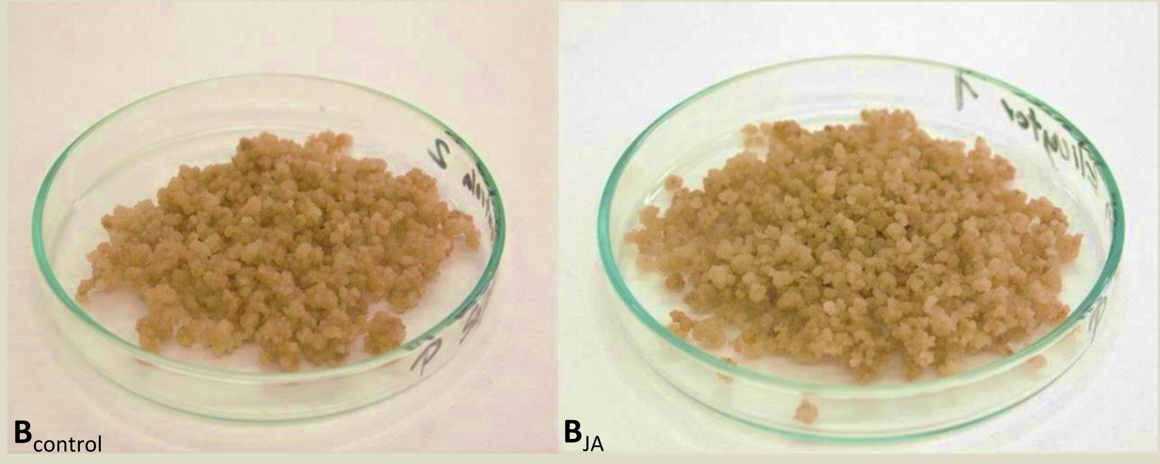
Triterpenic acid (TA) fractions were analyzed based on spectral data, chromatographic mobility, and retention times obtained by mass spectrometry. Analysis of triterpenoid saponin aglycones in the form of their methyl esters from soapwort suspension cultures revealed the presence of several peaks with relatively diverse retention times, ranging from 46.2 min to 85.9 min (Fig.2).

The methodology applied during sample preparation may indicate that the compounds forming the described peaks in the obtained chromatograms underwent partial degradation, for example as a result of acid hydrolysis. In order to compare the saponin content among the individual cultures, and particularly to evaluate the effect of elicitation on triterpenoid biosynthesis, these compounds were designated for further quantitative analyses as aglycones 1, 2, and 3 (1-methyl ester of oleanolic ester; 2-methyl ester of 3-acetyl-olean-12,15-dien-28-oic acid; 3-methyl ester of 2,3,23-trihydroxyolean-12-en-28-oic acid)

Content of TA (µg/g DW) in suspension culture elicited with JA

Origin of culture	Aglycone 1	Aglycone 2	Aglycone 3	Total TA content
B control	20,97	28,39	51,29	100,65
B JA	25,70	206,40	246,57	478,66
C control	38,25	69,55	22,69	130,49
C JA	229,21	441,08	118,74	789,04
H control	17,93	146,08	40,37	204,38
H JA	148,37	566,14	57,14	771,65
R control	36,01	51,64	27,40	115,05
R JA	87,01	182,59	62,74	332,34

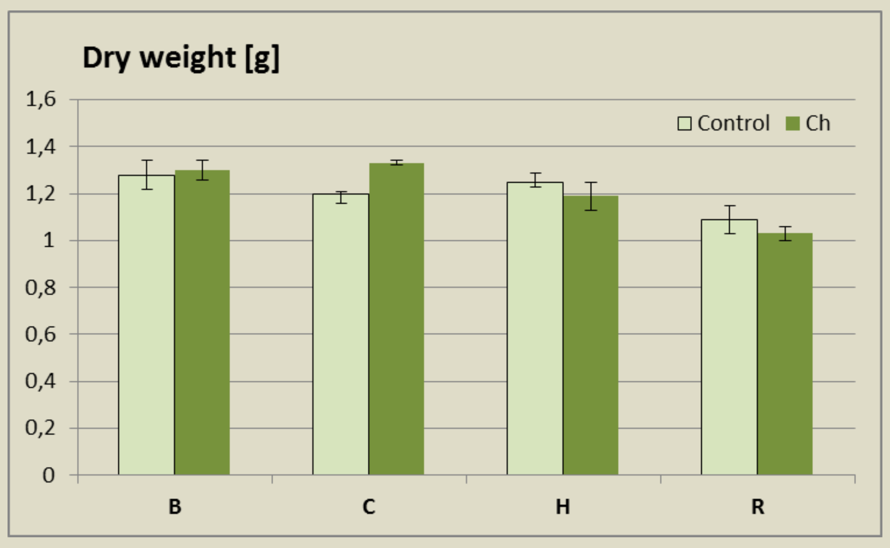


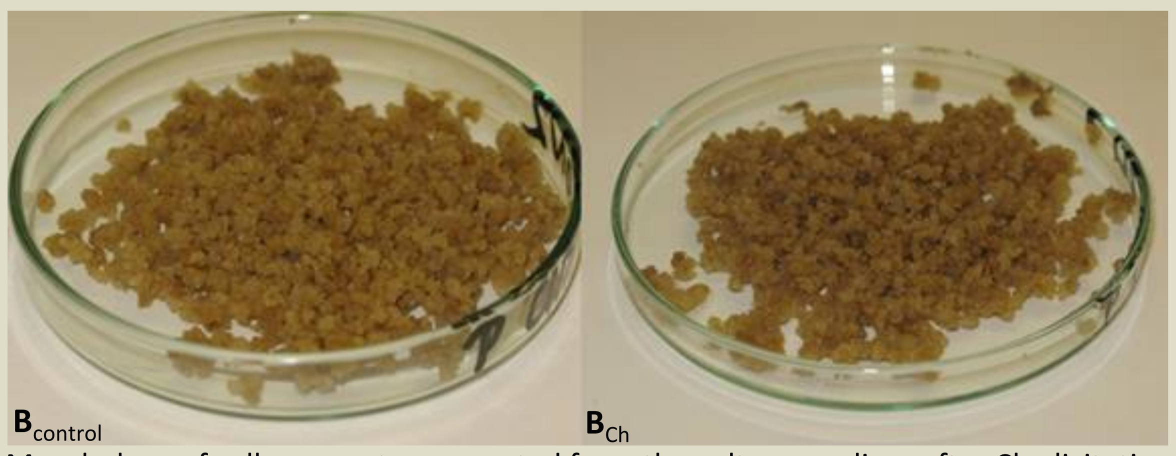


Morphology of cell aggregates separated from the culture medium after JA elicitation

Content of TA (µg/g DW) in suspension culture elicited with Ch

Origin of culture	Aglycone 1	Aglycone 2	Aglycone 3	Total TA content
B control	13,46	20,10	33,55	67,11
B Ch	14,43	37,06	61,53	113,02
C control	12,80	8,54	14,23	35,57
C Ch	12,94	12,38	20,03	45,35
H control	12,01	11,96	28,91	52,89
H Ch	17,43	12,91	25,22	55,55
R control	26,80	3,52	6,52	36,84
R Ch	34,15	4,06	8,47	46,68





Morphology of cell aggregates separated from the culture medium after Ch elicitation

Conclusions

- The most effective elicitation was obtained using 50 µM jasmonic acid, which resulted in a substantial increase in triterpenoid accumulation, ranging from 2.89- to 6.05-fold depending on the origin of the culture, while simultaneously showing no negative impact on growth dynamics or morphological characteristics of the cultures
- Treatment with 25 mg/L chitosan led to only a modest stimulation of triterpenoid biosynthesis, with an observed increase in their content ranging from 1.05- to 1.68-fold, indicating a considerably weaker elicitation effect compared to jasmonic acid
- Overall, jasmonic acid proved to be a significantly more potent elicitor than chitosan in enhancing triterpenoid production in the studied cultures, without compromising culture viability or morphology

Długosz M. Markowski M. & Pączkowski C. (2018) Source of nitrogen as a factor limiting saponin production by hairy root and suspension cultures of *Calendula officinalis* L. Acta Physiol Plant **40**, 35