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EXPERIMENTAL PAPER

Pentacyclic triterpenoids and polyphenols accumulation in cell suspension culture of *Chaenomeles japonica* (Thunb.) Lindl. ex Spach

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Summary

Introduction: Callus and cell suspension cultures are widely applied in investigation of production of high-value secondary metabolites, which may be used as cosmeceuticals, nutraceuticals and pharmaceuticals. Plant cell cultures are promising alternative to intact plant sources for the production of plant-derived drugs of industrial importance.

Objective: The aim of the study was to (i) initiate the cell suspension culture of *Chaenomeles japonica* from homogenous and uniform callus, (ii) stabilize the selected line and (iii) verify its ability to produce the desired groups of secondary metabolites – pentacyclic triterpenoids and polyphenols.

Methods: To establish a cell suspension culture, stabilized and homogeneous callus was selected. Cell cultures were systematically passaged every 2 weeks to fresh liquid medium with the same composition. Biomass from cultures at the growth phase and stationary phase was designated for phytochemical research. UHPLC-DAD-MS analyzes were performed. At the same time, their macroscopic and microscopic observations were carried out.

Results: Cells of suspension culture line A2 were characterized by the intense divisions. Cell culture extracts (both from the growth phase and stationary phase) contained pentacyclic triterpenoids. In addition, phe-

nolic compounds (chlorogenic acid and proanthocyanidins type B) and in a small amount also epicatechin are present in the extract of the cells harvested from the growth phase. In the present studies, three pentacyclic triterpenoids were detected and quantified in the extracts of cell suspensions and callus line A2. Ursolic and oleanolic acids were the main triterpenoids in the studied extracts. The cell suspension culture from the growth phase exhibited the highest content of ursolic, oleanolic, and betulinic acid (separately and together).

Conclusion: The cell suspension culture of *Chaenomeles japonica* is a promising source of pentacyclic triterpenoids.

Key words: ***Japanese quince, callus, cell suspension culture, chlorogenic acid, ursolic acid, oleanolic acid***

Słowa kluczowe: ***pigwowiec japoński, kalus, kultura zawiesinowa, kwas chlorogenowy, kwas ursolowy, kwas oleanolowy***

INTRODUCTION

Chaenomeles japonica (Thunb.) Lindl. ex Spach belongs to the subfamily *Maloideae* of the *Rosaceae*. *C. japonica*, a dwarf shrub naturally occurs in Central and South Japan [1]. In Europe, the plant was introduced in XIX century, and it has been appreciated for its ornamental value [2]. The high content of vitamin C, organic acids, phenolic compounds, dietary fiber, pectin, simple sugars of the fruits of *C. japonica*, make them well suited for industrial processing [3, 4].

Phytochemical studies have yielded secondary metabolites in selected raw materials of *C. japonica* – epicatechin, leucoanthocyanin, monoterpene glucosides, and roseoside in fruits; flavonol glycosides and epicatechin in leaves; and three pentacyclic triterpenoids (ursolic, oleanolic, and pomolic acids), daucosterol, prunasin and epicatechin in roots [5]. Among representatives of the genus *Chaenomeles*, in traditional medicine of Far East, the fruits of *Chaenomeles speciosa* were used for centuries as 'Mugua', while the fruits of *C. japonica* have been used as an astringent and in stomach diseases [6]. Moreover, the extract from seeds of *C. japonica* is on the list of cosmetic ingredients approved for use in the European Union acting as a supplement that nourishes the skin [7].

There is a growing interest in pentacyclic triterpenoids due to their interesting potential biological and pharmaceutical properties. Pentacyclic triterpenoids are a class of compounds occurring in several medicinal plants. Oleanolic acid and its isomer – ursolic acid have long been known to be anti-inflammatory, hepatoprotective, and anti-hyperlipidemic in traditional medicine of Asia. Moreover, recently there are many studies on the antiviral, antimicrobial and anticancer activities [8]. Betulinic acid is highly regarded for its anti-HIV-1 activity and cytotoxicity against cancer lines, but also was reported for its cosmetic properties [9].

Polyphenols are the most common bioactive compounds widely distributed in plants. They exhibit a great diversity and are divided into several classes i.e. phenolic acids, flavonoids, and proanthocyanidins. The phenolic constituents have a wide range of biological activities, mainly attributed to their antioxidant potential [10]. The main phenolic acid occurring in *C. japonica* is chlorogenic acid, which is well-known polyphenol exerting many biological activities i.e. antioxidant, antimicrobial, antipyretic, hepatoprotective, cardioprotective, anti-inflammatory, anticarcinogenic, and glucose and lipid metabolism modulatory [11].

Callus and cell suspension cultures are widely applied in investigation of high-value secondary metabolites production, which may be used as cosmeceuticals, nutraceuticals and pharmaceuticals. Plant cell cultures, with the continuous and reliable accumulation of desired bioactive compounds, are promising alternative to intact plant sources for the production of plant-derived drugs of industrial importance. In comparison with the conventional cultivation of plants, plant cell cultures offer an independent of geographical and environmental factors supply of uniform biomass with enhanced production of active constituents. Plant *in vitro* cultures ensures a rational utilization of biodiversity [12, 13].

The aim of the study was to (i) initiate the cell suspension culture of *Chaenomeles japonica* from homogenous and uniform callus, (ii) stabilize the selected line and (iii) verify its ability to produce desired groups of secondary metabolites – pentacyclic terpenoids and polyphenols. To our knowledge, this is the first report about production of polyphenols and pentacyclic triterpenoids in callus and cell suspension cultures of *Chaenomeles japonica*.

MATERIAL AND METHODS

Plant material

A voucher specimen of *C. japonica* was deposited in the herbarium of Department of Pharmaceutical Botany and Plant Biotechnology, Poznań, Poland, under the number 1526/2016.

The explants were taken from an old shrub growing in open environment conditions in Poznań. The seeds were surface disinfected and transferred to MS [14] medium to obtain the aseptic seedlings, which were the sources of explants for micropropagation (according to the paper [15]). Briefly, the mature seeds were washed under tap water for 1 min and then rinsed in distilled water for 5 min followed by submerging in 70 % (v/v) EtOH for 30 s. They were rinsed in sterilized water and placed on lignin in thermostat (26°C) for 24 h. The accurate disinfection step was to treat the seeds with commercial bleach at a concentration of 50% for 20 min. Then they were rinsed five times in sterilized distilled water and transferred to MS medium to obtain the aseptic seedlings. After four weeks *in vitro*-germinated seedlings were the sources of explants for proliferation of the new shoots. The media were enriched with BA 1.0 mg·l^{-1} and IAA 1.0 mg·l^{-1}. The shoots were multiplied by repetitive transfer of explants to the freshly prepared medium every six weeks.

The media variants for callus initiation and proliferation were initially tested and the four callus lines with the best growth indexes were selected for the further investigations (according to the paper [16]). Briefly, callus was inducted on young leaves (blades and petioles) of micropropagated plantlets. The media were enriched with 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1}, 2,4-D 1.0 mg·l^{-1} + KIN 1.0 mg·l^{-1}, 2,4-D 2.0 mg·l^{-1} + KIN 1.0 mg·l^{-1}, 2,4-D 0.5 mg·l^{-1} + NAA 0.05 mg·l^{-1}, 2,4-D 1.0 mg·l^{-1} + NAA 0.1 mg·l^{-1}, 2,4-D 2.0 mg·l^{-1} + NAA 0.2 mg·l^{-1}, 2,4-D 1.0 mg·l^{-1}, 2,4-D 2.0 mg·l^{-1}, DIC 1.0 mg·l^{-1}, or DIC 2.0 mg·l^{-1} (Table 1). Subcultures were performed at 4-week intervals. Callus initiation and its development were first observed visually, and then when callus culture became stabilized (5-7 passages), the growth callus index was calculated (Table 2) by the following equation: Growth index = (final cell weight – initial cell weight)/initial cell weight.

The four lines of stabilized callus were selected for cell suspension culture initiation: **A1** – callus from MS + 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1} (light), **A2** – callus from MS + 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1} (darkness), **B** – callus from MS + 2,4-D 2.0 mg·l^{-1} + NAA 0.2 mg·l^{-1}, and **C** – callus from MS + DIC 1.0 mg·l^{-1}.

Culture conditions

Each medium for callus and cell suspension growth (the same phytohormonal variant) was adjusted to pH 5.8 before autoclaving at 121°C/105 kPa for

Table 1

Induction of *Chaenomeles japonica* callus from leaves placed on various variants of MS medium (PGRs – Plant Growth Regulators)

MS with PGRs [mg·l^{-1}]		Callus parameters		
		Induction	Colour	Structure
2,4-D 1.0	-	+	yellow	friable, hard
2,4-D 2.0	-	+	yellow	friable, hard
2,4-D 1.0	KIN 0.1	+++	yellow, pink–yellow	friable, soft
2,4-D 1.0	KIN 1.0	++	yellow, yellow–brown	friable, hardened
2,4-D 2.0	KIN 1.0	++	yellow, yellow–brown	friable, hardened
2,4-D 0.5	NAA 0.05	++	yellow, white–yellow	slightly watery
2,4-D 1.0	NAA 0.1	++	yellow, white–yellow	friable
2,4-D 2.0	NAA 0.2	+++	white	downy, friable
DIC 1.0	-	+++	pink	slightly watery
DIC 2.0	-	++	pink, pink - brown	nodular

2,4-D – dichlorophenoxyacetic acid; DIC – dicamba; KIN – kinetin; MS – Murashige and Skoog medium; NAA – 1-naphthaleneacetic acid

Table 2

Growth parameters of selected lines of *Chaenomeles japonica* callus growing on various variants of MS medium (PGRs – Plant Growth Regulators)

Plant material	MS with PGRs [mg·l^{-1}]		Callus growth index [%] ± SE*			
			Passage 5	Passage 6	Passage 7	Mean
Callus line A1 light	2,4-D 1.0	KIN 0.1	404.04±58.90	831.51±48.83	867.43±13.58	854.26±13.58
Callus line A2 dark	2,4-D 1.0	KIN 0.1	503.94±58.60	907.21±1.82	978.59±14.45	960.46±14.24
Callus line B	2,4-D 2.0	NAA 0.2	372.32±30.67	804.36±44.15	824.28±12.66	808.56±38.56
Callus line C	DIC 1.0	–	418.82±16.66	458.66±9.77	480.59±5.98	480.59±5.98

2,4-D – dichlorophenoxyacetic acid; DIC – dicamba; KIN – kinetin; MS – Murashige and Skoog medium; NAA – 1-naphthaleneacetic acid

20 min. Plant growth regulators (PGRs) originated from Sigma-Aldrich (St. Louis, MO, USA). The cultures were maintained in a growth chamber (16/8 h photoperiod, 55 μmol m^{-2}s^{-1} light, temp. 21 ± 2°C).

Cell suspension culture initiation and maintenance

To establish a cell suspension culture, stabilized, homogeneous, friable, light and slightly hydrated callus was selected. Under aseptic conditions, the inoculum was experimentally selected (± 1 g) and transferred to a 250 cm^3 flask, with 50 ml of liquid medium (Fig. 1, 2). Callus was wiped through a sieve to crush the tissue. The flasks of cell suspension were placed in a growth chamber, on a rotary shaker (110 rpm), in the dark. Cell cultures were systematically passaged (5 ml inoculum) every 2 weeks to fresh liquid medium with the same composition. Biomass was collected between 8th and 10th passage for phytochemical studies. Biomass from cultures at the growth phase (GP; light colour; 8th-10th day) and stationary phase (SP; dark colour; 20th day) was designated for phytochemical research. The cells were frozen and then lyophilized to obtain material for further investigation. At the same time, their macroscopic and microscopic (optical microscope Leica IMS500 HD) observations were carried out.

Phytochemical analysis

Biomass of callus and cell suspension culture was taken for phytochemical analysis. In order to prepare samples, the *in vitro* culture material was frozen and then subjected to lyophilization. The samples (100 mg) were ground in a mortar and extracted with 80% (*v/v*) methanol (HPLC isocratic grade, Merck Millipore, Darmstadt, Germany) using the accelerated solvent extraction system (ASE 200, Dionex, Sunnyvale, CA, USA). Extraction was carried out at 100°C, operating pressure was 10.3 MPa. The extracts were evaporated to dryness, suspended in 5% MeOH, and subjected to solid phase extraction (SPE) on a Waters SepPak Classic cartridge equilibrated with 5% MeOH. The analytes were eluted with 95% MeOH, evaporated to dryness and reconstituted in 3.000 ml of 90% MeOH. The samples were then stored in -20°C and, before the analyses, they were centrifuged at 23000 x g for 15 min. UHPLC-DAD-ESI-MS analyzes were performed using an ACQUITY UPLC® chromatographic system (Waters Corp., Milford, MA, USA), equipped with a triple quadrupole mass detector. For the determination of the content of oleanolic, ursolic and betulinic acids, an ACQUITY HSS C18 column (2.1 × 100 mm, 100 Å, 1.8 μm; Waters) was used, flow rate was 0.400 ml min^{-1} (30°C), the injection volume was 2.5 μl, the isocratic elution with 80% methanol (HPLC gradient grade, Merck Millipore) containing 0.1% formic acid (LC-MS grade, Merck Millipore) was applied (11.9 min). The column was subsequently washed with 99% MeOH (0.1% formic acid) (12-13 min), then the initial conditions were restored (13.1-15.0 min). Detection of the acids by mass spectrometry was carried out in the negative ionization mode, using the Selected Ion Monitoring (SIM) method. The following MS settings were applied: capillary voltage was 2.8 kV; cone voltage 80 V; source temperature 140°C, desolvation temperature 350°C, cone gas flow (nitrogen) 100 l·h^{-1}, desolvation gas flow 800 l·h^{-1}. Content of ursolic and oleanolic acid in the investigated samples was determined by external calibration, using authentic standards (ursolic acid and oleanolic acid Sigma-Aldrich: 6 solutions

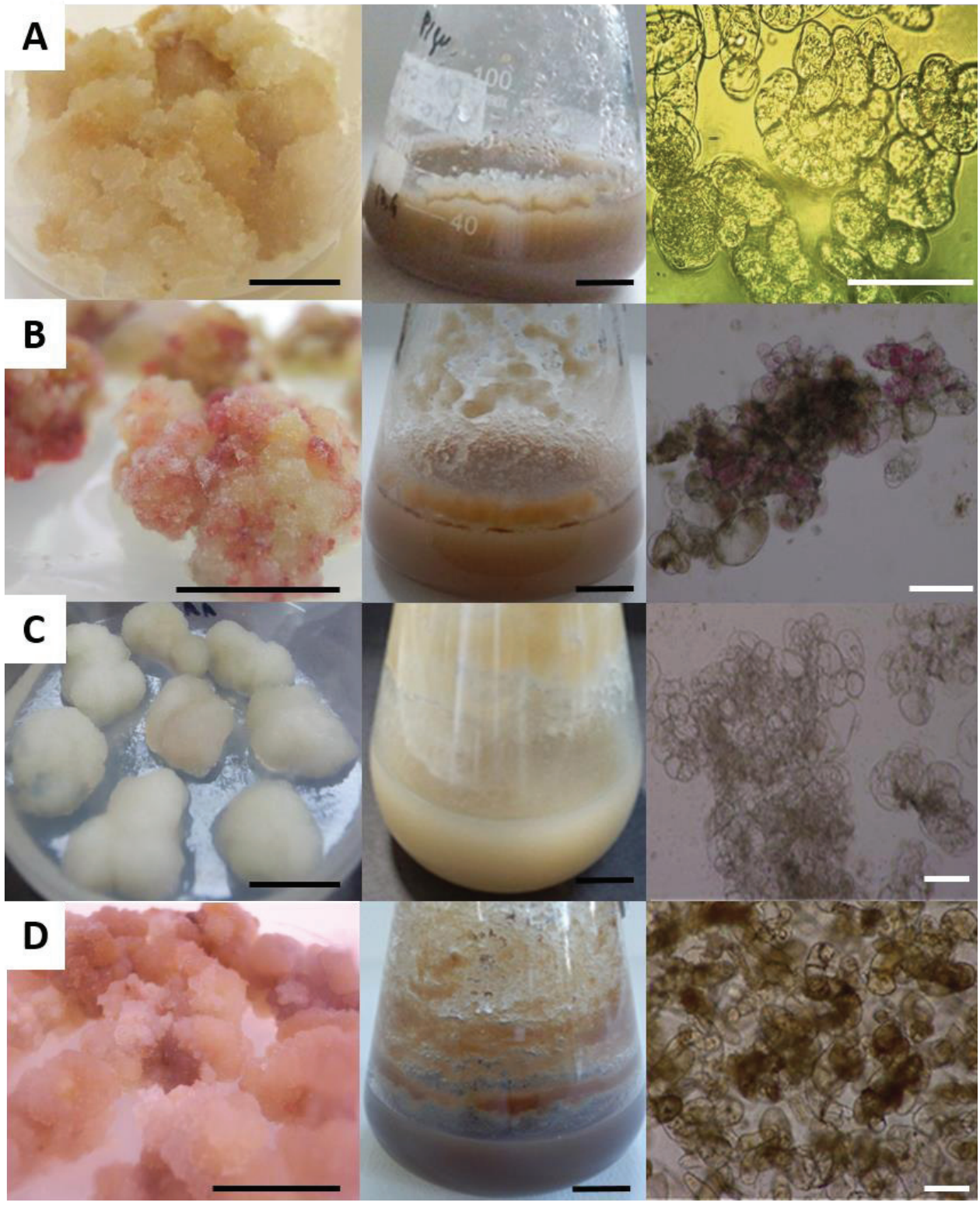


Figure 1

Macroscopic and microscopic images of callus and cell suspension cultures of *Chaenomeles japonica* growing (A) in the dark on MS with 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1}; (B) in the light on MS with 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1}; (C) in the dark on MS with 2,4-D 2.0 mg·l^{-1} + NAA 0.2 mg·l^{-1}; (D) in the dark on MS with DIC 1.0 mg·l^{-1} (black scale = 1 cm; white scale = 500 μm)

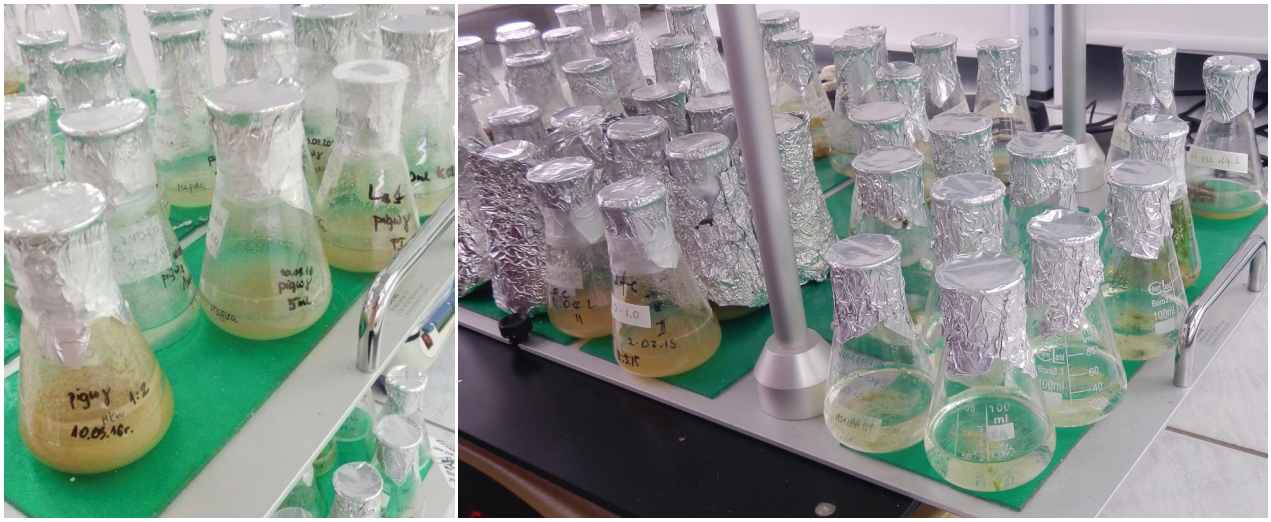


Figure 2

Cell suspension cultures of *Chaenomeles japonica* on a rotary shaker in a growth chamber

1.0 – 50 μg·ml^{-1}, y = -456.02x^2 + 52103x + 70463, R^2 = 0.991; ursolic acid: 6 solutions 1.0 – 50 μg·ml^{-1}, y = -339.1x^2 + 40079x + 67659, R^2 = 0.990). Betulinic acid content was expressed as oleanolic acid equivalent. For testing the qualitative composition of samples and determining the content of phenolic compounds, an ACQUITY BEH C18 column (2.1 × 100 mm, 130 Å, 1.7 μm; Waters) was used, gradient elution using acetonitrile (LC-MS grade, Merck Millipore) with 0.1% formic acid (as above) and 0.1% formic acid in water as mobile phase components (0.500 ml·min^{-1}, 50°C). The injection volume was 2.5 μl. The following elution program was applied: 0 – 0.5 min, 7% B; 0.5 – 11.9 min, 7-80% B; 11.9 – 12.0 min, 80-95% B; 12.0 – 13.0, 95% B; 13.0 – 13.1 min, 95-7% B; 13.1 – 15 min, 7% B. The MS analysis was carried out using the scanning method in the positive and negative ionization mode. The MS settings for the negative ionization mode were as follows: capillary voltage was 2.8 kV; cone voltage 45 V; source temperature 140°C, desolvation temperature 350°C, cone gas flow (nitrogen) 100 l·h^{-1}, desolvation gas flow 800 l·h^{-1}; positive ionization: capillary voltage was 3.1 kV, cone voltage 60 V, other settings – as above. Constituents of the investigated extracts were tentatively identified and classified on the basis of their UV spectra and/or MS data. Chlorogenic acid and other phenolics were quantified or semi-quantified on the basis of UV chromatograms, using a calibration curve of chlorogenic acid (Sigma-Aldrich; 6 solutions 2.3 – 184 μg·ml^{-1}, y = 182.94x - 560.94, R^2 = 0.9981). Contents of other phenolics were expressed as chlorogenic acid equivalents.

Extractions and analyzes were performed in triplicate, the presented results are means with standard deviation.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

Callus initiation took place from the cut edges of the leaf explants of *Chaenomeles japonica* on all tested media. Some combinations of plant growth regulators stimulated callus formation – this callus easily proliferated, was very soft and friable (Table 1). The growth parameters for the four fast-growing lines callus (5-7 subcultures) were calculated (Table 2). From those selected callus lines (Fig. 1), the cell suspensions were initiated (Fig. 1, 2).

The microscopic image of the cell culture established from the callus cultivated on MS with 2,4-D 1.0 mg·l^{-1} and KIN 0.1 mg·l^{-1} in the light (line A1) showed small, round and elongated cells, slightly pink in colour. The cells of the suspension culture established from the callus cultivated on the same medium in the dark (line A2) were characterized by the intense divisions. Small, round, dividing cells formed small aggregates. This culture presented the best biomass growth. The cell culture originated from the callus on MS + 2,4-D 2.0 mg·l^{-1} and NAA 0.2 mg·l^{-1} was characterized by the very high density of cells with different shapes, intensely dividing. The cell culture from the callus established on MS + DIC 1.0 mg·l^{-1} was the only cell culture

that had cells turning brown, mostly aging and elongated (Fig. 1).

For the first time, the cell suspension culture of *C. japonica* was maintained. The biomass obtained from the selected cell culture established from the callus A2 line growing on the medium MS + 2,4-D 1.0 mg·l^{-1} and KIN 0.1 mg·l^{-1} was collected for phytochemical studies. The line was chosen on the basis on fast biomass growth.

Callus and cell suspension cultures were tested in order to investigate their potential for the accumulation of two group of compounds – pentacyclic triterpenoids and selected polyphenols. UHPLC-MS analyses showed the presence of different putative triterpenoids in the callus and suspension cultures (Table 3, Fig. 3, 4). Most of them were tentatively identified on the basis of the MS data, as well as literature information [5, 17]. Three compounds, giving deprotonated ions of *m/z* 455, were selectively detected during additional UHPLC-MS analyses (SIM mode). Two of them were identified as oleanolic and ursolic acid, by comparison with authentic standards; the third compound was tentatively identified as betulinic acid, which was previously detected in *C. japonica*

Table 3

The content (mg·g^{-1}) of selected pentacyclic triterpenoids in methanol-water extracts of *Chaenomeles japonica* biomass from *in vitro* cultures

Pentacyclic triterpenoids [mg·g^{-1}] d. w.				
Plant material	Ursolic acid	Oleanolic acid	Betulinic acid *	SUM
Callus line A2 (MS+2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1}) dark	5.00±0.61	4.35±0.30	0.459±0.025	9.809±0.891
Cell suspension culture (MS+ 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1}) from growth phase (light)	9.37±0.49	7.54±0.52	0.757±0.054	17.667±1.061
Cell suspension culture (MS+ 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1}) from stationary phase (dark)	4.43±0.16	5.89±0.22	0.642±0.021	10.962±0.451

2,4-D – dichlorophenoxyacetic acid; DIC – dicamba; KIN – kinetin; MS – Murashige and Skoog medium
* expressed as oleanolic acid equivalent

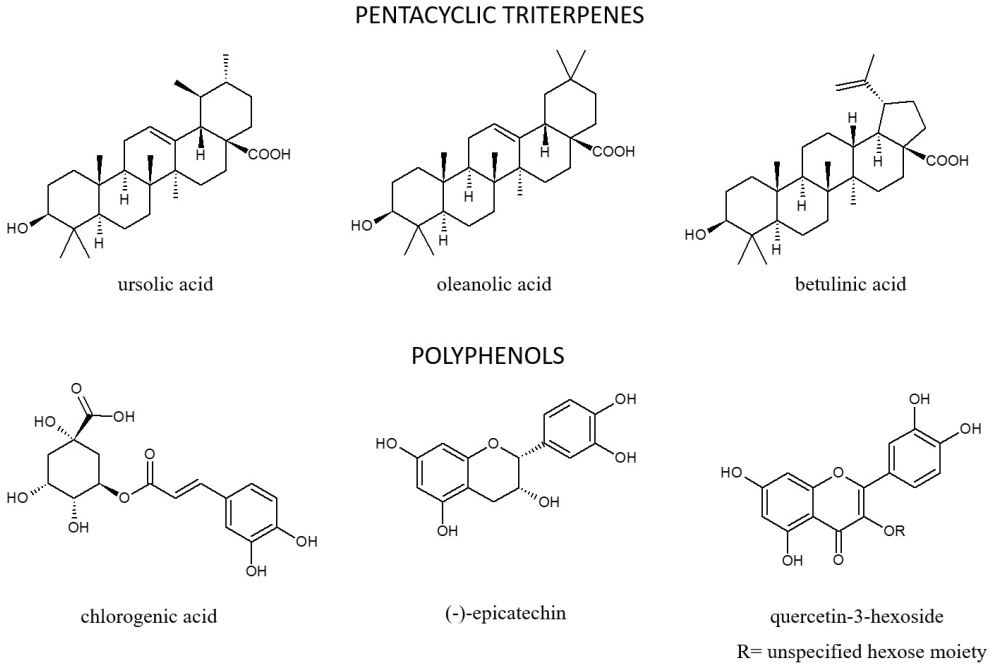
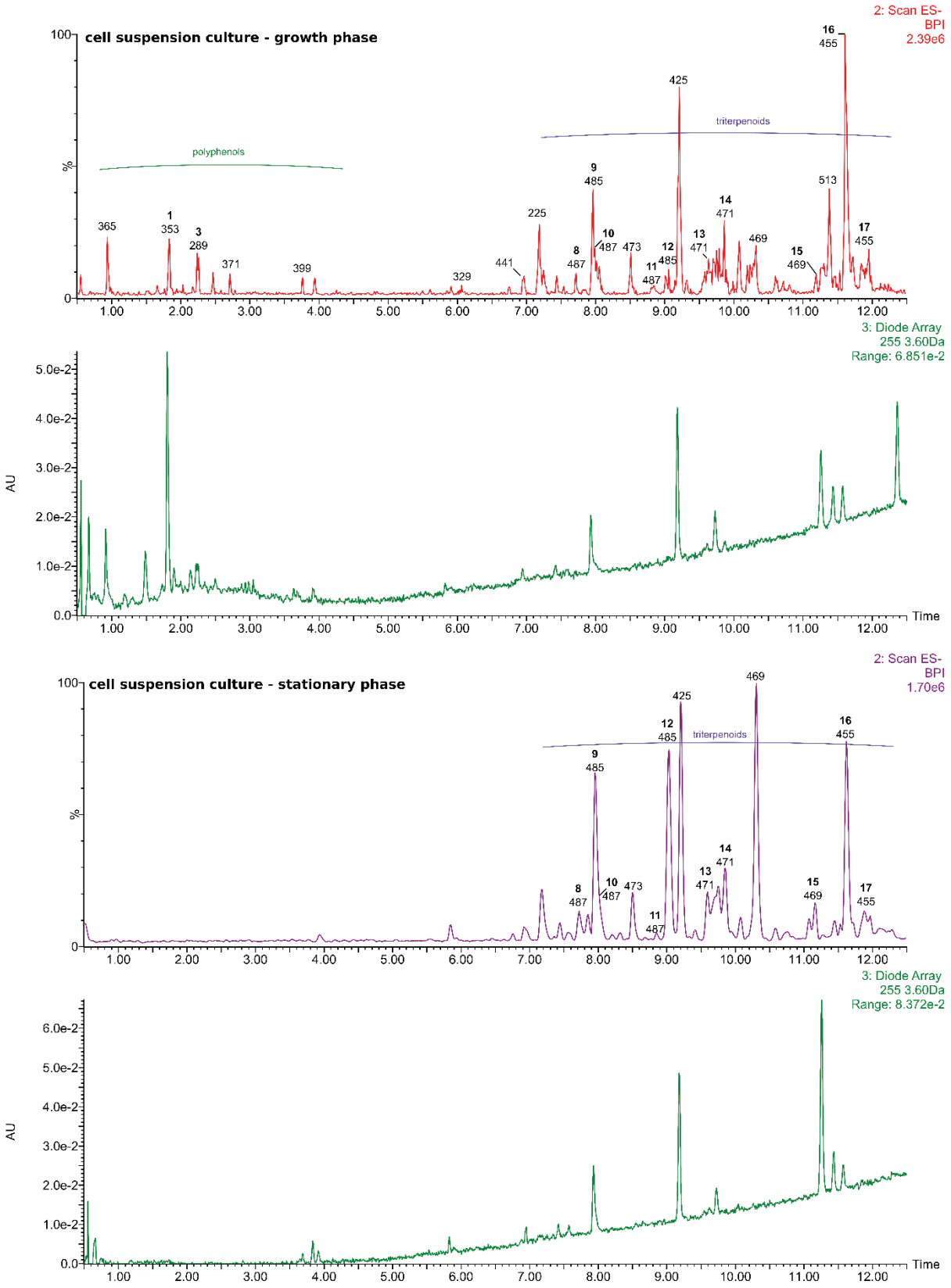


Figure 3

Chemical structures of main pentacyclic triterpenes and polyphenols of *Chaenomeles japonica* cell cultures


cell suspension culture - growth phase
2: Scan ES-
BPI
2.39e6
polyphenols
triterpenoids
365
353
289
371
399
329
441
225
485
487
473
485
487
425
471
469
513
455
3: Diode Array
255 3.60Da
Range: 6.851e-2
AU
Time
cell suspension culture - stationary phase
2: Scan ES-
BPI
1.70e6
triterpenoids
469
425
485
487
473
471
455
3: Diode Array
255 3.60Da
Range: 8.372e-2
AU
Time

No	tR (min)	[M-H]- (m/z)	identification
1	1.81	353	chlorogenic acid*
2	2.00	577	dimeric proanthocyanidin
3	2.22	289	epicatechin
4	2.28	337	coumaroylquinic acid
5	2.46	1153	tetrameric proanthocyanidin
6	3.05	463	quercetin hexoside
7	3.38	515	dicaffeoylquinic acid
8	7.71	487	triterpenoid
9	7.95	485	triterpenoid
10	8.00	487	triterpenoid
11	8.85	487	triterpenoid
12	9.05	485	triterpenoid
13	9.68	471	triterpenoid
14	9.86	471	triterpenoid
15	11.17	469	triterpenoid
16	11.62	455	betulinic acid
17	11.89	455	oleanolic & ursolic acid*

* confirmed by the comparison with an authentic standard

Figure 4

UHPLC-MS chromatograms of methanol-water extracts of cell suspension cultures of *Chaenomeles japonica* from growth phase and stationary phase maintained in MS medium with 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1} (ESI Base Peak chromatograms and UV chromatograms)

fruit by Yang *et al.* [17]. The highest amounts of ursolic acid (9.37 ± 0.47 mg·g^{-1} d.w.) and oleanolic acid (7.54 ± 0.52 mg·g^{-1} d.w.) were found in cell suspension biomass from growth phase (Table 3). In addition, some phenolic compounds, mainly chlorogenic acid, B type proanthocyanidins and epicatechin were present in the callus (Table 4) and cells harvested from the growth phase. In contrast, cells from the stationary phase were virtually devoid of phenolics (Fig. 3, 4). Among all phenolics in all tested systems, the main compound was chlorogenic acid. The highest accumulation was detected in the callus (1.094 ± 0.081 mg·g^{-1} d.w.) and cell suspension culture from growth phase (0.545 ± 0.040 mg·g^{-1} d.w.) (Table 4).

Production of pentacyclic triterpenoids in callus and cell suspension cultures of different plant species is available [18, 19]. In the present studies, the three pentacyclic triterpenoids were detected and quantified in the extracts of the cell suspensions and callus line A2, from which the cells cultures were obtained (Table 3). Ursolic and oleanolic acids were probably the main triterpenoids in the studied extracts. The cell suspension culture from the growth phase exhibited the highest content of ursolic (UA), oleanolic (OA), and betulinic acids (BA) (separately and as the sum). As mentioned in the literature, there are only few reports on the simultaneous presence of ursolic, oleanolic, and betulinic acids occurring together in cell cultures. Those three pentacyclic triterpenoids were determined in cell cultures of *Lantana camara* in an enhanced yield of UA - 4.12%, OA - 1.88% and BA - 3.1% [20, 21]. In cell cultures of *Hyssopus officinalis*, Skrzypek and Wysokińska [22] identified also the presence of UA and OA. The maximum production of terpenoides in cell suspension culture of *Centella asiatica* was observed in the stationary phase [23].

In addition, four polyphenolic compounds were analysed in the tested extracts. Chlorogenic acid, dicaffeoylquinic acid, epicatechin and a quercetin hexoside were detected in the callus line A2 and in a little amount in cell suspension culture at the growth phase. The main polyphenolic constituent

Table 4

The content (mg·g^{-1}) of selected pentacyclic triterpenoids in methanol-water extracts of *Chaenomeles japonica* biomass from *in vitro* cultures

Plant material	Pentacyclic triterpenoids [mg·g^{-1}] d. w.			
	Chlorogenic acid	Dicaffeoylquinic acid*	Epicatechin*	Quercetin hexoside
Callus line A2 (MS+2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1}) dark	1.094±0.081	0.077±0.006	0.068±0.008	0.064±0.008
Cell suspension culture (MS+ 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1}) from growth phase (light)	0.545±0.040	Not detected	0.067±0.009	Not detected
Cell suspension culture (MS+ 2,4-D 1.0 mg·l^{-1} + KIN 0.1 mg·l^{-1}) from stationary phase (dark)	Not detected	Not detected	Not detected	Not detected

2,4-D – dichlorophenoxyacetic acid; DIC – dicamba; KIN – kinetin; MS – Murashige and Skoog medium;
*expressed as chlorogenic acid equivalent

present in the tested callus was chlorogenic acid. Dicaffeoylquinic acid and quercetin hexoside were not detected in both cell cultures (Table 2). The production of chlorogenic acid in the cell suspension cultures was already studied for some medicinal plants i.e.: *Eryngium planum* [24], *Eucommia ulmoides* [25], *Lonicera macranthoids* [26], *Coffea arabica* [27].

CONCLUSIONS

The biosynthesis of secondary metabolites in cultured plant cells does not necessary yield compounds characteristic for the intact plant. In this context, the cell suspension culture of *Chaenomeles japonica* is a promising source of pentacyclic triterpenoids.

Abbreviations:

2,4-D – dichlorophenoxyacetic acid
BA – betulinic acid
DIC – dicamba
GP – growth phase
KIN – kinetin
MS – Muraschige & Skoog medium
NAA – 1-naphthaleneacetic acid
OA – oleanolic acid
SP – stationary phase
UA – ursolic acid

Conflict of interest: Authors declare no conflict of interest.

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