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ORIGINAL RESEARCH PAPER

In vitro shoot cultures of pink rock-rose (*Cistus ×incanus* L.) as a potential source of phenolic compounds

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Abstract

In vitro cultures of *Cistus ×incanus* (pink rock-rose) were maintained on two variants of Murashige and Skoog (MS) medium differing in terms of composition of plant growth regulators (PGRs): 6-benzyladenine (BA) and 1-naphthaleneacetic acid (NAA) at the following concentrations: 3 mg/L and 0, 3 mg/L and 1 mg/L, respectively, and on a variant without PGRs – a control. Cultures were maintained in a form of agar and agitated shoot cultures. The qualitative and quantitative analyzes of three groups of phenolic compounds (catechins, flavonoids, and free phenolic acids) were performed by using the HPLC-DAD technique in methanolic extracts of in vitro biomasses and of commercial plant raw material. In analyzed extracts from in vitro cultures, the presence of catechin [max. 197.80 mg / 100 g dry weight (DW)], epicatechin gallate (max. 30.74 mg / 100 g DW), gallic acid (max. 83.23 mg / 100 g DW), quercetin (max. 10.15 mg / 100 g DW), and quercitrin (max. 72.89 mg / 100 g DW) was confirmed. The quantities of accumulated compounds varied and depended on the type of in vitro culture and the concentration of PGRs in media. The highest amounts of all estimated compounds were obtained in biomasses from agar cultures cultivated on medium without PGRs in vitro. In extracts obtained from commercial raw material, gallic acid (max. 261.80 mg / 100 g DW) and quercetin (max. 255.96 mg / 100 g DW) were detected as being the dominant compounds.

Keywords

hairy rock-rose; *Cistus creticus*; catechin; free phenolic acids; flavonoids; plant biotechnology

Introduction

The genus *Cistus* is a taxon from the Cistaceae family, which belongs to the Malvales order. This genus includes more than 20 species [1,2], of which *Cistus ×incanus* L. (pink rock-rose, hairy rock-rose), *C. ladaniferus*, *C. laurifolius*, *C. monspeliensis*, and *C. salviifolius* are the most widely used in traditional therapies in the south of Europe and in the north of Africa. Pharmaceuticals available worldwide and food supplements of genus *Cictus* are mostly based on *C. ×incanus*. The name *C. creticus* is often used as a synonym for *C. ×incanus*. Recent findings on its taxonomy show that *C. ×incanus* L. is a hybrid of *C. albidus* L. and *C. crispus* L. [1,2]. A dried herb or leaf is the pharmaceutical raw material derived from *C. ×incanus*. These raw materials are famous for their high content of polyphenols, mainly flavonoids, e.g., quercetin and kaempferol, catechins, proanthocyanidins, and gallic acid. Moreover, fatty acids, phytohormones, vitamins, and terpenes were found in the herb extracts [3]. This rich chemical composition is responsible for the strong antioxidant potential of *C. ×incanus* and determines its profile

of biological activity. The use of pink rock-rose in therapy mainly results from its use in the traditional medicine of the Middle East, particularly Turkish medicine, where *C.* ×*incanus* was known as an anti-inflammatory agent in skin diseases, and as an antiulcer and antidiarrhea remedy; moreover, it was used in phytotherapy of rheumatism and nephritis [2,4–6]. In addition, *C.* ×*incanus* is recommended as a compound in dietary supplements for the prevention of chronic diseases such as hypertension, diabetes, or Alzheimer's disease [2,4,7–10].

The popularity of *C.* ×*incanus* as a medicinal plant, increasing recently in Central European countries and also in Poland, is reflected in its multidirectional pro-health activity confirmed by studies, especially into its antioxidant activity.

Current biotechnological studies on species of the genus *Cistus* relate mainly to micropropagation methods. Micropropagation protocols have been developed using buds or fragments of shoots as explants, Murashige and Skoog (MS) [11] medium, and different plant growth regulators (PGRs) [12–16].

Due to problems with taxonomic identification of material growing in the open air and the commonly reported phenomenon of the chemical composition variability of plant material in natural habitats, the goal was to establish in vitro cultures which would provide the possibility of controlling the culture conditions of selected plant material and which could potentially guarantee the production of highly valuable raw material with a high amount of specific medicinal compounds.

Our studies were aimed at establishing shoot cultures of *C.* ×*incanus* by analyzing the influence of the addition of PGRs: 6-benzyladenine (BA) and 1-naphthaleneacetic acid (NAA) on the type of culture (agar, agitated) and the growth of the biomass and biosynthetic potential of cells. Moreover, a comparative analysis of the secondary metabolite production of the in vitro cell biomass and plant raw material of soil-grown plants was performed. Groups of metabolites with polyphenolic structure (catechins, flavonoids, and free phenolic acids) were analyzed by using the DAD-HPLC technique in methanolic extracts of plant material cultured in vitro and in vivo.

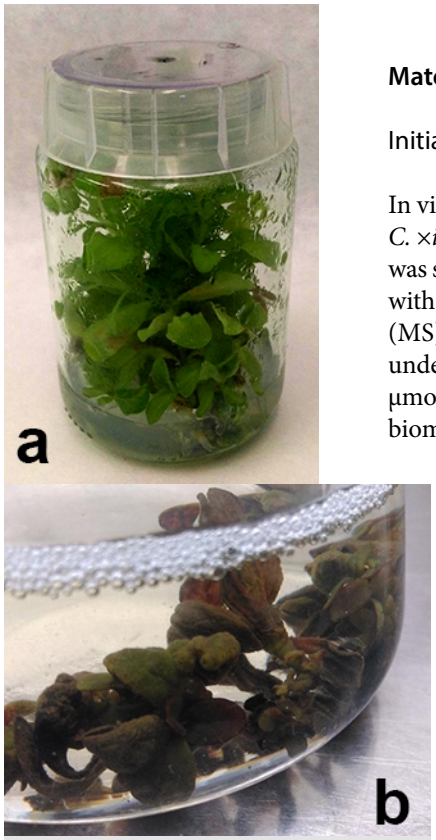

Fig. 1 Shoot in vitro cultures of *Cistus* × *incanus* cultivated on MS medium with 3 mg/L BA after 4-week growth cycle: agar culture (**a**), agitated culture (**b**).

Material and methods

Initiation of in vitro cultures

In vitro cultures were established in 2014 from hypocotyls of germinating seedlings of *C.* ×*incanus* obtained from the Sandeman Seeds company (England). The plant material was sterilized in 0.1% mercury(II) chloride ($HgCl_2$) for 10 min, then washed three times with sterile redistilled water. The initiating medium according to Murashige and Skoog (MS) [11] contained 1 mg/L BA and 0.5 mg/L NAA. The cultures were maintained under continuous artificial light (40 W m^{-2}, photosynthetic photon flux density = 12.15 µmol m^{-2} s^{-1}, LF-40 W fluorescent lamp, white light) at a temperature of 25 ±2°C. The biomass was passaged every 4 weeks until vigorous shoot cultures were established.

Experimental in vitro cultures

Experimental shoot cultures were maintained on the MS medium as stationary agar (Fig. 1a) and agitated cultures (Fig. 1b). Each culture type was cultivated using variants of MS medium with different contents of PGRs, cytokinin (BA) and auxin (NAA), as follows: 3 mg/L BA and 0 mg/L NAA as well as 3 mg/L BA and 1 mg/L NAA, and on one variant without PGRs. Experimental cultures were cultivated under continuous artificial light (40 W m^{-2}, photosynthetic photon flux density = 12.15 µmol m^{-2} s^{-1}, LF-40 W fluorescent lamp, white light). Agar cultures were cultivated in Magenta vessels (77 × 77 × 97 mm) (Sigma); the amount of inoculum was 1 g. Agitated cultures were cultivated in 300 mL Erlenmeyer flasks, containing 100 mL medium, and the amount of inoculum was 2 g. Biomass from agar cultures and biomass and experimental media from agitated cultures were collected after 4-week growth cycles (three series).

Fig. 2 Aboveground parts of *Cistus ×incanus* – parent plant material of studied in vitro cultures (Garden of Medicinal Plants, Faculty of Pharmacy, Jagiellonian University, Medical College, Cracow, Poland).

Soil-grown plant material

The part of *Cistus ×incanus* seeds from which in vitro cultures were initiated were planted to the soil in the greenhouse of the Garden of Medicinal Plants, Faculty of Pharmacy, Jagiellonian University, Medical College in Cracow (Poland), where they grew during the whole vegetable season (Fig. 2). *Cistus ×incanus* plants were taxonomically verified by scientific staff of the Garden of Medicinal Plants. These herbs constituted the parent plant raw material.

The commercial plant raw material comprised *C. ×incanus* herbs obtained from two different herbal companies (companies "A" and "B").

DAD-HPLC analysis

Air-dry crushed biomasses (each sample 0.5 g) and lyophilized media (50 mL) from tested in vitro cultures and soil-grown plant materials were extracted with 50 mL of methanol for 3 h under a reflux condenser. Qualification and quantification of the analyzed compounds in extracts was performed by using the HPLC-DAD (modified HPLC method according to Ellnain-Wojtaszek and Zgórka [17]. The method was validated by our team in collaboration with another team from the Faculty of Pharmacy in Cracow (Poland) [18]. HPLC-DAD equipment and conditions were as follows: HPLC system – Merck-Hitachi with a Purospher RP-18e analytical column (4 × 250 mm, 5 µm, Merck), mobile phase consisted of: (***i***) methanol with 0.5% acetic acid (1:4 v/v) and (***ii***) methanol, gradient program, flow rate at 1 mL/min, injection volume (10 µL), analytical wavelength (254 nm), temperature (25°C).

Within analyzes, the following standards from Sigma-Aldrich were used (in total 36 compounds): catechin and its isomer and derivatives (five compounds) – epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate; flavonoids (11 compounds): apigetrin, cynaroside, hyperoside, kaempferol, luteolin, myricetin, quercetin, quercitrin, rutoside, trifolin, vitexin; and phenolic acids (19 compounds): 3,4-dihydroxyphenylacetic, caffeic, chlorogenic, *o*-coumaric, *m*-coumaric, *p*-coumaric, ferulic, gallic, gentisic, hydrocaffeic, *p*-hydroxybenzoic, isoferulic, neochlorogenic, protocatechuic, rosmarinic, salicylic, sinapic, syringic, vanillic acids, and also a precursor of one group of these compounds – cinnamic acid.

Statistical analysis

The experiments and compound estimations have been repeated thrice. The results were presented as mean ± standard deviation (*SD*). The results of estimated compounds contents were compared with the one-way analysis of variance (one-way ANOVA). For comparison and contrast between different groups, post hoc Tukey HSD (honestly significant difference) test was used. The p value <0.05 was considered statistically significant. The STATISTICA version 12 PL software package (StatSoft) was applied for the analysis.

Results

Biomass increments

In this study, a successful initiation of in vitro *C. ×incanus* shoot cultures from hypocotyls of germinating seedlings was performed. Stable microshoot cultures were obtained after six passages. The vigorous cultures were grown on MS medium variants with different concentrations of PGRs, in two different types of in vitro systems: agar and agitated. During preliminary experiments, different amounts of BA and NAA were tested. An adverse effect related to the addition of NAA on the appearance and growth of *C. ×incanus* shoot cultures was observed; therefore, experimental in vitro cultures were

cultivated on the medium containing BA (3 mg/L) as the only PGRs, and one variant with addition of 1 mg/L NAA.

A significant influence of the composition of studied PGRs on biomass growth was observed. In agar cultures, the highest dry weight biomass increments, 7.8-fold, were observed on cytokinin-supplemented MS medium alone. The lowest biomass increments, 3.2-fold, were obtained on MS medium without PGRs. On medium supplemented with 3 mg/L BA and 1 mg/L NAA, biomass increments were equal to 5.5-fold.

High dry biomass increments on all tested media, in the range of 7.7 to 8.8-fold, were observed in agitated cultures. The highest increment was obtained on MS medium without PGRs, while the lowest was also obtained on MS medium with 3 mg/L BA and 1 mg/L NAA. The average biomass growth increments were shown for cultures maintained on medium with 3 mg/L BA as alone PGR.

Accumulation of estimated compounds

The applied DAD-HPLC analyzes allowed for simultaneous qualification and quantification of three groups of phenolic compounds (31 compounds): five catechins, 11 flavonoids and 19 phenolic acids and cinnamic acid. In the extracts from the soil-grown plant material of *C. ×incanus*, the following compounds were detected: catechin, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate, quercetin and its glycoside – quercitrin, and gallic acid. The DAD-HPLC analyzes of extracts from the studied shoot agar and agitated in vitro cultures showed the presence of: catechin, epicatechin gallate, quercetin and its glycoside – quercitrin, and gallic acid.

In the analyzed extracts from the in vitro culture media, no presence of the phenolic compounds was confirmed.

Accumulation of catechins

In the extracts from agar and agitated in vitro shoot cultures, the presence of catechin and epicatechin gallate was reported (Tab. 1). The amounts of the determined compounds were dependent on the MS medium variant used. In extracts from agar cultures, the content of catechin varied between 148.72 and 197.80 mg / 100 g dry weight (DW), while in extracts from agitated cultures, catechin content was smaller and varied between 39.39 and 69.84 mg / 100 g DW. The amounts of epicatechin gallate estimated in the tested biomass extracts from two types of in vitro cultures were lower, ranging from 8.93 to 30.74 mg / 100 g DW and from 2.60 to 6.17 mg / 100 g DW, respectively. The highest total content of these two compounds was obtained in extracts from biomass cultivated on the MS medium variant without PGRs (agar cultures – 228.54 mg / 100 g DW and for agitated cultures – 76.01 mg / 100 g DW, respectively).

In the analyzed parent and commercial plant raw material extracts from *C. ×incanus* of different origin, the presence of all five tested catechins was reported: catechin, epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (Tab. 1). In the parent plant material of the studied in vitro cultures of *C. ×incanus*, the total content of estimated catechins was equal to 78.28 mg / 100 g DW. The herb from company A was more abundant in catechins in comparison to that from company B, and the maximum total contents were equal to 183.42 mg / 100 g DW and 50.33 mg / 100 g DW, respectively (Tab. 1). The main differences in the contents of individual compounds were visible. The main compound in the parent plant material was epigallocatechin (51.72 mg / 100 g DW). But, in the extracts from herbs from company A the main compound was epigallocatechin gallate (68.29 mg / 100 g DW), while a plant material from company B exhibited epicatechin (28.90 mg / 100 g DW).

Accumulation of flavonoids

In all analyzed in vitro culture extracts, among eleven flavonoids, the presence of quercetin and one its glycoside – quercitrin (quercetin 3-rhamnoside) was confirmed. In agar cultures, the amount of accumulated quercetin ranged from 3.36 to 10.15 mg/g DW, while

Tab. 1 Amounts of estimated compounds (mg / 100 g DW ±*SD*) in extracts from in vitro cultivated biomasses and from soil-grown plants *Cistus ×incanus*.

Estimated compounds	In vitro cultures						Parent plant raw material	Commercial plant raw material	
	agar culture			agitated culture					
	MS medium variant BA/NAA (mg/L)								
	0	3/0	3/1	0	3/0	3/1		company A	company B
Catechin	197.80 ±12.74^{bcdefghi}	181.17 ±6.68^{acdefghi}	148.72 ±9.79^{abdefghi}	69.84 ±4.41^{abceghi}	54.78 ±2.06^{abcdfgi}	39.39 ±1.94^{abcdeghi}	7.16 ±0.21^{abcdefhi}	49.93 ±2.22^{abcdfg}	Trace^{sabcdefgh}
Epicatechin gallate	30.74 ±1.00^{defghi}	23.99 ±0.06^{acdefgi}	8.93 ±0.56^{abdfghi}	6.17 ±0.09^{abcfghi}	5.91 ±0.25^{abcfghi}	2.60 ±0.06^{abcdghi}	4.54 ±0.13^{abcdfhi}	8.46 ±0.29^{abdefgi}	19.43 ±0.10^{abcdefgh}
Epicatechin	nd*^{ghi}	nd^{ghi}	nd^{ghi}	nd^{ghi}	nd^{ghi}	nd^{ghi}	12.58 ±0.20^{abcdefi}	11.01 ±0.28^{abcdefi}	28.90 ±0.91^{abcdefgh}
Epigallocatechin	nd^{gh}	nd^{gh}	nd^{ghi}	nd^{gh}	nd^{gh}	nd^{gh}	51.72 ±1.18^{abcdefhi}	45.73 ±3.12^{abcdefgi}	Trace^{sgh}
Epigallocatechin gallate	nd^{ghi}	nd^{ghi}	nd^{abdefhi}	nd^{ghi}	nd^{ghi}	nd^{ghi}	2.28 ±0.04^{abcdefh}	68.29 ±1.52^{abcdefgi}	2.01 ±0.03^{abcdefh}
Quercetin	5.05 ±0.08^{bcdeghi}	10.15 ±0.40^{acdefghi}	3.36 ±0.04^{abdefghi}	1.91 ±0.02^{abcfghi}	1.45 ±0.10^{abcfghi}	5.06 ±0.13^{bcdeghi}	2.93 ±0.09^{abcdefhi}	nd^{abcdefg}	nd^{abcdefg}
Quercitrin	72.89 ±3.73^{bcdefghi}	62.16 ±2.05^{acdefghi}	70.60 ±3.43^{abdefghi}	13.88 ±0.93^{abcefghi}	15.85 ±1.07^{abcdfghi}	7.08 ±0.11^{abcdeghi}	25.91 ±1.54 ^{abcdefhi}	111.60 ±6.98^{abcdefgi}	255.96 ±7.81^{abcdefgh}
Gallic acid	40.51 ±0.25^{cdefghi}	41.42 ±2.82^{cdefghi}	83.23 ±2.11^{abdefghi}	10.98 ±0.65^{abcefghi}	20.47 ±0.03^{abcdfghi}	17.28 ±0.21^{abcdeghi}	2.48 ±0.11 ^{abcdefhi}	261.80 ±10.34^{abcdefgi}	178.89 ±3.39^{abcdefgh}

* nd – not detected. [a] p <0.05 vs. agar cultures, MS medium without PGRs. [b] $p < 0.05$ vs. agar cultures, MS medium with 3 mg/L BA. [c] $p < 0.05$ vs. agar cultures, MS medium with 3 mg/L BA and 1 mg/L NAA. [d] $p < 0.05$ vs. agitated cultures, MS medium without PGRs. [e] $p < 0.05$ vs. agitated cultures, MS medium with 3 mg/L BA. [f] $p < 0.05$ vs. agitated cultures, MS medium with 3 mg/L BA and 1 mg/L NAA. [g] $p < 0.05$ vs. parent plant raw material. [h] $p < 0.05$ vs. commercial plant raw material – company A. [i] $p < 0.05$ vs. commercial plant raw material – company B.

the amount of quercitrin was found to be between 62.16 and 72.89 mg / 100 g DW. The highest content of quercetin was found in biomass extracts cultivated on MS medium with 3 mg/L BA, while the accumulation of quercitrin was promoted by the variant without the addition of PGRs (Tab. 1). In agitated cultures, the amounts of quercetin and quercitrin were small and ranged between 1.45 and 5.06 mg/g DW and 7.08 and 15.85 mg / 100 g DW, respectively. Accumulation of quercetin was promoted by the medium variant containing 3 mg/L BA and 1 mg/L NAA, while in terms of quercitrin, the medium variant with 3 mg/L BA favored the accumulation of this compound.

In the parent plant material, the same flavonoids as in in vitro cultivated biomass were detected. The dominant compound was quercitrin (25.91 mg / 100 g DW). In the commercial plant raw material extracts, only the occurrence of glycoside, quercitrin, was reported: 111.60 mg / 100 g DW (company A) and 255.96 mg / 100 g DW (company B).

Accumulation of gallic acid

In extracts from biomass of both types of in vitro cultures and in extracts of plant raw material, out of the 19 phenolic acids analyzed only the presence of gallic acid was confirmed. In the biomass extracts from agar cultures, the amount of this acid ranged from 40.51 to 83.23 mg / 100 g DW. Its highest amount was found on the medium variant containing 3 mg/L BA and 1 mg/L NAA. In agitated cultures, the amount of gallic acid was lower and ranged from 10.98 to 20.47 mg / 100 g DW. The highest amount of this compound was confirmed in extracts of biomass cultivated on MS medium with 3 mg/L BA (Tab. 1).

In the parent herb material, a low amount of gallic acid was detected, while in commercial herb extracts, a high content of gallic acid equal to 261.80 mg / 100 g DW (company A) and 178.89 (company B) (Tab. 1) was determined.

Discussion

The dry biomass increments obtained during 4-week growth cycles on MS medium variants in shoot agar and agitated cultures were satisfactory and ranged from 3.2- to 7.8- and from 7.7- to 8.8-fold, respectively. The maximum biomass growth was observed on the MS medium variant containing 3 mg/L BA (agar culture) and on MS medium without PGRs (agitated culture). Our results for the biomass growth of in vitro cultures of different plant species demonstrated that these biomass increments are higher than the average obtained in in vitro cultures. In in vitro cultures (shoot cultures of *Ruta graveolens*, shoot-differentiating callus cultures of *Ruta graveolens* ssp. *divaricata* and *Schisandra chinensis*) we achieved about fourfold the biomass growth increments during 4 weeks of culture cycles [19–21].

During our studies, quantification of catechins, flavonoids, and phenolic acids was performed. In methanolic extracts from *C. ×incanus* in vitro cultivated biomass and from herb plant material, the following compounds were estimated: catechins – catechin, epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate; flavonoids – quercetin and quercitrin, and gallic acid. All estimated compounds have a common biogenetic pathway (Fig. 2) derived from the shikimic acid pathway, which confirmed the validity of the results. The in vitro cultured cells preserved the biosynthetic potential of parent plant; however, some differences were detected.

In vitro

During our analyzes, the qualitative differences between extracts of in vitro and in vivo material origin were confirmed. In comparison with plant raw material, in biomass extracts from in vitro cultures many times higher amounts of catechin and epicatechin gallate were estimated (Tab. 1). Nevertheless, no presence of epicatechin, epigallocatechin, or epigallocatechin gallate was detected in in vitro cultures. All these

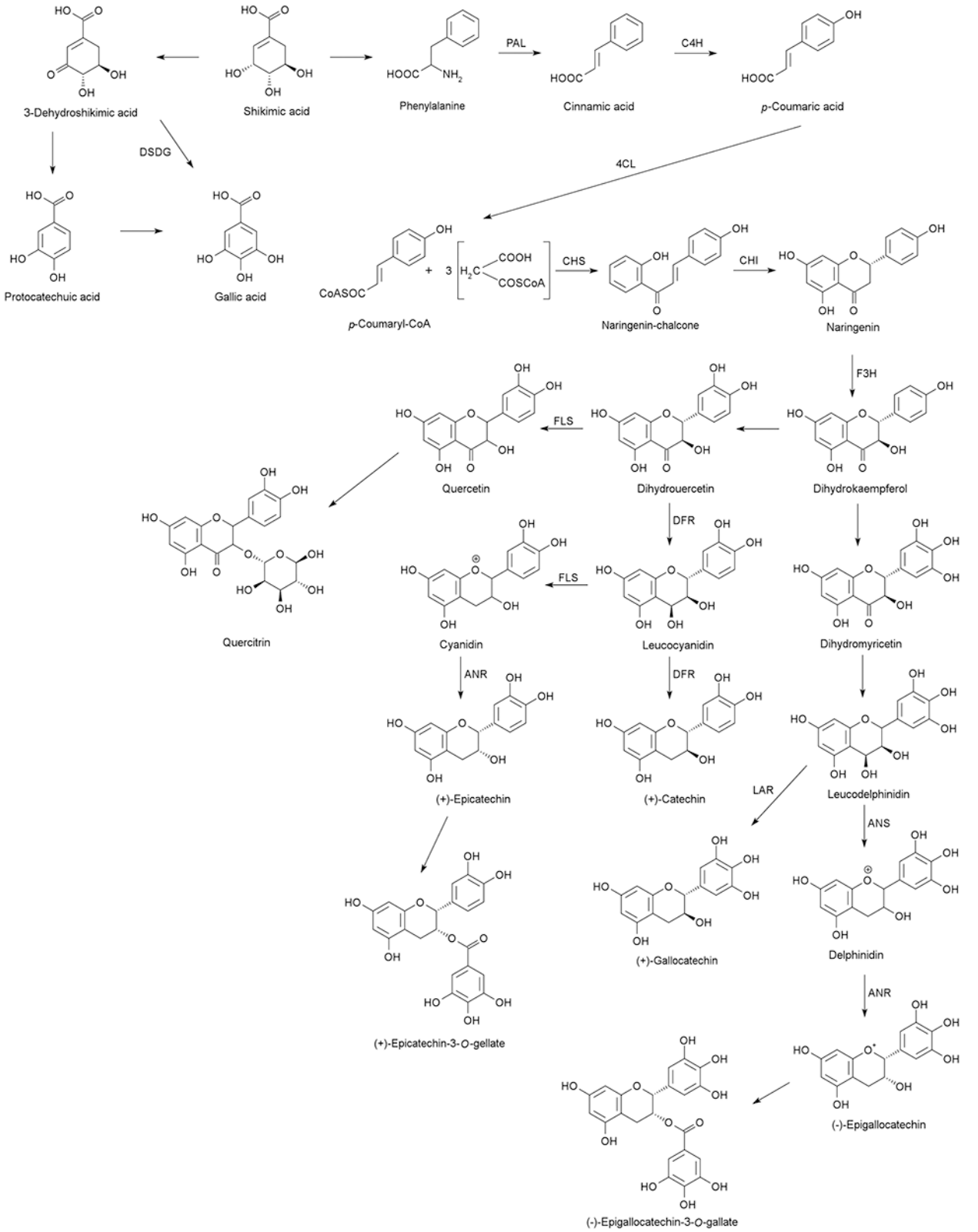

Fig. 3 Possible biosynthetic pathways of phenolic compounds (ANR – anthocyanidin reductase; ANS – anthocyanidin synthase; DSDG – dehydroshikimate dehydrogenase; C4H – cinnamate 4-hydroxylase; 4CL – 4-coumarate-CoA ligase; CHS – chalcone synthase; CHI – chalcone isomerase; F3H – flavanone 3-hydroxylase; DFR – dihydroflavonol 4-reductase; FLS – flavonol synthase; PAL – phenylalanine ammonia-lyase), according to [40].

compounds occurred in the studied herb extracts. The differences in the quantitative contents of each compound in the examined extracts were also found (Tab. 1).

Similar results have been observed in experiments on in vitro cultures of *Polygonum hydropiper*. In in vitro cultures of this plant species, higher amounts of catechin, epicatechin, and epicatechin gallate have been estimated in in vitro cultivated biomass than in tested comparative plant material [22]. Additionally, *Fagopyrum esculentum* and *Quercus acutissima* in vitro cultures are also indicated as rich sources of different catechins [23,24].

The *C. ×incanus* herb is known as a material rich in flavonoids [3,25,26]; nevertheless, in tested extracts from plant material, we detected only quercitrin. The quercetin occurred only in the parent plant raw material (Tab. 1). Higher contents of flavonoids (about 6 times) were found in extracts of shoots cultivated in the agar system when compared to the agitated system. Additionally, other shoot cultures, for example, of different species of *Hypericum*, e.g., *H. perforatum* [27,28] and *H. undulatum* [29], are able to accumulate quercetin and, in considerable amounts, quercitrin.

Gallic acid was the only compound identified by analyzing the studied extracts via HPLC. This compound occurred in high amounts in herb extracts (Tab. 1). Moreover, in extracts from agar cultures, high amounts of gallic acid were also determined (max. 83.23 mg / 100 g DW). The smallest amounts of gallic acid were found in extracts of agitated cultures (max. 20.47 mg / 100 g DW). Gallic acid is a simple phenolic acid formed during the early stages of the biosynthesis of phenolic compounds (Fig. 3). Ease of synthesis causes its frequent accumulation in the biomass of in vitro plant cultures, for example, in *Habenaria edgeworthii* [30], *Salvia officinalis* [31], and *Schisandra chinensis* [32]. Its significant amount in *C. ×incanus* is associated with the production of catechins. Gallic acid often occurs with catechins, for example, in *Quercus acutissima* [23], *Artemisia absinthium* [33] or in *Bergenia ciliate*, and *Bergenia ligulata* [34] in vitro cultures.

Within this study, two different types of in vitro cultures were studied. The cultivation system affected biomass secondary metabolite production. The stationary system of cultivation (agar medium) favored accumulation of all detected compounds. In comparison to parent plant material, the maximum amounts of catechin, epicatechin gallate, quercitrin, and gallic acid were 27.6-, 6.8-, 2.8-, and 33.6 times higher in agar cultures, respectively. The higher production of plant secondary metabolites in in vitro cultivated biomass than in parent plant material is an important phenomenon. Such a relationship has been confirmed, for example, in *Aronia melanocarpa* [35,36], *Cyclopia* sp. [37], *Genista tinctoria* [38], and *Schisandra chinensis* [32,39] in vitro cultures.

Due to their high potential of cell biosynthesis, the above-described in vitro *C. ×incanus* cultures may constitute a good subject for further biotechnology studies aimed at the accumulation of selected biologically active compounds – especially catechin, quercitrin, and gallic acid. These plant metabolites are known for their pharmacological activities, e.g., antioxidant, anticancer, and anti-inflammatory. Moreover, they are recommended in the prevention and treatment of heart diseases [6,8]. Further optimization of the conditions based on different strategies, e.g., precursor supplementation and elicitation, for in vitro cultures should enable an increase in the accumulation of these compounds in amounts that may be of interest from a practical viewpoint.

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