

DOI: 10.5586/asbp.3638

**Publication history**

Received: 2018-10-23

Accepted: 2019-10-07

Published: 2019-12-10

**Handling editor**

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**Authors' contributions**

EK: designed the study, obtained and maintained the hairy root cultures, prepared the extracts for the experiments, and wrote the manuscript; GS: determined the ginsenoside content by HPLC; IGK: performed the TPC analysis and antioxidant activity tests and verified the manuscript content; PS: performed the statistical analysis; MS: provided substantive consultations and took part in preparing the manuscript

**Funding**

The project was financed by the Medical University of Lodz from the research grant No. 502-13-754 and by The State Committee for Scientific Research grant No. 3P05F01523.

**Competing interests**

No competing interests have been declared.

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**Citation**

Kochan E, Szymańska G, Grzegorz-Karolak I, Szymczyk P, Sienkiewicz M. Ginsenoside and phenolic compounds in hydromethanolic extracts of American ginseng cell cultures and their antioxidant properties. *Acta Soc Bot Pol.* 2019;88(4):3638. <https://doi.org/10.5586/asbp.3638>

## ORIGINAL RESEARCH PAPER

# Ginsenoside and phenolic compounds in hydromethanolic extracts of American ginseng cell cultures and their antioxidant properties

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**Abstract**

The present study was performed to investigate the antioxidant properties of callus and suspension culture extracts of *Panax quinquefolium* (American ginseng). The ginsenoside content and the total phenolic content (TPC) in these cultures were also examined. The total amount of the nine studied saponins was found to be 2.08, 1.69, and 0.202 mg g<sup>-1</sup> dry weight in red callus line (RCL), green callus line (GCL), and suspension cultures, respectively, by HPLC analysis. The TPC was estimated using the Folin-Ciocalteu method. The TPC of the suspension culture extracts was approximately 36.7% and 17.6% higher than that of the RCL and GCL, respectively. The antioxidant activity of the extracts was evaluated using the in vitro ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] and FRAP (ferric reducing antioxidant power) assays; the methanolic *P. quinquefolium* suspension culture extracts demonstrated stronger antioxidant activity than that of the callus cultures.

**Keywords**

callus and suspension extracts; antioxidant activity; ginsenosides; phenolic compounds; *Panax quinquefolium*

**Introduction**

*Panax quinquefolium* is a herbaceous perennial plant of the family Araliaceae, and its roots possess medicinal properties. The slow growth of ginseng, requiring 3–4 years of cultivation, as well as the troublesome conditions of traditional cultivation and the sensitivity to fungal diseases, has prompted a search for alternative methods, such as in vitro cultures, of obtaining the plant material [1]. Ginseng plants contain pharmacologically active ingredients named ginsenosides (panaxosides) [2,3]. They are classified as triterpenoid saponins – derivatives of protopanaxadiol (e.g., Rb1, Rb2, Rc, and Rd), belonging to the Rb group, and protopanaxatriol (e.g., Rg1 and Re), belonging to the Rg group or derivatives of oleanolic acid (Ro). They regulate the function of the nervous, hormonal, cardiovascular, and immunological systems and have demonstrated adaptogenic properties [4–6]. In addition, a study on individual ginsenosides, such as Rb1, Rg1, and Re, found that they also possess antioxidant activity [7,8].

Phenolic compounds are the most widely-known metabolites with antioxidant activity, and their properties are well documented in the literature [9–11]. However, most of the studies have only examined extracts from the naturally grown *P. ginseng* and *P. quinquefolium* plants, and have focused only on their ginsenoside content [12–16]. We have established different organ, as well as cell, callus, and suspension cultures of

*P. quinquefolium* in our laboratory. In addition, our previous studies have investigated ginsenoside and total phenolic contents in the shoots [3] and hairy roots of *P. quinquefolium* [17]. Through, these studies, we also described the optimal growth conditions required by the culture for increased production of the bioactive compounds [2,18]. The present study evaluates the ginsenoside composition and total phenolic content (TPC) in selected lines of callus and suspension cultures of *P. quinquefolium*. The study also determines their antioxidant properties and scavenging potency, as not much is known about the antioxidant activity of these cultures.

## Material and methods

### Plant material

**Callus culture lines.** The leaf blades and stalks of 1-year-old soil-ground plants of *P. quinquefolium* were obtained from the Medicinal Plant Garden of the Medical University of Lodz, Poland and used as explants. They were sterilized with sodium hypochlorite solution (2%) for 2 min. After rinsing with sterile distilled water, the explants were cut into 0.5–1-cm-long fragments. For callus induction, the explants were placed on solid agar (0.7% Difco Bacto Agar) Woody-Plant (WP) medium [19] with 1 mg L<sup>-1</sup> 2,4-dichlorophenoxyacetic acid (2,4-D), 1 mg L<sup>-1</sup>  $\alpha$ -naphthalene acetic acid (NAA), and 0.1 mg L<sup>-1</sup> 6-benzylamino purine (BAP) at 26°C in light (40  $\mu$ E m<sup>-2</sup> s<sup>-1</sup>). The medium was maintained at a pH between 5.6 and 5.8 and was sterilized in an autoclave at 123°C (pressure 1 atm). The explants were placed in glass test tubes (19 × 2.5 cm, containing 25 mL of the medium). After three 5-week passages (15 weeks in total), the BAP concentration was increased to 0.2 mg L<sup>-1</sup>. Additionally, the growth medium of callus tissues derived from leaf blades was changed to Murashige and Skoog (MS) [20] with 1 mg L<sup>-1</sup> 2,4-D, 1 mg L<sup>-1</sup> NAA, and 0.2 mg L<sup>-1</sup> BAP; this medium was selected after preliminary attempts to optimize the growth of this callus line, which initially grew quite poorly (data not shown). Finally, green callus line (GCL) grew on WP medium supplemented with 1 mg L<sup>-1</sup> 2,4-D, 1 mg L<sup>-1</sup> NAA, and 0.2 mg L<sup>-1</sup> BAP, while RCL grew on MS medium with the same combination of phytohormones (Fig. 1). The callus tissues were subcultured every 5 weeks. The mean biomass of the inoculum was 0.275 g fresh weight (f.w.) and 0.0113 g dry weight (d.w.). The callus tissues used in this study were derived from 21–25 passages.

**Suspension culture growth condition.** The *P. quinquefolium* suspension culture was initiated from a three-week-old tissue of the red callus line (RCL), which was grown in continuous light (40  $\mu$ E m<sup>-2</sup> s<sup>-1</sup>) on solid MS medium [18] with 1 mg L<sup>-1</sup> 2,4-D, 1 mg L<sup>-1</sup> NAA, and 0.2 mg L<sup>-1</sup> BAP. About 3 g f.w. of callus from the twenty-third passage was transferred into Erlenmeyer flasks (300 mL) containing 50 mL of liquid MS medium with 0.1 mg L<sup>-1</sup> kinetin (kin) and 1 mg L<sup>-1</sup> 2,4-D. This combination of growth regulators was selected for the suspension culture based on the available literature [21] because the cells were found to clump together in large aggregates and grow poorly in the medium containing the same growth regulators used in the callus medium, from which the suspension was obtained. The pH and the sterilization conditions of the medium were the same as described above. The flasks were placed on a rotary shaker (100 rpm), at 26 ± 2°C, 90% humidity, and a photoperiod of 16 hours of light (40  $\mu$ E m<sup>-2</sup> s<sup>-1</sup>) and 8 hours of darkness. The suspension was subcultured every 3 weeks. The mean biomass of the inoculum was 2.66 g L<sup>-1</sup> f.w. and 0.232 g L<sup>-1</sup> d.w. The suspension culture used in the study was derived from 10–15 passages.

**Culture growth measurement.** The fresh weights of the tissues, after separating them from the medium, were measured. The dry weights were determined by drying the tissue at 100°C for 1 hour, and then at 80°C for 24 hours. The treatment was repeated thrice. The growth index (GI) was calculated according to the formula:

$$GI = (F_m - I_m) / F_m,$$

where *GI* is growth index, and *F<sub>m</sub>* and *I<sub>m</sub>* represent the final and initial (inoculum) biomasses, respectively (both for fresh and dry weights).

**Sample preparation.** The plant material was dried at room temperature and subjected to solid-phase extraction in 80% methanol as described Kochan et al. [2]. The dried hydromethanolic extracts were weighed and used for HPLC analysis.

#### Ginsenoside content determination by HPLC

The samples were tested for the presence of nine ginsenosides (Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, Rg2, and Rf, all purchased from Sigma Aldrich, Germany) by HPLC as per the method described by Kochan et al. [22]. The dried extracts were dissolved in 1 mL of methanol (HPLC-grade, J. T. Baker, the Netherlands) and filtered through 0.2- $\mu\text{m}$  pore diameter Millex-FG Hydrophobic Fluoropore filters (Sigma Aldrich). The liquid chromatography system, consisting of the Agilent Technology 1200 apparatus, a ZORBAX Eclipse XDB-C18 (150  $\times$  4.6 mm, 5  $\mu\text{m}$ ) column, Quat Pump and UV-VIS DAD type detector and autosampler, was used for qualitative and quantitative analysis of the samples. Agilent Technology set was combined with Agilent ChemStation 2001–2010 software. For the determination of ginsenosides, a two-component mobile phase composed of acetonitrile (A) and water (B) was used. The following gradient elution program was applied: 0–16 min: 18% A, 82% B; 16–28 min: 30% A, 70% B; 28–60 min: 32% A, 68% B; 60–64 min: 80% A, 20% B; and 64–68 min: 18% A, 82% B. The flow rate was 2 mL min<sup>-1</sup>. The ginsenosides were detected at a wavelength of 203 nm and quantified (mg g<sup>-1</sup> d.w.) by comparing retention time and peak areas between standards and samples.

#### Total phenolic content determination

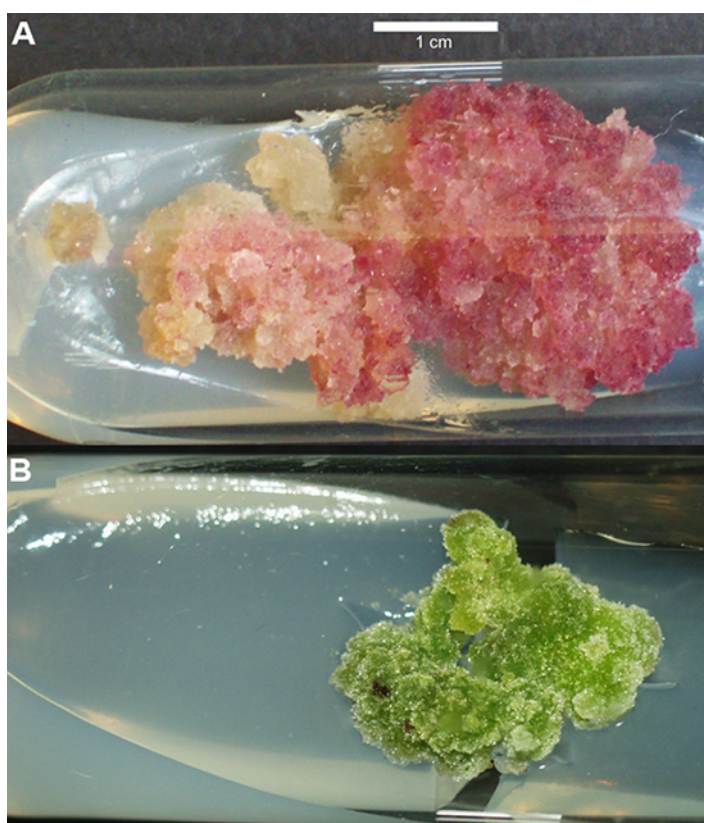
The TPC was determined using the Folin–Ciocalteu reagent as described by Kochan et al. [3]. The absorbance of the samples was measured at 765 nm on a Rayleigh UV-1601 spectrophotometer (Beijing Reyleigh Corp., China) versus a blank sample. The TPC was quantified as gallic acid milligram equivalents (GAE) per gram of dry extract.

#### Determination of antioxidant activity

The antioxidant potential of the extracts from callus and suspension cultures of *P. quinquefolium* was determined using two in vitro assays, ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical and ferric reducing antioxidant power (FRAP). Sample preparation and analysis were performed as described by Kochan et al. [3]. The results of ABTS analysis were expressed as IC<sub>50</sub> ( $\mu\text{g mL}^{-1}$ ). In addition, the results were calculated as Trolox  $\mu\text{M}$  equivalents per gram of dry extract using a calibration curve obtained from the Trolox standard at concentrations from 1–20  $\mu\text{M}$ . The antioxidant potential of the sample, measured by the FRAP method, was determined against a standard FeSO<sub>4</sub>·7H<sub>2</sub>O value calculated from a calibration curve plotted with concentrations from 0 to 2,000  $\mu\text{M}$ .

#### Statistical analysis

All the experiments were performed in triplicate. Data were analyzed using the Kruskal–Wallis test with STATISTICA (StatSoft, Inc. 2013, version 13.1) and  $p < 0.05$  was considered as statistically significant.



**Fig. 1** Callus cultures after 15 passages of subculturing (A) RCL tissue grown on Murashige and Skoog medium, (B) GCL tissue grown on Woody-Plant medium. Both media were supplemented with 2,4-dichlorophenoxyacetic acid ( $1 \text{ mg L}^{-1}$ ),  $\alpha$ -naphthalene acetic acid ( $1 \text{ mg L}^{-1}$ ), and 6-benzylamino purine ( $0.2 \text{ mg L}^{-1}$ ).

**Tab. 1** Growth of *Panax quinquefolium* callus tissue and suspension cultures.

Growth index $\pm$ SD	RLC (g)	GLC (g)	S ( $\text{g L}^{-1}$ )
Fresh	$8.309 \pm 2.05^a$	$2.466 \pm 0.15^b$	$18.169 \pm 1.88^c$
Dry	$13.993 \pm 0.02^a$	$6.608 \pm 1.33^b$	$9.799 \pm 0.05^c$

The letter in the top index indicates statistically significant differences according to the Kruskal–Wallis test. RCL – red callus line was grown in light on MS medium with the addition of  $1 \text{ mg L}^{-1}$  2,4-dichlorophenoxyacetic acid (2,4-D),  $1 \text{ mg L}^{-1}$   $\alpha$ -naphthalene acetic acid (NAA), and  $0.2 \text{ mg L}^{-1}$  6-benzylamino purine (BAP); passages 21–25. GCL – green callus line was grown in light on Woody-Plant (WP) medium with the addition of  $1 \text{ mg L}^{-1}$  2,4-D,  $1 \text{ mg L}^{-1}$  NAA, and  $0.2 \text{ mg L}^{-1}$  BAP; passages 21–25. S – suspension was grown in light on Murashige and Skoog (MS) medium with the addition of  $1 \text{ mg L}^{-1}$  2,4-D and  $0.1 \text{ mg L}^{-1}$  kinetin; passages 10–15.

The levels of the individual metabolites were also found to depend on the type of culture (Fig. 3). Ginsenoside Rb1 ( $0.899 \text{ mg g}^{-1} \text{ d.w.}$ ) dominated the seven saponins identified in the RCL tissues, and its level was twice as high as that in the GCL tissues. Additionally, GCL contained only four ginsenosides with Re being the dominant one. The saponin profile of *P. quinquefolium* suspension was completely different from that of the callus: the dominant compound was ginsenoside Rg1, accounting for about 54.5% of total examined saponins; however, its level in suspension tissue was significantly lower than that in both the callus cultures (Fig. 4).

## Results

### Morphology and growth of callus and suspension cultures

Callus tissue appeared on the cut surface of the explant after 10 days of culturing, and gradually covered the entire explant surface. The callus was not differentiated macroscopically. Initially, until the third passage, the cultures grew on WP medium with 2,4-D ( $1 \text{ mg L}^{-1}$ ), NAA ( $1 \text{ mg L}^{-1}$ ), and BAP ( $0.1 \text{ mg L}^{-1}$ ). Following this, BAP concentration was increased to  $0.2 \text{ mg L}^{-1}$  to maintain the vitality of the culture. In addition, the calli derived from leaf blades were transferred to MS medium. Both types of cultures, the tissue derived from leaf blades and stalks, were kept in the light; the tissue derived from leaf blades (RCL) was red, friable, and lumpy (Fig. 1A), whereas the tissue derived from stalks (GCL) was green and compact (Fig. 1B).

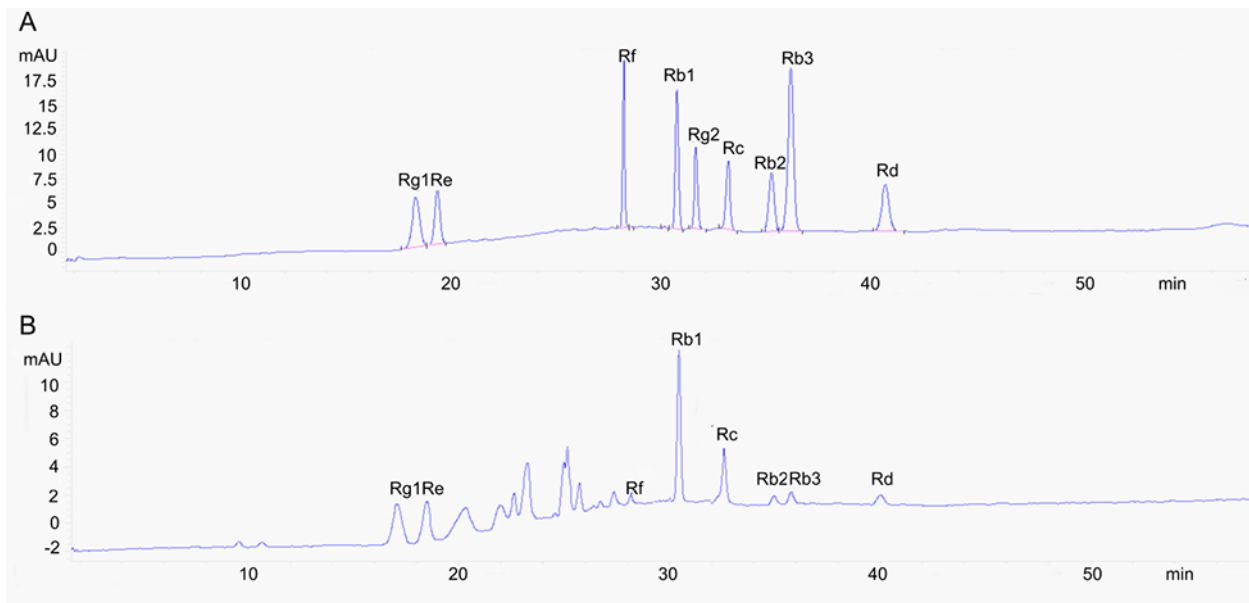
The callus cultures displayed differing growth intensities (Tab. 1). After 35 days of cultivation, the fresh and dry weights of the RCL tissue increased to ninefold ( $2.562 \text{ g}$ ) and 15-fold ( $0.169 \text{ g}$ ), respectively, from their initial inoculum weights (mean  $0.275 \text{ g f.w.}$  and  $0.013 \text{ d.w.}$ ). In contrast, the GCL grew slowly, achieving an increase of fourfold and eightfold in fresh ( $1.774 \text{ g}$ ) and dry ( $0.091 \text{ g}$ ) weights, respectively.

The cells of the suspension culture formed small aggregates and were red in color, like the RCL tissue. After 28 days of cultivation, a 19- and 11-fold increase in fresh and dry weight, respectively, were observed, with maximum levels of  $33.28$  and  $3.08 \text{ g L}^{-1}$  (Tab. 1).

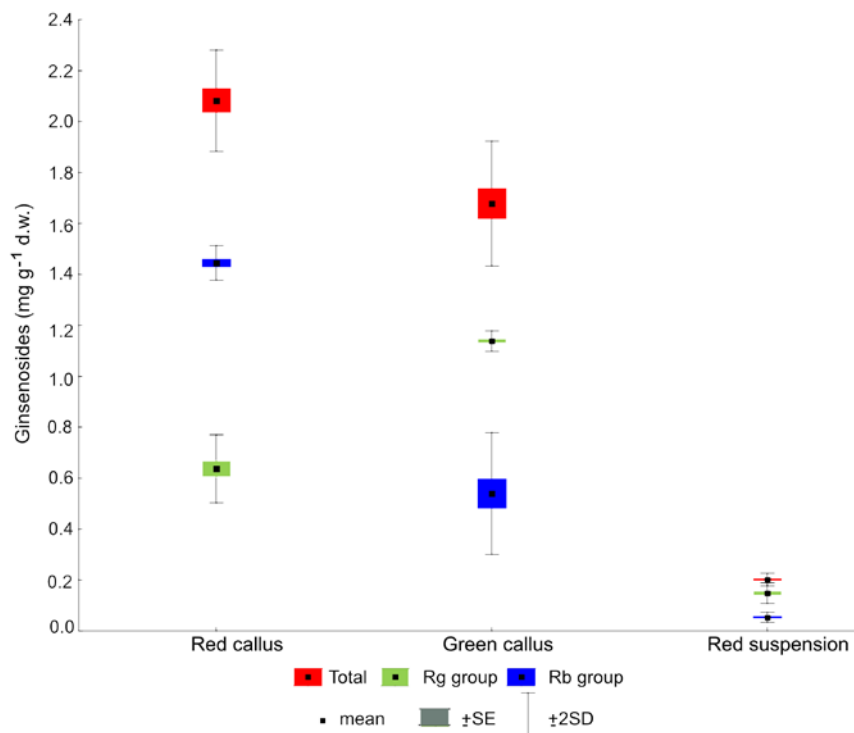
### Saponin content

In the studied cultures of *P. quinquefolium*, nine ginsenosides (Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3, and Rd) were analyzed qualitatively and quantitatively using HPLC. The representative chromatogram of standards and RCL studied extracts is given in Fig. 2.

The RCL tissue displayed the highest amounts of all determined compounds, totaling  $2.067 \text{ mg g}^{-1} \text{ d.w.}$  (Fig. 3). The green callus line contained almost 19% lower saponins than the red callus lines. In the suspension culture, the ginsenoside level was found to be tenfold lower than that in the RCL and eightfold lower than that in the GCL.



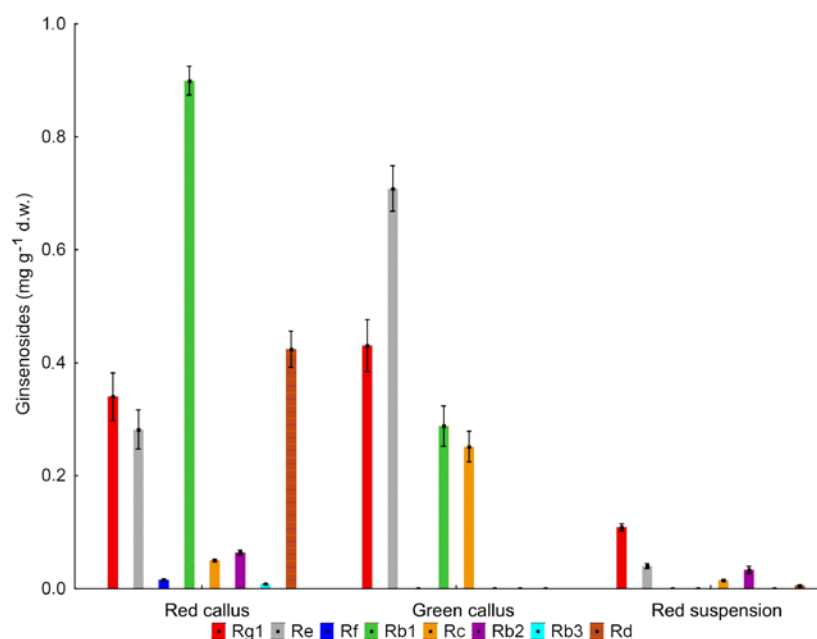
**Fig. 2** The representative chromatogram of standards and RCL extract. Rg1, Re, Rf, and Rg2 protopanaxatriol derivatives (ginsenosides of Rg group); Rb1, Rb2, Rc, Rb3, and Rd protopanaxadiol derivatives (ginsenosides of Rb group).



**Fig. 3** The content of protopanaxadiol (expressed as the sum of Rb1, Rb2, Rb3, Rc, and Rd) and protopanaxatriol (expressed as the sum of Rg1 and Re) derivatives, as well as total ginsenosides (sum all of the determined saponins) in cell cultures of *Panax quinquefolium*. Each value represents the mean of three replicates  $\pm$ SE.

### Total phenolic content

The TCP of the *P. quinquefolium* cell cultures was investigated using the FolinCiocalteu method (Tab. 2). The TPC was found to be higher in the suspension culture than that in the callus lines. It was about 37% and 18% higher than that in the RCL and GCL cultures, respectively. The results also demonstrated that the TPC was inversely related to the total ginsenoside content (TGC); the correlation coefficient between the parameters ( $r$ ) was  $-0.942$ .



**Fig. 4** The content of individual ginsenosides in RCL, GCL, and suspension cultures of *P. quinquefolium*. Each value represents the mean of three replicates  $\pm$ SE.

**Tab. 2** Total phenolic content (TPC) and antioxidant activity of *Panax quinquefolium* cell culture extracts.

Plant material	TPC* content	FRAP**	ABTS*	
			IC <sub>50</sub> ***	TEAC****
RLC	33.26 $\pm$ 0.40 <sup>a</sup>	385.87 $\pm$ 4.94 <sup>a</sup>	48.83 $\pm$ 0.47 <sup>a</sup>	218.75 $\pm$ 2.21 <sup>a</sup>
GLC	43.32 $\pm$ 0.07 <sup>b</sup>	419.14 $\pm$ 2.04 <sup>b</sup>	50.11 $\pm$ 0.22 <sup>b</sup>	215.71 $\pm$ 3.78 <sup>a</sup>
S	52.57 $\pm$ 0.11 <sup>c</sup>	423.26 $\pm$ 2.10 <sup>b</sup>	37.24 $\pm$ 0.12 <sup>c</sup>	274.96 $\pm$ 4.46 <sup>b</sup>

\* TPC – expressed as gallic acid equivalents in mg per gram of the *P. quinquefolium* cell culture dry extract.

\*\* Ferric reducing antioxidant power expressed in  $\mu$ mol Fe (II) g<sup>-1</sup> extract dry weight, 15 min of incubation.

\*\*\* IC<sub>50</sub> the concentration of the sample ( $\mu$ g mL<sup>-1</sup>) showing 50% of the maximal radical scavenging activity.

\*\*\*\* Micromolar Trolox equivalent antioxidant capacity (TEAC) expressed in  $\mu$ mol standard equivalents g<sup>-1</sup> extract dry weight.

The values are means  $\pm$ SE. The means with the same letter in the column do not differ significantly according to the Kruskal–Wallis test ( $p \leq 0.05$ ).

S – suspension culture was grown in light on MS medium with the addition of 1 mg L<sup>-1</sup> 2,4-dichlorophenoxyacetic acid (2,4-D), and 0.1 mg L<sup>-1</sup> kinetin (kin); passages 10–15. GCL – green callus line was grown in light on Woody-Plant (WP) medium with the addition of 1 mg L<sup>-1</sup> 2,4-D, 1 mg L<sup>-1</sup>  $\alpha$ -naphthalene acetic acid (NAA), and 0.2 mg L<sup>-1</sup> 6-benzylamino purine (BAP), derived from stalks; passages 21–25. RCL – red callus line was grown in light on Murashige and Skoog (MS) medium with the addition of 1 mg L<sup>-1</sup> 2,4-D, 1 mg L<sup>-1</sup> NAA, and 0.2 mg L<sup>-1</sup> BAP, derived from leaves; passages 21–25.

### The antioxidant activity

The antioxidant properties of the methanolic extracts of the RCL, GCL, and suspension culture extracts were determined using two in vitro tests based on single-electron transfer (the SET reaction). The free radical scavenging activity of the tested extracts was determined by the ABTS assay, expressed as the ability to scavenge 50% of free radical ABTS<sup>•+</sup> (IC<sub>50</sub>) and the TEAC (Trolox equivalent antioxidant capacity). The FRAP assay was performed to evaluate the ability to reduce ferric ions (Fe<sup>3+</sup> to Fe<sup>2+</sup>).

All the extracts demonstrated antioxidant potential (Tab. 2). The ABTS test indicated IC<sub>50</sub> values in the range of 37–50  $\mu$ g mL<sup>-1</sup> (Tab. 2); the highest activity (IC<sub>50</sub> of 37  $\mu$ g mL<sup>-1</sup>) was demonstrated by the suspension culture extracts, with the callus extracts showing more than 30% lower antiradical activity. The ABTS assay, expressed as the

TEAC, showed that suspension extracts possessed approximately 1.25 times higher TEAC value than the RCL and GCL. The FRAP test showed similar results to ABTS assay, with the suspension culture extracts demonstrating the best reducing ability [ $423 \mu\text{mol Fe(II) g}^{-1} \text{ d.w.}$ ]. The study results revealed that the green callus line presented a similar FRAP value [ $419 \mu\text{mol Fe(II) g}^{-1} \text{ d.w.}$ ] to the suspension culture. The least reducing ability was observed for the RCL extracts.

## Discussion

The analysis of biomass yield indicated that the suspension culture of *P. quinquefolium* underwent a 19- and 11-fold increase in their fresh and dry weights compared to their inoculum. These values are significantly higher than those observed in the suspension cultures of other ginseng species such as *P. ginseng* C. A. Meyer [23], *P. japonicus* (T. Ness) C. A. Mey [24], and *P. vietnamensis* Ha et. Grushv. [25]. Furthermore, the culture demonstrated better growth than *P. quinquefolium* callus as described by Obae et al. [26]. This difference in the growth of the callus observed for the same species of ginseng could be because of the choice of explants used to obtain cultures and their different growth conditions.

The suspension cultures in this study contained  $52.57 \text{ mg GAE g}^{-1} \text{ d.w.}$  of TPC, which was 15-fold higher than the level of total phenolic compounds in *Artemisia absinthium* L. suspension culture and sixfold than in *T. peruviana* suspension culture ( $3.57$  and  $9.46 \text{ mg GAE g}^{-1} \text{ d.w.}$  respectively) [27,28]. Lower content of phenolic compounds was found in both the callus cultures. However, previous studies indicate that the TPC in these cultures was still significantly higher than that in many calli described earlier [29–31]. For example, the phenolic compound content was found to be 4–10 times higher in the RCL and GCL cultures than *Habenaria edgeworthii*, *Hovenia dulcis*, and callus cultures [29,31]. In contrast, the callus culture of *Justicia gendarussa* included 2–2.5 times more phenolic compounds than the GCL and RCL cultures [32].

Our results indicate that the suspension culture, rich in phenolic compounds, contained relatively small amounts of saponins ( $0.202 \text{ mg g}^{-1} \text{ d.w.}$ ). Among the protopanaxadiol derivatives, only ginsenosides Rc and Rb2 were present in the culture, and among the protopanaxatriol derivatives, only Rg1 and Re were present in the culture. Significantly higher total ginsenoside levels have been observed in suspension cultures of *P. sikkimensis* R. N. Banerjee and *P. quinquefolium*, and similar to our study results, the dominant metabolite in these studies was also Rg1 and Rd was not found in any of the cultures [33]. In contrast, our culture produced saponin Rc, which was not detected by Biswas et al. [33].

Both callus tissues of *P. quinquefolium* contained higher levels of saponins than the suspension; however, both tissues were poorer sources of ginsenosides than another *P. quinquefolium* callus line previously derived from root explants [26]. On the other hand, Huang et al. [34] observed that *P. ginseng* callus, cultivated in the dark, showed less qualitative differentiation with regard to saponin accumulation and produced only three compounds, Re, Rg1, and Rb1. These differences in the findings may be attributed to the choice of plant species, the type of explant from which callus cultures were obtained, the age of callus cultures used for ginsenoside isolation, and the variation in the conditions of culture growth.

Comparative studies demonstrated that much higher levels of total saponin content were found in the leaves and stems of 1-year-old plants from which the calli were obtained ( $17.51$  and  $2.91 \text{ mg g}^{-1} \text{ d.w.}$ , respectively; unpublished data). Although the leaves of field-grown plants and callus tissues demonstrated significant differences in their saponin level, it is important to note that while the traditionally-cultivated, field-grown plants could be used as sources of plant material only once, after 1 year in soil, the callus tissues were able to supply raw material repeatedly, every 5 weeks.

It has been reported that the antioxidant properties can be related to the levels of phenolic compounds and ginsenosides in naturally-grown plant material [7,35–37]. The present study is the first to evaluate both the ginsenoside and total phenolic compound contents in *P. quinquefolium* cell culture extracts and determine their antioxidant capacity.

In the present study, the suspension culture extracts exhibited the highest antioxidant properties among the other extracts when tested using ABTS and FRAP assays; these extracts also demonstrated the highest TPC and the lowest ginsenoside level. These results indicate that the primarily phenolic compounds may affect the antioxidant capacity of the obtained cell cultures. Other investigations showed that the cell suspension cultures can constitute a good source of bioactive compounds with antioxidant potential [27,29,35]. Similarly, in a previous study, suspension of *A. absinthium* L. was found to produce phenolic compounds more efficiently and have a higher antioxidant activity than the callus culture [27]. In addition, it was found that *P. quinquefolium* suspension, demonstrated 2.5 times greater antioxidant capacity, expressed as Trolox equivalent, than *Thevetia peruviana* suspension [28].

The callus cultures described in the present paper characterized weaker antioxidant properties than the suspension cultures. Similar findings have been reported for the cultures of *J. gendarussa* [32] and callus of *Salvadora persica* [36].

However, FRAP analysis has found that the for ginseng, cell cultures have better antioxidant potential than embryogenic callus cultures, as well as shoots and hairy roots of *P. quinquefolium* growing in vitro [3,17].

The correlation coefficients between the antioxidant activity and TPC of *P. quinquefolium* cultures indicated a relationship between the two; the same was found for antioxidant activity and ginsenoside content (Tab. 3). Higher TPCs are positively associated with stronger antioxidant capacity, whereas TGC is inversely related. The strong correlations observed between the TPC and antioxidant ability, evaluated by ABTS and FRAP (Tab. 3), indicate that phenolic compounds that are stronger than ginsenoside also contribute to the antioxidant potential of the *P. quinquefolium* cell cultures. Our results confirm the findings of previous studies, which indicate that phenolic compounds are the main antioxidant components in plants and that their level is closely related to the plant antioxidant potential [35,37].

Similar observations were made for ginseng roots grown under different conditions; phenolic compounds were found to have a stronger influence on antioxidant activity than saponins [17].

**Tab. 3** Correlation coefficients between the antioxidant activity of *Panax quinquefolium* extracts and the total ginsenoside and total phenolic contents.

Metabolites	ABTS	FRAP
TGC	-0.969	-0.921
TPC	0.829	0.738

TGC – total ginsenoside content; TPC – total phenolic content.

## Conclusion

This is the first paper to examine the production of saponins and phenolic compounds in *P. quinquefolium* cell cultures and to evaluate the antioxidant potential of these cultures. The suspension culture grew more intensively than the callus cultures and was characterized by the highest total phenolic compounds; this resulted in higher antioxidant activity, as indicated by both the FRAP and ABTS tests. In contrast, ginsenoside biosynthesis was more effective in callus cultures, especially in RCL tissue.

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