

Ginsenoside Contents in *Panax quinquefolium* Organs from Field Cultivation

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Z. Naturforsch. **63c**, 91–95 (2008); received July 3/August 20, 2007

Quantitative composition of saponins (ginsenosides) in *Panax quinquefolium* originating from field cultivation in Poland and harvested at the beginning of the growing season, during the blossoming period and at the end of growth was determined. A colourimetric method gave lower values compared to results of HPLC analysis, but the tendency of change in the content of saponins was similar in both instances. Ginsenoside amounts in *P. quinquefolium* organs changed depending on the specific time during the vegetation season the samples were taken. This study found that the highest content of these metabolites – 66 mg/g d. w. – occurred in the roots of the plant at the time of bloom. Two among the six metabolites examined in our study were dominant independently of the vegetation season. These were Rb₁ and Re, with values of 25.4–33.8 mg/g d. w. and 16.4–19.7 mg/g d. w., respectively.

Key words: Ginsenoside, *Panax*, Field Cultivation

Introduction

Panax quinquefolium, one of the main medicinal species of the *Panax* genus, is a perennial plant which is found in forests of eastern regions of North America. In terms of chemical composition and medicinal properties it is most similar to *Panax ginseng*. Pharmacological and clinical investigations confirmed that ginseng extracts are beneficial to good health. They demonstrated multidirectional action, including antistress (Rai *et al.*, 2003), anticancer (Wang *et al.*, 2006; Li *et al.*, 2006), and anti-inflammatory activity (Larsen *et al.*, 2005; Ahn *et al.*, 2006), in addition to regulating metabolism as well as the nervous system (Cheng *et al.*, 2005; Rudakewich, 2001), the hormonal system (Lee *et al.*, 2006; Nocerino *et al.*, 2000) and the cardiovascular system (Dou *et al.*, 2001). For the medicinal effect of ginseng mainly ginsenosides (panaxosides), classified as triterpene saponins – derivatives of protopanaxadiol and protopanaxatriol or oleanolic acid – are responsible. Over 30 of these compounds are known until now. In this work, ginsenoside contents in different organs of *P. quinquefolium* were determined depending on the time of harvest, with regard to the period of vegetation.

Materials and Methods

Plant material

The material for investigation was harvested from an experimental field at the Agricultural University of Lublin. Organs of soil-grown 4-year-old *P. quinquefolium* plants were harvested at three key phases of growth: in the middle of May (beginning of vegetation), at the beginning of June (blossoming period), and in the middle of September 2003 (end of growth). The saponins were extracted from roots, stalks and leaves, harvested in the above-mentioned phases, and from fruits, harvested at the end of August 2003.

Saponin extraction

Extraction according to German Pharmacopoeia (DAB 10, 1991)

1 g of the dry raw material was weighed with the accuracy to 0.1 g, placed in a 250 ml flask together with 70 g of 50% methanol and then extracted for 1 h. Extract was cooled to room temperature and was supplemented with a 50% aqueous methanol solution. The methanol solution was weighed and evaporated to dryness using a vacuum evaporator under reduced pressure at 60 °C. The dry fraction was then weighed. The dried methanolic fraction

was dissolved in 20 ml 0.1 M HCl, and the flask was rinsed out twice with 5 ml 0.1 M HCl. 70 ml of the upper dividing phase were added to the solution, placed in a divider, and oscillated. The mixture was left for 30 min in order to separate the phases. In order to extract saponin from the methanol fraction according to the pharmacopoea method, a separating mixture composed of *n*-butanol, 0.1 M HCl and chloroform (6:3:1 v/v) was used. Two phases were employed separately. The upper layer was removed, and the next portion of 70 ml of the initial upper dividing phase was added to the bottom phase. The procedure was repeated three times, collecting the upper layers of separation each time. These three upper layers were combined, and 30 ml of the bottom dividing phase were added; subsequently the resulting solution was oscillated for 25 min. The lower layer was then eliminated, and the 30 ml lower initial separating phase (*n*-butanol/0.1 M HCl/CHCl₃) were again mixed with the upper one. The bottom layer of the final mixture was removed, while the upper layer was evaporated to dryness in a vacuum evaporator at 70 °C. The dry extract was placed into a desiccator for 24 h, and subsequently weighed. Extractions of saponin were carried out by 3–6 repetitions from every sample of the studied material.

Extraction of saponin with preliminary defatting of the raw material

Defatting with chloroform

1 g of dry crumbled raw preparation of ginsenoside was filled in a cartridge made of blotting paper and placed into a Soxhlet apparatus. In order to eliminate fatty compounds, this raw material was rinsed three times for 4 h with 50 ml chloroform at 70 °C.

Extraction with methanol

The defatted material was extracted three times in a 250 ml flask with 50 ml of 80% methanol for 30 min, at the boiling point of the solvent under reflux cooler. The combined methanolic extracts were filtered and evaporated by a vacuum evaporator under reduced pressure at 60 °C. The flask with the remaining dry residue was placed in a desiccator with a desiccant agent. The dry methanolic extract was weighed. Extractions of saponin were performed in 3–6 repetitions from every sample of the studied material.

Quantitative analysis of saponin

A colourimetric method according to the German Pharmacopoeia (DAB 10, 1991)

The dry methanolic extract was dissolved in 50 ml 80% acetic acid and filtered. The first 20 ml of filtrate were rejected. From the remaining part of the filtrate, 1 ml was placed into test tubes and 4 ml of reagent consisting of 98% CH₃COOH and 96% H₂SO₄ (1:1) were added. The tubes were shaken and warmed for 25 min at approximately 60 °C. After cooling to room temperature the absorbance of the solution was measured at 520 nm and compared with a standard reference. The reference mixtures were composed, in two stages, first by mixing 4 ml of the abovementioned reagent with 1 ml of 80% CH₃COOH, and then by adding different quantities of Rg₁ ginsenoside. The percentage contents of ginsenosides, converted to Rg₁ ginsenoside, were calculated according to the formula

$$\frac{301.7 \times A}{m_1 \times m_2},$$

where *A* is the measured absorbance, *m*₁ the filtrate mass (g), and *m*₂ the mass of raw material (g).

High pressure liquid chromatography

Dry extracts were dissolved in 2 ml of methanol for HPLC and filtered through 0.2 µm pore diameter Millex®-FG hydrophobic fluoropore filters (PTFE). Further analysis was carried out using a high pressure liquid chromatography system consisting of a LiChro CART® 250-4 column, Waters 600 controller pumps, and a UV-VIS Waters 996 detector, combined with the Pentium 60 PCI hardware equipped with Millennium software. Two different mixtures of acetonitrile with water were used as eluents. A 30:70 acetonitrile/water ratio was used for the determination of the ginsenosides Rb₁, Rb₂, Rc and Rd (flow rate 2 ml/min, time of analysis 45 min), and an 18:82 ratio was used for the determination of the ginsenosides Rg₁ and Re (flow rate 3 ml/min, time of analysis 40 min). The determination was made at 203 nm wavelength.

Statistical analysis

Standard error was enumerated according to

$$SE = \frac{SD}{\sqrt{n}},$$

where SD is the standard deviation and *n* the number of measurements. SD was calculated according to the pattern

$$SD = \frac{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2}}{n - 1},$$

where x_i is the value of the individual attempt and \bar{x} the arithmetic mean.

Results

The analysis of saponin using a spectrophotometric method proved that *P. quinquefolium* roots from the beginning of vegetation, the blossoming period and the end of vegetation differed in total ginsenoside contents, varying between 31 and 37 mg/g d. w. The highest amounts of saponins were found in roots harvested during the blossoming period, whereas the lowest occurred at the end of the vegetation season.

A more detailed analysis using the HPLC method for six ginsenosides revealed that the level of ginsenosides in these materials was much higher (Table I). Nevertheless, the highest ginsenoside contents (over 66 mg/g d. w.) were also observed in roots during the blossoming period, while the lowest occurred at the end of the vegetation period (over 55 mg/g d. w.).

Independently of the growing season the largest part had ginsenoside Rb₁ (approximately half of all determined saponins), followed by ginsenoside Re (comprising 26 to 31% of the sum of all determined saponins). The changes in individual saponin contents during the vegetation season showed variance. The contents of the metabolites Rb₁, Re and Rg₁ increased during the blossoming period

and decreased at the end of plant growth, especially for the Rg₁ saponin. An opposite dependence was found for Rc and Rd saponins – their content decreased strongly during the blossoming time and increased again at the end of vegetation growth. The content of ginsenoside Rb₂ decreased gradually during the vegetation season to only 44% of the content at the beginning of vegetation growth.

The content of ginsenosides in leaves of *P. quinquefolium* in bloom and at the end of growth was comparable, 23.72 mg/g d. w. and 24.8 mg/g d. w., respectively. This constitutes an increase of over 50% in relation to the beginning of growth; metabolite Rb₂ prevailed quantitatively. In these samples two metabolites, *i. e.* Rb₁ and Re, displayed clear dominance. Rb₁, Rb₂ and Rc contents increased during the blossoming period and decreased at the end of the vegetation period, but remained higher than at the start of the season. The contents of Rd and Rg₁ decreased during the blossoming period and increased at the end of vegetation again achieving the highest values (Table I).

Stalks contained considerably less saponins compared to roots and leaves; nearly 9.5 mg/g d. w. at the beginning of vegetation, below 6 mg/g d. w. during the blossoming period, and below 12 mg/g d. w. at the end of vegetation. In this organ, ginsenoside Re showed dominant occurrence at all phases of vegetation period. With regard to the contents of the saponins Rb₁, Rb₂ and Rg₁, a gradual increase during the growing season was observed. On the other hand, quantities of the ginse-

Table I. Individual ginsenosides found in *P. quinquefolium* organs by HPLC analysis depending on the vegetation season.

Organ	Period of growth	Saponin content ± SE [mg/g]								
		Rb ₁	Rb ₂	Rc	Rd	Rg ₁	Re	Rb group	Rg group	Total
Roots	Beginning	28.74 ± 1.75	0.81 ± 0.03	8.41 ± 1.88	4.00 ± 1.23	3.58 ± 0.13	16.38 ± 1.35	41.96	19.96	61.92
	Blossoming	33.76 ± 1.77	0.57 ± 0.04	4.66 ± 1.07	2.89 ± 0.76	4.68 ± 0.88	19.7 ± 1.32	41.88	24.38	66.26
	End	25.36 ± 1.67	0.3 ± 0.02	7.03 ± 2.15	3.16 ± 0.98	2.39 ± 1.01	17.45 ± 1.6	35.91	19.84	55.75
Leaves	Beginning	1.12 ± 0.65	4.04 ± 2.01	1.42 ± 0.33	1.37 ± 0.45	0.51 ± 0.02	5.15 ± 0.31	7.95	5.66	13.61
	Blossoming	2.26 ± 0.78	11.66 ± 1.98	5.35 ± 0.67	0.81 ± 0.07	0.39 ± 0.06	3.31 ± 0.80	20.02	3.7	23.72
	End	1.49 ± 0.38	8.41 ± 1.59	3.45 ± 0.22	4.27 ± 0.33	1.89 ± 0.11	5.29 ± 0.16	17.62	7.18	24.8
Stalks	Beginning	0.76 ± 0.06	0.69 ± 0.05	1.03 ± 0.12	2.11 ± 0.56	0.55 ± 0.04	4.29 ± 0.17	4.59	4.84	9.43
	Blossoming	0.97 ± 0.1	0.7 ± 0.04	0.32 ± 0.07	0.89 ± 0.09	0.63 ± 0.08	2.21 ± 0.23	2.94	2.82	5.78
	End	1.07 ± 0.08	3.54 ± 0.89	0.83 ± 0.09	1.47 ± 0.24	0.78 ± 0.07	4.06 ± 0.12	6.91	4.84	11.75
Fruits		0.86 ± 0.09	1.54 ± 0.95	1.51 ± 0.11	0.48 ± 0.1	0.53 ± 0.09	5.30 ± 0.54	4.39	4.83	9.22

nosides Rc, Rd and Re were variable: they decreased during the blossoming period and increased at the end of growth. The highest increase – fivefold – was noted for saponin Rb₂.

The fruits of *P. quinquefolium* contained a little over 9 mg/g d. w. total saponins, determined with high content of ginsenoside Re (over 5 mg/g d. w., about 55% of examined metabolites).

Discussion

Many authors (Wang *et al.*, 1999; Corthout *et al.*, 1999; Chuang *et al.*, 1995; von Breemen *et al.*, 1995; Cui, 1995) agree that the qualitative and quantitative compositions of ginsenosides in root extracts of these plants are characteristic for their taxonomic category and plant age. Mizuno *et al.* (1994) demonstrated that *P. ginseng* wild roots and cultivated roots had similar contents of total saponins (30 and 32 mg/g d. w., respectively), and that the ginsenosides Re and Rb₁ were the dominant metabolites. In other studies it was observed that *Panax ginseng* contains higher amounts of ginsenosides of the Rg group (Rg₁, Rf) than American ginseng, in which metabolites of the Rb group and their malonyl derivatives are dominant.

In our study, analyses of ginsenosides in different organs of *P. quinquefolium* plants showed that the highest contents of ginsenoside (56–66 mg/g) were found in the roots. Two ginsenosides, Rb₁ (protopanaxadiol derivative) and Re (protopanaxatriol derivative), dominated independently of the vegetation phase. A similar distribution of individual ginsenosides found in 4-year-old *P. quinquefolium* roots was described by Court *et al.* (1996), Li *et al.* (1996) and Li and Mazza (1999). However, the sum of the studied metabolites was on average only half as high as those obtained in our study.

Concerning the optimal period for harvest of *P. quinquefolium* roots, it would appear to be the blossoming time of the plant, but not the end of vegetation, as it is done usually.

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Acknowledgements

The authors thank Prof. George Gasyna for improving the language of this paper.

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