Foods and Raw Materials, 2020, vol. 8, no. 2

E-ISSN 2310-9599
ISSN 2308-4057

Research Article
Open Access

DOI: http://doi.org/10.21603/2308-4057-2020-2-369-376
Available online at http://jfrm.ru/en

# *Panax ginseng* callus, suspension, and root cultures: extraction and qualitative analysis

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Received May 29, 2020; Accepted in revised form June 11, 2020; Published August 25, 2020

**Abstract:**
*Introduction.* In recent years, scientists have been actively searching for medicinal plants containing biologically active substances with geroprotective properties to treat diseases of old age, in particular cancer, diabetes, cardiovascular diseases, and others. Ginseng (*Panax ginseng* L.) is a promising source of geroprotective compounds. We aimed to select optimal parameters for extracting organic compounds from ginseng callus, suspension, and root cultures and analyze their qualitative composition.
*Study objects and methods.* We studied ginseng callus, suspension, and root cultures, as well as their extracts. Biologically active substances were extracted with 30 to 70% ethanol. Organic compounds were determined by thin-layer chromatography. The results for each plant were archived and analyzed for the presence of quercetin, mangiferin, luteolin, rutin, quercetin-2-D-glucoside, malvidin, as well as caffeic, cinnamic, ferulic, and sinapinic acids.
*Results and discussion.* We developed a procedure for screening solvents and performed a fractional qualitative analysis of biologically active substances extracted from ginseng. As a result, we established the optimal parameters for extracting biologically active substances from the dried biomass of ginseng cultures. In all cases, temperature and the ratio of solvent to biomass were the same (50°C, 1:5). However, the extraction time and ethanol concentration differed, amounting to 60 min and 50% for callus cultures, 30 min and 60% for suspension cultures, and 60 min and 70% for root cultures. The qualitative analysis of organic compounds showed the presence of rutin (0.25), quercetin (0.75), and mangiferin (0.57), as well as caffeic and sinapinic acids in the extracts.
*Conclusion.* Our set of experiments to isolate biologically active substances from ginseng callus, suspension, and root cultures resulted in selecting the optimal extraction parameters and analyzing the extracts for the presence of organic compounds.

**Keywords:** Plant cultures, *Panax ginseng*, ginseng, plant extracts, geroprotective properties, gerontology

**Funding:** The research was financed by the Ministry of Science and Higher Education of the Russian Federation (Minobrnauka) No. FZSR-2020-0006 as part of the government task "Screening biologically active substances of plant origin with geroprotective properties and developing technology to obtain nutraceuticals that slow down aging". Thin layer chromatography was performed at Kemerovo State University's (KemSU) Core Facilities Center (CFC).

**Please cite this article in press as:** Dyshlyuk LS, Dmitrieva AI, Ivanova SA, Golubtsova YuV, Ostroumov LA. *Panax ginseng* callus, suspension, and root cultures: extraction and qualitative analysis. Foods and Raw Materials. 2020;8(2):369–376. DOI: http://doi.org/10.21603/2308-4057-2020-2-369-376.

## INTRODUCTION

Modern medicine and biology are actively searching for new drugs with geroprotective effects [1–8]. Highly useful in this regard are extracts of medicinal plants [8, 9].

A common extraction method involves using a reflux condenser, a Soxhlet extractor, mechanical stirring, and ultrasound. Soxhlet extraction takes place at 80–90°C and lasts from 20 to 24 h. Such parameters make it possible to efficiently extract biologically active compounds, such as saponins [10–13].

Modern extraction methods include ultra-high pressure extraction (UHPE), ultra-temperature extraction (UTE), pressurized liquid extraction (PLE), microwave-assisted extraction (MAE), pressurized hot-water extraction (PHWE), and supercritical fluid extraction (SFE) [9–12, 14–17].

Compared to traditional techniques, modern methods use smaller amounts of solvents, are easily automated, and take little time. However, they are hardly more effective than, for example, Soxhlet extraction or

mechanical mixing [13, 18]. Moreover, pressurized hot-water extraction and supercritical fluid extraction are technically quite difficult to perform [10, 14, 19, 20].

Among medicinal plants with geroprotective properties are *Schisandra chinensis* L., *Scutellaria baicalensis* L., *Rhodiola rósea* L., *Ginkgo biloba* L., and others [21–24]. The most highly valued geroprotective medicinal plants include *Panax ginseng* L., *Aralia mandshurica* L., and *Eleutherococcus senticosus* L. [25]. Since the 1980s, scientists have known of their antitumor effects [25–27].

Ginseng (*Panax ginseng* L.) is a slowly growing perennial plant that is often used as a functional component and a phytotherapeutic agent to prevent and treat various diseases, such as cancer, allergies, inflammatory diseases, and diabetes mellitus [26–31].

According to scientific literature, ginseng extract is used as an adaptogen to increase physical performance, vitality, immunity, as well as resistance to stress and aging [26, 32–34]. It also lowers total cholesterol and low-density lipoproteins, thereby improving a blood lipid profile [31, 32].

However, this plant is included in the Red Book of the Russian Federation and the collection of young roots is prohibited due to its depletion. In addition to low seed productivity and relatively slow growth, ginseng population is irreparably damaged by forest fires and human activities in its endemic areas [35, 36].

Therefore, a justified solution would be to use the plant's cell and organ cultures as an alternative source of renewable medicinal material [30, 32, 35–37]. In our study, we used ginseng callus, suspension, and root cultures – obtained in the early stages of research – as a source of biologically active substances.

We aimed to select optimal extraction parameters and perform a qualitative analysis of organic compounds isolated from ginseng callus, suspension, and root cultures.

## STUDY OBJECTS AND METHODS

Our study objects included callus, suspension, and root cultures of ginseng (*Panax ginseng* L.) obtained *in vitro*, as well as their extracts.

To determine the optimal parameters for extracting biologically active substances from ginseng by reflux extraction, we analyzed several extraction systems for their effectiveness. A water-ethanol mixture was selected as an extractant due to its safety (GRAS), economic efficiency, and the ability to extract a wide range of biologically active substances from plant materials [38, 39]. We screened the solvents and performed a qualitative analysis of organic compounds (Fig. 1). The percentage of ethanol in the solvents is indicated in mass fractions. The yield of extracts (%) is expressed in terms of 100 g of dry raw material.

To extract biologically active substances from ginseng callus cultures, we placed 3 ± 0.001 g of dry powdered callus culture in a 50 mL plastic tube and added 40 mL of 30, 40, 50, 60, or 70% solvent according to the screening scheme (Fig. 1). The tube was connected to a reflux condenser. After 60 min of extraction, we separated the dry mass from the solution by filtration. To remove suspended particles, we centrifuged the filtrate at 3900 rpm. Ethanol was evaporated from a 100 mL pre-weighed flask under reduced pressure. After evaporation, we weighed the flask and measured the extract yield.

Then, we dissolved the residue in a minimum amount of the solvent and determined the qualitative composition of organic compounds in the extract by thin-layer chromatography.

The chromatograms for each plant were archived and analyzed for the presence of quercetin (Sigma-Aldrich, USA, ≥ 95%), mangiferin (Sigma-Aldrich, USA, ≥ 98%), luteolin (Sigma-Aldrich, USA, ≥ 98%), rutin (Sigma-Aldrich, USA, ≥ 94%), quercetin-2-D-glucoside (Sigma-Aldrich, USA, ≥ 95%), caffeic acid (Sigma-Aldrich, USA, ≥ 98%), cinnamic acid (Acros Organics, Belgium,

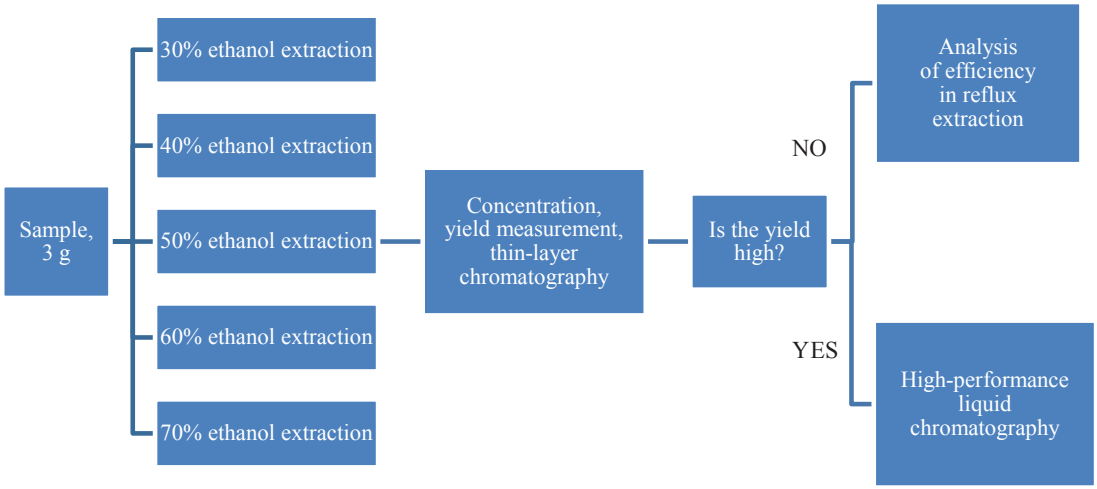


**Figure 1** Solvents efficiency in extracting biologically active substances from ginseng

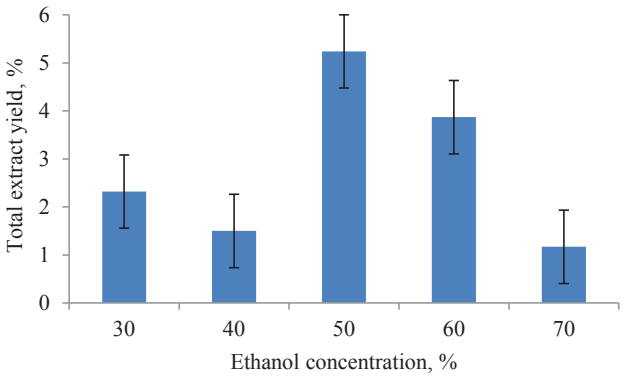


**Figure 2** Solvents efficiency in extracting biologically active substances from ginseng callus cultures

≥ 98%), ferulic acid (Sigma-Aldrich, USA, ≥ 99%), sinapinic acid (Honeywell, USA, ≥ 95%), and malvidin (Sigma-Aldrich, USA, ≥ 90%).

To prepare ginseng suspension and root cultures for the experiment, they were pre-dried to constant weight. Then, 0.5–2.0 g samples of dried cultures were extracted with solvents for callus cultures.

Thin-layer chromatography was performed as described in Pharmacopeia Article 1.2.1.2.0003.15. After evaporation of the solvent from the total extract, we dissolved the dry residue in 1 mL of a suitable extractant (methanol, methylene chloride or acetone) and applied it to the plate with a glass capillary for thin-layer chromatography.

Then, we placed the plate in a chamber and added a suitable eluent. When we used silica gel without modification, chromatography was performed in the $CH_2Cl_2$:MeOH system with a 0–10% methanol gradient, in increments of 1%. For reversed-phase chromatography, we used the $H_2O$:MeCN eluent system with a 0–20% acetonitrile gradient, in increments of 2%, and 0.1% trifluoroacetic acid as a modifier.

We separated the fractions with high-performance liquid chromatography (HPLC), using a Prominence LC-20 chromatograph with diode-array detection (Shimadzu, Japan) and a 250×4.6 mm Kromasil C18 chromatographic column with 5 µm sorbent particles. A mixture of water with o-phosphoric acid, pH = 4.6 (A) and acetonitrile (B) were used as a mobile phase. The gradient elution modes (% B) were 0–20 and 20–60 min with a gradient change of 10–20% and 20–50%, respectively. The eluent flow rate was 1.0 mL/min; the temperature of the column thermostat was 35°C. In the preparative accumulation mode, the eluent was used without the acid.

The instrument was calibrated with caffeine (Sigma-Aldrich, USA, ≥ 90%).

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian NMR System 400 spectrometer with a silent Ceccato OFCS 5/8 SD compressor (Varian, USA), with DMSO-D6 used as a solvent and tetramethylsipane as the internal standard.

## RESULTS AND DISCUSSION

To analyze the efficiency of various extraction systems, we obtained average yields of solids in total extracts. Total extract yields depending on the solvent's concentration are presented in Fig. 2.

Based on the results, we selected 50% ethanol as a solvent to extract biologically active substances from the dried biomass of ginseng callus cultures by reflux extraction. Further selection parameters are shown in Tables 1, 2.

According to Table 1, the maximum yield of biologically active substances extracted from dried ginseng callus cultures (5.88 ± 0.59%) at 45°C was provided by a 1:5 ratio of solvent to biomass and the

**Table 1** Dry extract yield of biologically active substances from dried ginseng callus culture biomass depending on extraction time (at 45°C)

| Solvent:culture ratio | Extract yield depending on duration, % | | | | | |
|---|---|---|---|---|---|---|
| | 10 min | 30 min | 60 min | 120 min | 180 min | 360 min |
| 1:1 | 0.50 ± 0.05 | 0.81 ± 0.08 | 1.22 ± 0.12 | 1.29 ± 0.13 | 1.38 ± 0.14 | 1.38 ± 0.14 |
| 1:2 | 0.80 ± 0.08 | 0.94 ± 0.09 | 1.35 ± 0.14 | 1.58 ± 0.16 | 1.67 ± 0.17 | 1.71 ± 0.17 |
| 1:5 | 1.20 ± 0.12 | 1.80 ± 0.18 | 2.78 ± 0.28 | 5.88 ± 0.59 | 5.95 ± 0.60 | 5.81 ± 0.58 |
| 1:10 | 1.40 ± 0.14 | 1.98 ± 0.20 | 2.98 ± 0.30 | 5.94 ± 0.59 | 5.97 ± 0.60 | 6.04 ± 0.60 |
| 1:20 | 1.40 ± 0.14 | 2.01 ± 0.20 | 3.01 ± 0.30 | 5.95 ± 0.60 | 6.01 ± 0.60 | 6.07 ± 0.61 |

**Table 2** Temperature selection for extracting biologically active substances from ginseng callus cultures

| Temperature, °C | Extract yield depending on duration, % | | | | | |
|---|---|---|---|---|---|---|
| | 10 min | 30 min | 60 min | 120 min | 180 min | 360 min |
| 25 | 1.20 ± 0.12 | 1.80 ± 0.18 | 2.78 ± 0.28 | 5.88 ± 0.59 | 5.95 ± 0.60 | 5.81 ± 0.58 |
| 40 | 1.55 ± 0.16 | 1.98 ± 0.20 | 3.92 ± 0.39 | 6.21 ± 0.62 | 6.18 ± 0.62 | 6.24 ± 0.62 |
| 50 | 1.79 ± 0.18 | 2.35 ± 0.24 | 6.98 ± 0.70 | 7.05 ± 0.71 | 7.01 ± 0.70 | 7.12 ± 0.71 |
| 80 | 1.62 ± 0.16 | 2.14 ± 0.21 | 6.04 ± 0.60 | 6.12 ± 0.61 | 6.14 ± 0.61 | 6.17 ± 0.62 |

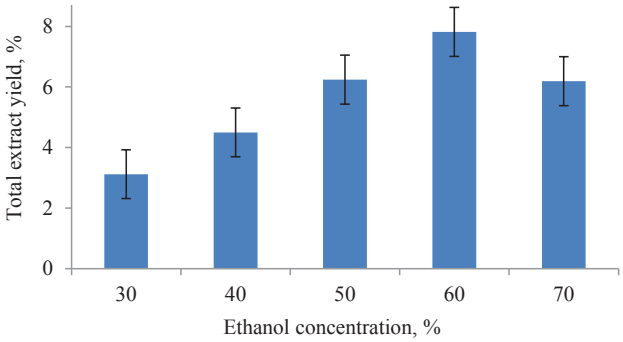


**Figure 3** Solvents efficiency in extracting biologically active substances from ginseng suspension cultures

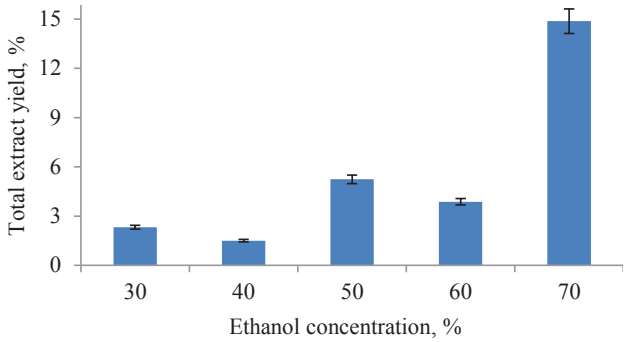


**Figure 4** Solvents efficiency in extracting biologically active substances from ginseng root cultures

extraction time of at least 120 min. Noteworthily, a further increase in duration had no effect on the yield of biologically active substances.

Next, we optimized the temperature and time of extraction (Table 2).

We found that the duration of 60 min and a temperature of 50°C produced the optimal yield of biologically active substances from the dried biomass of ginseng callus cultures (6.98 ± 0.70%).

Next, we determined the optimal parameters to obtain total extracts from the dried biomass of ginseng suspension cultures with various solvent concentrations (Fig. 3).

Based on the results, we selected 60% ethanol as the most optimal solvent to obtain total extracts of biologically active substances from the dried biomass of ginseng suspension cultures using the reflux extraction method. Further selection parameters are shown in Tables 3–4.

We found that the maximum yield of biologically active substances extracted from dried ginseng suspension cultures (8.78 ± 0.88%) at 45°C was provided by a 1:5 ratio of solvent to biomass and the extraction time of at least 120 min.

According to the results, the optimal parameters for extracting biologically active substances from ginseng with 60% ethanol (extract yield of 8.95 ± 0.90%) were 50°C, 30 min extraction, and a 1:5 solvent-to-biomass ratio.

At the next stage, we optimized the parameters for obtaining total extracts from *in vitro* ginseng root cultures. The total extract yield depending on the solvent is shown in Fig. 4.

According to Fig. 4, 70% ethanol produced the highest yield of biologically active substances from the dried biomass of ginseng root cultures by reflux extraction. Further selection parameters are shown in Tables 5, 6.

According to the results, the duration of 30 to 180 min and the solvent-to-biomass ratio of 1:5 and 1:10 provided the maximum yield of biologically active substances from the dried biomass of ginseng root cultures. In particular, the yield of 11.98% was produced at a ratio of 1:10 at 45°C during 30–60 min.

**Table 3** Dry extract yield of biologically active substances from dried ginseng suspension culture biomass depending on extraction time (at 45°C)

| Solvent:culture ratio | Extract yield depending on duration, % | | | | | |
|---|---|---|---|---|---|---|
| | 10 min | 30 min | 60 min | 120 min | 180 min | 360 min |
| 1:1 | 2.50 ± 0.25 | 2.51 ± 0.25 | 2.62 ± 0.26 | 2.92 ± 0.29 | 2.98 ± 0.30 | 2.83 ± 0.28 |
| 1:2 | 2.80 ± 0.28 | 2.94 ± 0.29 | 2.75 ± 0.28 | 2.85 ± 0.29 | 2.76 ± 0.28 | 2.91 ± 0.29 |
| 1:5 | 2.93 ± 0.29 | 8.78 ± 0.88 | 8.80 ± 0.88 | 8.68 ± 0.87 | 8.95 ± 0.90 | 8.21 ± 0.82 |
| 1:10 | 5.40 ± 0.54 | 8.98 ± 0.90 | 8.98 ± 0.90 | 8.94 ± 0.89 | 8.97 ± 0.90 | 8.84 ± 0.88 |
| 1:20 | 6.34 ± 0.63 | 8.61 ± 0.86 | 8.51 ± 0.85 | 8.95 ± 0.90 | 8.71 ± 0.87 | 8.77 ± 0.88 |

**Table 4** Temperature selection for extracting biologically active substances from ginseng suspension cultures

| Temperature, °C | Extract yield depending on duration, % | | | | | |
|---|---|---|---|---|---|---|
| | 10 min | 30 min | 60 min | 120 min | 180 min | 360 min |
| 25 | 2.20 ± 0.22 | 1.80 ± 0.18 | 2.78 ± 0.28 | 5.88 ± 0.59 | 5.95 ± 0.60 | 5.81 ± 0.58 |
| 40 | 2.55 ± 0.26 | 1.98 ± 0.20 | 3.92 ± 0.39 | 6.21 ± 0.62 | 6.18 ± 0.62 | 6.24 ± 0.62 |
| 50 | 2.79 ± 0.28 | 8.95 ± 0.90 | 8.40 ± 0.84 | 8.75 ± 0.88 | 8.21 ± 0.82 | 8.32 ± 0.83 |
| 80 | 3.62 ± 0.36 | 7.56 ± 0.76 | 7.34 ± 0.73 | 7.12 ± 0.71 | 7.14 ± 0.71 | 7.17 ± 0.72 |

**Table 5** Dry extract yield of biologically active substances from dried ginseng root culture biomass depending on extraction time (at 45°C)

| Solvent:culture ratio | Extract yield depending on duration, % | | | | | |
|---|---|---|---|---|---|---|
| | 10 min | 30 min | 60 min | 120 min | 180 min | 360 min |
| 1:1 | 2.35 ± 0.24 | 2.51 ± 0.25 | 2.62 ± 0.26 | 2.92 ± 0.29 | 2.98 ± 0.30 | 2.83 ± 0.28 |
| 1:2 | 2.38 ± 0.24 | 2.94 ± 0.29 | 2.75 ± 0.28 | 2.85 ± 0.29 | 2.76 ± 0.28 | 2.91 ± 0.29 |
| 1:5 | 2.30 ± 0.23 | 11.78 ± 1.18 | 11.80 ± 1.18 | 11.68 ± 1.17 | 11.95 ± 1.20 | 11.21 ± 1.12 |
| 1:10 | 5.34 ± 0.53 | 11.98 ± 1.200 | 11.98 ± 1.20 | 11.94 ± 1.19 | 11.97 ± 1.20 | 11.84 ± 1.18 |
| 1:20 | 6.54 ± 0.65 | 11.61 ± 1.16 | 11.51 ± 1.15 | 11.95 ± 1.20 | 11.71 ± 1.17 | 11.77 ± 1.18 |

**Table 6** Temperature selection for extracting biologically active substances from ginseng root cultures

| Temperature, °C | Extract yield depending on duration, % | | | | | |
|---|---|---|---|---|---|---|
| | 10 min | 30 min | 60 min | 120 min | 180 min | 360 min |
| 25 | 3.20 ± 0.32 | 1.80 ± 0.18 | 2.78 ± 0.28 | 5.88 ± 0.59 | 5.95 ± 0.60 | 5.81 ± 0.58 |
| 40 | 8.55 ± 0.86 | 1.98 ± 0.20 | 3.92 ± 0.39 | 6.21 ± 0.62 | 6.18 ± 0.62 | 6.24 ± 0.62 |
| 50 | 7.79 ± 0.78 | 11.95 ± 1.20 | 12.40 ± 1.24 | 11.75 ± 1.18 | 11.21 ± 1.12 | 11.32 ± 1.13 |
| 80 | 6.62 ± 0.66 | 11.56 ± 1.16 | 11.34 ± 1.13 | 11.12 ± 1.11 | 11.14 ± 1.11 | 10.17 ± 1.02 |

**Table 7** Optimal parameters for extracting biologically active substances from the dried biomass of ginseng callus, suspension, and root cultures

| Type of ginseng culture | Organic solvent | Ratio of solvent to biomass | Time, min | Temperature, °C |
|---|---|---|---|---|
| Callus | 50% ethanol | 1:5 | 60 | 50 |
| Suspension | 60% ethanol | 1:5 | 30 | 50 |
| Root | 70% ethanol | 1:5 | 60 | 50 |

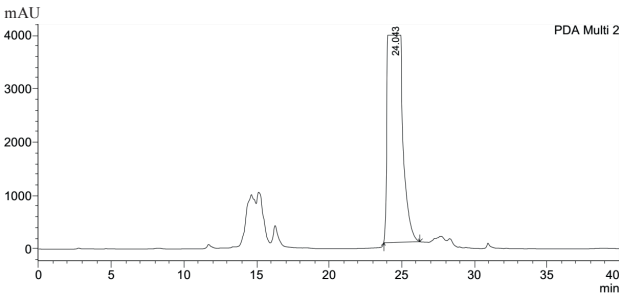


**Figure 5** Qualitative analysis of ginseng flavonoids

Based on the results in Table 6, we selected the following parameters for extracting biologically active substances from the dried biomass of *in vitro* ginseng root cultures: extraction at 50°C during 30 min at a 1:5 ratio of solvent to dried biomass. These parameters produced a yield of 11.95 ± 1.20%. We recommend to use 70% ethanol as a solvent.

Thus, we determined the optimal parameters (time, temperature, organic solvent, ratio of solvent to biomass) for extracting biologically active substances from ginseng callus, suspension, and root cultures (Table 7).

The qualitative analysis of standard compounds and biologically active substances extracted from dried ginseng callus, suspension, and root cultures showed the presence of rutin, quercetin, quercetin-glycoside, mangiferin, luteolin, apigenin, and caffeic acid.

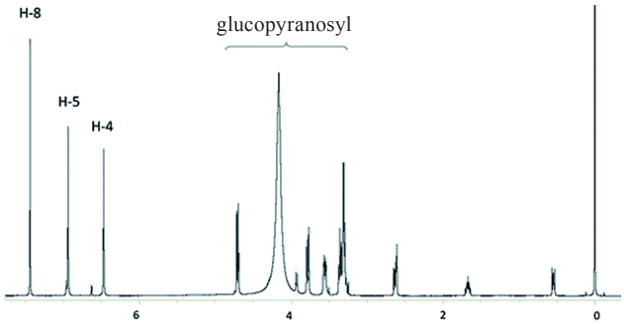


**Figure 6** NMR spectrum of mangiferin isolated from ginseng extracts

The fractions were separated by preparative HPLC (Fig. 5).

As a result, we isolated a basic substance with a retention time of 24 min, which was identified as mangiferin (Fig. 6).

Thus, rutin (0.25), quercetin (0.75), and mangiferin (0.57) were major biologically active substances found in the extracts of *in vitro* ginseng callus, suspension, and root cultures. We also identified caffeic and sinapinic acids in the extracts.

## CONCLUSION

We developed a solvent screening procedure and performed a qualitative analysis of biologically active substances extracted from ginseng (*Panax ginseng* L.).

The optimal parameters for extracting biologically active substances (organic solvent, ratio of solvent to biomass, time, and temperature) were 50% ethanol, 1:5 ratio, 60 min, 50°C for ginseng callus cultures; 60% ethanol, 1:5 ratio, 30 min, 50°C for suspension cultures; and 70% ethanol, 1:5 ratio, 60 min, 50°C for root cultures, respectively.

The qualitative analysis of the extracts of ginseng callus, suspension, and root cultures showed the presence of rutin (0.25), quercetin (0.75), and mangiferin (0.57) as predominant components. The extracts also contained caffeic and sinapinic acids.

Thus, the extracts obtained by water-ethanol extraction from ginseng callus, suspension, and root cultures can be used as biologically active ingredients in the production of functional geroprotective foods.

## CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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