J Ginseng Res 38 (2014) 289–292

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research note

In vitro cytotoxic activity of ginseng leaf/stem extracts obtained by subcritical water extraction

Kyoung Ah Lee [1], Kee-Tae Kim [2], Pahn-Shik Chang [3], Hyun-Dong Paik [1,2,*]

[1] *Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul, Korea*
[2] *Bio/Molecular Informatics Center, Konkuk University, Seoul, Korea*
[3] *Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea*

ARTICLE INFO

Article history:
Received 29 November 2013
Received in Revised form
16 May 2014
Accepted 22 May 2014
Available online 5 June 2014

Keywords:
cytotoxic activity
flavonoid
leaves and stems
Panax ginseng
subcritical water extraction

ABSTRACT

Ginseng leaf/stem extract produced by subcritical water extraction at high temperature (190°C) possess higher cytotoxic activity against human cancer cell lines than ethanol extract. Subcritical water extraction can be a great candidate for extraction of functional substance from ginseng leaves/stems.
Copyright © 2014, The Korean Society of Ginseng, Published by Elsevier. All rights reserved.

Ginseng (*Panax ginseng* Mayer) is a valuable agricultural product used in many traditional medicinal therapies [1]. Although ginseng root sells at a high price, growing ginseng in a forest environment is not a highly lucrative business strategy, because it requires a minimum growth of 5–8 years prior to harvesting [2].

Unlike ginseng root, it is possible to harvest ginseng leaves annually. Furthermore, ginseng leaves and stems were found to be rich in polysaccharides, phenolics, flavonoids, and ginsenosides [3,4]. Previous studies evaluated the bioactivity of extracts of ginseng leaves and stems; however, these extracts were prepared by traditional methods and using organic solvents such as methanol, n-hexane, chloroform, ethyl acetate, and n-butanol [5]. Also, these techniques involve a long extraction period and produce low yields.

Subcritical water (SW) extraction has been utilized extensively in various areas of green engineering and material cycling [6,7]. Under subcritical conditions, the dielectric constant of water can be altered [8]. This study aims to compare the antitumor efficacy of ginseng leaf and stem extracts, prepared by both traditional and SW extraction procedures, in human cancer cell lines.

Ginseng leaves and stems were extracted by ethanol, hot water, and SW extraction. For ethanol extraction, ginseng leaves and stems (20 g) were mixed with 200 mL of 70% (v/v) ethanol and heated for 3 hours at 60°C in a water bath. For hot water extraction, the sample (20 g) was dissolved in 200 mL distilled water and heated for 3 hours at 80°C in a water bath. After extraction, the slurry was filtered through filter paper (Whatman No. 2, GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, UK), and the solid residue was extracted twice more under identical conditions. The solvent was evaporated using a rotary evaporator (N-1000V; Eyela, Tokyo, Japan). After the evaporation was completed, the extract was transferred to a freeze-drying tube and lyophilized. The dried sample was then weighed and stored at −20°C prior to analysis [9].

SW extraction was performed using an SW extraction system (DIONEX ASE 100; Dionex Corporation, Sunnyvale, CA, USA). The extraction cell (34 mL) was filled with a mixture of ginseng powder and diatomaceous earth in the ratio of 1:3, and placed vertically in the extraction apparatus. And then distilled water flows in a Milli-Q

* Corresponding author. Department of Food Science and Biotechnology of Animal Resources, Konkuk University, 120 Neungdong-ro, Seoul 143-701, Korea.
E-mail address: hdpaik@konkuk.ac.kr (H.-D. Paik).

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

1226-8453/$ – see front matter Copyright © 2014, The Korean Society of Ginseng, Published by Elsevier. All rights reserved.
http://dx.doi.org/10.1016/j.jgr.2014.05.009

Table 1
Mobile phase gradient condition of high performance liquid chromatography analysis for flavonoid content in ginseng leaf and stem extracts

Time (min)	% mobile phase A[1)]	% mobile phase B[2)]
0	90	10
10	80	20
20	80	20
25	70	30
35	67	33
40	60	40
60	40	60

[1)] 0.1% formic acid (v/v), pH 2.7
[2)] 100% acetonitrile

system (Millipore, Bedford, MA, USA) into the apparatus. Working temperature and static time were set at 110°C, 165°C, and 190°C for 15 minutes. During extraction, the pressure was maintained at less than 500 psi. After extraction, fresh water was pumped through the entire pathway, including the cell, for washing. The SW extracts were freeze-dried and stored at −20°C until used.

Human cancer cell lines were purchased from the Korean Cell Line Bank (Seoul National University, Seoul, Korea). The AGS (human stomach adenocarcinoma), HT-29 (human colorectal adenocarcinoma), and MCF-7 (human breast adenocarcinoma) cell lines were maintained in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY, USA) containing 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT, USA), penicillin (100 U/mL), and streptomycin (100 μg/mL). SK-MES-1 (human lung carcinoma) cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin, and streptomycin. HeLa (human cervical adenocarcinoma) cells were grown in Minimum Essential Medium containing 10% heat-inactivated fetal bovine serum, penicillin, and streptomycin. All cell lines were cultured in a 37°C, 5% CO_2 incubator. For experimentation, adherent cells in the logarithmic growth phase were harvested using 0.25% Trypsin (Life Technologies, Inc., Carlsbad, CA, USA). Cells were counted using a hemocytometer (Hausser Scientific, Horsham, PA, USA). For cytotoxicity testing, cells were seeded in new dishes and grown to 80% confluence prior to treatment.

Cytotoxicity was determined by quantifying the relative cell number. In this study, cytotoxic activity was assessed by a modified MTT assay, as described previously [10]. Briefly, 100 μL aliquots of the cell suspension were transferred to 96-well microplates (1.5×10^4 cells/well) and incubated for 24 hours. Ginseng extracts were dissolved in phosphate-buffered saline, and the final concentrations were adjusted to 0.25 mg/well, 0.5 mg/well, 1 mg/well, and 2.5 mg/well. Cells were incubated with the extracts for 44 hours at 37°C. At the end of the incubation, MTT (2.5 mg/mL in phosphate-buffered saline) was added, and the plate was incubated for an additional 4 hours. The supernatant was then aspirated, and 100 μL of dimethyl sulfoxide was added to the wells, to dissolve the colored formazan crystals produced from the reaction of cells with MTT. Optical density values were then measured using a microplate reader at 570 nm. All conditions were performed in triplicate and the dose causing 50% cell death (IC_{50}) was calculated.

Flavonoid was extracted following a previous method, with some modifications [11]. The ginseng extracts (100 mg) were weighed and dissolved in 50 mL of 50% aqueous methanol containing 80 mg of ascorbic acid as an antioxidant. The mixture was sonicated for 5 minutes, and 2M HCl (10 mL) was slowly added to it within 5 minutes. This mixture was incubated at 90°C for 2 hours. After cooling, the mixture was filtered through a 0.45 μm syringe filter (25 mm i.d., GD/X 25 nylon syringe filter; Whatman Inc., Piscataway, NY, USA), and the filtrate was evaporated at 50°C using a rotary evaporator to remove the solvent, freeze-dried, and stored at −20°C until use.

Concentrations of flavonoids in the ginseng extracts were determined by high performance liquid chromatography (HPLC) [12]. The ginseng extract was dissolved in 50% dimethyl sulfoxide in methanol (5 mg/mL). After filtering through a 0.45 μm syringe filter, 20 μL was injected into an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA). The separation was carried out on a DuPont Zorbax ODS C_{18} column (150×4.6 mm^2, i.d. 5 μm). Table 1 shows the mobile phase condition of HPLC analysis. The peaks were identified using UV absorbance at 360 nm. Calibration curves were obtained by plotting peak area versus concentration of quercetin and kaempferol. The linear regression of calibration curve was more than 0.99.

The concentration of ginseng leaf and stem extracts affecting normal cell (Raw 264.7) viability was evaluated using an MTT assay. The extracts, at all concentrations, were found to have no cytotoxic activity on Raw 264.7 cells (<20%, data not shown). The IC50 value and cytotoxic activity of ginseng leaf and stem extracts were presented in Table 2 and Fig. 1., respectively. The extract prepared by the SW extraction method at 190°C showed the highest cytotoxicity. As shown in Table 2, the IC_{50} value of the SW 190°C extract was the lowest among all preparations. Importantly, the cytotoxic effect of this extract was the most pronounced in the AGS and MCF-7 cell lines, with the IC_{50} values for these cell lines being lower than 0.25 mg/mL.

First, the cytotoxic activity of the SW 190°C extract on AGS was high (>80%) at concentrations ranging between 0.25 mg/mL and 2.5 mg/mL (Fig. 1A). The extracts prepared by ethanol, hot water, or SW extraction at 110°C, 165°C, and 190°C, when added at a concentration of 2.5 mg/mL, inhibited the growth of HT-29 cells by 95.20%, 96.78%, 65.67%, 91.49%, and 85.51%, respectively (Fig. 1B). Although the SW 190°C extract exhibited slightly lower activity than ethanol, hot water, and SW 165°C extracts at a concentration of 2.5 mg/mL, it showed the highest activity at 0.5 mg/mL (80.53%), while interestingly, the other extracts, when used at this concentration, lost their cytotoxic activity (≤20%).

Table 2
Concentration of the ginseng leaves and stems extracts that inhibited 50% of the cell growth (IC50) of different cancer cell lines

Extract condition	IC_{50} value (mg/mL)				
	AGS	HT-29	HeLa	MCF-7	SK-MES-1
Ethanol	0.718 ± 0.04	0.979 ± 0.11	1.004 ± 0.14	1.034 ± 0.23	1.461 ± 0.05
Hot water	0.952 ± 0.09	0.990 ± 0.08	1.578 ± 0.29	1.094 ± 0.22	2.239 ± 0.28
SW 110°C	1.115 ± 0.03	1.914 ± 0.02	1.784 ± 0.23	2.707 ± 0.17	2.840 ± 0.19
SW 165°C	0.602 ± 0.08	1.062 ± 0.01	1.169 ± 0.20	1.563 ± 0.19	2.065 ± 0.17
SW 190°C	<0.25	0.327 ± 0.08	0.715 ± 0.05	<0.25	0.211 ± 0.11

Data are presented as mean ± standard deviation
AGS, human stomach adenocarcinoma; HeLa, human cervical adenocarcinoma; HT-29, human colorectal adenocarcinoma; IC_{50}, dose causing 50% cell death; MCF-7 human breast adenocarcinoma; SK-MES-1, human lung carcinoma; SW, subcritical water

Download English Version:

https://daneshyari.com/en/article/3099359

Download Persian Version:

https://daneshyari.com/article/3099359

Daneshyari.com