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Research article

Improved antimicrobial effect of ginseng extract by heat transformation

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ABSTRACT

Background: The incidence of halitosis has a prevalence of 22–50% throughout the world and is generally caused by anaerobic oral microorganisms, such as *Fusobacterium nucleatum*, *Clostridium perfringens*, and *Porphyromonas gingivalis*. Previous investigations on the structure-activity relationships of ginsenosides have led to contrasting results. Particularly, the antibacterial activity of less polar ginsenosides against halitosis-related bacteria has not been reported.

Methods: Crude saponins extracted from the *Panax quinquefolius* leaf-stem (AGS) were treated at 130°C for 3 h to obtain heat-transformed saponins (HTS). Five ginsenoside-enriched fractions (HTS-1, HTS-2, HTS-3, HTS-4, and HTS-5) and less polar ginsenosides were separated by HP-20 resin absorption and HPLC, and the antimicrobial activity and mechanism were investigated.

Results: HPLC with diode-array detection analysis revealed that heat treatment induced an extensive conversion of polar ginsenosides (-Rg1/Re, -Rc, -Rb2, and -Rd) to less polar compounds (-Rg2, -Rg3, -Rg6, -F4, -Rg5, and -Rk1). The antimicrobial assays showed that HTS, HTS-3, and HTS-4 were effective at inhibiting the growth of *F. nucleatum*, *C. perfringens*, and *P. gingivalis*. Ginsenosides-Rg5 showed the best antimicrobial activity against the three bacteria, with the lowest values of minimum inhibitory concentration and minimum bactericidal concentration. One major reason for this result is that less polar ginsenosides can more easily damage membrane integrity.

Conclusion: The results indicated that the less polar ginsenoside-enriched fraction from heat transformation can be used as an antibacterial agent to control halitosis.

1. Introduction

Panax quinquefolius (American ginseng), *Panax ginseng* (Chinese ginseng), and *Panax notoginseng* (notoginseng) are ginseng botanicals that have been used for thousands of years as important health food resources throughout the world. The saponins in ginseng, also called ginsenosides, are considered to be its main biological constituents. Thus far, at least 289 saponins have been reported from different Panax species [1]. The variety of ginsenosides is coincident in different parts of ginseng botanicals, but the contents show diversity [2]. Accordingly, ginsenosides from the stem and leaf of ginseng have similar pharmacological activity as those from the roots [3]. However, as the ginseng leaf and stem can be harvested every year as compared to every 4 yr for the roots, it is more economical to produce ginsenosides from the leaf and stem.

Most harvested ginseng roots are air-dried to obtain white ginseng, while others are steamed at 100°C for a few hours before drying, resulting in what is known as red ginseng. Red ginseng has a unique, less polar ginsenoside profile that is different from that of white ginseng, including ginsenoside-F4, -Rg3, -Rg5, -Rg6, -Rk1, -Rk2, -Rk3, and -Rs5, as well as a different biological activity [4,5]. Methods of bioconversion by enzymes and endophytes have been developed to transform saponins to less polar ginsenosides [6,7]. It was confirmed that it is possible to transform polar ginsenosides to less polar ginsenosides by a microwave and vinegar process [8]. However, transformation by heating in a reaction kettle is more

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conducive for application in a factory as compared to the other methods.

In our previous research, it was shown that steaming American ginseng roots at a high temperature could favorably change the ginsenoside structures, resulting in superior antibacterial activity against *Propionibacteria* and *Staphylococci* species [9]. However, to the best of our knowledge, the antibacterial activity of less polar ginsenosides against halitosis-related bacteria, such as *Fusobacterium nucleatum*, *Clostridium perfringens*, and *Porphyromonas gingivalis*, has not been reported.

The incidence of halitosis is 22–50% throughout the world [10]. Halitosis represents a global healthcare problem that greatly affects daily activity, hinders interpersonal communication, and can even cause psychological barriers in people of all ages [11]. Its cause is the oral production of volatile sulfide compounds, including hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethylsulfide (CH_3SCH_3), through proteolytic degradation by predominantly anaerobic Gram-negative oral microorganisms [12]. These bacterial species, such as *P. gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia*, and *F. nucleatum*, are the most likely to cause oral malodor [13]. Chlorhexidine, essential oils, metal ions, oxidizing agents, triclosan, and cetylpyridinium chloride are often used as active ingredients in clinical treatments [13,14]. However, there are some limitations regarding the use of these drugs, such as widespread periodontal anaerobe resistance and the risk of urticaria [15,16]. Therefore, in the present study, the profile of heat-transformed leaf-stem ginsenosides was characterized, and their potential as antibacterials for curing halitosis was investigated.

2. Materials and methods

2.1. Chemicals

American ginseng leaf-stem saponins (AGS) were purchased from Jilin Hongju Biotechnology Co., Ltd. (Jilin, China). Ginsenoside standards (-Rg1, -Re, -Rb1, -Rc, -Rb2, -Rd, -20(R)-Rg2, -20(S)-Rg2, -20(R)-Rg3, and -20(S)-Rg3) with > 98% purity were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ginsenoside-Rg6, -F4, -Rh4, -Rh2, -Rg5, and -Rk1 (> 98% purity) were isolated and identified in our laboratory by a previously reported method with some modifications [17]. Erythromycin, tetracycline, chlorhexidine, rhodamine 123, Coomassie Blue G250, and dimethyl sulfoxide (DMSO) were all purchased from Sigma-Aldrich (Shanghai, China). Agar, Gifu Anaerobic Broth (GAM broth), and CDC anaerobic blood agar base medium were purchased from Beijing Suolaibao Biotech Co., Ltd. (Beijing, China). The other chemicals are analytical or chromatographic grade.

2.2. Material and sample preparation

The AGS (500 g) was dissolved in distilled water (2,500 mL) and then subjected to an autoclave (MLS-3750; SANYO, Osaka, Japan) at 130°C for 3 h. The heat-transformed saponins (HTS, 400 g) were loaded onto a HP-20 column and sequentially eluted with an ethanol gradient from 0% to 30%, 60%, 80%, and 95%. The fractions were collected, evaporated using a rotary evaporator (Buchi, Flawil, St. Gallen, Switzerland) at 45°C to remove the ethanol, and were lyophilized to obtain a dry powder. The fractions were named HTS-1, HTS-2, HTS-3, HTS-4, and HTS-5.

2.3. HPLC analyses

Chromatographic analysis was performed using a SHIMADZU Prominence LC-20A HPLC instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a YMC-Pack ODS-AM column (4.6 mm × 250 mm; YMC Co., Ltd., Kyoto, Japan). The detection wavelength was set at 202 nm and the column oven at 25°C. The mobile phase consisted of water (A) and acetonitrile (B). A gradient elution was used as follows: 25% B at 0–5 min, 30–32% B at 5–14 min, 32–38% B at 14–28 min, 38–46% B at 28–30 min, 46–74% B at 30–50 min, 74–80% B at 50–51 min, 80–90% B at 51–60 min, 90–100% B at 60–65 min, and 100–25% B at 65–70 min. The flow rate was kept at 1 mL/min, and the injected volume was 10 μL.

2.4. HPLC-ESI-MS conditions

The column effluent of the HPLC was introduced into an Agilent-LC-1100 (Agilent, Santa Clara, CA, USA) mass spectrometer equipped with an ESI source 6,460 (Agilent). The parameters of the ESI were set according to a previous report with slight modifications [18]. Briefly, the collision-gas (N_2) rate was maintained at 10 mL/min and the column oven at 25°C. ESI-MS data were acquired in negative mode to generate $[M-H]^-$ ginsenoside ions by fully scanning m/z over 50–2,000. The spray voltage was 4.5 kV, the capillary voltage was 10 V, and the capillary temperature was 250°C.

Table 1
Analytical characteristics of ginsenosides (mg/mg)

Peak	Ginsenoside	Retention time	Calibration curve	R^2	AGS	HTS[1)]	HTS-1	HTS-2	HTS-3	HTS-4	HTS-5
1	Re(Rg1)	9.387	y = 481,999, x + 93,235	0.9985	0.41 ± 0.01	n.d	n.d	n.d	0.04 ± 0.01	n.d	n.d
2	Rg2(S)	28.011	y = 465,264, x + 200,215	0.9956	0.07 ± 0.01	0.05 ± 0.01	n.d	n.d	0.07 ± 0.01	n.d	n.d
3	Rb2	28.842	y = 108,262, x + 11,323	0.9933	0.16 ± 0.01	0.02 ± 0.01	n.d	n.d	0.11 ± 0.01	n.d	n.d
4	Rd	32.543	y = 271,969, x + 427,367	0.9837	0.16 ± 0.01	0.01 ± 0.00	n.d	n.d	0.03 ± 0.01	n.d	n.d
5	Rg6	38.751	y = 719,696, x + 188,528	0.9982	0.07 ± 0.01	0.10 ± 0.03	n.d	n.d	0.07 ± 0.01	0.06 ± 0.01	n.d
6	F4	39.378	y = 1E+06, x − 660,748	0.9863	0.01 ± 0.01	0.02 ± 0.01	n.d	n.d	0.04 ± 0.01	0.04 ± 0.01	n.d
7	Rh4	41.168	y = 774,675, x + 146,497	0.9995	n.d	0.08 ± 0.01	n.d	n.d	0.02 ± 0.01	0.07 ± 0.01	n.d
8	Rg3(S)	41.631	y = 177,999, x + 62,300	0.9926	0.01 ± 0.01	0.07 ± 0.00	n.d	n.d	0.02 ± 0.01	0.11 ± 0.01	n.d
9	Rg3(R)	42.105	y = 218,319, x − 56,590	0.9893	0.01 ± 0.01	0.04 ± 0.01	n.d	n.d	0.01 ± 0.01	0.07 ± 0.01	n.d
10	Rk1	48.011	y = 198,283, x − 56,590	0.9904	0.01 ± 0.01	0.20 ± 0.02	n.d	n.d	0.02 ± 0.01	0.27 ± 0.01	n.d
11	Rg5	48.653	y = 506,274, x + 31,631	0.9991	0.01 ± 0.01	0.25 ± 0.01	n.d	n.d	0.02 ± 0.01	0.27 ± 0.01	n.d
12	Rh2	51.384	y = 485,961, x + 194,713	0.9942	0.01 ± 0.01	0.01 ± 0.001	n.d	n.d	0.02 ± 0.01	0.02 ± 0.01	n.d
	Total content of				0.91 ± 0.03	0.83 ± 0.02	n.d	n.d	0.45 ± 0.04	0.91 ± 0.03	n.d
	less polar ginsenosides				0.10 ± 0.01	0.76 ± 0.04	n.d	n.d	0.20 ± 0.03	0.91 ± 0.03	n.d

Data are expressed as mean ± standard deviation of triplicate samples
AGS, *Panax quinquefolius* leaf-stem; HTS, heat-transformed saponins; n.d., not detected
[1)] HTS-1, HTS-2, HTS-3, HTS-4, and HTS-5 were from the 0, 30%, 60%, 80%, and 95% methanol-eluted fractions from an HP-20 column, respectively

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