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Original article

Quantitative Analyses of the Functional Constituents in SanYangSam and SanYangSanSam

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Key Words

SanYangSam, SanYangSanSam, LC-MS/MS. Polyacetylene compounds, Panaxidol

Abstract

Objective: SanYangSam and SanYangSanSam are traditional Korea-medical herbs that are grown from Panax ginseng C.A. Meyer. In our previous studies, we found that the functional compounds in SanYangSam and SanYangSanSam were different and depended on the type and the cultivation environment of ginseng. This study aimed to profile the functional constituents in SanYangSam and SanYangSanSam.

Methods: To profile the functional aspects of the many compounds that have therapeutic activities in SanYangSam and SanYangSanSam extracts, we used liquid chromatography tandem mass spectrometry and quadrupole orthogonal acceleration time-of-flight mass spectrometry.

Results: A total of four major compounds were detected; two of which were the natural flavonoids kaempferol and quercetin. Among others, two polyacetylene compounds, including panaxydol and panaxynol, were detected.

Conclusion: In this study, we found that panaxydol, one of the polyacetylene constituents of ginseng, is a candidate anti-cancer agent in SanYangSam and SanYangSanSam pharmacopuncture. In addition, we found that the panaxydol levels in the SanYangSanSam extract were over 30 times those in the SanYangSam extract.

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1. Introduction

Ginseng is widely regarded as the nation's most popular herbal supplement and is widely known throughout the world. In addition, ginseng has been found to have various beneficial activities, including antioxidant, anti-inflammatory, anti-diabetic, antibiotic resistance, and anti-cancer activities [1]. Ginseng's health benefits are related to the many compounds, including polyacetylenes, polysaccharides, sesquiterpenes, peptidoglycans, nitrogen - containing compounds, phenolic compounds, and which make up ginseng. In particular, the effects of treatment with ginseng are known to be largely attributed to more than 30 saponins, namely ginsenosides. However, the profiles of these compounds vary, depending on the type of ginseng (fresh, white, red) and the cultivation environment [2].

We found that red ginseng is the main product of the food hub manufacturing group. Furthermore, various commercial ginseng products exist, with four representative products being red ginseng (HongSam), white ginseng (BaekSam), black ginseng (HeukSam),

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and wild ginseng (SanSam) [3]. SanSam has a different appearance from normal cultivated ginseng. The roots of SanSam are silver-tinged and have a white nape, and the side roots are longer and tougher. Also, its body is much shorter, thinner and thicker. Four different varieties of SanSam are available: genuine SanSam, SanYangSam, SanYangSanSam, and NoeSam. SanYangSam is the name of ginseng that is grown in the mountains according to the Law in Korea; it is sown, but grows naturally in mountains without any chemicals or special attention. SanYangSanSam is the variety that grows in the woods from seeds deliberately planted by humans. SanYangSam and SanYangSanSam are both produced from wild ginseng plants, but less information is available on their health benefits or on their use as therapies for several diseases.

In our previous studies, we focused only on the ginsenosides of common ginseng; however, the organizations of the functional compounds that constitute ginseng depend on the type of SanSam and on its growth environment and cannot not be detected directly [4-6]. If the functional attributes of SanYangSam and SanYangSanSam are to be understood, comprehensive quantitative and qualitative, high-throughput analyses of their health characteristics are necessary [7]. Thus, the present study was undertaken to chemoprofile the two different extracts and to investigate their functional compounds by using quadrupole, orthogonal acceleration, time-of-flight (Q-TOF) mass spectrometry and liquid chromatography (LC)–mass spectrometry [8, 9]. Liquid chromatography - mass spectrometry (LC–MS) is an alternative analytical method with high specificity and is considered to be one of the most effective techniques for analyzing the components of SanYangSam and SanYangSanSam pharmacopuncture [7].

Q-TOF mass spectrometry provides the ability to collect both high-resolution precursor and fragmentation data, facilitating the characterization of metabolites. The advantages of tandem MS in Q-TOF mass spectrometry is that the collision energies can be regulated to enhance or decrease the degree of fragmentation, so more information about the compounds in SanYangSam and SanYangSanSam can be obtained. However, some compounds do not fragment well or fragment poorly when an adduct is present. An adduct can stabilize the ion and can lead to limited fragmentation, but different ionization strategies or solvent mixtures may improve fragmentation, and these protocols can be adjusted to other sample types [10].

We investigated the chemoprofiling of the biosynthetic flavonoids and polyacetylenes from the ginseng cultivars SanYangSam and SanYangSanSam pharmacopuncture by using LC tandem mass and Q-TOF mass spectrometry. We elucidated the variations in the major compounds, which were two flavonoids (kaempferol and quercetin) and two polyacetylenes (panaxydol and, panaxynol) , and we found that the contents of these compounds in SanYangSam and SanYangSanSam pharmacopuncture changed depending on the type of ginseng and the environment in which the ginseng had been grown [6]. In our research, we used the simple, selective, high-throughput method of high- performance Q-TOF mass spectrometry combined with LC to determine the compounds in SanYangSam and SanYangSanSam pharmacopuncture for chemoprofiling and therapeutic drug monitoring [11].

2. Materials and Methods

The process for preparing the SanYangSam and the SanYangSanSam extracts consisted of washing entire mountain-cultivated and wild ginseng plants, extracting the active ingredients by distillation for 4 hours (pH 7.35 to 7.45) in a clean room, filtering them twice, and sealing them in 20-mL aliquots in sterilized glass containers. An Agilent 6410B Triple Quadrupole LC/MS system (Agilent Technologies, Wilmington, USA) equipped with an electrospray ion (ESI) source was employed for the analysis. The four compounds in SanYangSam and SanYangSanSam, namely, kaempferol, panaxydol, panaxynol and quercetin, were purchased from (Chemface, Hubei, Wuhan, China) and used as reference standards. One hundred mg of each sample was mixed with 1 ml of methanol and centrifuged. Aliquots of 5 μl of the processed samples were injected into the high-performance LC (HPLC) system (1200 Series LC, Agilent Technologies, Wilmington, USA) fitted with a Phenomenex Synergi Hydro-RP 4 μm 80 Å 150 x 2 mm column maintained at 30°C. The ESI source was operated at +3000 V and a source temperature of 380 ° C. The capillary voltage, cone voltage, and source offset were set at 3 kV, 30 kV, and 30 V, respectively. The gas flows for desolvation and the cone were set at 650 L/h and 150 L/h, respectively, at a nebulizer pressure of 15 bars. A mobile phase composed of 0.1% formic acid in distilled water (buffer A) and 0.1% formic acid in acetonitrile (buffer B) was used to separate the material to be analyzed and was pumped into the ESI chamber at a flow rate of 0.5 mL/min for 20 min. The voltage of the fragmentor and the collision voltage were set at 90 V and 20 V, respectively. The detection of the ions of the four compounds kaempferol, panaxydol, panaxynol and quercetin was carried out in the multiple-reaction monitoring mode (MRM) by monitoring the transition pairs of m/z 252.1 → 136.1. Data acquisition was performed with the MassHunter Software (Version B.04.00).

The LC separation was performed using an Acquity I-Class UPLC system (Waters, Manchester, UK) with an Acquity UPLC BEH C18 column (1.7 um, 2.1 x 100 mm). The column temperature was 40°C. Mobile phase A was water with 0.1% formic acid, and mobile phase B was acetonitrile with 0.1% formic acid. The injection volume was 2 μl, and the flow rate was set at 0.4 μL/min. The MS detection was performed on a SYNAPT G2-Si system (Waters, Manchester, UK). The data acquisition mode was MS^E which the method for tandem mass spectrometry data acquisition using alternating low-energy collision-induced dissociation and high-energy collision-induced dissociation. The ionization mode was ESI- positive and ESI-negative. The source temperature was set at 120°C, and the reservoir temperature was set at 300°C. The lock mass compound used leucine enkephaline (556.2771 in positive, 554.2615 in negative) for an external standard. The operation parameters were as follows: The ESI positive capillary voltage was set at 3 kV, and the ESI negative capillary voltage was set at 2.5 kV. The cone voltage was set at 30 V. The col-

lision energies were set as 6-eV ramp (trap) for low-energy scans and 20- to 45-eV ramp (trap) for high-energy scans. The scan mass range was 100~1500 m/z. The LC-MS data acquisition was controlled by using the MassLynx 4.1 system (Waters, Manchester, UK). The acquisition data processing was performed by using UNIFI1.8 software with a traditional medicine library.

A triple quadrupole LC/MS (ESI) analysis was conducted to quantify the compositions of SanYangSam and SanYangSanSam. Figure 2 shows a qualitative evaluation of the components of SanYangSam and SanYangSanSam done using high-resolution mass spectrometer and a traditional medicine library; many of the components were found to be nucleotides. The Q-TOF mass spectrometry analyses of SanYangSam and SanYangSanSam were performed with 50% methanol or water extracts of SanYangSam or SanYangSanSam pharmacopuncture. The 50% methanol extract had 10 times the amount of SanYangSam and SanYangSanSam than the water extract in the range of 2 to 3 min.

3. Results

Four of the compounds that constitute SanYangSam and SanYangSanSam were identified (Fig. 1). Two of the compounds were analyzed by using triple quadrupole LC/MS (ESI), and two were analyzed using Q-TOF mass spectrometry. The four products that were identified were kaempferol (molecular weight: 286.23 g/mol), quercetin (molecular weight: 302.236 g/mol), panaxydol (molecular weight: 260.377 g/mol), and panaxynol (molecular weight: 244.378 g/mol) [12].

Triple quadrupole LC/MS (ESI) analyses were conducted to determine quantitatively the amounts of kaempferol and quercetin in SanYangSam and SanYangSanSam pharmacopuncture. The results obtained by using high-resolution mass spectrometer, along with a traditional medicine library, are shown in Figure 2. Kaempferol and quercetin were retained in the chromatography column within 13 min, with 12 ± 0.3 and 11.9 ± 0.3 min retention times, respectively, for SanYangSam and 11.3 ± 0.2 and 11.2 ± 0.2 min retention times, respectively, for SanYangSanSam respectively [13].

Q-TOF mass spectrometry analyses were used to determine the amounts of panaxydol and panaxynol in the SanYangSam and the SanYangSanSam extract solutions. As established in Figure 3, the SanYangSanSam extract has a larger amount of panaxydol than the SanYangSam in the range of 55 s. In contrast, the content of panaxynol was larger in SanYangSam than SanYangSanSam in the range of 53 - 55 s [14].

4. Discussion

NIn this study, we investigated the major functional compounds in SanYangSam and SanYangSanSam pharmacopuncture. A total of four metabolites, including two natural flavonoids and two polyacetylenes were detected in SanYangSam and SanYangSanSam by using LC-MS and Q-TOF-mass spectrometry (Fig. 1). Because the environments in which SanYangSam and SanYangSanSam are grown are different, their constituents and the amounts of those constituents are different. When the two biosynthetic flavonoids kaempferol and quercetin were analyzed using the LC-MS spectrometry method, kaempferol and quercetin were retained within 13 min, with 12 ± 0.3 and 11.9 ± 0.3 min retention times, respectively, for SanYangSam and 11.3 ± 0.2 and 11.2 ± 0.2 min retention times, respectively, for SanYangSanSam (Fig. 2). The amounts of the polyacetylenes panaxydol and panaxynol were different in the SanYangSam and the SanYangSanSam pharmacopuncture, with panaxydol having its highest levels and panaxynol having a very low level in the SanYangSanSam in the retention time range of 55 s. The difference in the amounts of panaxynol between the SanYangSam and the SanYangSanSam pharmacopuncture was not very much (Fig. 3).

From a diversity of ginseng cultivars, SanYangSam and SanYangSanSam are the most important for use as herbal medicines. As these results show, the biosynthetic compositions and the contents of SanYangSam and SanYangSanSam differ according to the type and the cultivation environment of the ginseng; therefore, a variety of medicinal herbs with different therapeutic aims can be developed from only one type of Korean ginseng [15, 16]. Recently, SanYangSam and SanYangSanSam pharmacopuncture have been used as anti-cancer agents in Korea. Clinical studies using SanYangSam and SanYangSanSam pharmacopuncture to treat patients with multiple metastatic hepatocellular carcinomas [17] and with various other cancers over stage III have been performed [17]. Also, a case series involving patients with non-small-cell lung cancer treated with mountain ginseng pharmacopuncture showed the potential of SanYangSam and SanYangSanSam pharmacopuncture as an effective intervention for the treatment of patients with cancer [18].

A previous study indicated that the polyacetylenes from the roots of cultivated-wild ginseng had a cytotoxic effect on various cancer-cell lines [19]. Panaxydol was also found to induce apoptosis in cancer cells through Epithelial Growth Factor Receptor (EGFR) activation and Endoplasmic Reticulum (ER) stress and to inhibit tumor growth in mouse models [20]. Based on these results and the results of this study, we recommend that the anti-cancer effects of SanYangSam and SanYangSanSam pharmacopuncture be established, especially for their standard components such as panaxydol, and that those results be translated to advanced clinical studies.

5. Conclusion

In this study, we found that panaxydol, one of the polyacetylene components from Ginseng, is a candidate anticancer agent in SanYangSam and SanYangSanSam pharmacopuncture. In addition, we found that the panaxydol levels in the SanYangSanSam extract were over 30 times those in the SanYangSam extract.

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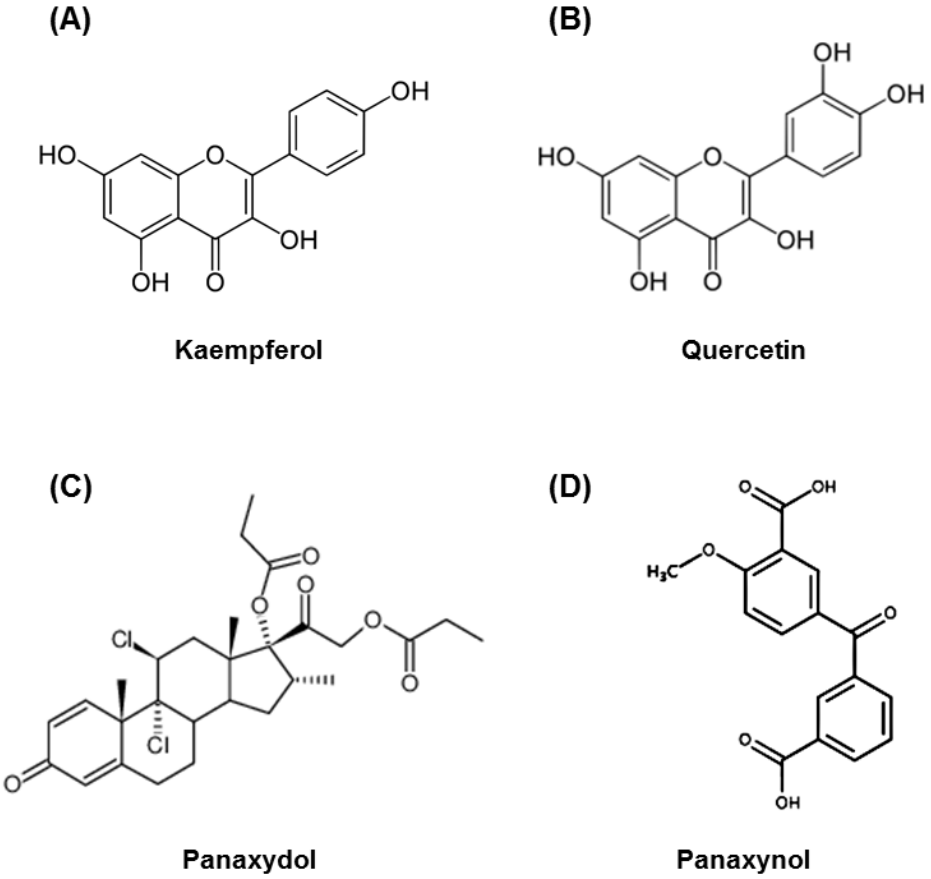
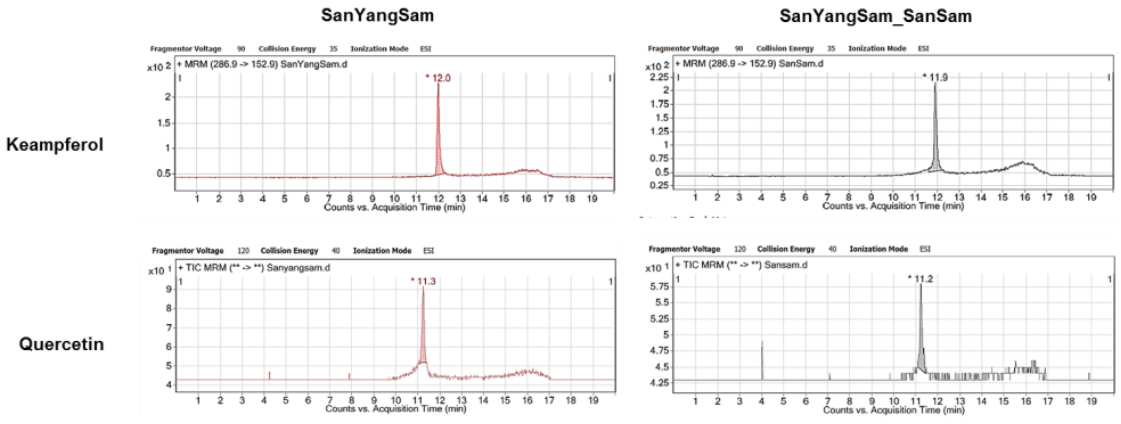


Molecular weight

#	Name	Molecular weight g/mol
1	Kaempferol	286.23
2	Quercetin	302.236
3	Panaxydol	260.377
4	Panaxynol	244.378

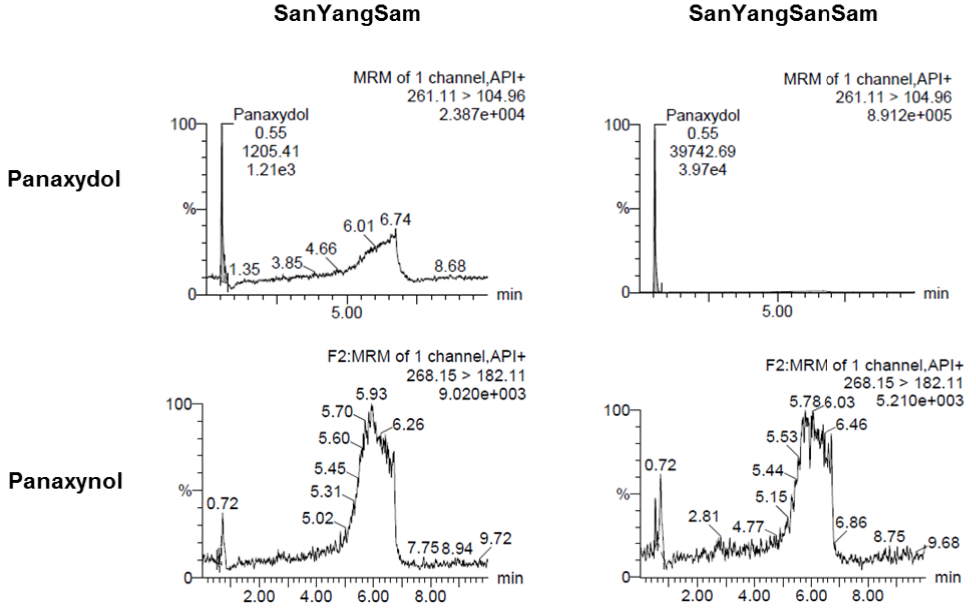
Figure 1 Chemical structures and molecular weights of four compounds found in SanYangSam and SanYangSanSam pharmacopuncture extracts: (A) kaempferol, (B) quercetin, (C) panaxydol, and (D) panaxynol.



Integration Peak List

#	Name	Start	RT	End	Height	Area	Area %
1	Kaempferol SanYangSam	11.7	12	12.3	181	1357	100
2	Kaempferol SanYangSam_SanSam	11.6	11.9	12.2	164	1395	100
3	Quercetin SanYangSam	11.1	11.3	11.4	40	274	100
4	Quercetin SanYangSam_SanSam	11.1	11.2	11.4	13	105	100

Figure 2 Quantitative liquid chromatography- mass spectrometry data for kaempferol and quercetin in SanYangSam and SanYangSanSam pharamcopunctures.



Description

#	Name	Type	Trace	RT	Area	Responce	Primer	ppb
1	Panaxydol SanYangSam	Analyte	261.11 > 104.96	0.55	12.3	1205.413	MMI	N.D.
2	Panaxydol SanYangSam_SanSam	Analyte	261.11 > 104.96	0.55	39742.691	39742.691	bb	1806.1
3	Panaxynol SanYangSam	Analyte	268.15 > 182.11	0.55	17.139	17.139	MMI	
4	Panaxynol SanYangSam_SanSam	Analyte	268.15 > 182.11	0.53	59.140	59.140	MMI	

**ND: not detected

Figure 3 Quantitative quadrupole orthogonal acceleration time-of-flight (Q-TOF) mass spectrometry data for panaxydol and panaxynol in SanYangSam and SanYangSanSam pharmacopuncture.

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