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Hydrolysis of Ginseng Saponins and Quantifications of Saponins, Prosapogenins and Sapogenins in Crude Drug Extracts for Quality Control

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Abstract : Ginseng saponins have been known as main active principles and are quantified as the index components of ginseng and its products for quality control. However ginseng saponins are easily hydrolyzed in acidic solutions of crude drug preparations. Due to the hydrolysis of saponins in acidic condition, it is generally difficult to determine ginseng saponins in crude drug preparations. Ginseng saponins, prosapogenins and sapogenins of crude drug extracts were quantified by HPLC. Ginseng saponins were quantified by HPLC on Lichrosorb-NH_2 column with acetonitrile/water/1-butanol(80:20:10, v/v). Ginseng prosapogenin-Rg_3 and -Rg_2 were extracted with ethyl acetate from 50% acetic acid hydrolyzates of saponin fractions and quantified by HPLC on Lichrosorb-NH_2 column with acetonitrile/water(90:10, v/v). Ginseng sapogenins, panaxadiol and panaxatriol, were extracted with diethyl ether from 7%-sulfuric acid hydrolyzates of saponin fractions and quantified by HPLC on μ-Bondapak C_{18} column with acetonitrile/methanol/chloroform(83:10:7, v/v). These methods of analyses of sapogenins and prosapogenins were more useful for quality control than those of ginseng saponins in some of crude drug preparations.

Key words : Ginseng saponins, hydrolysis, prosapogenins, sapogenins, quantification, crude drug extracts

INTRODUCTION

Ginseng saponins, which have been known to occur in the plants of Panax genus, have many pharmacological efficacies and have attracted a great deal of attention as the effective components[1-3]. Ginseng saponins, ginsenosides are generally different from those of other plants not only in chemical structures but also pharmacological efficacies[2,3]. Ginsenoside-Rb_1,-Rb_2,-Rc,-Rd,-Re and -Rg_1 etc.[2,5,6] were quantified for the quality control of ginseng and its products[7,8]. But ginseng saponins[9,10] are liable to be hydrolyzed on glycosidic bonds at C-20 position of sapogenin on the preparation procedure of ginseng extract and crude drug preparations. From the ancient times, oriental people have traditionally used crude drug preparations, mostly as tang(decoction), to prevent or cure their diseases. Moreover many kinds of crude drug preparations have been recently prepared as the instant types of preparations, drinks and extract granules etc. for the convenient application of the preparations. But few studies were reported about the hydrolyzates of ginseng saponin compounds for the quality control of crude drug preparations[10-12]. In this paper, we were carried out comparing the analytical conditions for ginseng saponins and its acidic hydrolyzate such as prosapogenins and sapogenins for the quality control of crude drug preparations.

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MATERIALS AND METHODS

Materials

Ginseng and crude drugs

The 6 year old red ginseng was kindly donated from Korea Ginseng Corporation and crude drugs were purchased from wholesale medicinal herb store under the confirmation of professional advice to use for the studies.

Reagents

HPLC grade(E. Merck Co.) of acetonitrile, 1-butanol distilled water for HPLC analysis, silica gel 60 precoated aluminum sheet(E. Merck, Art. 5554, layer thickness 0.2 mm) for TLC and silica gel(E. Merck Co., 70-230 mesh) for column chromatography were used.

Crude drug preparations with different pH solutions

Red ginseng, Zizyphi Fructus, Zingiberis Rhizoma, Cinnamomi Cortex, Lycii Fructus and Epimedi Herba (3:1:0.27:0.26:0.1:0.05, w/w) were mixed in a vessel according to the ratios described in chinese traditional prescription. These mixture was extracted with 10 times volume of water(v/w) in 75°C water bath for 8 hours. And then the extract solution was evaporated and dissolved in pH 2.4-5.1 ranges of citric acid buffer solution.

Ginseng and crude drug extracts

Same amount of red ginseng and each of 17 kinds of crude drugs were mixed and extracted with 10 times volume(v/w) of water. These extraction was done in 80°C water bath for 4 hours. The extracted solutions were centrifuged at 10,000rpm (9,200×g), and then the supernatants were evaporated under 60°C to give ginseng and crude drug extracts.

Authentic standards of ginseng saponins, prosapogenins and sapogenins

The standards of ginseng saponin, prosapogenin and sapogenin were prepared in KT&G Central Research Institute by the same methods previously reported [2,10].

Identification and quantification of ginseng saponins, prosapogenins and sapogenins in crude drug extracts

Fractionation of saponins, prosapogenins and sapogenins: Crude saponins[10,11] were prepared from 1-butanol soluble fraction of 80% methanol extract of ginseng. Prosapogenins[2,10] were prepared by refluxing crude saponin at 70°C for 2 hours with 50% acetic acid and then extracting with ethyl acetate. Sapogenins[2,10] were prepared by refluxing crude saponin at 100°C for 6 hours with 50% ethanolic-7% sulfuric acid and then extracting with diethyl ether.

TLC of saponin, prosapogenin and sapogenin : Saponin and prosapogenin fractions[10] were chromatographed on silica gel plate using chloroform/methanol/water(65/35/10, lower phase) and sapogenin fraction was chromatographed on silica gel plate using chloroform/acetone(1:1, v/v). The spots were visualized by spraying with 30% sulfuric acid and heating the plate at 110°C for 5 min.

HPLC of saponin, prosapogenin and sapogenin : For the analysis of saponin, prosapogenin and sapogenin HPLC was used with Waters Associates Model 510 and Differential Refractometer RI 410 detector. For the analysis of saponin and prosapogenin, Lichrosorb-NH_2 column (10 μm, 4.6 mm×25cm, Merck Co.) was used with the mobile phase of acetonitrile/water/1-butanol(80/20/10, v/v) and acetonitrile/water(90/10, v/v), respectively[10]. For the analysis of sapogenin, μ-Bondapak C_{18} column(3.9 mm ×30cm, Waters Co.) was used with the mobile phase of acetonitrile/water/methanol/chloroform(73/20/6/1, v/v)[10].

Quantification of organic acids in ginseng and crude drugs

The methylesters of organic acids from ginseng and crude drugs were prepared by the method of Court and Hendel method using 12% sulfuric acid/methanol (w/v). The methylesters of glutaric acid internal standard and another organic acid standards from Sigma chemical company were prepared by the same method[13] using 12% sulfuric acid/methanol(w/v).

The organic acid methylesters were analyzed by GLC[13] using a supelco wax 10 fused silica capillary column (30 m×0.32 mm ID, 0.25 μm film thickness). Column temperature was held at 100°C for 3 minutes and then increased to 230°C by 3°C/min., and then was held for 10 minutes. GC injector temperature and FID detector temperature were 230°C and 240°C, respectively. N_2 carrier gas of GC was flowed at the rate of 1.85 ml/min. and carrier gas mode was split mode(split ratio=40:1).

RESULTS AND DISCUSSION

Hydrolytic degradation of ginseng saponins in various pHs of crude drug preparations

The crude drug preparations were heated at 85°C on the various pHs for 30-240 minutes. As shown on Fig. 1, the total ginseng saponins of major ginsenosides, ginsenoside-Rb_1, -Rb_2 ,-Rc, -Rd, -Re and -Rg_1 decreased considerably in the acidic solutions with the decrease of pH in the crude drug preparations. In accordance with these results, ginseng saponins are more easily hydrolyzed in the acidic solutions of crude drug preparation[10]. The major hydrolyzates were identified with authentic standards of ginseng saponin and prosapogenins[10]. As previously reported[10], ginseng saponins are easily hydrolyzed on the glucosidic bonds at C-20 position of sapogenins in the acidic solution[10]. To prevent or cure their diseases, oriental people have traditionally used crude drug preparations since the ancient times. Moreover, crude drug preparations have been prepared to the instant types of extracts, extract granules and drinks, etc. for the convenient dosage of the preparations.

Organic acid contents of ginseng and crude drugs

Oxalic acid, malonic acid, fumaric acid, succinic acid,

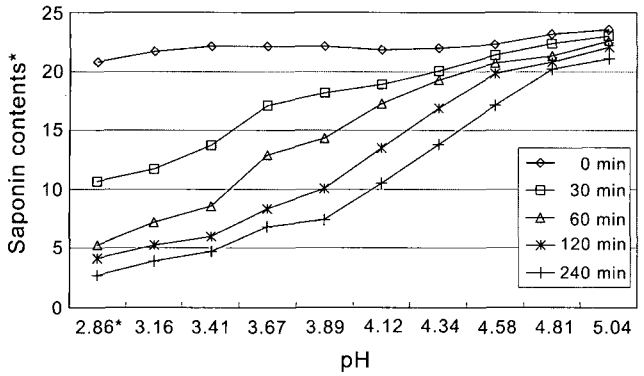

Fig. 1. Hydrolytic degradation of total ginseng saponins, "ginsenoside-Rb_1, -Rb_2, -Rc, -Rd, -Re and -Rg_1" in crude drug preparations of various pHs by heating times at 85°C

*Citric acid buffer solutions were respectively added to the crude drug preparation. The pHs of crude drug preparations after adding of citric acid buffer solutions were as follows; 2.4→2.86, 2.7→3.16, 3.0→3.41, 3.3→3.67, 3.6→3.89, 3.9→4.12, 4.2→4.34, 4.5→4.58, 4.8→4.81, 5.1→5.04.

**Saponin contents of crude drug preparation : Saponin contents of water extract from red ginseng, zizyphi fructus, zingiberis rhizoma, cinnamomi cortex, lycii fructus and epimedi herba(3:1:0.27:0.26:0.1:0.05, w/w)

malic acid and citric acid of ginseng and crude drugs were determined by GLC. As shown on Table 1, the contents of organic acids were quite various in ginseng and crude drugs. The contents of total organic acid(80.6 mg/g), citric acid(57.8 mg/g) and malic acid(22.2 mg/g) were very high in schizandrae fructus. The contents of total organic acid(26.4 mg/g), malic acid(8.8 mg/g) and oxalic acid(3.7 mg/g) were higher in red ginseng than in another crude drugs except schizandrae fructus.

As seen on the Table 2, major ginseng saponins, "ginsenoside-Rb_1, -Rb_2, -Rc, -Rd, -Re and -Rg_1" can not detected in the mixture of schizandrae fructus and ginseng extract. And these major ginsenosides decreased more greatly in various crude drug preparation than in ginseng extract itself. It means that these ginsenosides were hydrolyzed by the organic acids in schizandrae fructus extract, another crude drug extract and ginseng extract itself. And these ginsenosides were considerably decreased or not separated from other components of crude drugs in HPLC analysis.

Identification and quantification of ginseng saponin in ginseng and crude drug extracts

Major ginseng saponins, ginsenoside-Rb_1, -Rb_2, -Rc, -Rd, -Re and -Rg_1 in water extract of red ginseng were identified and quantified clearly by TLC and HPLC. However, these saponin in several mixtures of ginseng and crude drugs extracts could not distinctly quantified due to hydrolysis with organic acids of red ginseng with other components. Especially these major ginsenosides were not detected in ginseng and shizandrae fructus extract. Shizandrae fructus has high amounts of citric acid and other organic acids as shown in Table 1. As seen on Table 2, ginsenoside contents were decreased remarkably in ginseng and crude drug extracts compared with ginseng extract itself. According to the combination ratios of ginseng and crude drugs, the decrease amounts of ginsenosides were different in ginseng and crude drug preparations[10].

Table 1. Organic acid contents in ginseng and crude drugs (Unit : mg/g)

Crude Drugs	Oxalic	Malonic	Fumaric	Succinic	Malic	Citric	Total
Red Ginseng(紅蔘)	3.70	2.13	-	0.44	8.78	13.34	26.39
Glycyrrhizae Radix(甘草)	10.80	0.93	0.63	0.08	2.00	0.73	15.17
Bupleuri Radix(柴胡)	4.30	1.48	-	-	1.49	2.57	9.84
Pinelliae Tuber(半夏)	12.03	-	-	-	0.12	0.10	12.25
Hoelen(茯苓)	1.07	-	-	-	-	-	1.07
Cnidii Rhizoma(川芎)	0.76	-	-	-	1.08	3.07	4.91
Angelicae Radix(當歸)	2.25	1.54	0.03	0.08	5.47	3.43	12.80
Rehmanniae Radix(地黃)	1.86	0.03	0.02	0.25	1.96	1.17	5.29
Atractylodis Rhizoma(蒼朮)	20.14	0.12	0.13	0.04	2.14	1.14	23.71
Puerariae Radix(葛根)	12.52	-	-	0.06	1.76	4.19	18.53
Zingiberis Rhizoma(乾薑)	6.70	0.41	0.07	-	0.49	0.99	8.66
Cinnamomi Cortex(桂皮)	17.40	-	0.03	-	0.25	0.43	18.11
Schizandrae Fructus(五味子)	0.25	0.05	-	0.36	22.17	57.77	80.60
Lycii Fructus(枸杞子)	0.98	0.66	-	0.71	3.45	3.57	9.37
Scutellariae Radix(黃芩)	1.83	0.22	-	-	2.69	1.60	6.34
Liriopis Tuber(麥門冬)	1.78	-	-	0.06	0.57	1.00	3.41
Epimedii Herba(淫羊藿)	10.39	0.51	0.06	0.09	4.01	0.69	15.75
Zizyphi Fructus(大棗)	2.38	0.08	0.10	0.05	1.93	1.76	6.30

Table 2. Determinations of ginsenosides, prosapogenins and sapogenins in ginseng and crude drug extracts (Contents : mg/g)**

Extracts	Saponin(ginsenoside-)						Prosapogenin		Sapogenin	
	Rb_1	Rb_2	Rc	Rd	Re	Rg_1	Rg_3	Rg_2	PD	PT
Ginseng(G)	3.44	1.43	1.63	0.67	1.25	2.68	2.98	0.58	4.39	1.25
Glycyrrhizae Radix+G#	2.83	1,23	1.55	NS*	NS	NS	2.50	0.49	2.52	1.00
Bupleuri Radix+G#	1.95	0.90	1.13	0.58	NS	NS	1.69	0.36	2.57	1.03
Pinalliae Tuber+G#	2.20	0.93	1.09	0.45	1.20	2.00	1.82	0.46	2.61	0.97
Hoelen+G#	2.61	1.10	1.31	NS	1.23	2.58	2.61	0.50	4.11	1.23
Cnidii Rhizoma+G#	1.98	0.75	0.93	0.37	1.05	1.60	1.61	0.41	2.43	0.95
Angelicae Radix+G#	2.35	0.90	1.03	0.38	1.24	1.91	1.65	0.43	2.39	0.89
Rehmanniae Radix+G#	1.88	0.73	0.84	0.40	0.83	NS	2.38	0.52	3.70	1.16
Atractylodis Rhizoma+G#	2.58	1.00	1.20	0.53	1.15	2.14	2.07	0.51	3.44	1.01
Puerariae Radix+G#	2.53	0.98	1.15	0.50	1.20	2.18	0.90	0.32	3.85	1.09
Zingiberis Rhizoma+G#	2.18	0.85	1.05	0.43	1.25	1.70	0.55	0.22	2.61	0.89
Cinnamomi Cortex+G#	2.85	NS	1.35	0.64	NS	2.24	0.64	0.27	3.67	1.23
Schizandrae Fructus+G#	-	-	-	-	-	NS	0.62	0.15	2.40	0.81
Lycii Fructus+G#	2.33	0.88	1.10	0.48	0.98	1.95	0.54	0.17	2.95	0.63
Scutellariae Radix+G#	2.35	1.03	1.30	NS	1.33	NS	0.73	0.43	2.47	0.92
Liriopis Tuber+G#	2.95	1.14	1.28	0.53	1.18	2.05	0.59	0.22	3.82	1.17
Epimedii Herba+G#	2.75	1.16	1.48	NS	NS	NS	0.58	0.24	2.83	0.73
Zizyphi Fructus+G#	2.65	1.28	1.53	0.64	NS	2.43	2.64	0.51	3.84	1.23

#Crude drug and ginseng extracts were prepared from same amounts of ginseng and each crude drug with water extraction method
*NS : not separated from other components of crude drugs
**The contents of saponins, prosapogenins, and sapogenins mentioned on the table are those per 1 g of ginseng

Among the 4 kinds of crude drug preparations[10] In-Sam-Tang(人蔘湯) "the simplest composition of crude drugs" showed the highest quantity of ginseng saponin, while So-Shi-Ho-Tang(小柴胡湯) did the lowest quantity of ginseng saponin. Ginsenoside-Rc, -Rd, -Re and -Rg_1 couldn't be quantified by being overlapped with other crude drug components in So-Shi-Ho-Tang which contains high amounts of Bupleuri Radix and Pinniae Tuber, etc.[4,10]. As shown on Table 2, several kinds of ginsenosides could not analyzed due to overlapping of other components from Glycyrrhiza Radix, Bupleuri Radix, Cinnamomi Cortex, Scutellariae Radix or Epimedii Herba, etc.

Identification and quantification of ginseng prosapogenins in ginseng and crude drug extracts

Prosapogenins, prosapogenin-Rg_3 and -Rg_2[9,10] were distinctly identified and quantified by TLC and HPLC on 50% acetic acid hydrolyzates of saponin fractions from ginseng and crude drug extracts. This method also enabled the analysis of ginseng saponin compounds in the granules of In-Sam-Tang, So-Shi-Ho-Tang, Sa-Kun-Ja-Tang(四君子湯) and Yook-Kun-Ja-Tang(六君子湯) by quantifying the prosapogenins with HPLC[10]. These prosapogenins in the extract granules of those preparations can be quantified by HPLC and the analytical results of prosapogenins were clear than those of ginseng saponins[10]. The transfer rates of prosapogenins from saponin in the preparations were 66-94% except the So-Shi-Ho-Tang granule. Choi[10] reported that ginsenoside-Rb_1 was transferred to prosapogenin by only 13.1% and the transfer rates of prosapogenin-Rg_3 was higher than saponin in So-Shi-Ho-Tang. The quantified contents of prosapogenins-Rg_3 and -Rg_2 were 57% and 62%, respectively in ginseng and Bupleuri Radix extract as shown on Table 2. These result suggest that the analysis of prosapogenin could be a good methods for quality control in ginseng and crude drug preparation.

Identification and quantification of sapogenins in ginseng and crude drug extracts

Ginseng sapogenins, panaxadiol and panaxatriols[2,10] were distinctly identified and quantified by TLC and HPLC from 7% sulfuric acid hydrolyzates of saponin fractions from ginseng and crude drug extracts. As Shibata[2] reported, ginseng saponins can be converted into two types of aglycones, resulting from the cyclization of side chain at C-20 position by hydrolyzing at the sugars bonded to C-3, C-6 and C-20 positions with 7% sulfuric acid in 50% ethanol. Namely, all the protopanaxadiol saponins give panaxadiol sapogenin, and all the protopanaxatriol saponins give panaxatriol sapogenin[2]. The variations of transfer ratios of ginsenosides in the ginseng and crude drug extracts were 0-82.3%, while those of prosapogenins in the extracts which was hydrolyzed with 50% acetic acid[10] were 19.9-88.5% and those of sapogenins in the extracts which was hydro-

lyzed with 7% sulfuric acid[10] were 56.9-94.7%. The sapogenins and prosapogenins was separated well in HPLC analysis more than saponin in ginseng and various crude drug preparation extract mixtures. These result suggests that the identification and quantification of sapogenins and prosapogenins could be useful than to do saponins in crude drug extracts for quality control in these crude drug preparations.

REFERENCES

1. Brekhman, I. I. and Dardymov, I.V. : New substances of plant origin which increase nonspecific resistance. *Ann. Rev. Pharm.* **9**, 419-443 (1969).
2. Shibata, S.: Some chemical studies on ginseng. *Proceedings of international ginseng symposium*. The central Research Institute, Office of Monopoly, Seoul, p.69-76 (1974).
3. Takagi, K.: Pharmacological studies on ginseng. *Proceedings of international ginseng symposium*. The central Research Institute, Office of Monopoly, Seoul, p.119-127 (1974).
4. Shibata, S., Isokawa, H., Sankawa, U., Shoji, J. and Takido, M. : Natural products for medicinal use, Nanzando Company, Limited, Tokyo, p.386-445 (1982).
5. Kasai, R., Besso, H., Tanaka, O., Saruwatari, Y. and Fuwa, T. : Saponins of red ginseng. *Chem. Pharm. Bull.*, **31**, 2120-2125 (1983).
6. Kitagawa, I. : Chemical studies on crude drug processing red ginseng and white ginseng. *Proceedings of the 4th international ginseng symposium*, Korea Ginseng and Tobacco Research Institute, Daejeon, Korea, p.159-168 (1984).
7. Soldati, F. : HPLC seperation and quantitative determination of ginsenosides from *Panax Ginseng, Panax Quinquefolium* and from ginseng drug preparations. *Proceedings of the 3rd international ginseng symposium*, Korea Ginseng and Tobacco Research Institute, Seoul, Korea, p.59-69 (1980).
8. Choi, K.J., Ko, S.R., Kim, S.C. and Park, J.D. : Saponin and ginsenoside content in korean red ginseng products. *Korean J. Ginseng Sci.* **13**, 178-182 (1989).
9. Kaku, T. and Kawashima, Y. : Isolation and characterization of ginsenoside-Rg_2, 20R-prosapogenin, 20S-prosapogenin and Δ^{20}-prosapogenin. *Arzneim. -Forsh./Drug Res.*, **30**, 936-943 (1980).
10. Choi, K.J., Ko, S.R., Kim, S.C. and Kim, M.W. : Idetification and determination of ginseng saponins, prosapogenins and sapogenins from crude drug preparations for quality control. *Proceedings of the 6th international ginseng symposium*, Korea Ginseng and Tobacco Research Institute, Seoul, Korea, p.206-214 (1993).
11. Choi, K.J., Ko, S.R., Jeon, B.S. and Sung, H.S. : Identification of ginseng sapogenin and quantitative determination of ginsenoside-Rb_1 from crude drug preparation containing Bupleuri Radix. *Kor. J.Pharmacogn.* **20**, 175-179.(1989).
12. Choi, K.J., Ko, S.R., Kim, N.M. and Sung, H.S. : Identification of ginseng saponin and quantitative determination of ginsenoside-Rb_1 from crude drug preparation drink. *Korean J. Ginseng Sci.* **14**, 112-116 (1990).
13. Kwak, Y.S., Choi, K.J. and Kim, N.M. : Effect of ozone treatment on the changes of fatty acids, organic acids, and sensory properties in ginseng powder. *J. Fd. Hyg. Safety* **11**, 51-55 (1996).