Journal of Medicinal Plants Research Vol. 5(23), pp. 5513-5516, 23 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

The evaluation of contents of nine ginsenoside monomers in ginseng hairy roots by high performance liquid chromatography (HPLC)

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Accepted 8 September, 2011

Nine ginsenoside monomers (Rg1, Re, Rf, Rb1, Rg2, Rc, Rb2, Rb3, Rd) of ginseng hairy roots and cultivated ginsenoside monomers were simultaneous determined by high performance liquid chromatography (HPLC) to assay chemical components of ginseng hairy roots. The results demonstrated that there were no significant difference between Rg2 of ginseng hairy roots and Rg2 of six-year-old cultivated ginseng (P<0.05), and the other eight kinds of ginsenoside monomers from six-year-old cultivated ginseng were higher than that of ginseng hairy roots (P<0.05); Rg1, Rb2 and Rb3 of ginseng hairy roots were significantly lower than that of four-year-old cultivated ginseng (P<0.05), and there were no significant difference of other six kinds of ginsenoside monomers from ginseng hairy roots and four-year-old cultivated ginseng (P<0.05). The total content of ginsenosides Rgl and Re were 0.3188%, Rgl and Rb1 0.4848% in ginseng hairy roots, which was comply with quality standards in Chinese Pharmacopoeia (2010) and European Pharmacopoeia, which provide a basis and reference for quality control of ginseng hairy root.

Key words: Ginseng hairy roots, ginseng, reversed phase-high performance liquid chromatography (HPLC), ginsenoside monomer.

INTRODUCTION

Asian ginseng (Panax ginseng) belonging to Araliaceae family, is an important medicinal plant for its restorative properties, which has been a valuable herbal medicine used in Oriental countries for thousands of years (Attele et al., 1999; Ang-Lee et al., 2001; Xie et al., 2005). Previous studies have demonstrated that ginseng root and its major bioactive components (ginsenosides) have complex constitutions and multifaceted pharmacological functions, such as anticancer properties (Helms et al., 2004; Wang et al., 2007). The known biochemical and pharmcological activities of ginseng include antiaging, antidiabetic, anticarcinogenic, analgesic, antipyretic, antistress, antifatigue, and tranquilizing properties as well as the promotion of DNA, RNA, and protein synthesis (Attele et al., 1999; Kitts et al., 2000; Radad et al., 2006). Ginsenosides, the main components of ginseng, are the main functional ingredients of the ginseng-type functional foods (Liu et al., 1991; Yun et al., 1991), which were denominated R0, Ra, Rb1, Rb2, Rb3, Rc, Re, Rf,Rg1, Rg2, Rg3, Rh1, Rh2, Rh3 and so on (Gao et al., 2010; Zhao et al., 2001; Sung et al., 2004). Most of the reports, however, have focused upon the root of ginseng for the ginsenosides studies so far. There is very limited data available in the literature with regard to hairy root ginsenosides studies. The present study aims to investigate ginsenoside monomers in ginseng hairy roots by high performance liquid chromatography (HPLC).

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MATERIALS AND METHODS

Equipment and materials

High performance liquid chromatograph (Shimadzu, LC-2010A) bought from Japanese. LC-2010A Shimadzu Liquid Chromatography Pumps and Autosampler LC-2010A bought form

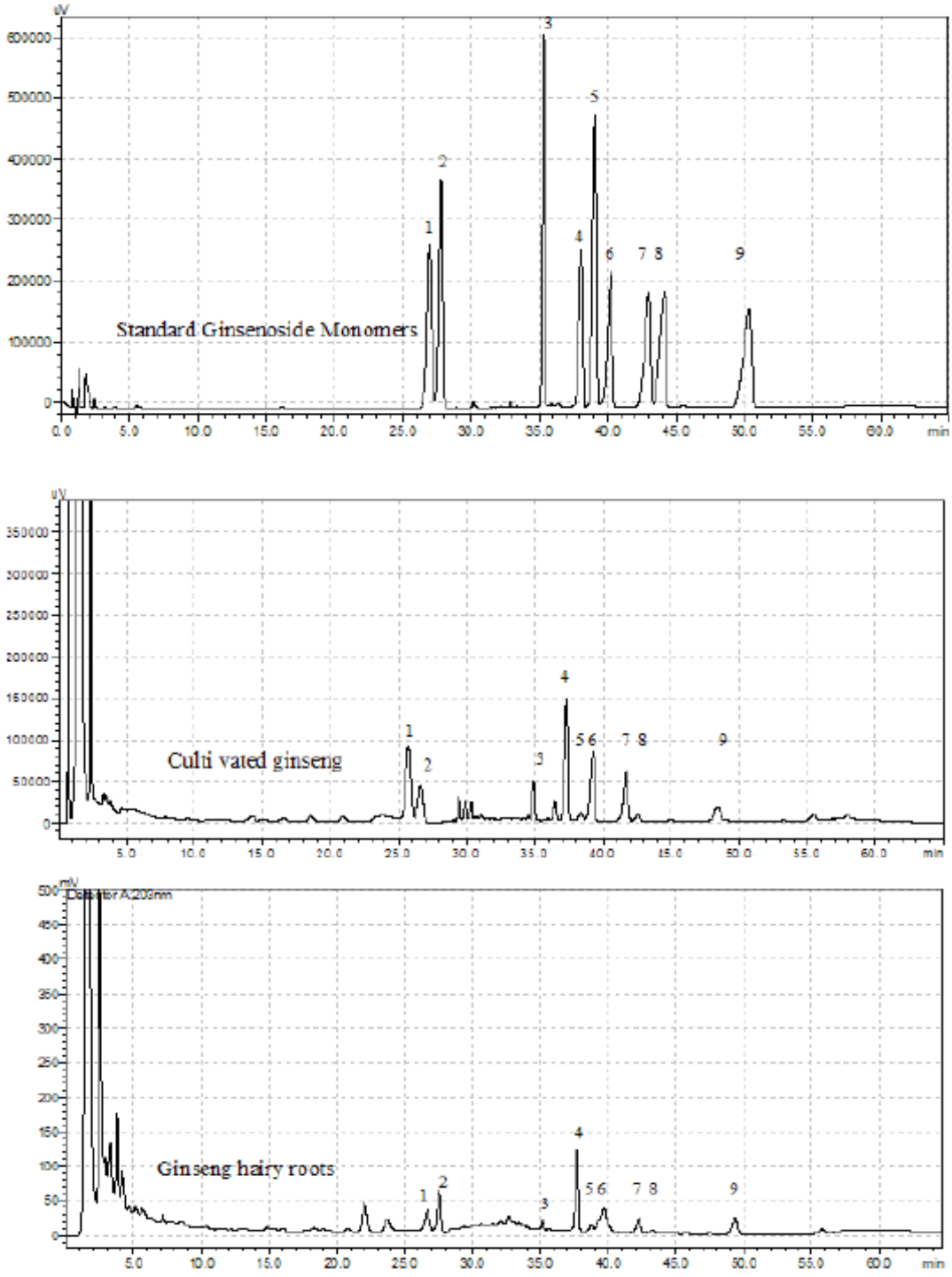

Figure 1. The chromatogram of standard Ginsenoside Monomers and ginseng sample. 1: Rg1, 2: Re, 3: Rf, 4: Rb1, 5: Rg2, 6: Rc, 7: Rb2, 8 :Rb3, 9: Rd.

USA. Nine ginsenosides (Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3 and Rd) standards were purchased from Jilin university. The acetonitrile and methanol were bought form Fisher Scientific (USA). Water used in the experiment was from a Millipore pure water system (Millipore, USA). Four-year-old cultivated ginseng and six-year-old cultivated ginseng obtained from Jingyu county of Jilin province in China. Ginseng hairy root came form Jilin Agricultural University.

HPLC conditions

The gradient elution system consisted of water (A) and acetonitrile (B). Separation was achieved using the following gradient: 0 min (18% A), 24 min (22% A), 26 min (26% A), 30 min (32% A), 50 min (33.5% A), 55 min (38% A), 65 min (38%A). The column temperature was set at 35°C. The flow rate was 1 ml/min.

Table 1. The comparison of contents of nine ginsenoside monomers between ginseng hairy roots and cultivated ginseng (%).Different letter represent the significant difference at p<0.05.

Sample	Rg1	Re	Rf	Rb1	Rg2	Rc	Rb2	Rb3	Rd
Ginseng hairy root (n=26)	0.1281^{a}±0.0237	0.1906^{a}±0.0287	0.0299^{a}±0.0207	0.3566^{a}±0.1093	0.0221^{a}±0.0113	0.2101^{a}±0.1044	0.0509^{a}±0.0475	0.0191^{a}±0.0166	0.1112^{a}±0.0324
six years ginseng (n=13)	0.3587^{c}±0.0508	0.2936^{b}±0.0262	0.0848^{b}±0.0120	0.6040^{b}±0.0755	0.0243^{a}±0.0022	0.4746^{b}±0.0502	0.2725^{b}±0.0409	0.0390^{b}±0.0060	0.2000^{b}±0.0363
4 years ginseng (n=6)	0.2020^{b}±0.1216	0.1606^{a}±0.0436	0.0434^{a}±0.0203	0.3889^{a}±0.1156	0.0216^{a}±0.0044	0.2783^{a}±0.0505	0.2278^{b}±0.0241	0.0339^{b}±0.0036	0.0991^{a}±0.0233

Table 2. The comparison of contents of ginsenoside monomers between ginseng hairy roots and cultivated ginseng (%). Different letter represent the significant difference at p<0.05.

sample	Rg1+ Re	Rg1+ Rb1	Rg1+ Re+Rf+ Rb1+Rg2+Rc+Rb2+Rb3+Rd
Ginseng hairy roots (n=26)	0.3188±0.0389^{a}	0.4848±0.1068^{a}	1.1186±0.2677^{a}
6 years ginseng (n=13)	0.6523±0.0708^{b}	0.9627±0.1120^{b}	2.3515±0.2588^{b}
4 years ginseng (n=6)	0.3626±0.1429^{a}	0.5909±0.2283^{a}	1.4556±0.3652^{a}

The UV detection wavelength was 203 nm. The injection volume was 20 µl. The mean values of three replicates were calculated.

Control solution preparation

Standard ginsenosides (Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3 and Rd) were mixed and diluted with methanol to get a blended standards solution containing each ginsenoside at 2.1, 2.16, 1.94, 2.14, 1.96, 1.9, 2.1, 2.14 and 2.02 mg/ml, respectively, and a single standards solution containing each ginsenoside at 0.98, 1.05, 1.02, 1.08, 0.98, 0.99, 1.01, 1.08, 0.99 and 1.04 mg/ml, respectively.

Sample preparation for HPLC analysis

For ginseng hairy roots samples preparation, 1.0 g of powder (40 mesh) was weighed accurately and refluxed for 4 h in moderate ether in a soxhlet extractor. After discarded ether and added moderate methanol, the sample solution was refluxed for 4 h and discarded methanol. Then, added chromatographic grade methanol dissolve and metered volume to 10 ml, the sample solution was obtained by filtering the supernate with a nylon filter membrane (0.45 µm) prior to the HPLC analysis.

Statistical analysis

To calculate the statistical differences among groups, the statistical package SPSS13.0 (SPSS Incorporated, Chicago) was used for all analysis. All values were expressed as mean ± SD. In general, p values less than 0.05 were considered statistically significant.

RESULTS

The analysis of chromatographic peaks

Ginsenosides were identified by the retention time of the main peak defined by the chromatogram of standard ginsenoside and the characteristic absorption spectra from DAD. As shown in Figure 1, retention time of nine ginsenosides (Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3 and Rd) were 26.946, 27.413, 35.196, 37.724, 38.840, 39.812, 42.429, 43.445 and Rd: 49.581 min, respectively. Nine known ginsenosides were found in ginseng hairy root and cultivated ginseng by HPLC. Moreover, hairy root had two kinds unknown ginsenosides ginseng before Rg1, and cultivated ginseng had one kind unknown ginsenoside before Rb1.

Analysis of ginseng samples

The nine ginsenosides contents of sample were 0.5 to 40 µg by HPLC determination. As shown in Table 1, there were no significant difference between Rg2 of ginseng hairy roots and Rg2 of six-year-old cultivated ginseng (P<0.05), and the other eight kinds of ginsenoside monomers from ginseng hairy roots were lower than that of six-year-old cultivated ginseng (P<0.05); Rg1, Rb2 and Rb3 of ginseng hairy roots were significantly lower than that of four-year-old cultivated ginseng (P<0.05), and there were no significant difference of other six kinds of ginsenoside monomers from ginseng hairy rootsand four-year-old cultivated ginseng (P<0.05). As shown in Table 2, The total content of ginsenosides Rgl and Re were 0.3188%, Rgl and Rb1 0.4848% in ginseng hairy roots, which was comply with quality standards in Chinese Pharmacopoeia (2010) and European

Pharmacopoeia.

DISCUSSION

The present study determined the nine ginsenosides in ginseng hairy root. The sum content of Rg1 and Re were no less than 0.3% and Rb1 content was no less than % in Chinese Pharmacopoeia (2010), respectively as the index of physical and chemical for ginseng quality control.Rg1 and Rb1 content were no less than 0.2 and 0.1% respectively in American Pharmacopoeia (USP-25-NF20), the sum content of Rgl and Rbl was no less than 0.40% in European Pharmacopoeia. The current study showed that he sum of Rg1 and Re content were 0.3188% in ginseng hairy root, the content of Rb1 was 0.3566%, the sum of Rg1 and Re were 0.4848%, which met or exceeded quality standards in Chinese Pharmacopoeia (2010) and European Pharmacopoeia.

So far, more than 60 kinds of ginsenosides had been isolated from ginseng (Li et al., 2010). Many study on Rg1, Re, Rf, Rg2, Rb1, Rc, Rb2, Rb3 and Rd in cultivated ginseng had been conducted (Xiao et al., 2004; Wei Shi et al., 2007; Zhu et al., 1998; Sun et al., 2010; Yin et al., 2010; He et al., 2010; Chong Liu et al., 2007; Bin et al., 2009; Yijun et al., 2011; Erin et al., 2006), However, these study mostly focused on less than eight kinds of ginsenosides, simultaneous determination of nine ginsenosides was scarce, especially, nine ginsenosides in ginseng hairy roots simultaneous identified had not reported until now. In conculsion, the present study determined the nine ginsenosides of ginseng hairy root, which provide a basis and reference for quality control of ginseng hairy root.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support provided by China Postdoctoral Science Foundation (20090461042), Supported by National Key Technology Program for the 12th five-year plan (2011BAI03B01), Supported by Ministry of Science and Technology of China (2009GJB10031); Supported by National Natural Science Foundation of China (30300256, 31070316, 30570185) supported by a grant from the Major State Basic Research Development (2007BAI38B01).

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