

Identification of Major Saponins from Jiaogulan Extract (*Gynostemma pentaphyllum*)

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ABSTRACT

Jiaogulan (*Gynostemma pentaphyllum*; GP) contained a saponin glycoside which was related to ginseng saponin. The purpose of this study was to identify the main saponin in water extract GP compared to methanol and ethanol extract. The sample was prepared by alkaline cleavage, trimethylsilyl (TMS) derivatization and gas chromatographic-mass spectrometric (GC-MS) analysis comparison with standard ginsenoside Rb1. The results showed that there were two main dammarane type aglycones in water, methanol and ethanol extracts GP, 20(S)-dammar-24-ene-3b, 12b, 20 triol and 20(S)-dammar-24-ene-3b, 6a, 12b, 20 tetrol, which were the same compound as ginsenoside Rb1 and Rg1 from ginseng (*Panax spp.*), representatively. The other peak, 20(S)-dammar-24-ene-3b, 12b, 19, 20 tetrol, was not obvious in water extract as in methanol and ethanol extracts. In conclusion, the water extract GP contained two main saponin compounds, which were the same compounds as in ginseng.

Key words: *Gynostemma pentaphyllum*, Jiaogulan, saponin, Gas chromatography mass spectrophotometry

INTRODUCTION

Jiaogulan (*Gynostemma pentaphyllum*, GP) is a Chinese medicinal herb which is called "Panjakun" in Thai and "Amachazuru" in Japanese (Blumert and Liu, 2003). *G. pentaphyllum* is in the family *Cucurbitaceae*, and is a perennial liana with stems that grow like vines and has leaves that are oval-shaped with saw tooth edges and white hairs and grow in cluster of five leaves. GP can be cultivated either by seed or from plant cuttings (Takemoto, 1984) and harvested in 4-5 months. *G. pentaphyllum* grows abundantly in Southern

China, Japan, and Korea. In Thailand, it can be grown easily with good quality. At present, GP is cultivated widely in Northern Thailand such as Chiangmai, Chiangrai, and Maehongson. The important active components of GP are saponin glycosides and antioxidant. Phytochemical studies of this plant have identified about 90 dammarane-type glycosides (called gypenoside) closely related to ginseng saponins (Cui *et al.*, 1999). Pharmacological studies of *G. pentaphyllum* and the isolated saponin have shown a variety of interesting activities such as antitumor, cholesterol-lowering, immunopotentiating,

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anticancer, antioxidants and the others (Blumert and Liu, 2003). Compared to ginseng, Jiaogulan is cheaper and more readily available. Therefore, GP has high potential as raw material for nutraceutical and functional foods.

It is difficult to establish practical analytical methods for the separation and identification of such a great number of individual saponins as occur in *G. pentaphyllum*. On the other hand, this task could be considerably simplified by measurement of their main and specific aglycones if suitable procedures were available. This present study is purposed to identify main saponin from water extract *G. pentaphyllum* compare to methanol and ethanol extracts.

MATERIALS AND METHODS

Plant

Gynostemma pentaphyllum from the Royal Pangda Station in Amphur Samern, Chiangmai, Thailand, was used for this research. The plants were harvested in the early morning before being transported to the Royal plant-packing house in Chiangmai and moved to laboratory before noon in the same day. Fresh GP leaves were dried by microwave dryer until the moisture content was below 10%. Dried GP was vacuum packed in aluminium foil and kept in -20 °C until used.

Chemicals

The chemicals used for GC-MS analysis; Trimethylchlorosilane (Fluka Chemical Corp., U.S.A.), Trimethyl-silylimidazol and N,N-bis-trimethylsilyl-trifluoro acetamide (Macherey-Negel, Germany), and standard ginsenoside Rb1 (MP Biomedicals, LLC, U.S.A.). The solvent, methanol, ethanol and butanol, were analytical grades from Merck, Germany.

Sample preparation

Extraction from microwave dried GP

used three methods, hot water, methanol and ethanol extractions. The dried GP were extracted with solvents at 1:30 proportions. The water extract method used double distilled water, heating in water bath shaker at 90 °C for 10 min at 100 rpm. The methanol extract method used 80% methanol with Soxhlet extraction for 6 hours (Kwon *et al.*, 2003 and Korean Ginseng & Tobacco Research Institute, 1991). The ethanol extract method was conducted by shaking 95% ethanol at 200 rpm for 6 hours (Kawpinit, 1993; Poomecome, 1999; Maisutisakul and Pongsawatmanit, 2004). The extracts were centrifuged at 1000 rpm for 5 min, then the supernatant was filtrated by filter paper (Whatman No. 1). The filtrate was dried by rotary evaporator at 50 °C, then lyophilized to the dried extract for chemical component identification.

Gas Chromatography - Mass Spectrometry was used to characterize saponin in GP extract (Cui *et al.*, 1998 and Cui *et al.*, 1999). A 40 mg extract sample and 5 ml of distilled water were mixed in a 15 ml test tube. The mixture was heated on a heating block for 30 min at 75 °C and then the extract was sonicated for 15 min prior to being centrifuged at 1000 g for 5 min. The supernatant was applied to a Sep-Pak C₁₈ cartridge. The column was washed with 10 ml of water followed by 10 ml of 30% methanol in water. The saponin fraction was eluted with 5 ml of methanol and collected in a 13 ml test tube with a Teflon cap. The solvent was evaporated at 65 °C under a nitrogen stream.

Alkaline cleavage and derivatization

The dried fraction was dissolved in 3 ml of n-butanol and 75 mg of NaOCH₃. The alkaline cleavage reaction was carried out at 85 °C for 8 hours. After washing with d.i. water (2×1.2 ml), 600 µl of the butanol phase was evaporated to dryness at 80 °C under a nitrogen stream. The residue containing the released aglycones was trimethylsilylated with 100 µl of a mixed reagent (BSTFA: TMSI: TMSCI = 3:3:2 v/v/v) at 70 °C

for 20 min and the derivatives was analyzed by GC-MS.

GC-MS analysis.

A GCQ (Finnigan, MAT) ion trap gas chromatography mass spectrometer (electron impact ionization, 70 eV) was used in this study. A SPB 1701 column (column length 15 m., 0.25 mm. I.D., film thickness 0.25 μ m) was used. The column flow rate was 0.8 ml/min by helium gas. The conditions for the SPB1701 column were 150 °C for 0.1 min to 270 °C at 10 °C/min with a hold for 10 min. Injector temperature was 250 °C. Ion source temperature was 200 °C. The aglycones obtained from the samples were identified by comparing of the retention time, relative retention time and mass spectra those obtained from the authentic saponin.

Ginsenoside Rb1 (Reference grade, MP Biomedicals, LLC, Ohio, U.S.A.) from ginseng (*Penax spp.*) was used for a reference standard. The standard (0.5 mg) was prepared in the same assay as the sample. The reference substance was subjected to alkaline cleavage, derivatization and GC-MS analysis.

RESULTS AND DISCUSSION

Chromatographic procedure resulting in profiles of active constituents is a more objective method for such authentication than the traditionally based morphological studies. Cui (1995) reported that alkaline cleavage of ginsenosides, followed by trimethylsilyl (TMS) derivatization and gas chromatographic-mass spectrometric analysis was a suitable method for analysis and authentication of *Panax* drugs. Compared to acidic cleavage, alkaline cleavage did not produce as many artifacts (Cui *et al.*, 1993). The preparation of *Gynostemma Pentaphyllum* extract used alkaline cleavage, followed by TMS derivatization and analysis by GC-MS. The GC-MS chromatograms of 3 GP extracts; water extract,

methanol extract and ethanol extract, as determined by SPB 1701 columns are shown in Figure 1 (a, b, c). Three peaks are determined in methanol and ethanol extract GP but the water extract GP presents only two peaks. The reference standard ginsenoside Rb1 chromatogram is shown in Figure 1 (d).

The mass spectra of three aglycone compounds found in the GP extract are as follow (Figure 2-4);

1. 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol : the ion characters; 199, 321, 411, 501, 584 m/z
2. 20(S)-dammar-24-ene-3 β , 12 β , 20 triol : the ion characters; 199, 323, 413, 503, m/z
3. 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol : the ion characters; 199, 321, 411, 501 m/z

Table 1 presents molecular weight and the retention time of the aglycones obtained from GP extracts and the standard Rb₁. The chromatograms show that the peak corresponding to the standard ginsenoside Rb₁ in the same retention time as the second peak found in the GP extracts. Table 2 shows the characteristic ions of the trimethylsilylated aglycones from the mass spectral data. The structures of three aglycone saponins are shown in Figure 5. The first aglycone, 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol, is not as pronounced in the water extract as in the methanol and ethanol GP extract which consists of CH₂OH in the structure, so this compound is less polarity than the others. Cui *et al.* (1998) identified these aglycones from gypenosides LXII, LXIV, LXV, LXVI, LXXII, and LXXXVI standards. The second dammar aglycone, 20(S)-dammar-24-ene-3 β , 12 β , 20 triol, is the most intense peak in all GP extract in the same retention time as the important compound from ginsenoside Rb₁ (Cui *et al.*, 1998). The third aglycone, 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol, is supported by Cui *et al.* (1998) who confirmed that it was a

ginsenoside Rg₁. Previously, Cui *et al.* (1993) analyzed ginsenoside Rb₁ by chromatography and mass spectrometry which could produce two sapogenins, 20S-protopanaxadiol and 20S-protopanaxatriol, that support same characteristics ion as our results. Cui *et al.* (1999) identified the major sapogenins from *Gynostemma pentaphyllum* compared to *Panax* species. The results support our study that 20(S)-dammar-24-

ene-3 β , 12 β , 19, 20 tetrol was found in *G. pentaphyllum* and 20(S)-dammar-24-ene-3 β , 12 β , 20 triol was found both in *G. pentaphyllum* and *Panax* species. But the 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol was found only in *P. ginseng*, *P. quinquefolium* and *P. notoginseng* (Cui *et al.*, 1999). Shen (2000) reported that ginsenoside Rb₁, one of the main 20(S)-protopanaxadiol group saponin, showed effective anti-inflammatory

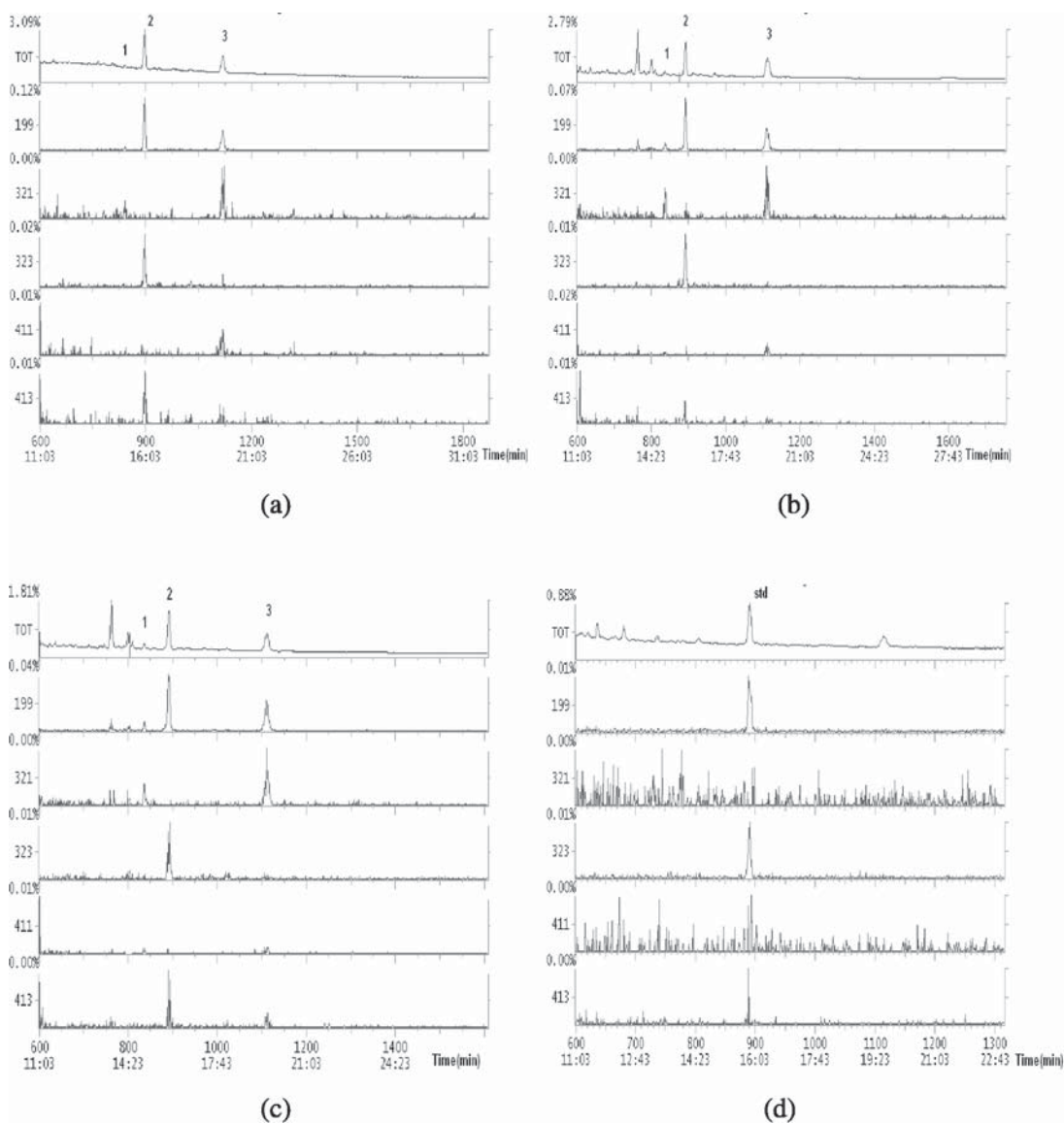


Figure 1 GC-MS ion chromatogram of GP extracts; water extract (a), methanol extract (b), ethanol extract (c), and standard ginsenoside Rb₁ (d).

action, obvious vaso-dilating effect, and tranquilizing function to the central nervous system, moreover ginsenoside Rb1 protected the brain from ischemic and reperfusion injuries (Zhang and Liu, 1996). The saponin, 20 (S)-

protopanaxatriol group, represented by ginsenoside Rg1, possessed the properties of exciting the central nervous system, anti-fatigue and hemolysis (Shen, 2000).

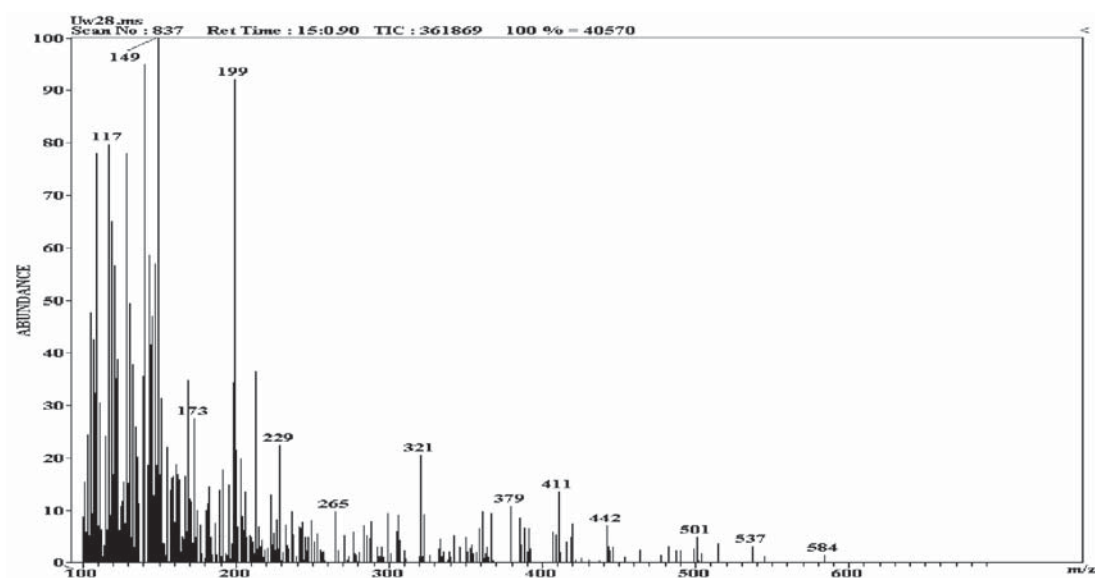


Figure 2 Mass spectrum of peak 1: 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol.

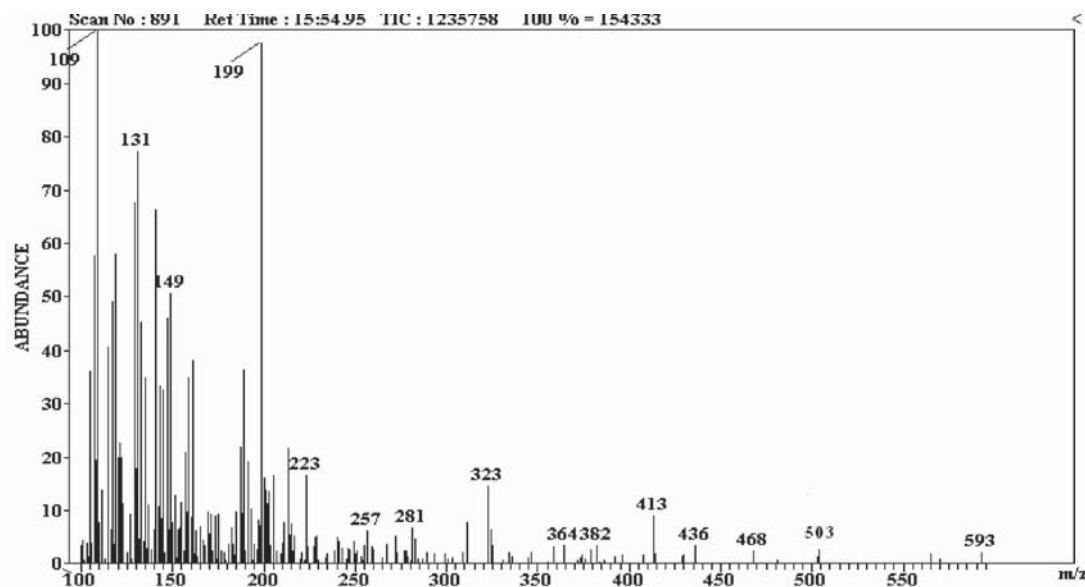


Figure 3 Mass spectrum of peak 2: 20(S)-dammar-24-ene-3 β , 12 β , 20 triol.

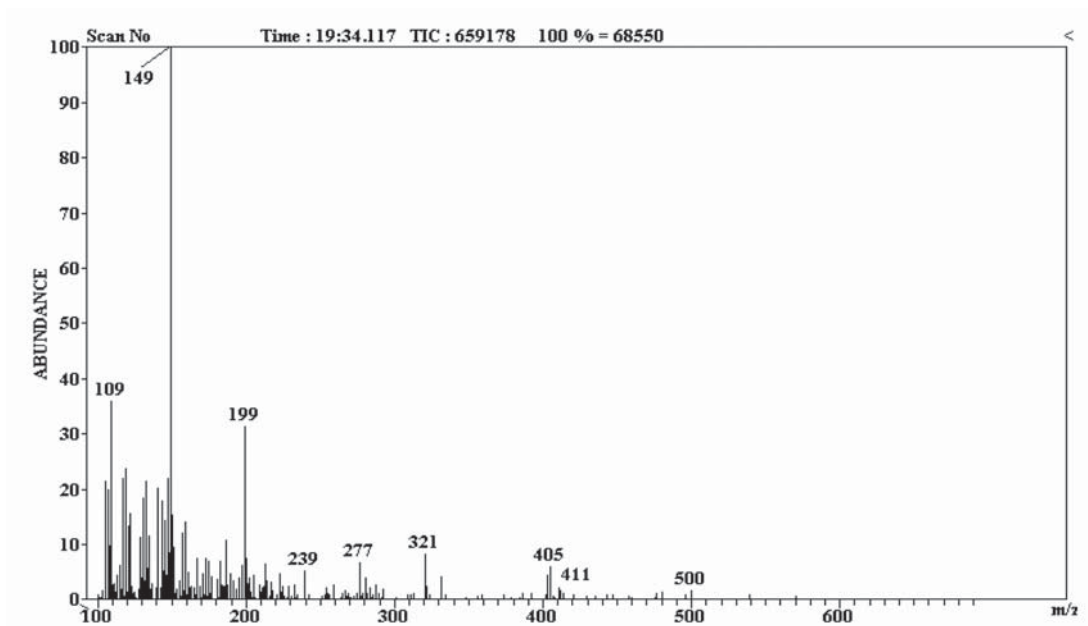


Figure 4 Mass spectrum of peak 3: 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol.

Table 1 Aglycones obtained from GP extracts by alkaline cleavage.

Aglycones	MW ¹	t _R ² (min)	Base peak (m/z) ³
1. 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol	476	15.01	199
2. 20(S)-dammar-24-ene-3 β , 12 β , 20 triol	460	15.55	199
3. 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol	476	19.34	199
4. Standard Ginsenoside Rb1	460	15.53	199

¹ Molecular weight of aglycones.

² t_R = retention time

³ Base peak above 150 m/z

Table 2 Characteristic ion of trimethylsilylated aglycones obtained from GP extracts.

Aglycone derivative	MW ¹	Characteristic ion (m/z) ²
TMS-1 (20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol)	764	199, 321, 411, 501, 584
TMS-2 (20(S)-dammar-24-ene-3 β , 12 β , 20 triol)	676	199, 323, 413, 503
TMS-3 (20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol)	764	199, 321, 411, 501
TMS-4 (Ginsenoside Rb1)	676	199, 323, 413, 503

¹ Molecular weight of trimethylsilylated derivatives of aglycones

² mass-to-charge ratio

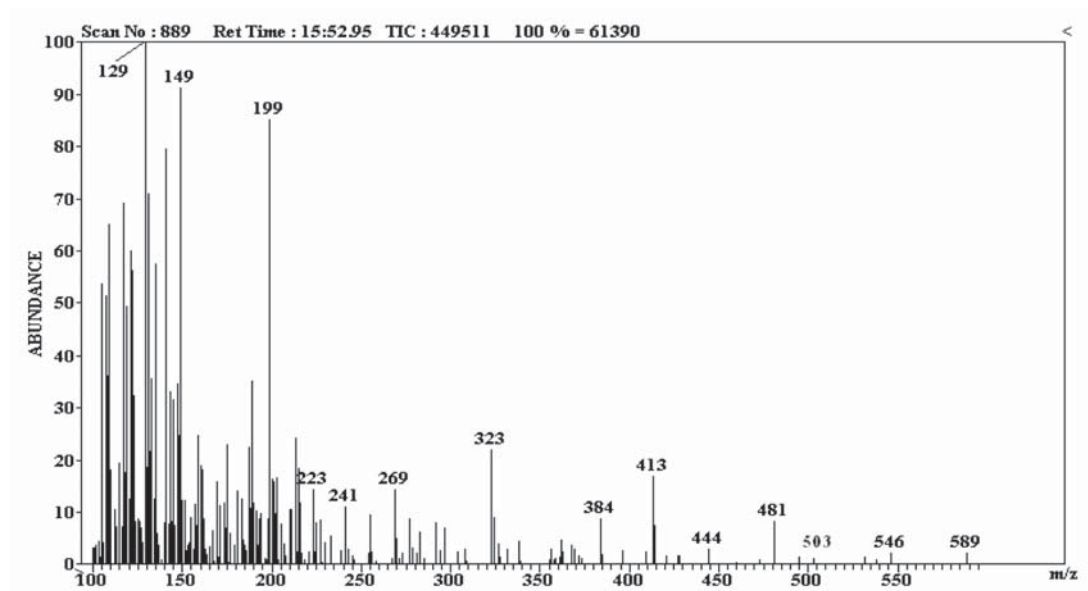


Figure 5 Mass spectrum of standard ginsenoside Rb1.

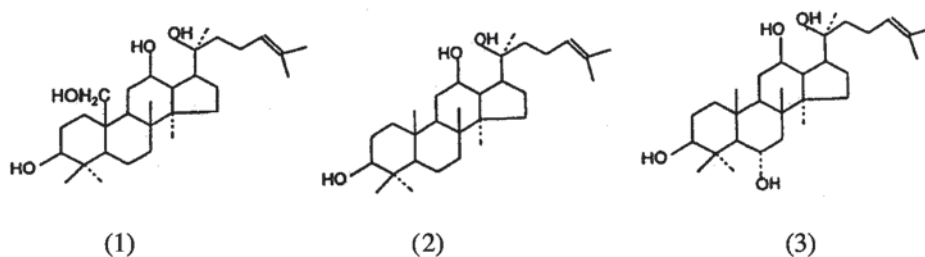


Figure 6 Structures of aglycones obtained after alkaline cleavage of some dammarane saponins isolated from GP extracts. (1) 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol (2) 20(S)-dammar-24-ene-3 β , 12 β , 20 triol (3) 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol.

CONCLUSIONS

This study shows that two major saponins in *Gynostemma pentaphyllum* water extract are the same compounds as in *Panax spp.* Two main saponins in GP water extract are 20(S)-dammar-24-ene-3 β , 12 β , 20 triol and 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol which are the same compound from ginsenoside Rb1 and Rg1 from ginseng (*Panax spp.*). Not only these two compounds, but also one more compound identified from the methanol and ethanol extract; which is 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol.

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