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Chemical Specification of *Gynostemma pentaphyllum* (Thunb.) Makino

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ABSTRACT *Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae) is of interest as a potential crude drug. Its chemical constituents were identified as gypenosides, dammarane-type saponins, which possessed various activities : antitumor, antilipemic, and anti-inflammatory activities etc. In this investigation, the quality of *G. pentaphyllum* collected from 13 various areas, both wild and cultivated, was examined. The chemical identifications were provided by colour reaction, froth test and TLC fingerprint. The efficient methods to determine the total saponins which are pharmaceutically valuable have been developed. Subsequently, the chemical specifications for the aerial parts of this herb were provided.

Key words : *Gynostemma pentaphyllum*, Chemical specification, Total saponins

Introduction

Gynostemma pentaphyllum (Thunb.) Makino belongs to the family Cucurbitaceae. It is widely distributed in the South Shaanxi and the southern area of the Yangtze River of China, Japan, India, Indo-China, and Indonesia. In Thailand, this plant is found growing wild on highlands in the northern region such as Doi Chiang Dao, Chiang Mai province where it is also cultivated for commercial purposes. *G. pentaphyllum* is a herbacious climber with slender stem and 2-branched tendril. The leaves are compound, consisting of 3 - 5 leaflets, rarely 7 leaflets, ovate-orbicular in outline. The male and female flowers grow on separate plants. Flowers are very small, greenish yellow and arranged in a panicle. Fruits are small, globose, and black when ripe[1-4].

The aerial parts are of interested as potential crude drug. In Japan, it is known as Amachazuru which is used as antilipemic[5,6], antitumor[7,8], and health food supplements[9]. The utilities of this plant in China is anti-inflammatory, antitussive, treatment of cough, and chronic bronchitis[10]. *G. pentaphyllum* is known as Panchakhan in Thailand. Although it is said to be one of the most commonly medicinal plants in many countries in Asia, there has been no report for its traditional use in Thailand except a well-known herbal tea in the recent year.

Earlier phytochemical studies of *G. pentaphyllum* were reported the dammarane-type

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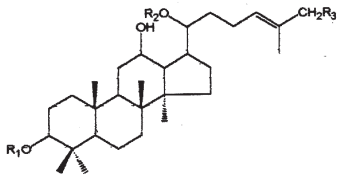
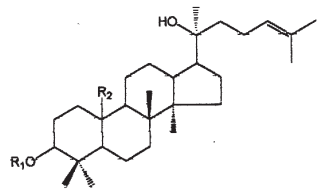
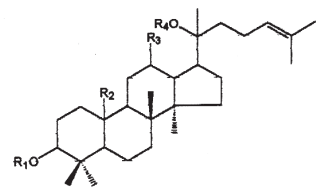


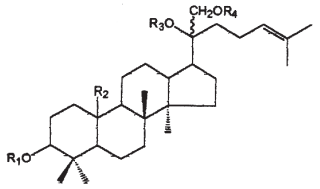
Gypenosides	R_1	R_2	R_3
I	-glc-(glc)-rham	-glc-glc	H
II	-glc-(glc)-rham	-glc-rham	H
III	-glc-glc	-glc-glc	H
IV	-glc-glc-	-glc-xyl	H
V	-glc-glc	-glc-rham	H
VI	-glc-(glc)-rham	glc	H
VII	-glc-rham	-glc-rham	H
VIII	-glc-glc	glc	H
IX	glc	-glc-xyl	H
X	glc	-glc-rham	H
XI	-glc-rham	glc	H
XII	glc	glc	H
XIII	H	-glc-xyl	H
XIV	H	-glc-rham	H
XV	-glc-xyl	-glc-xyl	H
XVI	-glc-xyl	-glc-rham	H
XVII	glc	-glc-glc	H
XVIII	-glc-(glc)-rham	-glc-rham	OH
XIX	-glc-glc-	-glc-rham	OH
XX	-glc-(glc)-rham	-glc-glc-	OH
XXI	H	-glc-xyl	OH



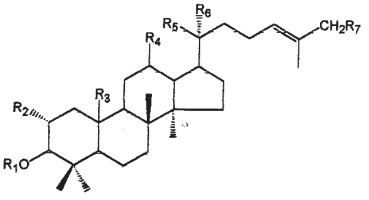
Gypenosides	R_1	R_2
XXVII	-glc-glc	CH_2OH
XXVIII	-glc-glc	CHO
XXIX	-ara-glc	CHO



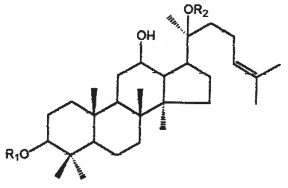
Gypenosides	R_1	R_2	R_3	R_4
XXXVI	-ara-glc	CHO	H	-glc-rham
XXXVII	-ara-glc	CHO	H	-glc-xyl
XXXVIII	-glc-glc	CH_2OH	H	H
XXXIX	-glc-glc	CH_2OH	OH	H (20R)
XL	-glc-glc	CHO	OH	H (20R)
XLI	-glc-glc	CH_2OH	H	H (20R)



Gypenosides	R_1	R_2	R_3	R_4
XXII	-glc-glc	CH_2OH	-glc-xyl	H
XXIII	-glc-glc	CH_2OH	H	glc
XXIV	-glc-glc	CHO	H	Glc
XXV	-ara-glc	CHO	H	glc
XXVI	-ara-glc	CHO	glc	H
XXX	glc	CH_2OH	glc	H
XXXI	-glc-glc	CH_2OH	H	H
XXXII	glc	CH_2OH	H	glc
XXXIII	-glc-glc	CHO	H	H
XXXIV	-glc-glc	CHO	-glc-rham	H
XXXV	-glc-glc	CHO	-glc-xyl	H



Gypenosides	R_1	R_2	R_3	R_4	R_5	R_6	R_7
XLII	-glc-glc	OH	CH_3	OH	-oglc-glc	CH_3	H
XLIII	-glc-glc	OH	CH_3	OH	-oglc-rham	CH_3	H
XLIV	glc	OH	CH_3	OH	-oglc-glc	CH_3	H
XLV	glc	OH	CH_3	OH	-oglc-rham	CH_3	H
XLVI	-glc-glc	OH	CH_3	OH	-oglc	CH_3	H
XLVII	-glc-glc	OH	CH_3	OH	-oglc-rham	CH_3	OH
XLVIII	-ara-(glc)-rham	H	CHO	H	OH	$-CH_2$oglc	H
XLIX	-ara-(xyl)-rham	H	CHO	H	OH	$-CH_2$oglc	H



Gynosaponins	R_1	R_2
A	-glc-(rham)-glc	-glc-glc
B	-glc-(rham)-glc	-glc-rham
E	-glc-glc	-glc-rham
F	-glc-(rham)-glc	glc
G	-glc-rham	-glc-rham
I	glc	-glc-xyl
J	glc	-glc-rham
K	-glc-rham	glc
M	H	glc
N	H	-glc-rham

Figure 1 Structure of active constituents in aerial parts of *G. pentaphyllum* (Thunb.) Makino[5-9, 11-12, 14]

saponins, gypenosides (Figure 1) of which the structures were related to ginsenosides from the Ginseng root, as the major constituents[11]. Eighty-two saponins have been isolated and identified[12]. The structures of gypenosides III, IV, VIII and XII were identical to ginsenosides Rb_1, Rb_3, Rd and F_2, respectively[11, 13]. Antilipemic gynosaponins were reported as gynosaponins A, B, E, F, G, I, K, M, N, O[5, 6]. Gypenosides I-III, V, VII-VIII, X-XIII, XIX-XX, XXII-XXIX had antitumor activity[7, 8], while gypenosides XXX-XLI had anti-peptic ulcer activity[14]. Total saponins isolated from this plant had anti-inflammatory[15], anti-platelet aggregation[16], anti-atherosclerosis and anti-aging activities[17]. Furthermore, *G. pentaphyllum* contained many minerals such as calcium, magnesium, manganese, copper, potassium, sodium, iron[18], and amino acids[19].

Since *G. pentaphyllum* becomes a popular health food supplement in Thailand and there is still no information presently available that specifically deal with chemical specifications of this crude drug. In this investigation, two wild samples and eleven cultivated samples were analyzed and its appropriate chemical specifications are established to convince the ultilization and quality control of this crude drug.

Materials and Methods

Materials

1. Crude drug sample : Eleven samples and 2 samples of *Gynostemma pentaphyllum* (Thunb.) Makino were taxonomic collected from various cultivated areas and 2 different wild areas, respectively, in Chiang Mai province during December 1999 to March 2000. The voucher specimen (Bansiddhi 43-21) was deposited at the Botanical Laboratory, Medicinal Plant Research Institute of Department of Medical Sciences. The botanical identifications were determined using description of Aymonin[1], Backer[2] and Wu[3], and compared with the authentic specimen (Kerr 6555) at the Bangkok Herbarium (BK), Department of Agriculture, Ministry of Agriculture and Cooperative. The foreign matters were removed. The aerial parts were washed thoroughly, then cut into small pieces, dried in the hot-air oven at 50°C, ground to powder, passed through sieve with mesh no. 80 and kept in the well-closed containers.

2. Sieve : Aperture 180 microns, mesh no. 80 from Endecotts Ltd., London, England.

3. Sep-Pak C-18 Cartridges from Waters Corp., USA.

4. TLC Plate Silica gel 60, precoated, 0.25 mm thickness, Art. 5721 from E. Merck, Germany.

5. Antimony trichloride from E. Merck, Germany.

6. Conc. Sulfuric acid from Farmitalia Carlo Erba, Italy.

7. Solvents and chemicals used in this investigation were all analytical grade, and water was distilled water.

Methods

I. Chemical Identification

A. Preliminary Test

1. The powdered drug (0.5 g) was heated with 10 ml of water on a water-bath for 15 min, then filtered. The filtrate was transferred to a separatory funnel and extracted with the equal

volume of butanol. Activated charcoal (0.1 g) was added to the butanol layer, stirred and filtered. The final filtrate was proceeded as following :

1.1 Two-ml of the butanol extract was evaporated to dryness in porcelain dish, added dropwise saturated solution of antimony trichloride in chloroform, evaporated to dryness again and the colour was noted [20].

1.2 Two-ml of butanol extract was evaporated to dryness in porcelain dish, added a few drop of conc. sulfuric acid and the colour was noted.

2. The powdered drug (0.5 g) was heated with 10 ml of water on a water-bath for 15 min, then filtered. One-ml of the filtrate was transferred to a test-tube and shaked for a while, a long lasting foam was observed [21].

B. Thin Layer Chromatography (Confirmatory Test)

Sample preparation : The powdered drug (1 g) was refluxed with 50 ml of water for 2 hr, filtered and washed with hot water, then transferred to 100-ml volumetric flask and adjusted to volume. Ten-ml portion of the solution was transferred to a separatory funnel, 15 ml of water was added and the mixture was extracted with three 10-ml portions of butanol. The butanol extract was evaporated to dryness, then dissolved in 10 ml of water. Five-ml portion of the resulting solution was applied to Sep-Pak C-18 and washed with 10 ml of water, 5 ml of 50% methanol, 2 ml of 60% methanol and eluted with 2 ml of absolute methanol. The methanol eluate was concentrated to 1 ml.

Adsorbent : Silica gel 60, precoated 0.25 mm thickness, E. Merck

Developing solvent : Chloroform : methanol : water (65 : 35 : 10 lower phase)

Developing distance : 10 cm

Spotting amount : 10 µl

Detection : Spray with excess amount of 20% sulfuric acid and activating at 105°C for 5 min.

II. Quality Evaluation

A. Determination of Ash

Total ash and acid-insoluble ash content were determined as described in Thai Pharmacopoeia[22].

B. Determination of Extractive Values

Ethanol soluble and water soluble extractive were carried out using the methods described in British Pharmacopoeia[23].

C. Determination of Water Content

Water content was carried out by the azeotropic method described in The United States Pharmacopoeia XXII[24].

D. Determination of Foaming Index

Foaming index was determined as described in Thai Herbal Pharmacopoeia Vol. II[25].

E. Determination of Fixed Oil Content

Fixed oil content was carried out using method described in The Quantitative Analysis of Drugs[26].

The powdered drug (5 g) was accurately weighed, placed in an extraction thimble and extracted with light petroleum (b.p. 40°C to

60°C) in a soxhlet apparatus for 3 to 4 hours. The thimble was removed and dried. The contents were ground finely in a mortar, returned into the thimble and continued the extraction for another hour. After evaporation of solvent, the oil was dried to constant weight at 100°C. The percentage of the fixed oil was calculated on the water free basis.

F. Determination of Total Saponins Content by Modified Method

The analytical conditions of the determination procedure for total saponins content in crude drug was modified by the following principle of Takemoto's method [27].

The fine powdered (180 μm) 0.5 g of *G. pentaphyllum* was accurately weighed and placed in a 250-ml round bottom flask, then refluxed with 50 ml of water for 2 hours and filtered. The marc was washed with a proper volume of hot water. The washing and the filtrate were combined, transferred to 100-ml volumetric flask and adjusted to volume. Twenty-ml portion of this solution was transferred to a separatory funnel and extracted with three 10-ml portions of butanol. The butanol extracts were combined and washed with two 10-ml portions of water. After evaporating to dryness, the residue was dissolved in water and made up to volume of 10.0 ml. Five-ml portion of the resulting solution was applied to Sep-Pak C-18, then washed with 10 ml of water, 5 ml of 50% methanol, 2 ml of 60% methanol, and eluted with 2 ml of absolute methanol, respectively. The eluate was evaporated to dryness and dried to constant weight at 105°C. The total saponins content was calculated on the water free basis.

Results

I. Chemical Identification

The chemical identification of 11 samples of *G. pentaphyllum* collected from heterogenous cultivated areas was performed compared to both samples obtained from different wild areas by preliminary test and Thin-layer chromatographic analysis. The results are shown in Table 1, Figure 2 and Table 2, respectively.

Table 1 Chemical identification of aerial parts of *G. pentaphyllum* (Thunb.) Makino.

Sources	Preliminary test			Confirmatory test
	Saturated antimony trichloride/chloroform	conc. Sulfuric acid	Froth test	
Cultivated areas	All samples gave violet colour	All samples gave red colour	All samples produced persisting foam for over 30 min	The samples showed 4-14 unidentified saponins
Wild areas (Both samples)	violet colour	red colour	produced persisting foam for over 30 min	showed 9 unidentified saponins

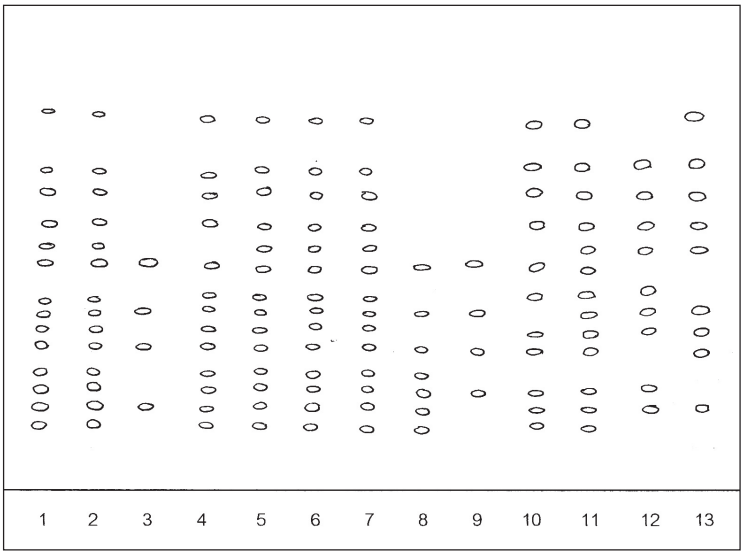


Figure 2 TLC chromatograms of the water extracts of aerial part of *G.pentaphyllum* (Thunb.) Makino
Developing solvent : Chloroform:methanol:water (65:35:10 lower phase)
Remarks : 1-11 = samples of *G.pentaphyllum* collected from cultivated areas
12-13 = samples of *G.pentaphyllum* collected from wild areas
O = detected after spraying with 20% sulfuric acid and activating at 105°C for 5 min gave violet colour.

Table 2 hRf Values of components in water extract of aerial parts of *G. pentaphyllum* (Thunb.) Makino.

Spot	hRf Value	Detection with 20% sulfuric acid
1	11-12	Violet
2	14-15	Violet
3	17-18	Deep violet
4	20-21	Deep violet
5	25-26	Violet
6	28-29	Violet
7	31-32	Violet
8	34-35	Violet
9	39-41	Deep violet
10	43-44	Violet
11	47-48	Violet
12	53-54	Violet
13	58-59	Violet
14	67-69	Violet

II. Quality Evaluation

To estimate the value of *G. pentaphyllum* crude drugs, quality evaluation were performed as follows : determination of ash, extractives, moisture content, fixed oil content, foaming index, and total saponins content. The results are shown in Table 3, Figure 3, 4, 5, 6, 7, 8, 9, and 10. The quality evaluation of *G. pentaphyllum* collected from cultivated areas compared to the samples from wild areas is demonstrated in Table 4.

Table 3 Quality evaluation of aerial parts of *G. pentaphyllum* (Thunb.) Makino.

Quality evaluation	Range (%) (n=13)	$\bar{X}$ ± S.D. (n=13)
Total ash content	7.50 - 17.96	11.21 ± 2.27
Acid-insoluble ash content	0.06 - 1.86	0.85 ± 0.59
Ethanol-soluble extractive	6.96 - 12.11	10.10 ± 1.00
Water-soluble extractive	21.24 - 30.26	24.13 ± 2.57
Moisture content	4.90 - 8.83	7.10 ± 1.38
Fixed oil content	1.40 - 3.78	1.94 ± 0.28
Foaming index	166 - 2000	735 ± 763
Total saponins content	1.30 - 6.59	4.66 ± 1.31

Table 4 Comparison of quality evaluation of aerial parts of *G. pentaphyllum.* (Thunb.) Makino. collected from cultivated areas and wild areas.

Quality evaluation	Sources	
	Cultivated areas range in % (n=11)	Wild areas range in % (n=2)
Total ash content	10.15 - 17.96	7.50 - 8.51
Acid-insoluble ash content	0.33 - 1.86	0.06 - 0.08
Ethanol-soluble extractive	6.96 - 12.11	8.32 - 9.81
Water-soluble extractive	21.24 - 30.26	23.78 - 25.78
Moisture content	4.90 - 8.83	4.90 - 8.82
Fixed oil content	1.79 - 3.78	1.40 - 1.96
Foaming index	166 - 2000	2000
Total saponins content	1.30 - 6.59	3.16 - 6.08

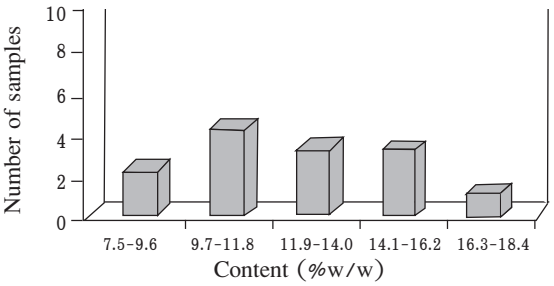


Figure 3 Total ash content in aerial parts of *G.pentaphyllum*

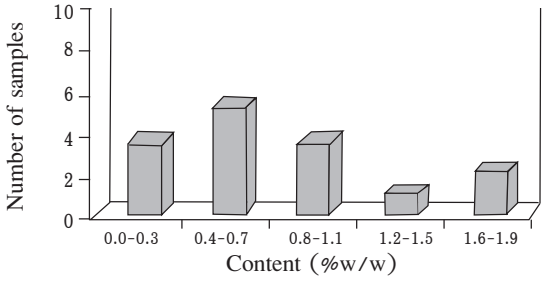


Figure 4 Acid insoluble ash content in aerial parts of *G.pentaphyllum*

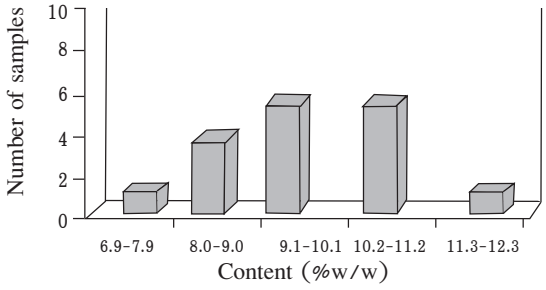


Figure 5 Ethanol soluble extractive content in aerial parts of *G. pentaphyllum*

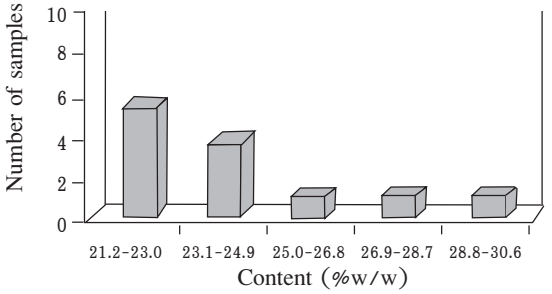


Figure 6 Water soluble extractive content in aerial parts of *G. pentaphyllum*

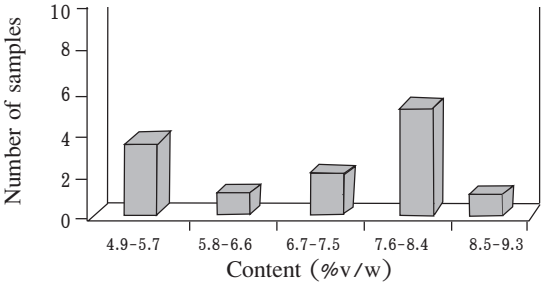


Figure 7 Moisture content in aerial parts of *G. pentaphyllum*

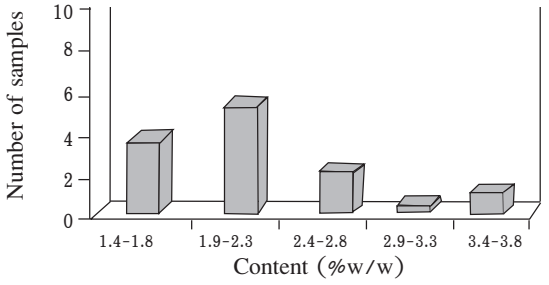


Figure 8 Fixed oil content in aerial parts of *G. pentaphyllum*

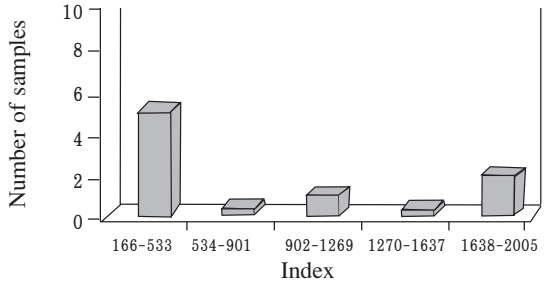


Figure 9 Foaming index of the aerial parts of *G. pentaphyllum*

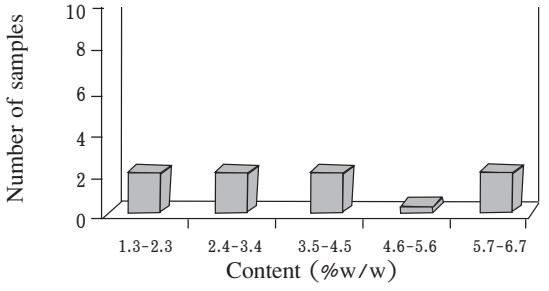


Figure 10 Total saponins content in aerial parts of *G. pentaphyllum*

Discussion

The purpose of drug quality control and evaluation is to assess the value of raw materials and to assure the phytopharmaceutical products to the standard requirement. Since there is no report of *G. pentaphyllum* distribution in Thailand and it is found both wild and cultivated areas in Chiang Mai province, all samples of *G. pentaphyllum* in this investigation is collected from Chiang Mai province. In the current study, the chemical identification of various samples of *G. pentaphyllum* collected from cultivated areas was performed comparing to the samples from wild areas by preliminary test and TLC fingerprint. Since the active compounds were dammarane-type saponins, the preliminary test was emphasized on detection of the total saponins by colour reaction with saturated solution of antimony trichloride and conc. sulfuric acid, and by froth test. TLC fingerprint was also particularly valuable for the qualitative determination. At double concentration, the TLC chromatograms of sample number 3, 8 and 9 showed the same pattern as the others from cultivated areas. The TLC chromatogram illustrated that the pattern of the contents in *G. pentaphyllum* obtained from cultivated areas and wild areas were quite similar.

According to the Quality Control Methods for Medicinal Plant Materials[21], the determination of ash content, total ash and acid insoluble ash, are intended to measure the amount of residual substance remaining after ignition of the crude drugs. The values of total ash and acid insoluble ash content are varied from 7.50 - 17.96 and 0.06 - 1.86%, respectively. The rise of these values is dued to the presence of the high minerals content in *G. pentaphyllum*[18].

To attain the efficient phytotherapy, the determination of active constituents plays the most important part of quality control. Previous study in China reported that the total saponins content in the leaves and stems of *G. pentaphyllum* were 6.65% and 4.05%, respectively[28] while the results obtained from this investigation indicated that the total saponins content varied from 1.30% to 6.59%. There were 4 samples, in comparison with the other samples and the previous report of China, having very low content; 1.30, 1.90, 2.29, and 2.77%. Therefore, these samples may not be counted for the specification. Besides, most of the variations of active content and ash content occurred in the cultivated samples. These are probably the result from many factors such as varieties, climate, physical features of the growing area, irrigation, fertilizer and harvesting time, etc. So further study should be carried on to evaluate the factor affected the saponins content.

Conclusion

From the results of the study, the appropriate chemical specification of aerial parts of *Gynostemma pentaphyllum* (Thunb.) Makino are proposed as follows :

Total ash content not more than 14.0% w/w

Acid-insoluble ash content not more than 2.0% w/w

Moisture content not more than 8.0% v/w

Ethanol-soluble extractive not less than 9.0% w/w

Water-soluble extractive not less than 21.0% w/w

Fixed oil content not less than 1.0% w/w

Foaming index not less than 242

Total saponins content not less than 4.0% w/w

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บทคัดย่อ สมุนไพรปัญจขันธ์มีชื่อวิทยาศาสตร์ว่า *Gynostemma pentaphyllum* (Thunb.) Makino วงศ์ Cucurbitaceae สมุนไพรชนิดนี้มีศักยภาพที่จะนำมาพัฒนาเป็นผลิตภัณฑ์สมุนไพร สารออกฤทธิ์เป็นสารประเภท dammarane-type saponins ชื่อ gypenosides ซึ่งมีฤทธิ์ยับยั้งการเจริญเติบโตของเนื้องอก ลดไขมันในเลือด และฤทธิ์ต้านอักเสบ ฯลฯ จึงได้ศึกษาคุณภาพของสมุนไพรปัญจขันธ์ ซึ่งเก็บจากแหล่งธรรมชาติและแหล่งปลูกต่าง ๆ จำนวน 13 ตัวอย่าง โดยจัดทำข้อกำหนดเอกลักษณ์ทางเคมี เพื่อตรวจหาสารประเภทซาโปนินซึ่งเป็นสารออกฤทธิ์ ด้วยปฏิกิริยาการเกิดสี การเกิดฟอง และรงคเลขผิวบาง (Thin layer chromatography) นอกจากนี้ ยังได้พัฒนาวิธีวิเคราะห์ปริมาณสารออกฤทธิ์ และจัดทำข้อกำหนดทางเคมีของส่วนเหนือดินของสมุนไพรชนิดนี้ด้วย