

Comarison of Major Constituents in *Acanthopanax* Taxa and Variety Cheongsong in Korea by GC-MS

Kyung Soon Cho, Pyung Tae Ku and Man Kyu Huh^{1*}

Busan Institute of Health & Environment, Busan 613-806, Korea,

¹Department of Molecular Biology, Donggeui University, Busan 614-714, Korea

Received April 15, 2007 / Accepted May 14, 2007

Species of genus *Acanthopanax* (Araliaceae) are long-lived trees primarily distributed throughout East Asia. These species are regarded as medically and ecologically important in Korea. A variety of Cheongsong in Korea is one of these cultivated varieties, however this variety is much longer (>100 years) than those of other cultivated groups. The components of variety of *Acanthopanax* in Cheongsong were analyzed for the first time and were compared to those of all *Acanthopanax* taxa in Korea. Nineteen components were specific to variety in Cheongsong. The main components of this variety were β -caryophyllene, hexadecanoic acid and ethyl stearate. Although some components are differ from each other, variety Cheongsong was similar to *A. senticosus* at phenetic topology with content of the chemicals; In addition, six species of genus *Acanthopanax* were investigated to compare the major chemical components by GC-MS.

Key words – Variety of *Acanthopanax* in Cheongsong, components, GC-MS

Introduction

Some species belonging to the genus *Acanthopanax*, currently used as a Chinese herbal medicine and very significant in pharmacology, particular *Acanthopanax senticosus* (Araliaceae). *A. senticosus* is sold in the United States as "Siberian Ginseng". It is known in China as ci wu Jia [9]. It is also more expensive medicine materials than other *Acanthopanax* species [7]. Siberian Ginseng has used for bronchitis, heart ailments, and rheumatism, and as a tonic to restore vigor, increase stamina [1,4,5].

A great number of studies refer to the biologically active components isolated from eleutheroside A-M, acanthoic acid, lignan, saponin, chiisanoside, syringoside, diterpene compounds, daucosterol, polyacetylenes, ziliodendrin, β -sistosterol, campesterol, ethoxy-hydroxy-benzoic acid, and flavonoids [7,10,12,14]. The essential components are not used independently in medicine. This is probably the reason for the smaller number of studies on the composition of the essential components of species belonging to this genus.

The genus *Acanthopanax* is comprised of about 5-7 species in Korea [11]. *A. senticosus* and *A. sessiliflorus* have wide range of geographic distribution in the Northern

Hemisphere including East Asia, whereas, *A. seoulense*, *A. koreanum*, *A. chiisanensis* and *A. rufinerve* were native to Korea [11]. The taxonomy of *Acanthopanax* has processed mainly through morphological characteristics. However morphological characteristics are restricted to their resolving power mainly because of the small number of variables available [8]. Especially, *Acanthopanax* variant is an endemic to Cheongsong province in Korea. One of the most striking features between both taxa was spine (or thorn) which is sharp and stiff outgrowth of a stem. Although The plants of *Acanthopanax* are covered with many spines, this variant "Cheongsong" (therefore variant Cheongsong) has few or rare spines in stems and branches. Although plants grow high in the mountains on fertile soil, recently (< 20 years) they are also extensively cultivated as medicine crops. Variety Cheongsong in Korea is one of these cultivated varieties, however this variety is much longer (>100 years) than those of other cultivated species.

The aim of this paper was to determine the compositions of the components of variety Cheongsong for the first time and to compare it with the composition of *Acanthopanax* taxa from the near locations.

Materials and Methods

Plant materials

Leaves were collected from natural (wild) populations of

*Corresponding author

Tel : +82-51-890-1529, Fax : +82-51-890-1521

E-mail : mkhuh@deu.ac.kr

six species of *Acanthopanax* in Korea (*Acanthopanax sessiliflorus*, *A. senticosus*, *A. koreanum*, *A. chiisanensis*, *A. seoulense*, and *A. rufinerve*) and one variety Cheongsong. In addition, the species of same genus, Chinese *A. siebolianus* was provided for the outgroup and used to compare the phylogenetic relationship.

Extraction

Thirty mature trees (≥ 5 yr) were randomly collected from each species. 50 g of *Acanthopanax* leaves were added to 50 ml distilled water and homogenized in homogenizer with 3,000 rpm for 2 minutes. Samples were treated in an ultrasonic bath for 30 min. Samples were dried and ground hydro-distilled for 2.5 h using a Clevenger-type apparatus. The components were extracted from the distillate with ether, and then dried with anhydrous Na_2SO_4 . The solvent was removed by distillation at atmosphere pressure, and the pure oil was kept at 4°C until analysis.

Analysis by GC-MS

A gas chromatography was equipped with a fused silica capillary column (30 m x 0.25 mm), with a 0.25 μm film thickness of PTE-5 and FID was used for GC measurements [13]. The injection volume was 5 ml. The operating conditions were as follows: temperature programme was 260°C at 4.3 min for injector. Detector temperature was 280°C and chromatographic elution was carried out at a flow rate 1.0 ml/min using the carrier gas (He). GC-MS analyses were performed on a Hewlett Packard (model 6860GC/5972MSD) equipped with fused silica HP-5MS capillary column (30 m x 0.25 mm) of film thickness 0.25 μm . Constituents were identified by comparison of their mass spectra to those from MS libraries (Adams89, Nist92, and Amdis32) and the results obtained were correlated with calculated and Adam's retention indices. Area percent was obtained electronically from the GC-FID response without the use of an internal standard or correction factors.

Phenetic analysis

Phenetic relationships among taxa of genus *Acanthopanax* were used to construct a dendrogram, using UPGMA (unweighted pair group method with arithmetic average) in the neighbor algorithm of the Phylogeny Inference Package (PHYLIP ver. 3.57)[3].

Results and Discussion

The mean content of the aerial dried parts was 0.42% across all species, varying from 0.35% for *A. rufinerve* with the lowest content and 0.51% for *A. senticosus* with the highest content.

Results of GC and GC-MS are shown in Figures 1, 2, 3, and 4. One hundred and thirty-three components of six all *Acanthopanax* species and one variety Cheongsong were identified. There was, however, significant product-to-product variability in the amount of many components present. For example, eleutheroside concentrations varied 5-fold (0.124-0.624 by g/L) in the liquid extracts.

Our main focus is to determine specific-components for variety Cheongsong. Out of the 97 components, nineteen are specific for variety Cheongsong and do not show at other *Acanthopanax* taxa (Fig. 4). The components with the highest quality in this variety were β -caryophyllene, hexadecanoic acid and ethyl stearate (Table 1). It was not registered in the previously examined all *Acanthopanax* taxa. Interesting, β -caryophyllene was also the main components in the previously examined oil of *Acanthopanax*. We have found 19 components in a very high level in our variety Cheongsong (>72% quality).

Further investigations of the composition and biological activity of 19 components of variety Cheongsong, along with more data about different species of the *Acanthopanax*, could be helpful in chemotaxonomy and might possibly be considered for eventual medical use.

Although the species are very distant geographically isolated, the content of the *A. senticosus* was close to content of the leaf extractions of *A. siebolianus* from China (Figs. 1-4). The cluster analysis was performed with UPGMA phenogram among *Acanthopanax* taxa and variant Cheongsong based on content of the chemicals (Fig. 5). The phenetic topology with content of the chemicals, *A. seoulense* was similar to *A. sessiliflorus*, while *A. rufinerve* was more distinct. *A. seoulense*, *A. sessiliflorus*, *A. koreanum*, and *A. chiisanensis* clade, *A. senticosus* clade, and *A. rufinerve* clade were supported with bootstrap values of 92%, 78%, and 66%, respectively. At phenetic topology with content of the chemicals, variety Cheongsong was similar to *A. senticosus*, although some components are differ from each other.

With many countries contemplating the regulation and standardization of herbal products, reliable and accurate

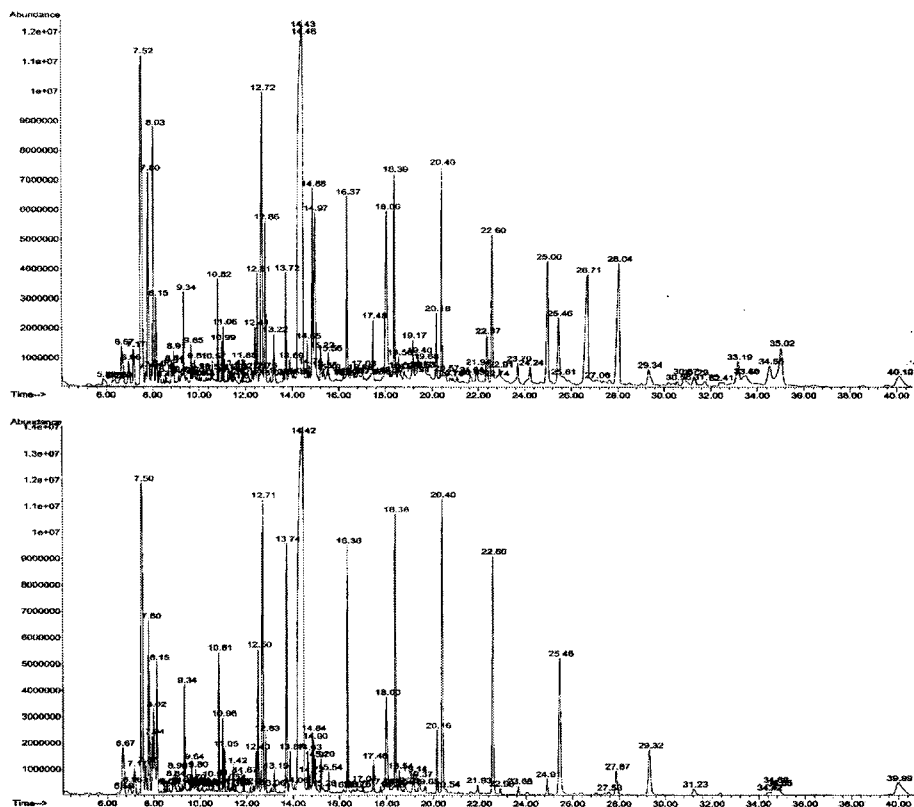


Fig. 1. GC/MS profiles of the extracts from *Acanthopanax senticosus* (upper) and *Acanthopanax sessiliflorus* (lower).

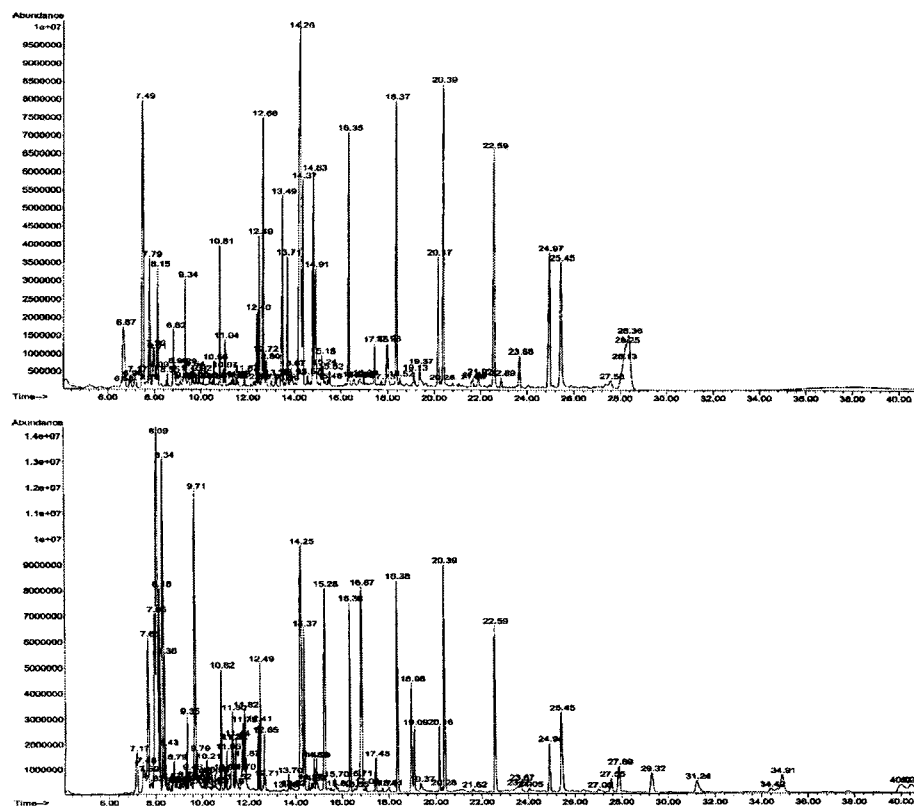


Fig. 2. GC/MS profiles of the extracts from *Acanthopanax koreanum* (upper) and *Acanthopanax seoulense* (lower).

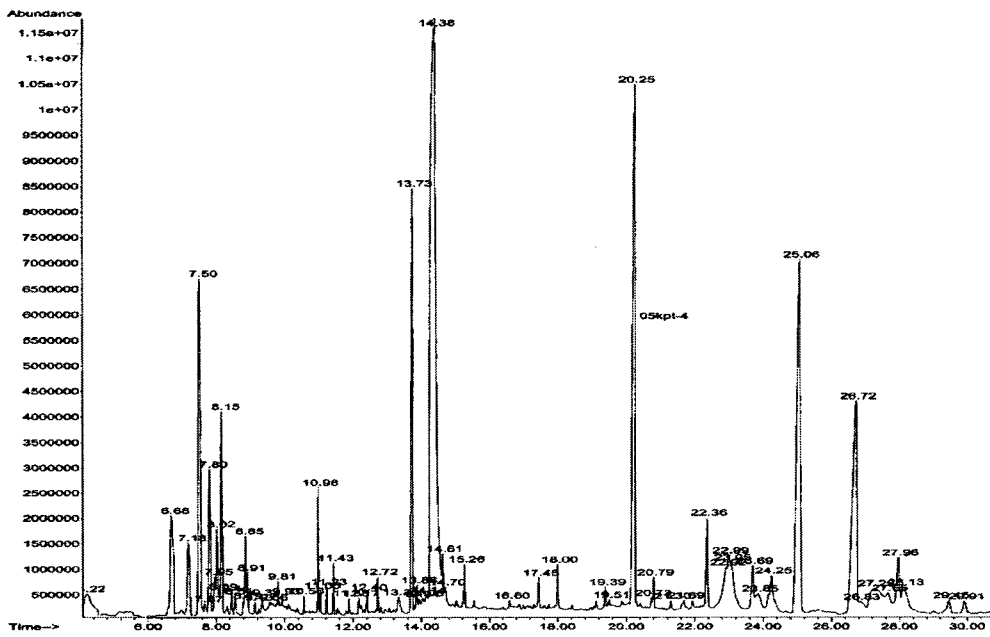
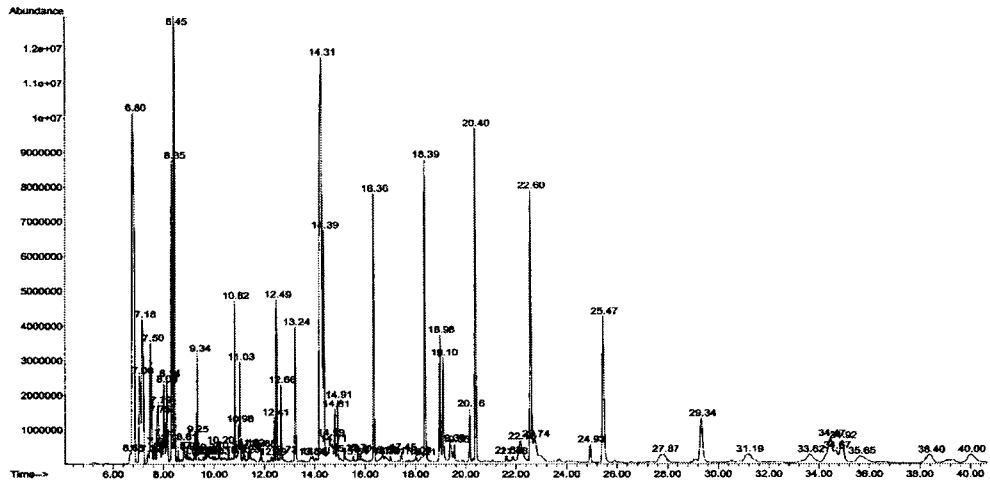


Fig. 3. GC/MS profiles of the extracts from *Acanthopanax chiisanensis* (upper) and *Acanthopanax rufinerve* (lower).

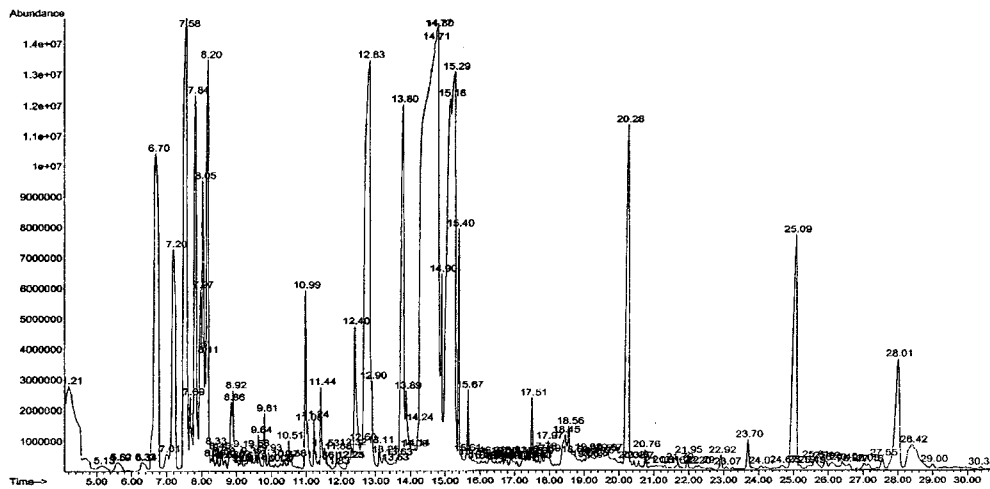
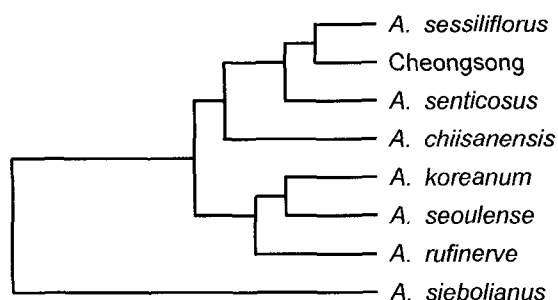


Fig. 4. GC/MS profile of the extracts from *Acanthopanax senticosus* variety (Cheongsong).

Table 1. The isolated compounds from variety Cheongsong

RT	Compound name	CAS. No	Quality(%)
6.70	γ -Elemene	003242-08-8	91
7.58	β -Caryophyllene	000087-44-5	99
7.84	α -Humulene	006753-98-6	98
8.05	Germacrene	023986-74-5	98
8.20	Bicyclogermacrene	024703-35-3	98
9.81	Hexadecanal	000629-80-1	95
12.41	Ethyl linoleolate	000544-35-4	87
12.83	Hexadecanoic acid, ethyl ester	000628-97-7	99
13.80	Kaurene	000562-28-7	96
14.71	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	000150-86-7	97
15.16	9,12,15-Octadecatrienoic acid, ethyl ester	001191-41-9	89
15.40	Ethyl stearate	000111-61-5	99
17.97	Octadecanoic acid, ethyl ester	000111-61-5	86
20.28	Bis(2-ethylhexyl)phthate	000117-81-7	91
20.76	Pentadecanoic acid, ethyl ester	041114-00-5	93
23.69	13-(fur-3-yl)-1-(4'-methyl-3'-hydroxy-2',5'-dihydro-5'-oxo-furan-2'-yl)-2,6,10-trimethyltrideca-6,8-diene	113994-72-2	72
25.08	2,6,10,15,19,23-hexamethyl	000111-02-4	96
28.01	(E,E)-farnesylacetone	001117-52-8	80
28.42	4-(4,5-Dihydro-5-oxo-3-methyloxazole-4-ylidenehydrazino)-3-methyl-N,N-diethylaniline	078023-72-0	83

Fig. 5. A phenogram showing the relationships among eight taxa of *Acanthopanax*, based on chemical analysis.

methods for analysis and quantification are required [2]. MS detection was only completed to evaluate assay specificity. Assay validation (accuracy and reproducibility and quantitative analysis) was not completed with MS detection. LC-fluorescence was also more specific than LC-UV at either wavelength and may actually be as specific direct probe MS-MS. In the present study, the major constituents mainly caryophyllene, hexadecanoic acid, and ethyl stearate of genus *Acanthopanax* in Korea were identified by GC-MS.

References

- Cai, X. F., I. S. Lee, N. T. Dat, G. Shen and Y. H. Kim. 2004. Diterpenoid with inhibitory activity against NFAT transcription factor from *Acanthopanax koreanum*. *Phytother. Res.* **18**, 677-680.
- Draves, A. H. and S. E. Walker. 2000. Determination of hypericin and pseudohypericin in pharmaceutical preparations by liquid chromatography with fluorescence detection. *J. Chromatography* **747**, 57-66.
- Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5s, Distributed by the author. Department of Genetics, Univ. of Washington, Seattle, USA.
- Foster, S. 1996. Siberian Ginseng-*Eleutherococcus senticosus*. Botanical Booklet Series, No 302. Austin: American Botanical Council.
- Glatthaar-Saalmuller, B., F. Sacher and A. Esperster. 2001. Antiviral activity of an extract derived from roots of *Eleutherococcus senticosus*. *Antiviral Research* **50**, 223-228.
- Gudzin, B., S. Dordevic, R. Palic and G. Stojanovic. 2001. Essential oils of *Hypericum olympicum* L. and *Hypericum perforatum* L. *Flavour. Frangr. J.* **16**, 201-203.
- Harkey, M. R., G. L. Herderson, M. E. Gershwin, J. S. Stern and R. M. Hackman. 2001. Variability in commercial ginseng products: an analysis of 25 preparations. *Am. J. Clin. Nur.* **73**, 1101-1106.
- Huh, M. K. and H. W. Huh. 2005. Classification of genus *Acanthopanax* in Korea and genetic diversity using allozymes. *Silva Genetica* **54**, 206-210.
- Jiao, S. D. 2003. Ten Lectures of the Use of Medicinals. pp. 711. Paradigm Publications, MA, USA.
- Kim, Y. H., B. S. Chung and U. Sankawa. 1988. Pimaradine

diterpenes from *Acanthopanax koreanum*. *J. Nat. Prod.* **51**, 1080-1083.

11. Lee, Y. N. 1997. Flora of Korea. Kyo-Hak Publishing Co, Seoul, Korea.

12. Park, E. J., Y. Z. Zhao, Y. H. Kim, J. J. Lee and D. H. Sohn. 2004. Acanthoic acid from *Acanthopanax koreanum* protects against liver injury induced by tert-butyl hydroperoxide or carbon tetrachloride in vitro. *Planta Med.* **70**, 321-327.

13. Petersen, I. L., K. E. Andersen, J. C. Sørensen and H. Sørensen. 2006. Determination of shikimate in crude plant extracts by micellar electrokinetic capillary chromatography. *J. Chromatography* **1130**, 253-258.

14. Takeshi, D., N. Sansei and N. Yoshisa. 2001. Constituents and pharmacological effects of *Eucommia* and Siberian ginseng. *Acta Pharmacol. Sin.* **22**, 1057-1070.

초록 : GC-MS에 의한 오갈피나무 분류군과 청송 변종의 주요 성분 비교

조경순 · 구평태 · 허만규^{1*}

(부산광역시 보건환경연구원, ¹동의대학교 분자생물학과)

오갈피나무속 식물종은 주로 동아시아에 분포하는 다년생 목본류이다. 이 속의 식물 종은 한국에서 약 6종으로 알려져 있으며 일부 종은 약용으로 사용되어 경제적으로 매우 중요하다. 청송변종은 청송에서 100년 이상 재배되고 있는데 그 기원이 불분명하여 주요 생화학적 성분을 GC-MS로 분석하여 다른 종과 비교하였다. 분석한 화학성분 중 19개가 청송변종에 특이하였는데 β -caryophyllene, hexadecanoic acid, 그리고 ethyl stearate 등이다. 청송변종은 오갈피나무와 비록 일부 성분에 있어 차이가 나지만 전체적인 분류학적 위상에서 다른 종에 비해 가장 유사하여 오갈피나무에서 분지한 것으로 판단된다. 또 오갈피나무속 6종에 대한 성분 차이가 GC-MS로 잘 구분되어 화학성분에 의해 종간 차이가 있음이 밝혀졌다.