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# 吴茱萸五加不同部位挥发性成分及其抗炎活性和细胞毒活性研究

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**摘　要**:以水蒸气蒸馏法分别提取吴茱萸五加叶和茎皮的挥发性成分,并采用气相色谱-质谱联用技术(GC-MS)对其进行成分分析。结果从叶中鉴定出 28 种成分,占叶总挥发性成分的 97.31%;从茎皮中鉴定出 36 种成分,占茎皮总挥发性成分的 92.61%。以脂多糖(LPS)刺激 RAW 264.7 细胞,分别用叶和茎皮的挥发性成分进行干预,测定细胞存活率和一氧化氮(NO)的含量,结果表明在 0 ~ 20 μg/mL 浓度范围内两个部位的挥发性成分均表现出轻微的细胞毒性,但叶挥发性成分表现出了显著的一氧化氮抑制活性。本研究为开发利用吴茱萸五加这一丰富的民间药物资源并从中寻找新的天然抗炎物质提供科学基础。

**关键词**:吴茱萸五加;挥发性成分;抗炎;一氧化氮;细胞毒活性;气相色谱-质谱联用技术

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# Chemical Composition, Anti-inflammatory and Cytotoxic Activities of Essential Oils from Different Parts of *Acanthopanax evodiaefolius*

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**Abstract**: The essential oils from leaves and stem barks of *Acanthopanax evodiaefolius* Franch were extracted by hydrodistillation, and their chemical composition were analyzed by GC-MS. Twenty-eight compounds were identified in leaves, representing 97.31% of the total oil of the leaves, while 36 compounds were identified in stem barks, representing 92.61% of the total oil of stem barks. Their cytotoxic activity and inhibitory effect on nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells were tested. The essential oils from leaves and stem barks were shown slightly cytotoxic activity while the oil from the leaves showed significant NO inhibition activity. This result provided scientific basis for screening new natural anti-inflammatory substances and better utilization of *A. evodiaefolius*.

**Key words**: *Acanthopanax evodiaefolius*; essential oils; anti-inflammatory; nitric oxide; cytotoxic activity; GC-MS

## Introduction

Natural essential oils constituents, including monoterpenes, sesquiterpenes, oxygenated monoterpenes, oxygenated sesquiterpenes, phenolics, have been shown to possess anti-microbial, anti-fungal, anti-bacterial and anti-inflammatory properties. Recently more and more attention has been focused on inflammatory treatment of essential oils [1-3].

Inflammation is a complex response of host defense against microbial infection, endotoxin exposure, or cell injury, and ultimately leads to the restoration of normal cell structure and function. Inflammation has been

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shown to be associated with a number of diseases, including rheumatoid arthritis, chronic bronchitis, chronic nephritis, inflammatory bowel disease, and meningitis which are common worldwide [4,5]. During inflammation, high levels of nitric oxide (NO) plays a key role in pathophysiology of the disease as a free radical [6,7]. NO is known to be responsible for the vasodilation and hypotension observed during septic shock and inflammation[8,9]. It is well known that overproduction of NO contributes to the development and courses of various inflammatory diseases. The view down-regulation of NO has been used to treat such diseases in clinical.

*Acanthopanax* species plants are richly distributed in Asia, growing about 26 kinds and 18 varieties in China, 11 kinds and 3 varieties in Korea, and 9 kinds in Japan. They have been used as medical plants for centuries. The root bark of *A. gracilistylus* W. W. smith (Araliaceae) has been used to relieve rheumatic conditions, tonify liver and kidney, and strengthen tendons and bones in China [10]. It has been listed in the Chinese pharmacopoeia as Cortex Acanthopanacis (Wujiapi) [11]. *A. sessiliflorus* (Rupr. et Maxim) Seem, growing widely in Korea, is traditionally used as anti-rheumatoid arthritis, anti-inflammatory and anti-diabetic drugs [12]. *A. sieboldianus*, a member of the Araliaceae family which is richly distributed in Japan, has antioxidant effects and suppresses the postprandial rise in the blood glucose levels [13]. *A. evodiaefolius* Franch (Araliaceae) is widely distributed in the south of the Yangtze River in China, its root has been traditionally used for the treatment of rheumatism, edema, cough, asthma as folk medicine. However, only one phytochemical investigation on the stem barks of *A. evodiaefolius* have been reported in the literature [14]. The chemical composition and biological activity from leaves of *A. evodiaefolius* have never been reported.

In the current study, the essential oil from leaves (EOL) and from stem barks (EOSB) of *A. evodiaefolius* extracted by hydro-distillation were tested for their inhibitory effect on LPS-induced NO production in RAW 264.7 mouse macrophages and their chemical composition were analyzed by GC-MS.

## Materials and Methods

### Plant materials and essential oils preparation

The plant materials were collected at Yongzhou, Hunan province in China, Oct 2012. It was identified by Prof. Changsoo Yook of College of Pharmacy in KyungHee University. The voucher specimen (20121003) of plant materials was kept in Herbarium of Hunan University of Chinese Medicine. The leaves and stem barks of *A. evodiaefolius* were air-dried and smashed into powder and then subjected to hydro-distillation for 8 hr using a Clevenger-type apparatus for the extraction of essential oil. The oil was stored in a refrigerator until analyzed.

### Gas chromatography-mass spectrometry analysis

GC-MS analysis involved an Shimadzu GC-MSQP2010 (Japan) and an Rtx-5ms column (i. d., 0.25 mm; length, 30 mm; film thickness, 0.25 μm). Oven temperature were maintained at 80 ℃ for 1 min initially and raised to 150 ℃ at a rate of 10 ℃/min, then sequentially raised to 260 ℃ where the temperature were finally held for 10 min. The MS conditions were: ionization voltage, 70 eV; scan rate, 1 scan/s; mass range, 35-550 M/Z; trap temperature, 150 ℃; transfer line temperature, 230 ℃. Operating conditions were: injector temperature, 260 ℃; carrier (He) flow rate, 1.0 mL/min.

### MTT assay for cell viability

RAW264.7 cells were cultured in 96-well plates for 4 hr, followed by treatment with the essential oils at different concentrations (10 μg/mL, 20 μg/mL). After 24 hr incubation, MTT were added to the medium for 4 hr. Finally, the supernatant was removed and the formazan crystals were dissolved in dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm.

### Nitric oxide (NO) assay

The RAW 264.7 cells were dispensed to 96 well plate and cultured for 4 hr. Then the essential oils were adhered and incubate for 1 hr. After pre-incubation of RAW 264.7 cells ($5 \times 10^4$ cells/mL) with LPS (0.5 μg/mL) for 24 hr, the quantity of nitrite in the culture medium were measured as an indicator of NO production. Amounts of nitrite, a stable metabolite of NO, were measured using Griess reagent. Briefly, 100 μL of cell

culture medium were mixed with 100 μL of Griess reagent. Subsequently, the mixture were incubated at room temperature for 10 min and the absorbance were measured at 540 nm in a microplate reader. The quantity of nitrite were determined from a sodium nitrite standard curve.

### Statistical analysis

All values are expressed as the mean ± SEM. Differences between mean values of normally distributed data were assessed with one-way ANOVA (Newman Keuls t-test). Statistical significance was accepted at $P < 0.05$.

## Results and Discussion

### Chemical composition of the essential oils

Hydro-distillation of the leaves of *A. evodiaefolius* gave pale yellow oil, with a strong pleasant aromatic odor, while hydro-distillation of the stem barks gave brown oil. The ingredient of the oil of *A. evodiaefolius* was analyzed by GC-MS. Chemical compositions were separated by programmed temperature gas chromatography, and then individual MS fragments were analyzed. The percentage compositions of the extract were computed by normalization to the GC peak areas. Identification of constituents was based on the MS fragmentation pattern with reference compositions in the database of the NIST 08 Mass Spectral Search Program. The GC-MS total icon chromatogram of EOL and EOSB were showed in Fig. 1.

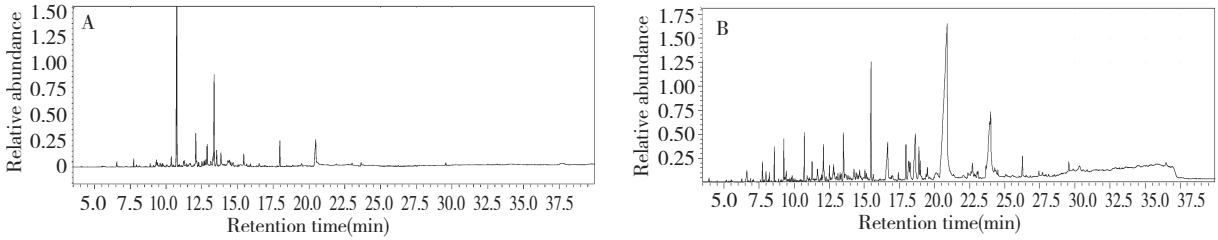


**Fig. 1 GC-MS total icon chromatograms of EOL (A) and EOSB (B)**

Twenty-eight compounds were identified, representing 97.31% of the total oil of the leaves, while 36 compounds were identified, representing 92.61% of the total oil of the stem barks. The identified compounds were listed in Table 1.

**Table 1 Chemical composition of the essential oil identified by GC-MS**

| No. | Component | leaves(%)[a] | Stem barks(%)[a] |
|---|---|---|---|
| 1 | Hexanoic acid | – | 0.12 |
| 2 | n-Octaldehyde | 0.08 | 0.03 |
| 3 | D-Limonene | – | 0.04 |
| 4 | n-Nonaldehyde | – | 0.04 |
| 5 | (E)-2-Nonenal | – | 0.10 |
| 6 | Octanoic Acid | 1.08 | 0.74 |
| 7 | n-Decaldehyde | – | 0.11 |
| 8 | (E)-2-Decenol | 1.05 | 0.61 |
| 9 | n-Nonanoic acid | 0.38 | 0.59 |
| 10 | (E,E)-2,4-Decadiena | – | 0.28 |
| 11 | Ethanone | 0.54 | – |
| 12 | alpha. -Cubebene | 0.19 | – |
| 13 | 2-Undecenal | – | 1.33 |
| 14 | trans-2-Decenoic acid | 1.13 | – |

| | | | |
|---|---|---|---|
| 15 | n-Decanoic acid | 0.33 | – |
| 16 | Copaene | 0.52 | – |
| 17 | Cyclobuta[1,2:3,4]dicyclopentene, decahydro-3a-methyl-6-methylene-1-(1-methylethyl)-, [1S-(1. alpha. 3a. alpha. ,3b. beta. ,6a. beta. ,6b. alpha. )] | 0.41 | – |
| 18 | Caryophyllene | 1.87 | – |
| 19 | 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a. alpha. ,3b. beta. ,4. beta. ,alpha. ,7aS *)]- | 0.28 | – |
| 20 | (Z)-. beta. -Farnesene | 34.01 | 1.97 |
| 21 | (E,E,E)-2,6,6,9-tetramethyl-1,4,8-cycloundecatriene | 0.26 | – |
| 22 | 1-Isopropyl-4-methyl-7-— methylene-1,2,3,4,4a,5,6,7-octahydronaphthalene | 0.45 | – |
| 23 | α-curcumene | 0.28 | – |
| 24 | β-selinene | 0.25 | – |
| 25 | Tetradecanal | – | 0.62 |
| 26 | Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)- | 5.06 | 1.75 |
| 27 | Naphthalene,1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-,(1S-cis)- | – | 0.49 |
| 28 | Naphthalene,1,2,3,4,4a,7-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)- | 0.86 | – |
| 29 | alpha. -Calacorene | 1.11 | 0.71 |
| 30 | Benzene,1-(2-butenyl)-2,3-dimethyl- | 0.82 | – |
| 31 | 8,11,14-Eicosatrienoic acid,(Z,Z,Z)- | 1.11 | – |
| 32 | Dodecanoic acid | – | 1.00 |
| 33 | Furan,3-(4,8-dimethyl-nonadienyl)-,(E)- | 3.74 | – |
| 34 | Caryophyllene oxide | 19.37 | 0.86 |
| 35 | Tetradecanal | 2.28 | 2.53 |
| 36 | 2-(1,4,4-Trimethyl-cyclohex-2-enyl)-ethanol | 3.82 | – |
| 37 | 1. beta. -Cadin-4-en-10-ol | – | 0.72 |
| 38 | 3-Eicosene,(E)- | – | 0.50 |
| 39 | Tetradecanoic acid | – | 4.14 |
| 40 | 2-Pentadecanone,6,10,14-trimethyl | 5.44 | 1.73 |
| 41 | 6-Octadecenoic acid,(Z)- | – | 1.67 |
| 42 | Cyclopentadecanone,2-hydroxy- | – | 1.07 |
| 43 | Pentadecanoic acid | – | 5.12 |
| 44 | 9,17-Octadecadienal,(Z)- | – | 1.70 |
| 45 | cis-9-Hexadecenal | – | 0.67 |
| 46 | n-Hexadecanoic acid methyl ester | – | 0.64 |
| 47 | n-Hexadecanoic acid | 10.44 | 43.62 |
| 48 | n-Heneicosane | – | 0.62 |
| 49 | 2(3H)-Furanone,dihydro-5-tetradecyl- | – | 0.67 |
| 50 | 1-Cyclopropyl-1-dodecanone | – | 0.75 |
| 51 | 9,12-Octadecadienoic acid (Z,Z)- | – | 9.22 |
| 52 | Oleic Acid | – | 4.63 |
| 53 | n-Eicosane | – | 0.44 |

%[a]: Percentage of the oil; "-", not detected.

It was obviously that there were marked difference between the constituent of EOL and EOSB from our stud-

y. The results showed the most abundant constituent detected in EOL was (Z)-beta-farnesene (34.01%) and other major components were caryophyllene oxide (19.37%), n-hexadecanoic acid (10.44%) and 2-pentadecanone, 6, 10, 14-trimethyl (5.44%). The most abundant constituent of EOSB was n-hexadecanoic acid (43.62%), and other major components were (Z,Z)-9,12-octadecadienoic acid (9.22%), pentadecanoic acid (5.12%) and oleic acid (4.63%). It can also be concluded that the content of sesquiterpenes was high in EOL, which comprised 63.53% of the total; While the content of fatty acid was high in EOSB, which comprised 75.44% of the total.

**Effect of essential oils on cell viability**

As shown in Fig. 2, there were slightly effects of essential oils on cell viability at the concentration lower than 20 μg/mL.

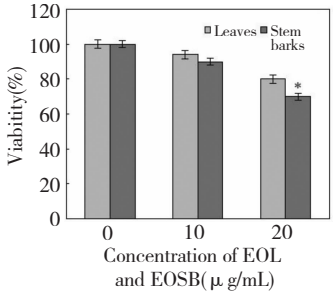


**Fig. 2 Cell viability of murine macrophage RAW 264.7 cells**

92 × 54 mm (300 × 300 DPI)

$n = 3$, * ($P < 0.05$), ** ($P < 0.01$) when compared to the control group by one-way ANOVA

**Effect of essential oils inhibition NO production in LPS-stimulated RAW 264.7 cells**

As shown in Fig. 3, the level of NO production induced by LPS in RAW cells decreased significantly when treated with different concentrations of the EOL. Pretreatment with 10 μg/mL EOL could inhibit NO production by 20.82%. The oil significantly inhibited NO production by 52.57% at 20 μg/mL, while the cell viability did not seem to be seriously affected. However, EOSB almost could not show the inhibitory activity at any concentrations we tested. The component differences of the oil may contribute to the significantly different inhibitory effects from different parts of the plant.

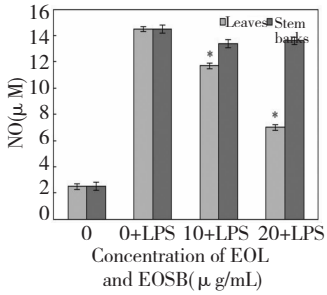


**Fig. 3 Inhibitory effect of essential oils on NO production in a culture medium of LPS-stimulated RAW 264.7 cells**

93 × 56 mm (300 × 300 DPI)

$n = 3$, * ($P < 0.05$), ** ($P < 0.01$) when compared to the control group by one-way ANOVA

In fact, many researchers had investigated the anti-inflammatory activities of individual chemical constituents of the essential oils. Sesquiterpenes, the main constituent of the oil of leaves, has been provided with certain activities in inflammatory by different mechanisms in last decade. Caryophyllene[15] were shown as a natural agonist of endogenous cannabinoid 2 receptors, which are expressed in immune cells and mediate anti-inflammatory effects. Alpha-iso-cubebene[16] significantly inhibited NF-κB transcription factor activation in TNF-α-stimulated human umbilical vein endothelial cells. Arcurcumene[17] could reduce lipopolysaccharide (LPS)-induced secretion of the pro-inflammatory chemokine interleukin 8 (IL-8) and RANTES (regulated upon activation, normal T-cell expressed and secreted). Other theories[18] on the anti-inflammatory effects of sesquiterpenes included activation of p53 and an increase in ROS as cytotoxic effects of sesquiterpenes. Hence, sesquiterpenes, main active compounds identified in EOL, may be related to the inhibitory activity; However, their action mechanism of anti-inflammatory need be tested in the future researches.

## Conclusion

In conclusion, our present findings indicated that EOL were abundant in sesquiterpenes while EOSB were abundant in fatty acid by GC-MS analyses. EOL can inhibit LPS-induced NO production in RAW 264.7 cells and EOSB almost was not observed NO inhibitory activ-

ity while both of them were shown slightly cytotoxic activity. The anti-inflammatory effect of EOL may be related to the sesquiterpenes compounds such as (Z)-beta-farnesene, caryophyllene, alpha-iso-cubebene, arcurcumene, *et al*, which were of high content in the leaves. Therefore, the leaves and stem barks should be used separately and EOL may be considered as a potential anti-inflammatory substances for in-depth study. Our study gives a rational support to the traditional use of *A. evodiaefolius* for treating inflammatory diseases as a folk medicine.

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