



## Determination of Antioxidant Compounds from Leaf and Stem of *Angelica keiskei* by Gas Chromatography-Mass Spectrometry

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In this paper, the antioxidant activities of leaf extract and stem extract from *Angelica keiskei* were investigated employing 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Leaf extract showed significant radical scavenging activity which was close to that of rutin, an excellent antioxidant compound while the radical scavenging activity of stem extract was a little weak. Subsequently, gas chromatography-mass spectrometry (GC-MS) was employed for determination of antioxidant compounds of the leaf extract and stem extract. Results indicated that 3,5-dihydroxy-6-methyl-2H-pyran-4(3H)-one was the main antioxidant compound in leaf extract and stem extract, fatty acid/ester and unsaturated fatty acid/ester are other kind of antioxidant compounds present in leaf extract.

**Keywords:** *Angelica keiskei*, Antioxidant, Free radical scavenging activity, Gas chromatography-mass spectrometry.

### INTRODUCTION

Free radical mediated reactions and reactive oxygen species are the major reasons in the pathological events such as aging, cancer, coronary heart ailment and Alzheimer's disease<sup>1</sup>. While the antioxidant defense mechanisms declined or exposure to environmental factors such as smoke, pollution, ultraviolet radiation, high-fat diet, *etc.* or under other pathological conditions, the levels of free radical and reactive oxygen species in living systems ascended, resulting in oxidative stress which can damage key organic substrates such as DNA, lipids and proteins, compromising cells physiological function thus led to pathological events<sup>2</sup>. So much attention has been focused on antioxidants which could protect the human body from oxidative damage by free radicals and reactive oxygen species. However, some synthetic antioxidants used in foods may accumulate in the body, resulting in liver damage and carcinogenesis<sup>3</sup>, therefore the development and utilization of the safety antioxidant without toxicity are desired. With proved excellent free radical scavenging activities<sup>4</sup>, direct plant extracts or compounds from them are regards as good candidates to search for new antioxidant rather than synthetic ones.

*Angelica keiskei* is a perennial plant belonging to umbelliferae and has been taken as a health-promoting vegetable grown originally in Japan. Putative beneficial effects of *Angelica keiskei* reported including the antitumor, anti-metastatic activities<sup>5</sup> and inhibition effects on hepatic lipid peroxide<sup>6</sup>. The most important compounds from *Angelica keiskei* is chalcones, which

reported to have inhibition of IL-6 production in TNF- $\alpha$ -stimulated MG-63 cell<sup>7</sup>, antidiabetic activities<sup>8</sup>, cancer chemopreventive effects<sup>9</sup>, superoxide-scavenging activity<sup>10</sup> and inhibition on influenza virus neuraminidase<sup>11</sup>. Moreover, there are some other kinds of bioactivities compounds isolated from *Angelica keiskei*, such as Ashitabaol A<sup>12</sup>, phytonutrients<sup>13</sup>. Recently, isobavachalcone, xanthoangelol and 4-hydroxyderricin were also isolated from the 95 % ethanol extract of *Angelica keiskei* which have notable NQO1 induction activity<sup>14</sup>. However, the trace of antioxidant compounds from leaf extract and stem extract of *Angelica keiskei* has been seldom reported to the best of our knowledge. In order to elucidate the roles of antioxidant compounds in health-promoting functions of *Angelica keiskei*, in present study, the antioxidant activities of leaf extract and stem extract of *Angelica keiskei* were evaluated employing 2,2'-diphenyl-1-picrylhydrazyl radical scavenging assay, then GC-MS of leaf extract and stem extract was processed to determined antioxidant activities compounds.

### EXPERIMENTAL

2,2'-Diphenyl-1-picrylhydrazyl was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals were purchased from China National Medicine Group Shanghai Corporation (Shanghai, China). All chemicals and solvents used were of analytical grade.

The following instruments were used: UV-1100 spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation, Beijing, China); RE-52AA rotavapour (Shanghai Yarong

Biochemistry Instrument Factory, Shanghai, China); DZF-1B vacuum drier (Shanghai Yuejin Medical Instrument CO., Ltd., Shanghai, China); SHB- $\beta$ A water-circulation multifunction vacuum pump (Zhengzhou Great Wall Scientific Industry and Trade CO., Ltd., Zhengzhou, China). Agilent 6890-5973N GC/MS (Agilent Technologies, Shanghai, China).

**Preparation of extracts:** *Angelica keiskei* was supplied by Guizhou biological characteristics technology company. The leaf and stem of *Angelica keiskei* were ground (max particle size 0.4 mm) after dried in room temperature, then extracted (100 g) with methyl alcohol (300 mL, 60 %) with ultrasonic assisted extraction (500 W, 100 MHz) at 40 °C for 40 min after soak for 12 h. Subsequently, extract solution was filtered with Whatman No. 1 filter paper and then evaporated using a RE-52AA rotavapour at 35 °C and a SHB- $\beta$ A water-circulation multifunction vacuum pump. Extracts were finally dried in a DZF-1B vacuum drier at 30 °C and 0.07 MPa. The dried sample of each extract was weighted to determine the yields and stored in a freezer until use. The yield of leaf extract and stem extract was 8.64 and 9.33 %, respectively.

**Scavenging activity on 2,2'-diphenyl-1-picrylhydrazyl free radical:** To evaluate the free radical scavenging activity, leaf extract and stem extract were allowed to react with a stable free radical, 2,2'-diphenyl-1-picrylhydrazyl radical compared to rutin. Briefly, samples solution (0.1 mL) in distilled water at different concentrations (0.2, 0.5, 0.8, 1.2 mg/mL) was added to 3.9 mL 0.004 % (w/v) solution of DPPH in ethanol. The reaction mixture was incubated at room temperature. The scavenging activity on DPPH radical was determined by measuring the absorbance at 517 nm after 0.5 h. The antioxidant activity was expressed as a percentage of scavenging activity on DPPH radical:  $SC\% = [1 - (\text{absorbance of sample}) / (\text{absorbance of control})] \times 100\%$ . The control contains all reagents except the extract.

**Analysis of GC/MS:** Analyses were performed with a Agilent 6890-5973N GC/MS. Chromatographic condition are below: HP-5MS 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m quartz flexibility capillary column, initial column temperature is 50 °C, keep warm for 2 min, then heat to 260 °C by 10 °C/min<sup>-1</sup>. Injection port's temperature is 260 °C, carrier gas flow rate is 1 mL/min, the way inject simple into it is splitless. MS with electron-impact (EI) ionization (electron energy, 70 eV) was performed in the selected ion monitoring (SIM) mode. Mass spectrometry conditions are: Electronic multiplier voltage 1.42 V, EI source electronic energy for 70 eV, ion source temperature of 200 °C, scanning time interval 0.43 s, quality scanning range ( $m/z$ ) 40-600 amu.

## RESULTS AND DISCUSSION

Antioxidant activities, especially radical scavenging activity, are very important due to the deleterious role of free radicals in foods and in biological system. In present assay, a concentration-dependent 2,2'-diphenyl-1-picrylhydrazyl radical scavenging assay was carried out with leaf extract and stem extract comparing to rutin. The results are normalized and expressed as EC<sub>50</sub> values ( $\mu$ g various extracts per mL) for comparison. Effectiveness of antioxidant properties are inversely correlated with their EC<sub>50</sub> values. Fig. 1 illustrates that radical

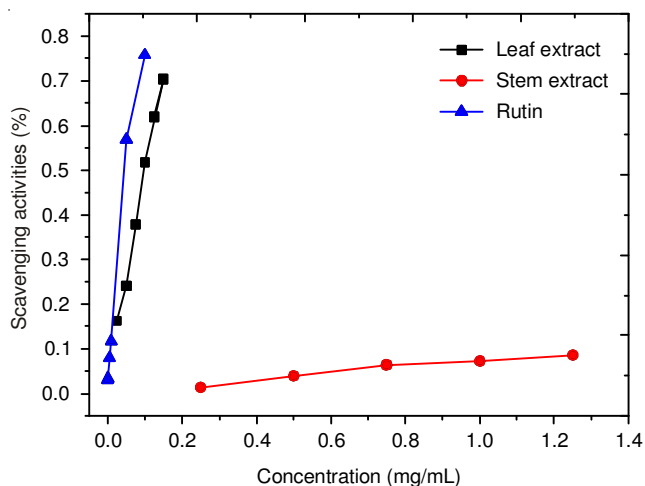


Fig. 1. 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities of rutin, leaf extract and stem extract

scavenging activities of leaf extract and stem extract increased with increasing concentration. The EC<sub>50</sub> values of leaf extract was 0.10 mg/mL which was close to rutin with EC<sub>50</sub> values was 0.06 mg/mL, indicating excellent DPPH radical scavenging activity. However, the DPPH radical scavenging activity of stem extract was not so good with the EC<sub>50</sub> only 7.08 mg/mL.

In order to identify free radical scavenging activities compounds in leaf extract and stem extract, GC-MS were performed. The retention time and the relative content of each compound contained in leaf extract and stem extract are given in Tables 1 and 2, respectively. With the assistance of the NIST's pesticide library, 10 compounds (**a-j**) were identified from leaf extract with the total peak area of 61.22 % and the most high relative content % of (E)-3,7,11,15-tetramethylhexadec-2-en-1-ol (**i**) reach to 19.87 %. For stem extract, compounds 3,5-dihydroxy-6-methyl-2H-pyran-4(3H)-one (**c**) and 2-dimethyl-8-oxo-2,3,4,8-tetrahydropyrano[3,2-g]chromen-3-yl-3-methylbut-2,3-noate (**j**) were identified which also present in leaf extract with the total peak area of 67.59 %. The relative content % of compounds **c** and **j** in stem extract was 31.75 and 1.86 %, respectively.

As shown in Fig. 2, the structures of these compounds from leaf extract and stem extract include furan, pyran, coumarin, fatty acid/ester and unsaturated fatty acid/ester. It should be noted that compound **c** was reported to be a strong antioxidant recently which should contribute to the antioxidant activities of stem extract and leaf extract<sup>15</sup>. Interestingly, the antioxidant activity of stem extract was not so good as leaf extract although the relative relative content % of compound **c** in stem extract reach to 31.75 % which is much higher than that in leaf extract. This might due to the synergistic or antagonism effect of compound **c** with other compounds. In addition, fatty acid/ester and unsaturated fatty acid/ester should also play important roles because they have been proved to exhibit antioxidant potential in other antioxidant plants<sup>16</sup>.

## Conclusion

In conclusion, the leaf extract of *Angelica keiskei* exhibits excellent antioxidant potential which is close to that of rutin but better than stem extract. Therefore, *Angelica keiskei* could

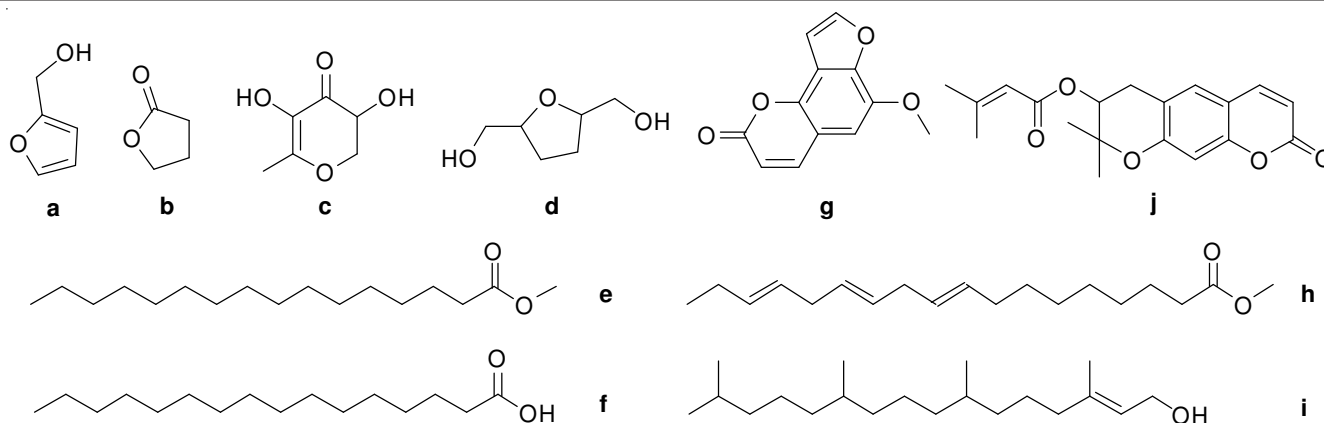
Fig. 2. Compounds from *Angelica keiskei*'s leaf and stem identified by GC-MS

TABLE-1  
PEAKS IDENTIFIED IN THE MASS SPECTRAL  
CHROMATOGRAMS OF LEAF EXTRACT SAMPLES

Peak No.	Retention time (min)	Name of compounds	Relative content (%)
1	3.664	<b>a:</b> Furan-2-ylmethanol	3.92
2	4.075	<b>b:</b> Dihydrofuran-2(3H)-one	2.33
3	8.813	<b>c:</b> 3,5-Dihydroxy-6-methyl-2H-pyran-4(3H)-one	8.54
4	10.433	<b>d:</b> (Hydroxymethyl)tetrahydrofuran-2-carbaldehyde	7.58
5	21.248	<b>e:</b> Methyl palmitate	1.18
6	21.694	<b>f:</b> Palmitic acid	2.66
7	22.079	<b>g:</b> 6-Methoxy-2H-furo[2,3-h]-chromen-2-one	2.72
8	23.316	<b>h:</b> (9E, 12E, 15E)-Methyl octadeca-9,12,15-trienoate	11.19
9	23.598	<b>i:</b> (E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	19.87
10	28.14	<b>j:</b> 2-Dimethyl-8-oxo-2,3,4,8-tetrahydropyran[3,2-g]chromen-3-yl-3-methylbut-2,3-noate	1.23

Peak area normalization method determine the component relative content, all the peak area as 100 %, the total content of substance was 61.22 %

TABLE-2  
PEAKS IDENTIFIED IN THE MASS SPECTRAL  
CHROMATOGRAMS OF STEM EXTRACT SAMPLES

Peak no.	Retention time (min)	Name of compounds	Relative content (%)
1	8.90	<b>c:</b> 3,5-dihydroxy-6-methyl-2H-pyran-4(3H)-one	31.75
2	28.14	<b>j:</b> 2,2-dimethyl-8-oxo-2,3,4,8-tetrahydropyran[3,2-g]-chromen-3-yl-3-methylbut-2,3-noate	1.86

The peak area normalization method determine the component relative content, all the peak area as 100 %, the total content of substance was 33.61 %

be used as a readily accessible source of the natural antioxidant in food and pharmaceutical industry. GC-MC analysis indicated that the main antioxidant activities compounds in leaf extract were 3,5-dihydroxy-6-methyl-2H-pyran-4(3H)-one,

fatty acid/ester and unsaturated fatty acid/ester. Moreover, 3,5-dihydroxy-6-methyl-2H-pyran-4(3H)-one was also determined in stem extract. These results provide a useful information for the further isolation of antioxidant active compounds from *Angelica keiskei*.

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