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Antioxidant activity and total phenolic contents of eight umbelliferae herbs

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Aim. Identification of antioxidant activity and total phenolic contents of eight species of Umbelliferae herbs including *Angelica dahurica*, *Ligusticum chuanxiong*, *Ligusticum sinensis*, *Ledebouriella seseloides*, *Angelica sinensis*, *Notopterygium incisium*, *Cnidium officinale* and *Cnidium monnieri*.
Methods. DPPH and ABTS methods are used to analyze the radical scavenging activity. Folin-Denis method was used to identify the total phenolic content in eight experimental species.
Results. From the above analysis the highest total phenolic contents were found in *N. incisum* (154.2±22.6 mg CE/g dry weight). The ethyl acetate extracts obtained from *A. sinensis* and *N. incisum* showed higher DPPH (1,1-Diphenyl-2-picrylhydrazyl radical 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) radical scavenging activity and ABTS (2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate)) radical scavenging activity.
Conclusion. The ethyl acetate extract will enrich the total phenolic content and radical scavenging activities from the experimental species, and they may be used as cosmetic additives such as a natural antioxidant.

KEY WORDS: Apiaceae - Antioxidants - Plants, medicinal.

Eight species of Umbelliferae (Apiaceae) herbs including *Angelica dahurica*, *Ligusticum chuanxiong*, *Ligusticum sinensis*, *Ledebouriella seseloides*, *Angelica sinensis*, *Notopterygium incisium*, *Cnidium officinale*, and *Cnidium monnieri* are widely distributed in China. They have anti-inflammatory and antimicrobial properties, and have been used in traditional Chinese herbal medicine as the major compounds for antioxidant activities.[1-4] However, no comparative analysis of antioxidant activities and total phenolic contents has been conducted yet for these eight Apiaceae Chinese herbal medicines (ACHMs).

Due to their hydroxyl free radical scavenging ability, phenols are used as the antioxidants. Phenolic compounds are very important plant constituents and have been widely exploited in different areas of plant research.[5, 6] Moreover, they also have been reported to be responsible for the antioxidant property of plants.

The total phenolic contents were measured by the Folin-Denis method. Previous report has shown that phenolic phytochemicals inhibit oxidation of skin collagen metabolism in aged and photoaged human skin.[7] Phenolic compounds are effective antioxidants that play an important role in human nutrition as preventive agents against several diseases and protecting the body tissues from oxidative stress. The aim of this study is using the total phenolic contents and DPPH and ABTS radical scavenging

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activity as index to search for potential antioxidants from ACHMs. The results of this study show that the total phenolic contents of these eight herbs' extracts can be assumed to be cosmetic additives such as a natural antioxidant.

Materials and methods

Plant materials

Angelica dahurica, *L. chuanxiong*, *L. sinensis*, *L. seseloides*, *A. sinensis*, *N. incisium*, *C. officinale*, and *C. monnieri* Cuss herbs were purchased from a traditional Chinese medicine pharmaceutical company in Taiwan. They were authenticated by one of authors, Dr. Gang Zhang. The voucher species were deposited in Research center of Natural Cosmeceuticals Engineering, Xiamen Medical College, China.

Preparation of plant extracts

Eight species of ACHMs were ground, respectively. A quantity of 200 g of each plant material was placed in conical flask and soaked in 1000 mL ethanol at 8 °C for 48 hours. The extraction was carried out with ethanol by using exhaust filter and the filtrate was marked as the first extract. The residues were extracted in the same way for 72 hours and the second extract was obtained after filtration. Then the two extracts were combined together and concentrated into 30-60 mL at 45 °C under vacuum concentration. The ethanol crude extract was mixed with ethyl acetate/aqueous (50:50, v/v, 600 mL) in separatory funnel to partition the mixture into ethyl acetate phase and aqueous phase. The clear two phases were collected in flasks and concentrated in vacuo to 30-60 mL at 45 °C. Then the concentrates were placed in -20 °C refrigerator for 2 days to obtain dried extract powders. The dried powders were reserved in the oven for posterior studies.

Determination of the antioxidant capacity

DPPH radical scavenging activity

For the analysis, the dried extracts were resuspended in methanol to a concentration of 0.5 mg/mL. A quantity of 4 mg DPPH was dissolved in 95% methanol and quantified to 25 mL. Both of them were stored in dark glass bottle, covered with aluminum foil and placed in ice bath. The method of determination was based on the report of Pellegrini (1999).[8] A quantity of 50 µL of herbal extract was mixed with 150 µL of 400 µM DPPH solution in 96 well plate, homogenized at 25 °C for 90 min and the absorbance was recorded at the wavelength of 517 nm before and after reaction. The scavenging activity of the reference method was calculated as follows: Scavenging activity % = [(The absorbance of blank 517 nm) – (The absorbance of sample 517 nm)]/(The absorbance of blank 517 nm) ×100%.

ABTS cationic scavenging activity

The ABTS aqueous solution was prepared freshly. ABTS 1000 µM, H_2O_2 500 µM, peroxidase 44 unit/mL, and deionized aqueous were mixed homogeneously in a ratio of 1:1:1:6 and kept for 1 h in dark at room temperature, which produced blue-green ABTS cation radical after reaction. The herbal extracts were diluted into a series of concentration 0, 25, 50, 100, 200, and 400 ppm. At room temperature, 180 µL ABTS solution was added to 20 µL herbal extracts homogeneously in 96 well plate. Then the absorbance was measured at 620 nm after 10 min. When the absorbance was lower, the scavenging activity of the sample was stronger. The scavenging effects could be obtained by the following equation: Scavenging activity % = [The absorbance of the samples at 734 nm/the absorbance of the control group could not add in the samples] × 100%.[9]

Determination of total phenolic contents

The total phenolic contents were measured by Folin-Denis method. A quantity of 4 mg gallic acid was dissolved in 10 mL methanol and diluted to a series of concentrations (0, 25, 50,100, 200, and 400 ppm), each of which added 100 µL 1N-Folin-Ciocalteu phenol reagents. After 20 min, 200 µL of 20% sodium carbonate was added. The mixture was shaken vigorously and allowed to stand at room temperature for 10 min, and then centrifuged with 10,000 rpm for 5 min. A quantity of 150 µL of clean solution was removed into 96 well plates, and the absorbance was measured at 750 nm to obtain the standard curve and regressive equation. The samples were processed and detected in the same way. After measuring the absorbance, the total phenolic

contents of samples could be determined by the regressive equation. The total contents of phenols have been presented by the gallic acid equivalent of per gram of Apiaceae herbal extracts.[4]

Statistical analysis

Data were calculated as mean±SD. Statistical analyses were carried out by analysis of variance (ANOVA) followed by appropriate post tests including multiple comparison tests (LSD). All analyses were made using the SPSS statistical software package and a probability value of less than 0.05 was considered as statistically significant.

Results and discussion

Determination of scavenging activity with the DPPH radicals

The DPPH radical scavenging activity of extracts increased with the rising of concentrations (Figures 1-3). To further characterize, the DPPH radical scavenging activity of different extracts from eight Umbelliferae herbs at the concentration of 400 μg/mL was presented in Table I. The data showed that the extraction solvent could affect the antioxidant activity. In these eight species, most of them had stronger DPPH radical scavenging activity in their ethyl acetate extracts, such as *L. chuanxiong, L. sinense, A. sinensis, N. incisum*, and *C. monnieri*. However, *C. officinale*'s (a special case) strongest scavenging activity was detected in the aqueous extract. The DPPH radical scavenging ability of aqueous extract was 43.5%, which was much higher than ethyl acetate extract (20.1%) and ethanol extract (11.3%). For the rest two, *A. dahurica* and *L. seseloide*, the DPPH radical scavenging activity did not show consider-

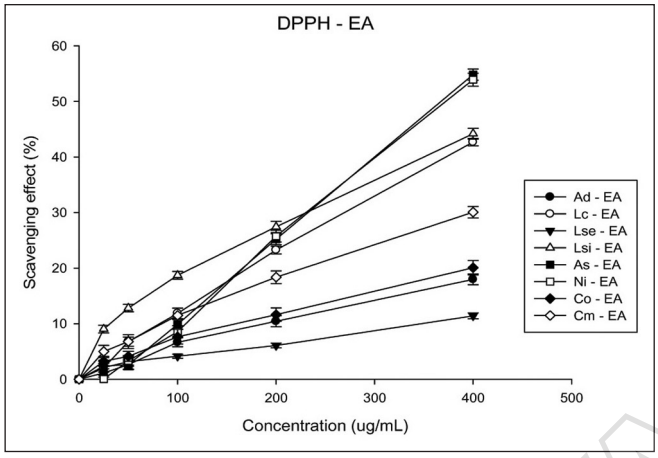

Figure 1.—Scavenging effects of ethyl acetate extracts on DPPH radical. A) *A. dahurica* (●); B): *L. chuanxiong* (○); C): *L. seseloides* (▼); D): *L. sinense* (◇); E): *A. sinensis* (■); F): *N. incisum* (□); G): *C. officinale* (◆); H): *C. monnieri* (◆).
Values are represented as mean±standard deviation (N.=3).

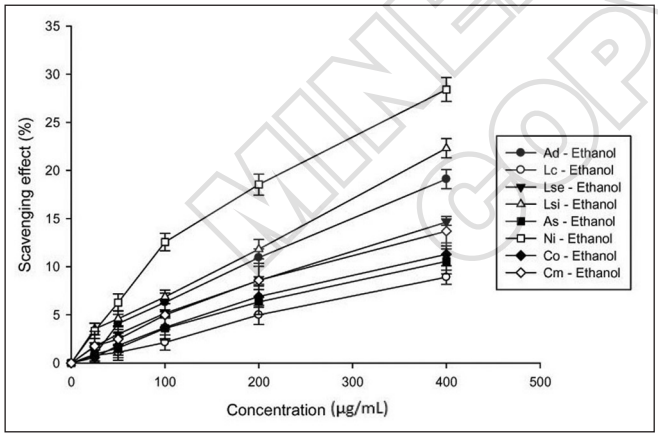

Figure 2.—Scavenging effects of ethanol extracts on DPPH radical. A) *A. dahurica* (●); B) *L. chuanxiong*; C) *L. seseloides* (▼); D) *L. sinense* (◇); E) *A. sinensis* (■); F) *N. incisum* (□); G) *C. officinale* (◆); H) *C. monnieri* (◇).
Values are represented as mean±standard deviation.

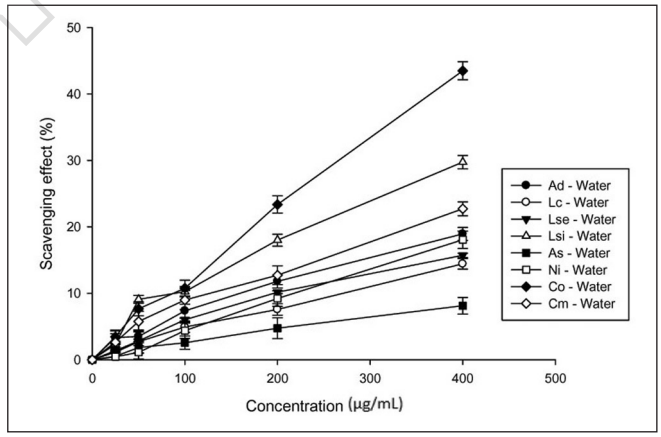

Figure 3.—Scavenging effects of aqueous extracts on DPPH radical. A) *A. dahurica* (●); B) *L. chuanxiong*; C): *L. seseloides* (▼); D) *L. sinense* (◇); E) *A. sinensis* (■); F) *N. incisum* (□); G) *C. officinale* (◆); H) *C. monnieri* (◇).
Values are represented as mean±standard deviation.

TABLE I.—*DPPH radical scavenging activity of different extracts from eight Umbelliferae herbs at the concentration of 400 μg/mL.*

	DPPH radical scavenging activity (%)		
	Ethanol extract	Ethyl acetate extract	Aqueous extract
A. dahurica	19.1	18.0	18.0
L. chuanxiong	8.9	42.7	10.6
L. seseloides	14.6	11.4	14.4
L. sinense	22.3	44.2	29.7
A. sinensis	10.6	54.8	10.6
N. incisum	28.4	53.9	15.7
C. officinale	11.3	20.1	43.5
C. monnieri	13.7	30.0	18.9

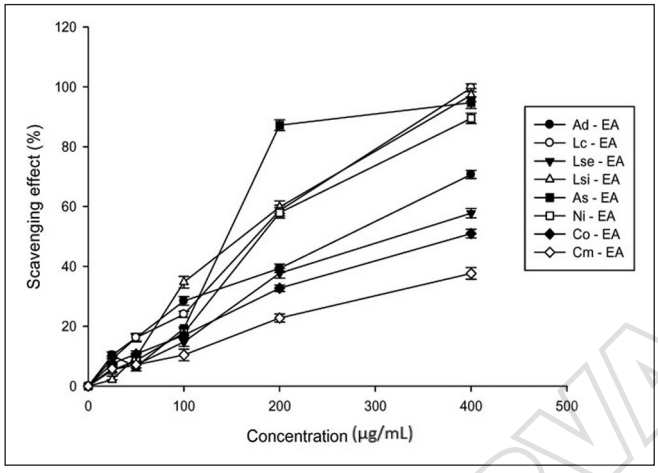

Figure 4.—Scavenging effects of ethyl acetate on ABTS radical. A) *A. dahurica* (●); B) *L. chuanxiong* (○); C) *L. seseloides* (▼); D) *L. sinense* (◇); E) *A. sinensis* (■); F) *N. incisum* (□); G) *C. officinale* (◆); H) *C. monnieri* (◇).
Values are represented as mean±standard deviation (N.=3).

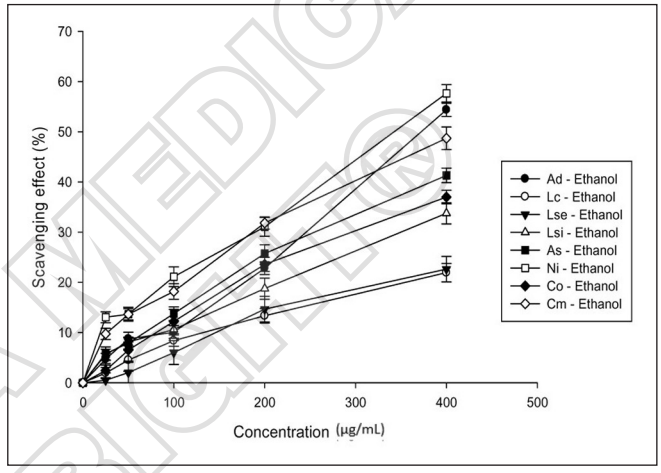

Figure 5.—Scavenging effects of ethanol extracts on ABTS radical. A) *A. dahurica* (●); B) *L. chuanxiong* (○); C) *L. seseloides* (▼); D) *L. sinense* (◇); E) *A. sinensis* (■); F) *N. incisum* (□); G) *C. officinale* (◆); H) *C. monnieri* (◇).
Values are represented as mean±standard deviation (N.=3).

able differences among ethanol, ethyl acetate, and aqueous extracts.

Determination of scavenging activity with the ABTS radicals

This study displayed the ABTS cation radical scavenging activity for the extracts of eight ACHMs. The ABTS radical scavenging activity with different concentrations of trolox was used as the calibration curve. The samples were tested in the same process and presented by trolox equivalent antioxidant capacity (TEAC). The ABTS radical scavenging activity of eight ACHMs also increased with different concentrations (Figures 4-6). The ethyl acetate extract of *N. incisum* at the concentration of 400 μg/mL had high antioxidant properties. Its ABTS cation radical scavenging activity reached to 89.4%, while the DPPH radical scavenging activity reached to 53.9%. The ethyl acetate extract of *L. chuanxiong* had very strong scavenging activity on ABTS cation radical. The scavenging activity achieved 99.6% at 400 μg/mL, 58.7% at 200 μg/mL, and the extract rate reached to 3.1%. The ethyl acetate extract of *A. sinensis* at 400 μg/mL had strong scavenging activity that reached to 94.7% for ABTS cation radical and 54.8% for DPPH radical. *L. Sinense* performed good scavenging activity in both ethyl acetate extract and aqueous extract, which was up to 97.2% and 61.2%, respectively. The ABTS cation radical

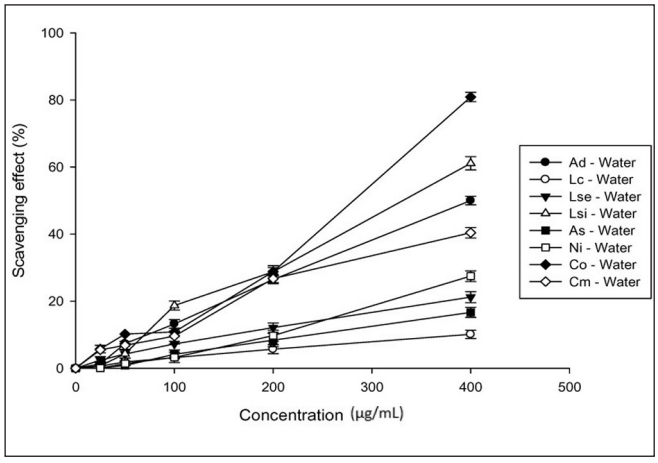

Figure 6. Scavenging effects of aqueous extracts on ABTS radical. (A): *A. dahurica* (●) (B): *L. chuanxiong* (○) (C): *L. seseloides* (▼) (D): *L. sinense* (◇) (E): *A. sinensis* (■) (F): *N. incisum* (□) (G): *C. officinale* (◆) (H): *monnieri* (◇)
Note: Values are represented as mean±standard deviation (n = 3).

scavenging activity of *A. dahurica* was measured at the same concentration of 400 μg/mL, and the scavenging activity of ethyl acetate, ethanol, and aqueous extracts exhibited 70.7%, 54.4%, and 50%, respectively. The scavenging activity of *C. monnieri* at 400 μg/mL was approximately 40-50%, even though any type of extraction solvent was used. *L. Seseloides* had a medium ABTS radical scavenging activity at 57.8% in ethyl acetate extract and did not have any special performance in others. Notably, *C. officinale* revealed distinctive property again. Its aqueous extract showed good ABTS radical scavenging activity at 400 μg/mL, which was up to 81.2%. The ABTS radical scavenging ability of aqueous extract was stronger than the other two extracts.

Determination of total phenolic contents

Polyphenols have been shown to exhibit antioxidant properties in vitro. This investigation is the first report on the comparative analysis of total phenol and antioxidant activity of the ethyl acetate layer, aqueous layer, and ethanol extract of *A. dahurica, L. chuanxiong*, *L. sinensis*, *L. seseloides, A. sinensis, N. incisium, C. officinale*, and *C. monnieri*. The total phenolic contents were determined by the regressive equation and presented by the gallic acid equivalent of per gram of Apiaceae herbal extracts. Quantitative differences of the total phenols among ethyl acetate extract, ethanol extract, and aqueous extract of eight ACHMs had been shown in Table II. Ethyl acetate layer contained both the nonpolar and polar compounds (aglycones and glycosides) in the ACHMs. Except *A. dahuric*, the others contained higher total phenolic contents in ethyl acetate extract. The special case may be due to more strong polar compounds contained in *A. dahurica*.

Conclusions

The results of the present study have revealed that using ethyl acetate as extraction solvent could further enrich the total phenolic contents. This may be responsible for the higher DPPH and ABTS radical scavenging activities performed in ethyl acetate extracts. In addition, the abnormal exhibition of *C. officinale* could be a new project. Its aqueous extract showed higher antioxidant activities than the other two extracts. Overall, the ethyl acetate extract of *A. sinensis* and *N. incisum* had higher DPPH anion radical and ABTS cation radical scavenging activities than the others, and they could be candidates for the potential natural cosmetic additives.

TABLE II.—*Total phenolic contents (mg CE/g dry weight).*

Chinese herbal name	Ethanol extract	Aqueous layer partition	Ethyl acetate layer partition
A. dahurica	50.2±6.3	345.8±32.5	218.7±22.5
L. chuanxiong	32.2±2.6	438.2±22.6	502.6±23.7
L. seseloides	10.2±2.3	7.2±2.3	113.8±19.2
L. sinense	89.2±3.1	176.2±13.3	208.4±23.8
A. sinensis	30.0±1.3	7.2±1.2	159.0±22.3
N. incisum	154.2±22.6	118.2±10.6	603.6±20.3
C. officinale	69.0±13.3	44.2±5.7	130.2±11.2
C. monnieri	114.2±19.3	30.2±6.3	107.5±12.3

Values represented as mean±standard deviation (N.=3).

References

1. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B, Rahmani Z. Antioxidant and Antihaemolytic Activities of the Leaves of *Kefe cumin* (Laser trilobum L.) Umbelliferae. Trop J Pharm Res 2010;9:441-9.
2. Wang J, Xu L, Yang L, Liu Z, Zhou L. Composition, antibacterial and antioxidant activities of essential oils from *Ligusticum sinense* and *L. Jeholense* (Umbelliferae) from China. Rec Nat Prod 2011;5:314-8.
3. Taherkhani M, Rustaiyan A, Rasouli I, Taherkhani T. Chemical constituents, antimicrobial activity, antioxidant and total and total phenolic content in the leaves of *Thecocarpus Meifolious* Boiss. Umbelliferae herbs growing wild IRAN. Pharm Chem J 2014;48:104-8.
4. Ahmed J, Guvenc A, Kucukboyaci N, Baldemir A, Coskun M. Total phenolic contents and antioxidant activities of Prangos Lindl. (Umbelliferae) species growing in *Konya province* (Turkey). Turk J Biol 2011;35:353-60.
5. Chen YH, Chang FR, Lin YJ, Wang L, Chen JF, Wu MJ *et al*. Identification of phenolic antioxidants from Sword Brake Fern (*Pteris ensiformis* Burn.) Food Chem 2007;105:48-56.
6. Chen YH, Chang FR, Lin YJ, Hsieh PW, Wu MJ, Wu YC. Identification of Antioxidants from Rhizome of *Davallia solida*. Food Chem 2008;107:684-91.
7. Chung JH, Seo JY, Choi HR, Lee MK, Youn CS, Rhie G *et al*. Modulation of skin collagen metabolism in aged and photoaged human skin in vivo. J Invest Dermatol 2001;117:1218-24.
8. Pellegrini R, Proteggent N, Pannala A, Yang A, Rice-Evans C. Antioxidant activity applying an improved Abts radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
9. Guohua C, Emin S, Ronald LP. Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. Free Radic Biol Med 1977;22:749-60.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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