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Quantitative Determination of Psoralen and Angelicin from Some Medicinal Herbs by High Performance Liquid Chromatography

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A reversed-phase high performance liquid chromatographic method was developed to determine the contents of psoralen and angelicin from some medicinal herbs. The optimum eluent for chromatography was 20 v/v% acetonitrile in water on a Zorbax 300SB C_{18} column. The identification was carried out by comparing the retention time and mass spectra of the relevant peaks with their standards. The variation of the concentration of psoralen and angelicin was wide between different species. The seeds of *Psoralea corylifolia* showed the highest contents of psoralen (7.8 mg/g) and angelicin (2.3 mg/g) among the tested herbs.

Key words: Psoralen, Angelicin, Medicinal herb, HPLC

INTRODUCTION

Psoralen is used in the treatment of vitiligo (Marianoa *et al.*, 2002), which is a condition causing the skin to lack pigment, bald patches of hair, and also in dermatology, such as psoriasis, mycosis fungoides, atopic eczema, etc. (Milesi *et al.*, 2001). It is also used in sunscreen to increase tanning. Side effects of oral psoralen was known to include sunburn, nausea and vomiting, itching, abnormal hair growth, and hyperpigmentation. Oral psoralen photochemotherapy may increase the risk of skin cancer. Psoralen is the effective ingredient extracted from a Chinese herb, bu-gu-zhi (*Psoralea corylifolia*), also known as po-gu-zi. With regard to *Psoralea corylifolia* some review comments that psoralen related compounds (furanocoumarins) may be irritant and photosensitizing and that their use in treatment may require specialized medical expertise and standardized products.

Angelicin (isopsoralen) is a photosensitizing agent and used for determining DNA/RNA structures in cells and microorganisms (Kittler *et al.*, 1980). Angelicin and its methoxy derivatives occur in a number of plants belonging to the Umbelliferae family. These compounds have been tested clinically in combination with ultraviolet A radiation for use in the treatment of psoriasis. Angelicin, in the presence of ultraviolet A radiation, bound covalently to isolated DNA and to DNA in bacteria, yeast and cultured mammalian cells (Natarajan *et al.*, 1981).

Several methods such as HPLC (Murali *et al.*, 2002; Cardoso *et al.*, 2002), GC (Yao *et al.*, 1996) and micellar electrokinetic capillary chromatography (Wang *et al.*, 1999) were presented for the analysis of psoralen and related components. The analysis of active components in plants revealed that the concentration of psoralen and related components vary widely both between different species and between different parts of the plant species. High concentrations are associated in particular with the surfaces of leaves and fruits (Zobel *et al.*, 1988) and sometimes with seeds, though in some species ripening is associated with the content (Zobel *et al.*, 1991; Zobel, 1990). The content varied also by the preparation of herbal drugs such as drying and storage. Due to the wide variability of concentrations of the components reported it is difficult to give quantitative estimates for medicinal herbs and it is also important to set the range of content for those components from the view of the quality control of the medicinal herb.

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MATERIALS AND METHODS

Instruments and chemicals

The chromatographic system for quantitative analysis

consisted of a SCL-10A system controller, an LC-10AD pump, a SPD-10MVP diode array detector (Shimadzu, Japan), column temperature controller (Waters, CA, USA) and a Rheodyne 7725 injector with a 20 μL sample loop. For quantitative analysis a 5989B LC/MSD system (Hewlett-Packard, USA) with a 59987A electrospray interface, G1312A binary pump and G1315A photo diode array detector were used. Methanol (Tedia, OH, USA), acetonitrile (J. T. Baker, NJ, USA.) used in this work were of HPLC grade and other reagents were of analytical grade. Milli-Q (Millipore, MA, USA) treated water (with resistivity more than 18 MΩ cm) was used throughout the experiment. Psoralen and angelicin standards were purchased from Sigma Co. (MI, USA).

Plant materials

The seven plant materials, *Angelica keiskei* (CNU-A11), *Angelica pubescens* (CNU-A12), *Dystaenia takeshimana* (CNU-D02), *Glehnia littoralis* (CNU-G01), *Heracleum moellendorffi* (CNU-H11), *Peucedanum japocicum* (CNU-P11), *Ruta graveolens* (CNU-R05)] were collected from the medicinal plant garden in the College of Pharmacy, Chungnam National University (CNU). *Daucus carota* (CNU-D01) and *Psoralea corylifolia* (CNU-P12) were purchased from the herbed market in Daejeon and Kumsan herbal area, respectively. *Aquilaria agallocha* (CNU-A13) was kindly obtained from the Institute of Natural Product Chemistry in Vietnam. All the plant samples were identified by Prof. KiHwan Bae in the College of Pharmacy, CNU. The collected samples were separated in leaves, stems and roots, when it was possible, and stored at a cool and dark place. The voucher specimens were deposited at the herbarium in the College of Pharmacy, CNU.

Sample preparation

The air dried and coarsely powdered sample (2 g) was extracted with 80 mL of mixed solution (dichloromethane-water = 1:1, v/v) for 1 h in the ultrasonic bath. The dichloromethane layer was separated into the round bottomed flask. The remaining water layer and residue were washed twice with 20 mL of dichloromethane. The dichloromethane layer was collected and evaporated to dryness under nitrogen gas stream. The residue was dissolved in methanol and the volume was adjusted to 10 mL. The solution was then centrifuged at 15,000 g for 10 min (Microspin, Hanil Co., Korea) and 10 μL of the supernatant was injected to the HPLC. When the concentration of the components was higher than the upper limit of the calibration, the solution was diluted with methanol.

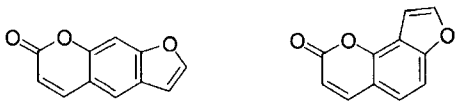
Chromatography and identification

The HPLC separation of psoralen and angelicin for qualitative and quantitative analysis was performed using a reverse phase system. A Zorbax 300SB C_{18} (4.6×150 mm, Hewlett-Packard Co., CA, USA) chromatographic column was used and column temperature was maintained to be 25°C. The mixed solution of acetonitrile and water was employed as mobile phase at a flow rate 1.0 mL/min. Detection was carried out at UV 245 nm. The identification of psoralen and angelicin in the sample was carried out by analyzing the mass spectrum of a peak corresponding to the components in the HPLC effluent, respectively.

RESULTS AND DISCUSSION

Resolution of psoralen and angelicin

The psoralen and angelicin are structural isomers and the systematical approach was necessary to resolve the structural analogous isomers. To select an optimal mobile phase composition for the analysis of psoralen and angelicin, several eluents in different ratio of acetonitrile and water were prepared. The higher resolution between psoralen and angelicin could be achieved with the lower concentration of acetonitrile (Fig. 2). But with the concentration of acetonitrile lower than 20 v/v% the retention time was dramatically increased, indicating that the appropriate mobile phase was 20 v/v% acetonitrile in water. The chromatograms of standard mixture and an extract of *P. corylifolia* separated using the selected mobile phase (Fig. 3) showed that psoralen and angelicin could be separated in 15.8 and 17.6 min, respectively. The two peaks designated **1** and **2** in the chromatogram of *Psoralea corylifolia* extract (Fig. 3b) were confirmed as psoralen and angelicin,



psoralen angelicin

Fig. 1. Chemical structures of psoralen and

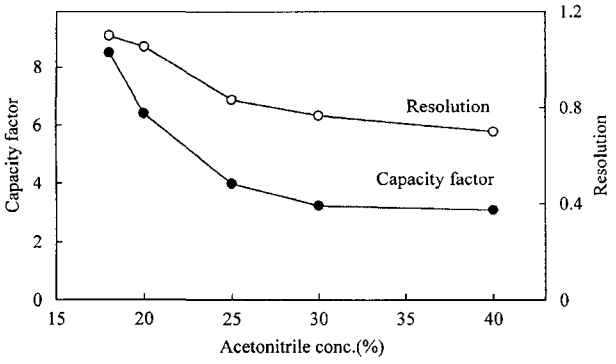


Fig. 2. The effects of acetonitrile concentration in the mobile phase on the capacity factor (closed circle) of psoralen and the resolution (open circle) between psoralen and angelicin.

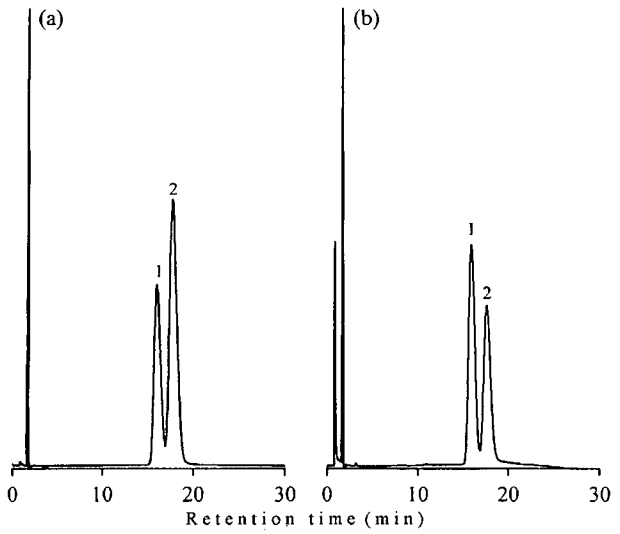


Fig. 3. Chromatograms of (a) standard mixture of psoralen (**1**) and angelicin (**2**) and (b) an extract of *Psoralea corylifolia* separated on Zorbax 300SB C_{18} column with 20 v/v% acetonitrile in water as mobile phase. Detection; UV 245 nm.

respectively, by comparing retention times and mass spectra with those of authentic samples (Fig. 4). The base peak of psoralen and angelicin was found both at *m/z* 187 $[M+H]^+$.

Linearity, recovery, accuracy and precision

The calibration functions of psoralen and angelicin standard calculated with peak height (*y*, mAU) and concentration (*x*, μg/mL) were $y = 1.07x - 0.69$ (r=0.9999) and $y = 1.57x - 0.85$ (r = 0.9999), respectively, over the concentration range 5 to 200 μg/mL. For recovery testing, known amounts

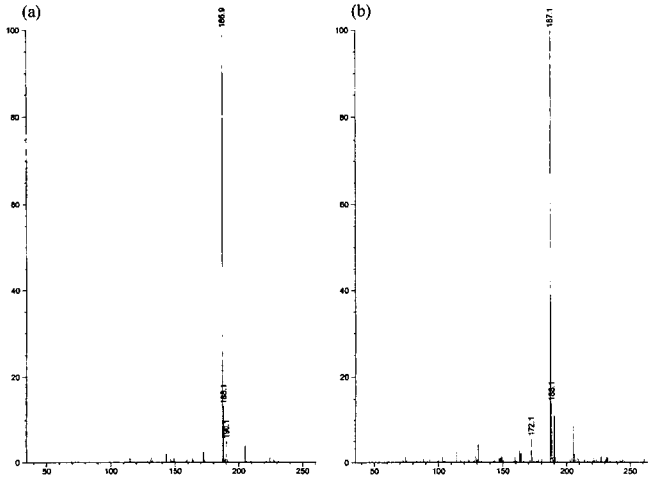


Fig. 4. Mass spectra of separated peaks of *Psoralea corylifolia* at 15.8 (a) and 17.6 min (b). MS conditions: Ionization; ESI-Positive, Quadrupole temp.; 100 °C, Fragment voltage; 80 V, Nebulizer; nitrogen 9 L/min, 350 °C, 40 p.s.i.

Table I. The recovery of psoralen and angelicin from the roots of *Heracleum mollendorffi* by the HPLC method (mean of n=3)

Added (mg/g)	Psoralen		Angelicin	
	Found (mg/g)	Recovery (%)	Found (mg/g)	Recovery (%)
0.030	0.028	94.1	0.027	91.0
0.150	0.156	104.1	0.132	88.0
0.700	0.766	109.6	0.639	91.2

of psoralen and angelicin standards were added to the root of *H. mollendorffi*, which contained no psoralen and angelicin, and analyzed by HPLC. The efficiency of recovery by the HPLC analysis was shown in Table I. The average recovery of psoralen (102.6%) was higher than that of angelicin (90.1%). The detection limit of psoralen and angelicin was 0.1 μg/g for both at a signal to noise ratio of 3.

The accuracy, the percentage of the measured mean concentration divided by known concentration, for psoralen and angelicin was in the range of 96.7-107.8%. The intraday precisions of psoralen and angelicin were in the range of 0.7-4.4% and 0.8-4.7%, respectively. The inter day precision was about 0.6-1.7% higher than the intraday precision.

The observed linearity, recovery, accuracy and precision indicated this method is suitable and applicable for

Table II. The intraday and inter day precisions of the standard analysis.

Concentration (μg/g)	Psoralen			Angelicin		
	Accuracy (%)	Intraday CV(%)	Inter day CV(%)	Accuracy (%)	Intraday CV(%)	Inter day CV(%)
5.0	107.0	4.0	4.8	107.8	4.3	5.6
20.0	96.9	4.4	4.2	96.7	4.7	5.2
50.0	99.7	1.2	2.9	99.4	1.1	2.6
100.0	100.1	0.7	1.3	100.1	0.8	2.1

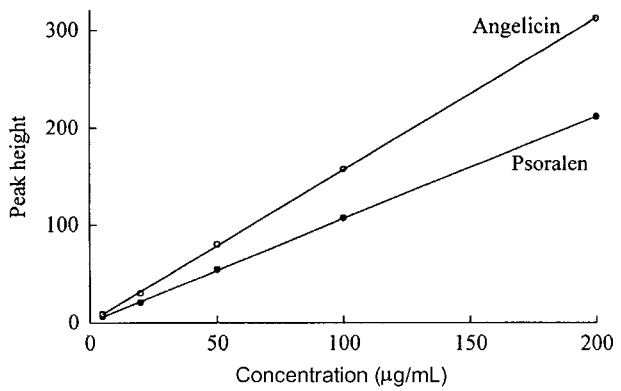


Fig. 5. Calibration curve of psoralen (closed circle) and angelicin (open circle) in the range of 5 to 200 μg/mL.

Table II. Concentration[1] of psoralen and angelicin in plant samples

Scientific name	Family	Analyzed parts	Psoralen	Angelicin
Psoralea corylifolia	Leguminosae	Seeds	7.809±0.361	3.703±0.204
Aquilaria agallocha	Thymelaeaceae	Woods	2.283±0.141	n.d.[2]
Ruta graveolens	Rutaceae	Roots	1.005±0.086	n.d.
		Aerial parts	0.091±0.008	n.d.
Glehnia littoralis	Umbelliferae	Roots	0.023±0.004	n.d.
		Aerial parts	0.024±0.003	0.016±0.002
Angelica keiskei	Umbelliferae	Roots	0.022±0.003	0.012±0.002
		Aerial parts	n.d.	n.d.
Daucus carota	Umbelliferae	Seeds	0.005±0.002	0.002±0.001
		Roots	n.d.	n.d.
Dystaenia takeshimana	Umbelliferae	Whole plants	n.d.	n.d.
Peucedanum japocicum	Umbelliferae	Whole plants	n.d.	n.d.
Angelica pubescens	Umbelliferae	Whole plants	n.d.	n.d.
Heracleum mollendorffi	Umbelliferae	Whole plants	n.d.	n.d.

[1]Data are given as mean±SD (n=3-4) in mg/g dried sample.
[2]Not determined (<0.5 μg/g dried sample).

qualitative and quantitative evaluation of the psoralen and angelicin in the plant samples.

Application to the medicinal herbs

The psoralen and angelicin from some medicinal herbs were analyzed by the validated HPLC method. The contents of psoralen and angelicin were presented in Table III. The *P. corylifolia* showed the highest contents of psoralen (7.8 mg/g) and angelicin (2.3 mg/g) among the tested herbs. Both psoralen and angelicin were found from the herbs belong to Leguminosae and Umbelliferae, while only psoralen was found from the herbs of Thymelaeaceae and Rutaceae. The concentration of angelicin in the *P. corylifolia* (Leguminosae) recorded about 230 times higher than that in *G. littoralis* (Umbelliferae), though it is generally known that angelicin is contained in the plants belong to Umbelliferae. There were some differences of the contents of psoralen and angelicin not only between the species but also between the parts. About 7 ppm of psoralen and angelicin has been recorded in the seeds of *D. carota*, but there was no detectable amount of these components in the roots. The concentration of psoralen in the roots of *R. graveolens* and *A. keiskei* was higher than that in aerial parts. Together, these results provided the tendency of the contents of psoralen and angelicin in the medicinal herbs and indicated that this method could be used for evaluation of the psoralen containing plants.

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