CHEMICAL AND ECOLOGICAL EVALUATION OF A GENUINE CHINESE MEDICINE: *ATRACTYLODES MACROCEPHALA* KOIDZ

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Abstract

The study was designated to complete a chemical and ecological evaluation of a genuine Chinese medicine *Atractylodes macrocephala* Koidz (*A. macrocephala*) and to explore the existence of correlation between chemical ingredients of different *A. macrocephala* populations and ecological factors. Different samples of *A. macrocephala* collected from six Chinese habitats were analyzed using chemical and ecological methods. The results showed the bioactive ingredients of *A. macrocephala* to be closely related to ecological factors. The study can serve the purposes of preservation and development of genuine Chinese medicines.

Keywords: *Atractylodes macrocephala* Koidz (*A. macrocephala*); chemical and ecological evaluation; genuine medicine; HPLC.

Introduction

Atractylodes macrocephala Koidz (*A. macrocephala*), a Chinese medicinal herb, has been extensively used to treat digestive diseases in China and most other Asian countries (PPRC, 2005; Lee et al, 2007). Dry rhizomes of *A. macrocephala* are rich in sesquiterpenes and acetylenic compounds (Endo et al, 1979; Chen 1987; Huang et al, 1992; Lin et al, 1997). Typical polysaccharides atractan A, B, and C, present in *A. macrocephala*, have been reported to exhibit hypoglycaemic activities (Wang et al, 2000; Jia et al, 2003). Although atractylenolide I, atractylenolide II and atractylenolide III are all bioactive substances present in *Atractylodes macrocephalae*, the majority of research studies carried out in the recent years have focused on atractylenolide II and atractylenolide III (Kang et al., 2011b). Atractylenolide II is a marker substance present in *Atractylodes macrocephalae* which exhibits well-documented gastrointestinal inhibitory effects and anticancer activity (Zhang et al., 1999; Liu et al., 2005). Atractylenolide II is one of the main constituents present in the effective volatile oil fraction (Li et al., 2001), potentially effective in treating senile dementia (Ge et al., 2007). Atractylenolide III was shown to inhibit the secretion and expression of IL-6 in phorbol 12-myristate 13-acetate- and calcium ionophore A23187-stimulated human mast cells (HMC)-1 (Kang et al., 2011b). In addition, atractylenolide III inhibited histamine release in stimulated HMC-1 cells, in which it may control immunological reactions by virtue of regulating cellular functions of IL-6 in mast cells (Kang et al., 2011b). Atractylenolide III can also induce apoptosis of lung carcinoma cells by inhibiting cell growth and increasing lactate dehydrogenase release (Dong et al., 2008). Moreover, atractylenolide III is a possible candidate for the treatment of human lung carcinoma (Kang et al., 2011a).

The term "genuine herb" refers to the traditional Chinese herb featured by region-specific characteristics, an outstanding

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clinical efficacy, fixed processing method, and high quality. Ecological factors may play an important role in genuine herb formation, particularly when it comes to secondary metabolites' accumulation (Huang et al, 2007).

The study was designated to complete a chemical and ecological evaluation of *A. macrocephala*, a genuine Chinese medicine, and to explore the existence of correlation between chemical ingredients present in *A. macrocephala* populations and ecological factors..

Materials and Methods

Plant materials

Samples of *A. macrocephala* (20090829, 20090912, 20090811, 20091005, 20090803, 20091017) were collected from six different habitats in China: Zhejiang Province, Anhui Province, Jiangxi Province, Chongqing City, Hunan Province and Hebei Province.

Chemicals and reagents

Analytical grade-methanol was purchased from Sigma Aldrich, UK. HPLC-grade water, acetonitrile and tetrahydrofuran (THF) were purchased from Fisher Scientific UK. The liquid chromatographic system used was a Waters HPLC System (Waters, Milford, MA, USA) equipped with a 2996 photodiode-array (PDA) UV detector, Waters 626 Pump, and Waters 600 Controller (LCD), Waters 717 plus Auto-sampler. Chromatographic separation of the three compounds was achieved using a reversed-phase HPLC column (Agilent ZORBAX Rx-C18 2.1 x 150 mm, 5 μm).

Contents' Determination, Extraction and isolation

Ten (10) g of *A. macrocephala* was refluxed for 60 minutes with 100 ml of water remained in the flask by means of Soxhlet-extraction. After filtration, the extraction procedure was repeated using 80 mL of water. The filtrate was concentrated *in vacuo* and freeze-dried. Each freeze-dried sample was weighed with the maximal precision, dissolved in water, and filtered through a membrane filter (0.45 μm). The samples were injected into HPLC. The extraction flowchart is presented in Figure 1.

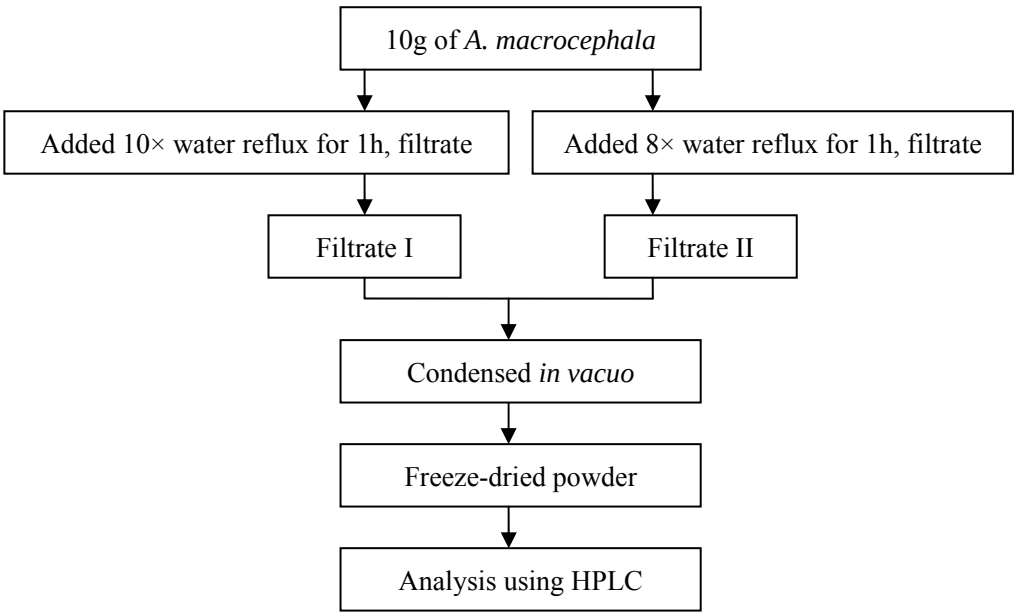

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Figure 1 Flowchart descriptive of the conventional extraction of *A. macrocephala*

HPLC setting

Gradient elution employed in HPLC analysis consisted of two solvent compositions: 0.5% tetrahydrofuran (THF) dissolved in water (solvent A) and acetonitrile (solvent B). Gradient elution was carried out according to the following program: 0-5 min, 0-40%; 5-15 min, 40-55%; 15-60 min, 55-90%. The flow rate was 0.2 µl/min. The sample injection volume was 3 µl. Both the column and the auto-sampler were set at the ambient temperature. The eluent was monitored by an UV detector operating at the wavelengths of 220 nm and 230 nm so as to detect atractylenolide II and III.

Preparation of standard solutions and calibration curves

Standard stock solutions (730.0 µg/ml of atractylenolide II and 470.0 µg/ml of atractylenolide III) were prepared separately in 50%-methanol. Standard working solutions of atractylenolide II and atractylenolide III were prepared by virtue of diluting their original stock solutions with 50%-methanol so as to reach the final concentrations of 73.0 µg/ml, and 47.0 µg/ml, respectively. Mixed standard solution was prepared using 200 ml of atractylenolide II and atractylenolide III mixed together so as to reach the final concentrations of atractylenolide II and atractylenolide III of 15.0 µg/ml and 16.0 µg/ml, respectively. Working standard solutions and quality control sample solutions were prepared in high, medium and low concentrations by diluting the standard stock solutions with 50%-methanol in order to retrieve atractylenolide II and atractylenolide III. These solutions were all kept in brown glass bottles and stored at −20 °C.

Calibration curves were obtained by plotting the peak-area ratio of each analyte standard versus analyte concentration. The studied concentration ranges were 0.73–73.0 µg/ml for atractylenolide II and 0.48–48.0µg/ml for atractylenolide III.

Validation of the method

Validation of the HPLC method was performed by determining the intra-day and inter-day accuracy and precision. Quality control samples were analyzed in a set of five on a single assay day so as to determine intra-day precision and accuracy. They were also analyzed in duplicate on three separate days so as to determine inter-day precision and accuracy. Quality control samples were used in low, medium and high concentrations (atractylenolide II: 18.3, 36.5, 73 µg/ml; atractylenolide III: 11.8, 23.5, 47.0µg /ml).

Statistics

Data were analysed by an independent university-based statistician by virtue of hierarchical cluster analysis using the Statistical Package for Social Sciences (SPSS 13.0 for Windows). Multiple stepwise linear regression analysis was performed to demonstrate the correlation between the contents of atractylenolide II and atractylenolide III present in *A. macrocephala* and ecological factors. The content of atractylenolide II and atractylenolide III acted as a dependent,variable, while ecological factors acted as independent variables.

Results and Discussion

Calibration curve

The regression equation was calculated as Y = AX+B, where Y represents the peak area and X represents the content of the constituent. The regression equations of atractylenolide II and atractylenolide III were

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Y=1464851.82X-81346.00 (r^2=0.9997), and Y=498989.66X+13987.51 (r^2=0.9990), respectively.

Content determination

HPLC sample chromatogram obtained at 230 nm and 3-dimensional HPLC chromatogram are presented in Figure 2. HPLC fingerprints of the samples harvested on six different habitats and analysed at 220 nm are presented in Figure 3. The contents of atractylenolide II and atractylenolide III present in *A. macrocephala* originating from different habitats are shown in Table 1.

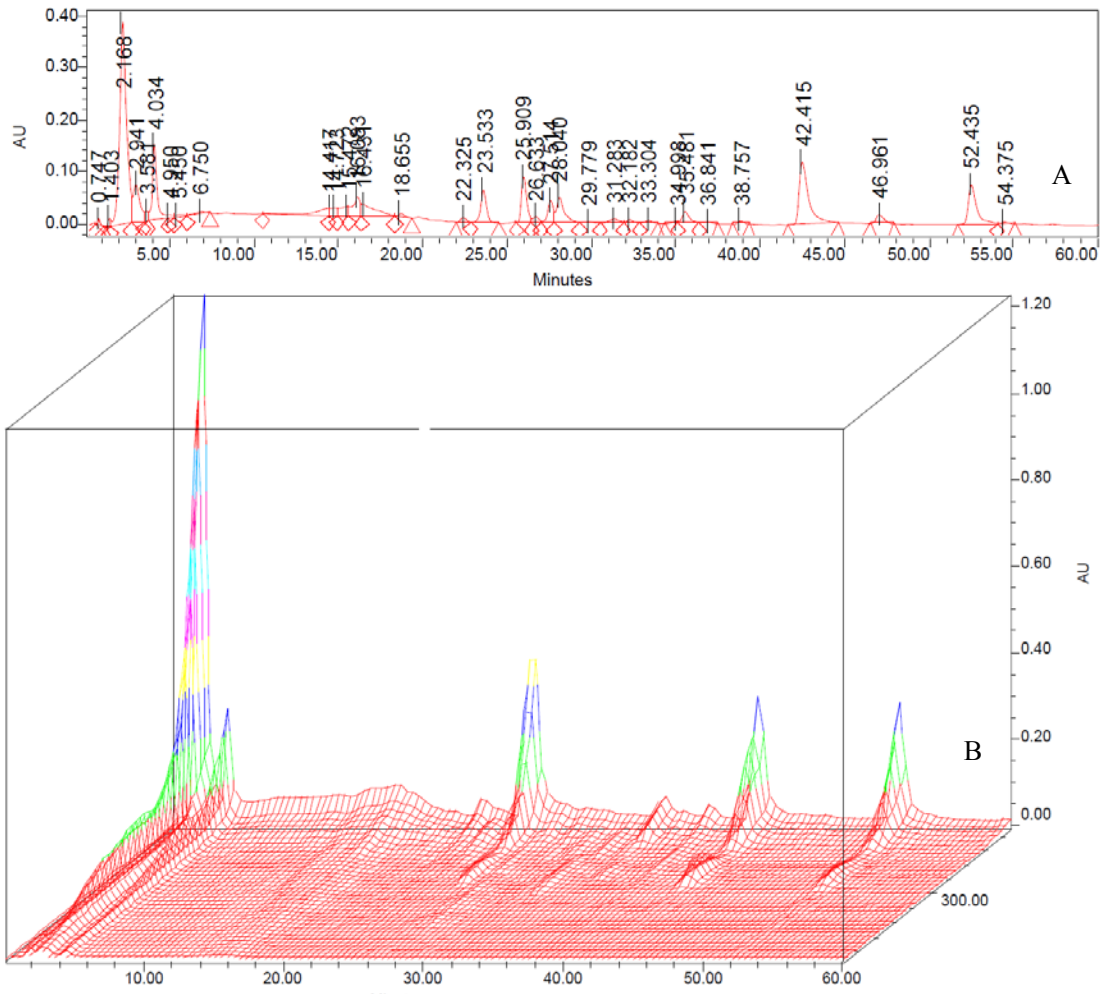

Figure 2 HPLC chromatogram of *A. macrocephala*.

Note:

(A) chromatogram of *A. macrocephala* (wavelength=230 nm)

(B) 3-dimensional chromatogram of *A. macrocephala*

Chromatographic setting:

Solvent A: 0.5% tetrahydrofuran (THF) dissolved in water; solvent B: acetonitrile.

Gradient elution program: 0-5 min, 0-40% (Solvent A); 5-15 min, 40-55% (Solvent A); 15-60 min, 55-90% (Solvent A).

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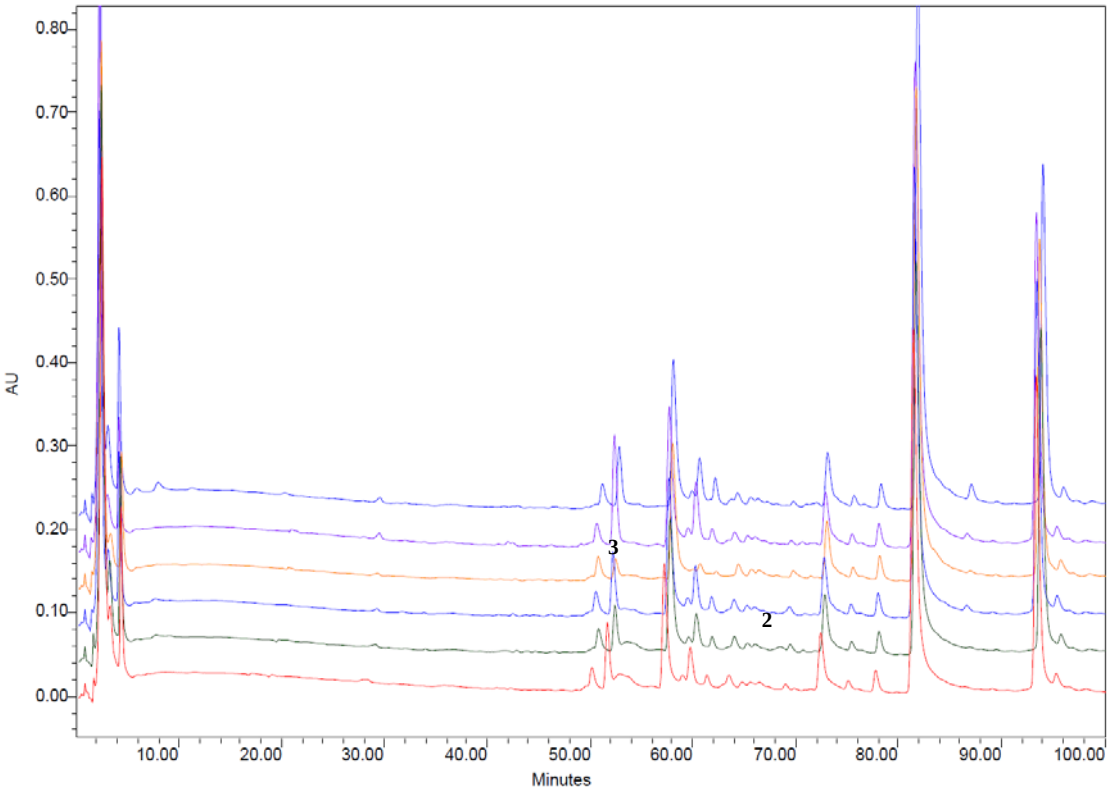

Figure 3: HPLC fingerprints of *A. macrocephala* from different habitats (220 nm).

Note: From bottom to top, the samples were harvested in Zhejiang Province, Anhui Province, Jiangxi Province, Chongqing City, Hunan Province and Hebei Province.

3= atractylenolide III, 2= atractylenolide II

Table 1: Contents of atractylenolide II and atractylenolide III present in *A. macrocephala* originating from different habitats (mean ± SD)

Habitats	Atractylenolide II (µg/ml)	Atractylenolide III (µg/ml)
Zhejiang Province	13.66	31.89
Anhui Province	10.65	23.53
Jiangxi Province	11.90	23.99
Chongqing City	12.08	26.48
Hunan Province	9.32	22.61
Hebei Province	8.40	19.77

Based on the content of atractylenolide II and atractylenolide III present in *A. macrocephala* samples, the six habitats were finally classified as group 1 (Zhejiang Province), group 2 (Anhui Province, Jiangxi Province, Chongqing City) and group 3 (Hunan Province, Hebei Province). The sample from Zhejiang Province was proven to be the best source of *A. macrocephala*.

Ecological Evaluation

A. macrocephala samples were collected from six different Chinese habitats. Ecological factors descriptive of these six locations are presented in Table 1. Based on ecological factors relevant for atractylenolide II and atractylenolide III

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contents present in *A. macrocephala* samples, the six habitats were finally classified as group 1 (Zhejiang Province), group 2 (Anhui Province, Jiangxi Province, Chongqing City) and group 3 (Hunan Province, Hebei Province).

Table 2: Ecological factors descriptive of six *A. macrocephala* habitats

Habitats	Longitude E	Latitude N	Annual average Temperature℃	Annual Precipitation (mm)	Soil
Zhejiang Province	120.59	20.99	16-22	800-1500	Red
Anhui Province	117.48	30.56	14-20	800-1500	Red
Jiangxi Province	115.58	29.43	16-21	800-1500	Red
Chongqing City	106:33	29:33	16-21	2000-2500	Yellow
Hunan Province	112.59	28.12	15-20	800-1500	Red
Hebei Province	115.28	38.53	7-12	500-700	Dark Brown

In the present study, ecological factors including longitude, latitude, annual precipitation, annual average temperature and soil were considered. Amongst these, longitude, latitude and annual average temperature positively correlated with *A. macrocephala* atractylenolide II and atractylenolide III contents. The result of the multiple linear stepwise regression analysis showed the longitude, latitude and annual average temperature to be capable of influencing the accumulation of atractylenolide II and atractylenolide III. The study showed *A. macrocephala* samples collected in Zhejiang Province to have higher contents of atractylenolide II and atractylenolide III as compared to those harvested on other habitats; the credit should be given to ecological factors of Zhejiang Province which favour the accumulation of chemical ingredients in *A. macrocephala*. A close correlation between chemical ingredients present in different *A. macrocephala* populations and ecological factors was uncovered.

Various ecological settings, as well as harvesting and processing methods, often lead to differences in quality of traditional Chinese medicines. The term “genuine herb” refers to the traditional Chinese herb featured by region-specific characteristics, an outstanding clinical efficacy, fixed processing method and high quality. Dried rhizome of *A. macrocephala* is a common Chinese medicinal herb widely used in most of the Chinese formula (Lin et al, 2008). Understanding of the scientific basis of traditional genuine medical knowledge is an essential requirement for maintaining and preserving these remedial herbs (Said, 2002). The present study was designated to complete a chemical and ecological evaluation of *A. macrocephala*, a genuine Chinese medicine, and to explore the existence of correlation between chemical ingredients present in different *A. macrocephala* populations and ecological factors. The present research evaluated the quality of six different original samples of *A. macrocephala* using chemical and ecological methods. This study primarily revealed the scientific basis of genuine herbs’ remedial activity, potentially to the goal of preservation and development of genuine Chinese medicines.

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