



The role of chemical fingerprinting: application to *Ephedra*

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Abstract

Ephedra sinica, known as *Ma Huang*, is one of the oldest medicinal herbs in Traditional Chinese Medicine (TCM). Preparations, namely teas, of *E. sinica* have been used for over 5000 years as a stimulant and as an antiasthmatic. In the West, extracts of *E. sinica*, *E. intermedia* or *E. equisetina* are most commonly used in dietary supplements as a stimulant and to promote weight loss. More than 50 species of *Ephedra* are native to both hemispheres, but the detection of ephedrine alkaloids has been limited to species in Eurasia. Currently, methods exist to quantitate the ephedrine alkaloids in extracts of plant material or dietary supplements, but the methods are not able to verify the extract is of an *Ephedra* species. Reverse phase high performance liquid chromatography with photodiode array detection was applied for the chemical fingerprinting of the *Ephedra* species. Two regions of comparison were determined in the chromatograms at 320 nm. The series of peaks between 52 and 64 min confirms an *Ephedra* species is being analyzed. The aforementioned peaks also could distinguish between *Ephedra* species from Eurasia, North America and South America. Peaks at ca. 57 and 59 min were isolated and determined to be two new compounds, 4-(2-eicosyloxycarbonyl-vinyl)-benzoic acid and 4-(2-docosyloxycarbonyl-vinyl)-benzoic acid respectively. Authentication of ground plant material as *Ephedra* can be achieved by this chemical fingerprinting method.

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1. Introduction

During the mid 1990s, the botanical dietary supplement market grew exponentially. This increased demand for herbal products was met by a flood of new companies wanting their share of this billion dollar industry. Unfortunately, the scientific support behind these products has not been able to keep up with the industries rapid expansion (Cardellina, 2002). The lack of science has created an industry where quality is sometimes compromised. One common method used by the industry for quality control is analyzing the product for the presence of chemical markers known to be present in the specific herbal they happen to be marketing, whether the markers are the cause of the physiological affect or not. Even though this has been the acceptable method for quality control, the presence of the chemical

markers do not always guarantee an individual is getting the actual herbal stated by the product label, especially if the product has been spiked with the chemical markers. The quantitation method for the chemical markers will confirm the compounds presence, but it may not confirm the presence of the plant material known to contain the chemical markers. Authentication of the plant material may be possible by a chemical fingerprint of the botanical. Chemical fingerprinting is an additional method that must be included as a quality control method in order to confirm or deny the plant material being used for the manufacturing of a product. Products claiming to contain *Ma Huang* are a prime example of a botanical where chemical fingerprinting is a must for authentication purposes.

Ephedra sinica, known as *Ma Huang*, is one of the oldest medicinal herbs in traditional Chinese medicine (TCM). Preparations, namely teas, of *E. sinica* have been used for over 5000 years as a stimulant and as an antiasthmatic (Bensky and Gamble, 1993; Chen and Schmidt, 1926). The genus of *Ephedra*, which contains

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over 50 species, belongs to the family Ephedraceae (Caveney et al., 2001). The shrubs, which reach approximately one meter in height, grow in semiarid and desert conditions in both hemispheres across six continents (Price, 1996).

In the West, dietary supplements containing *Ma Huang* extracts have become one of the top selling weight loss and endurance enhancing products on the market. Currently, it is thought that the number of people consuming *Ephedra* is in the millions. However, this growth has not been without its controversy, over

the last decade state and federal governments have regulated *Ephedra* containing products due to a growing number of reported adverse events caused by misuse or abuse of the herb (Food Drug Administration, 1997; Department of Health and Human Services, 2000; Haller and Benowitz, 2000; Zaacks et al., 1999). These adverse events are thought to be due to a series of optically active alkaloids.

Six optically active alkaloids, the ephedrine alkaloids (Fig. 1), are considered the active constituents in *Ma Huang*. The concentration of ephedrine alkaloids can vary from 0.02 to 3.40% in the aerial parts of the plant (Leung and Foster, 1996). (–)-Ephedrine (**1**) is the major isomer. The minor ephedrine alkaloids include (+)-pseudoephedrine (**2**), (–)-methylephedrine (**3**), (+)-methylepseudoephedrine (**4**), (–)-norephedrine (**5**) and (+)-norpseudoephedrine (**6**). The pharmacological studies have shown **1** to be a sympathomimetic agonist at both the α - and β -adrenergic receptors, which leads to an increased cardiac rate and contractility, to peripheral vasoconstriction, to bronchodilation, and to central nervous system (CNS) stimulation (Fouad-Tarazi et al., 1995; Walker et al., 1998). Ephedrine (**1**) is not the only alkaloid used in products, over the counter decongestant preparations commonly contain **2**. Weight loss and enhanced performance in endurance training and body building may be due to the CNS stimulation and thermogenic properties of **1** (Walker et al., 1998). Due to the ephedrine alkaloid activities indicated in previous studies, contraindications are given for individuals with hypertension or other cardiovascular diseases, glaucoma, diabetes and hyperthyroidism (Fetrow and Avila, 1999; Tyler, 1999).

Although *E. sinica* has been the primary source for ephedrine alkaloids, other species of *Ephedra* throughout Eurasia contain the active constituents: *E. equisetina*,

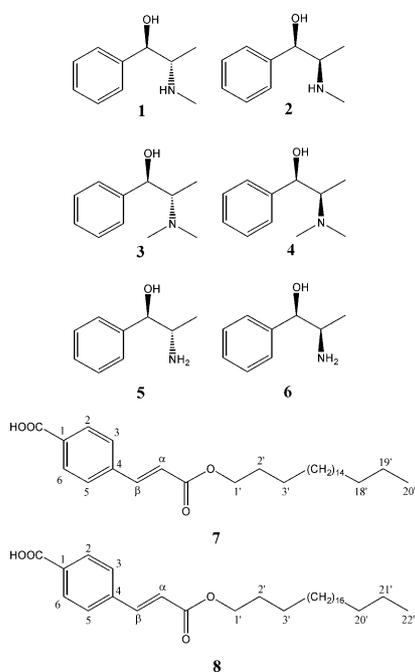


Fig. 1. The structures of the ephedrine alkaloids and two new compounds isolated from *E. sinica*.

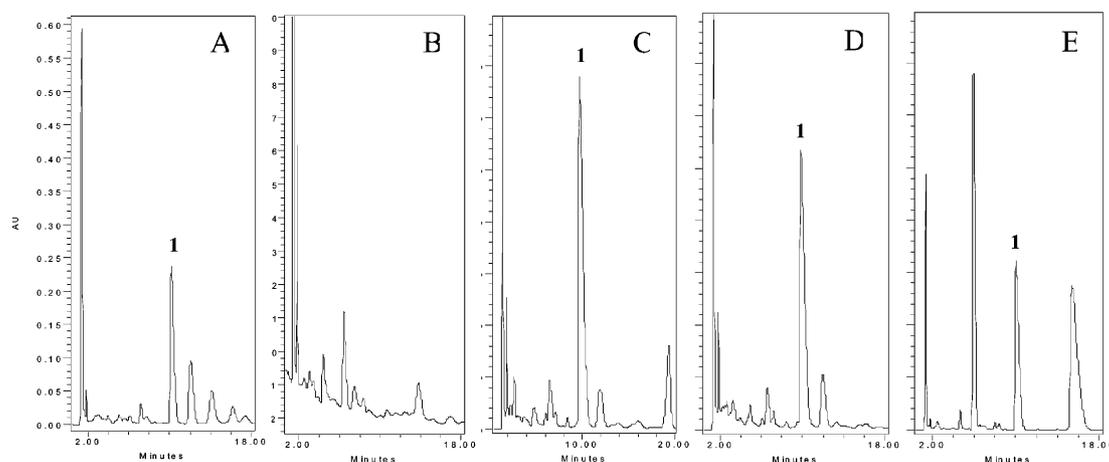


Fig. 2. The HPLC chromatograms obtained by an alkaloid method: an extract of *E. sinica* (A), an extract of *E. aspera* (B), an extract of a dietary supplement only containing *E. sinica* (C), an extract of a dietary supplement containing *E. sinica* as part of a blend of herbals (D) and an extract of *G. biloba* spiked with ephedrine alkaloids (E).

E. intermedia, *E. gerardiana*, *E. alata*, *E. distachya*, *E. botschantzevii*, *E. fragilis*, *E. major*, *E. minuta*, *E. monosperma*, *E. pachyclada*, *E. likiangensis*, *E. saxatilis*, *E. lomatolepis*, *E. lepidosperma*, *E. przewalskii* and *E. regeliana* (Caveney et al., 2001). Although three articles have been published which claim the presence of ephedrine alkaloids in some North and South American species of *Ephedra*, the results remain unconfirmed (Caveney et al., 2001; Willaman and Schubert, 1964; Wink, 1998). Currently, the *Ephedra* species of the Americas are considered devoid of the ephedrine alkaloids (Caveney et al., 2001).

Due to the number of people consuming *Ephedra* and the number of adverse events reported to the Food and Drug Administration (FDA), continued research is needed to ensure quality of the products being sold. Several factors may have contributed to the adverse effects. These include consumer misuse, manufacturer abuse and contraindication, hypersensitivity and/or drug interaction. Of the three, manufacturer abuse deals with the spiking of *Ephedra* plant material and/or products with synthetic stimulants or the synthetic ephedrine alkaloids (Ross et al., 1999). Methamphetamine (MDMA) is easily synthesized from ephedrine, and may be one of the spiking adulterants present in a product (Skinner, 1990).

At this time a number of methods have been reported for the quantitation of the ephedrine alkaloids present in some *Ephedra* species: chiral gas chromatography

(Betz et al., 1997), capillary electrophoresis (Chinaka et al., 2000), high performance liquid chromatography with UV detection (Gurley et al., 1998) and liquid chromatography with mass spectrometry detection (Gay et al., 2001). Although important, these methods are each limited in their ability to ensure identity of the plant material being used in the product. Thus, an HPLC method is here in reported where a chemical fingerprint was developed for a number of *Ephedra* species, some known to contain ephedrine alkaloids and some known to be ephedrine alkaloid free. The creation of the chemical fingerprint also led to the isolation of two new compounds, **7** and **8**.

2. Results and discussion

For illustrative purposes, five different samples were analyzed with an in house HPLC ephedrine alkaloid quantitative method. The five samples were *E. sinica* ground plant material (A), *E. aspera* ground plant material (B), a dietary supplement claiming to contain only *E. sinica* (C), a dietary supplement containing *E. sinica* as part of a herbal blend (D) and a dietary supplement containing *Ginkgo biloba* spiked by us with **1** (E). As shown in Fig. 2, it is difficult to detect which of the products contains *Ginkgo* spiked with **1**. The peak representing **1** is marked in each chromatogram. On the

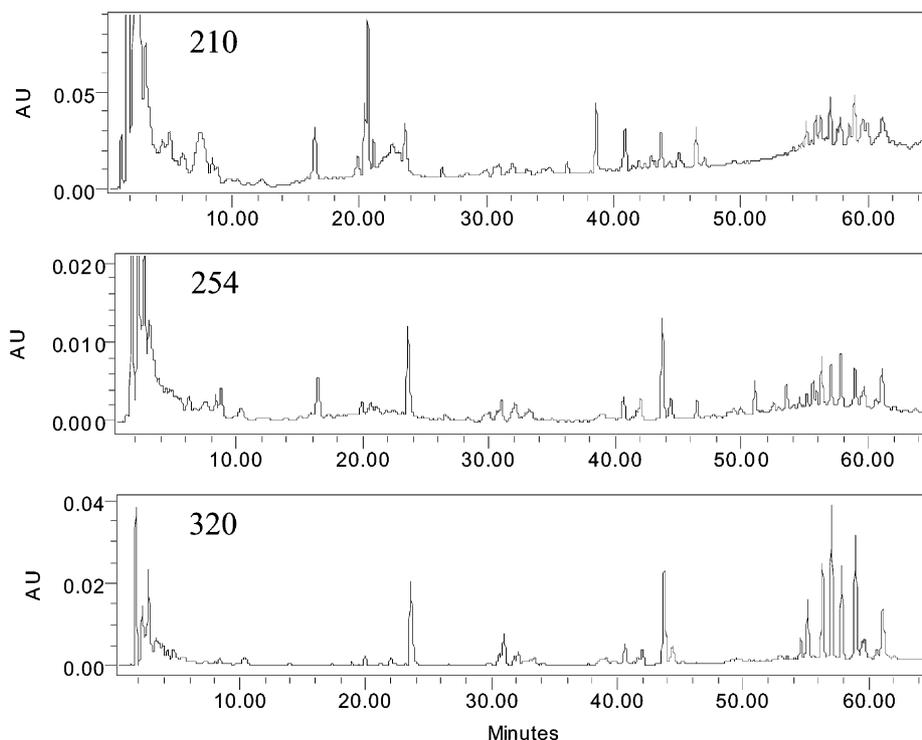


Fig. 3. HPLC chromatograms of *E. sinica* detected at 210, 254 and 320 nm.

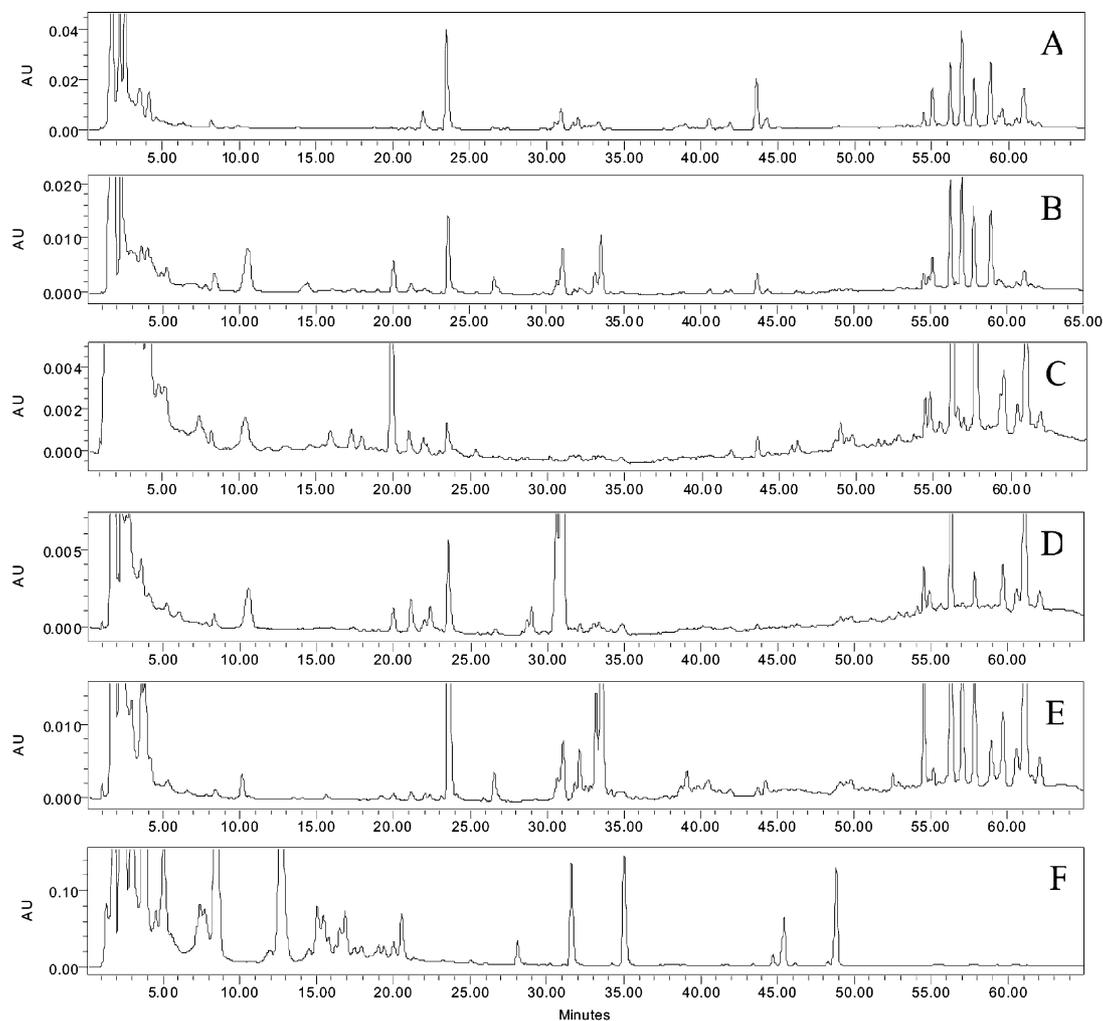


Fig. 4. HPLC comparison of chromatograms at 320 nm of *E. sinica* (A), *E. gerardiana* (B), *E. nevadensis* (C), *E. fominea* (D), *E. distachya* ssp. *helvetica* (E) and *G. biloba* spiked with ephedrine alkaloids (F).

same note, most would not realize chromatogram B was indeed an *Ephedra* species due to the lack of ephedrine (I) detection.

Although the quantitation method is useful for the standardization of the ephedrine alkaloids to ensure quality in a product by making sure the ephedrine alkaloids are present and not in quantities above the maximum daily dose, the method falls short of *Ephedra* plant authentication. Many companies purchase bulk ground plant material from an outside source, but do not have a method for the authentication of the material or do not receive authentication information from the supplier. Although they will analyze the material for ephedrine alkaloid content, they may not have a method for *Ma Huang* confirmation. Quality concerns can be overcome by simple testing of the starting plant material. It can determine if the plant is in fact *Ephedra*, which then can be used to determine if another plant species had been spiked with ephedrine alkaloids. At the same time a plant authentication method can distinguish

between species within the genus. The chemical fingerprint method developed in this study was able to verify an *Ephedra* species present in ground plant material and it was able to distinguish between the *Ephedra* species grown in North America, South America and Eurasia.

Initial fingerprint development began by extracting authenticated *E. sinica* with ethanol. The extract was then analyzed on a series of stationary phases with a gradient solvent system (95 water/5 acetonitrile to 100 acetonitrile) over 1 h. Three UV wavelengths were analyzed: 210, 254 and 320 nm. Regardless of the stationary phase, a level baseline could not be achieved. This was also the case after changing the mobile phase gradient and percentages. Since a level baseline was not achieved with ethanol, the extraction solvent was changed to acetone. Due to the decrease of co-extractives with acetone, an optimal system for HPLC was achieved. The acetone extract was analyzed on a Waters XTerra RP₁₈ column. A level baseline was achieved. An optimal mobile phase gradient was then determined for the fingerprint. A

comparison of the chromatograms at 210, 254 and 320 nm found the absorbance at 320 nm to be the most advantageous (Fig. 3).

Once the fingerprint method had been developed through the use of authenticated plant material, other species of *Ephedra* had to be tested to ensure its usefulness. All species of *Ephedra* tested are listed in the experimental section. A comparison of the fingerprints of a number of the *Ephedra* species obtained is shown in Fig. 4. The first five chromatograms are of *Ephedra* species, while the sixth chromatogram is of an ephedrine alkaloid spiked *G. biloba* extract. A series of peaks

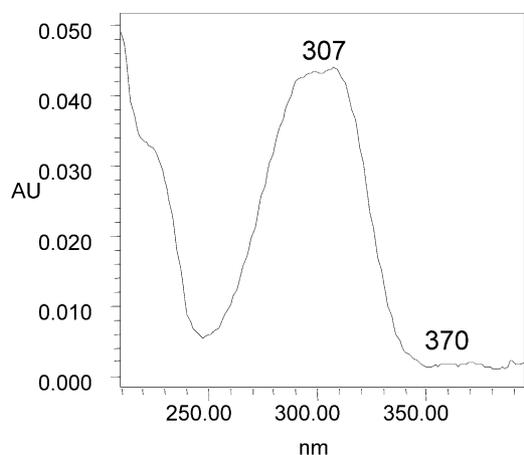


Fig. 5. The UV spectrum of compounds 7 and 8.

between 52 and 64 min are of interest. The UV spectrum for peaks 7 and 8 are the same and shown in Fig. 5. All *Ephedra* species analyzed contained this series of peaks between 52 and 64 min with slight variation between the species in the Americas and Eurasia. More importantly, those peaks present at 320 nm in the *Ephedra* species were not present in the ephedrine alkaloid spiked *G. biloba* extract (Fig. 4). The region was also absent in the acetone extract of Ginseng (data not shown). This shows the fingerprint is capable of determining if ground plant material is from an *Ephedra* species. The North American and South American species of *Ephedra* could also be distinguished from the Eurasian species of *Ephedra* (Fig. 6). The differences are marked in Fig. 6.

The fingerprint method was validated by testing a number of populations within a single species of *Ephedra*. Fig. 7 compares the chromatograms obtained from four different populations of *E. trifurca*. The key regions are present in all four chromatograms, and the chromatograms all have the same relative features. *E. trifurca* was collected in populations from Texas and New Mexico.

The development of the fingerprint method led to the isolation of two new compounds, 7 and 8 from *E. sinica*. Both compounds were isolated by high performance flash chromatography and prep HPLC. The representative peaks are shown in Fig. 6 and the structures in Fig. 1.

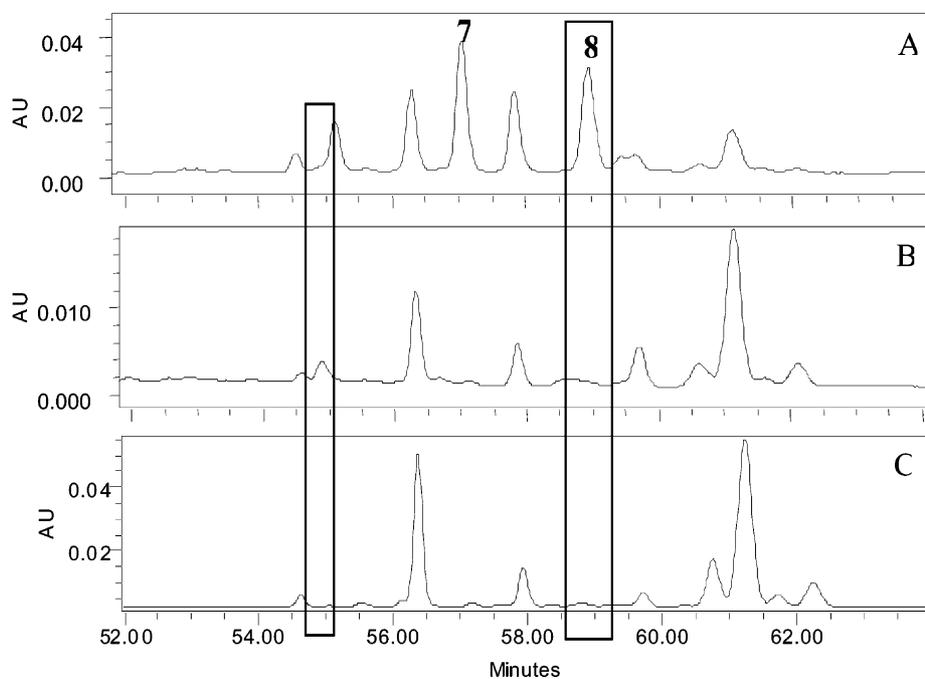


Fig. 6. HPLC chromatogram comparison of the expansion of the region between 52 and 64 min of *E. sinica* from Eurasia (A), *E. trifurca* from North America (B) and *E. ochreata* from South America (C).

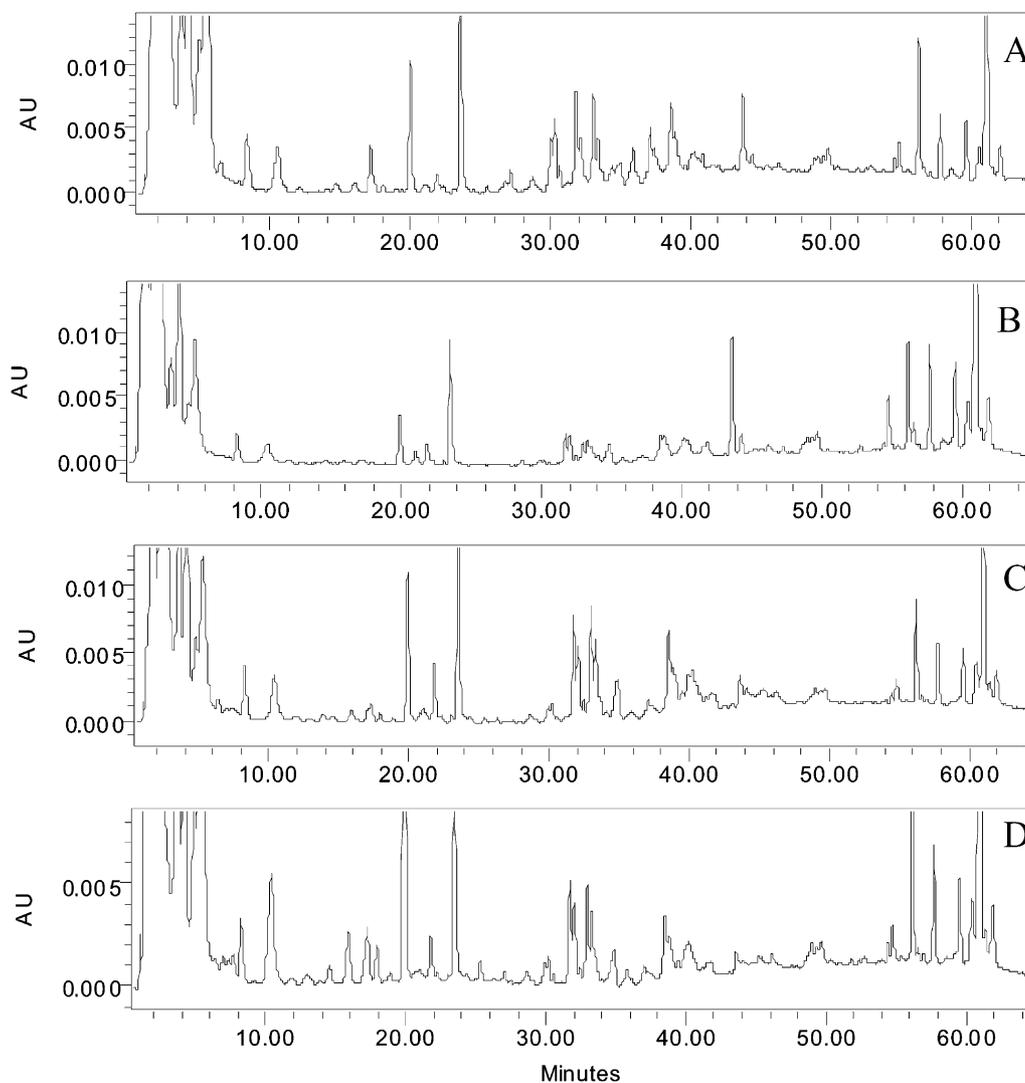


Fig. 7. HPLC chromatograms of four *E. trifurca* populations.

3. Conclusions

The availability of analytical methods for the analysis of ground plant material is paramount in ensuring quality in dietary supplement and herbal products. It is possible a manufacturer will purchase in bulk, ground plant material, but will only have the word of the supplier for sample authentication. As a powder, visual authentication of the plant is not possible. With this fingerprint method, manufacturers as well as consumer groups can test the ground plant material to confirm or deny the presence of an *Ephedra* species. This will help stop possible spiking of plant material other than *Ephedra* with synthetic ephedrine as well as spiking of a species of *Ephedra* known to be devoid of the ephedrine alkaloids. Chemical fingerprinting is only one step towards ensuring quality in dietary supplements.

4. Experimental

4.1. Plant material

E. antisiphilitica, *E. aspera*, *E. californica*, *E. foeminea*, *E. coryi*, *E. cutleri*, *E. equisetina*, *E. fasciculata*, *E. nevadensis*, *E. pendunculata*, *E. torreyana* ssp. *powelliorum*, *E. torreyana*, *E. trifurca*, *E. viridis*, *E. aspera* × *E. trifurca*, *E. distachya*, *E. distachya* ssp. *helvetica*, *E. equisetina*, *E. gerardiana*, *E. intermedia*, *E. major*, *E. ochreatea*, *E. sinica* and *E. triandra* were studied and voucher specimens are stored at The University of Mississippi Herbarium (MISS).

4.2. Solvents

Acetonitrile, acetone, absolute ethanol and methanol were purchased from Fisher Scientific (Fair Lawn, NJ)

and nanopure water was prepared by filtering distilled water through a 45 μm filter.

4.3. Sample preparation

Approximately 500 mg of ground plant material was placed in a Falcon Blue Max Jr. 15 ml polypropylene conical tube (Becton Dickinson Labware, Franklin Lakes, NJ) with 6.0 ml of acetone and sonicated 15 min in a Fisher Scientific Ultrasonic Cleaner (Pittsburgh, PA). After sonication, the sample was centrifuged for 10 min in a Centricone (Precision Scientific, Chicago, IL). The supernatant was transferred by pipette to a borosilicate 6 Dram sample vial (VWR, South Plainfield, NJ). The extraction was repeated two more times and the respective supernatants were combined and the acetone removed (Savant Speed Vac Plus SC210A with a Savant Refrigerated Vapor Trap, Holbrook, NY). To the dried extract were added 5.0 ml of absolute ethanol. Once the extract had dissolved, 2.0 ml were filtered (the first 0.5 ml were discarded) through a 45 μm Nylon filter (Phenomenex, Torrance, CA) into an HPLC borosilicate sample vial (VWR, South Plainfield, NJ) for analysis.

4.4. HPLC fingerprinting

HPLC/PDA analysis was performed on a Waters Alliance 2695 Separations Module with a Waters 996 Photodiode Array Detector (Waters, Milford, MA, USA). The column was a Waters XTerra RP₁₈ 5 μm (150 \times 4.6 mm, 5 μm particle size) (Waters, Milford, MA, USA). The mobile phase consisted of nanopure water (A) and acetonitrile (B). After the column was equilibrated to 40 °C, the gradient mobile phase began by holding at 75A/25B for 10 min. Then the system mixture changed to 0A/100B over 45 min and was held at 100B for 10 min. Total run time was 65 min. The injection volume was 10 μl and the flow rate was 1.00 ml/min. Sample analysis was processed by Waters Millennium³² software (Waters, Milford, MA, USA).

4.5. Isolation method

Plant material (*E. sinica*; 450 g) was extracted with acetone (1 l) by sonication for 30 min and filtered. The extraction procedure was carried out a total of four times and the filtrates were combined. The filtrate was concentrated to dryness in vacuo (14.1 g). An aliquot of the extract (1.50 g) was applied to Horizon Biotage Inc. (Biotage, Inc., Charlottesville, VA, USA), HPFC system (High Performance Flash Chromatography) using normal phase silica gel (Si 40M; Flow rate: 15 ml/min), eluted with *n*-hexane (200 ml), *n*-hexane–EtOAc mixtures (90:10; 80:20; 70:30; 60:40; 50:50; each 200 ml), *n*-hexane–EtOAc–MeOH mixtures (50:50:5; 50:50:10:

50:50:20; each 200 ml), and MeOH (0.5 l). According to HPLC profiles, the fractions 1–20 were pooled together and subjected to Prep HPLC. The new compounds **7** and **8** were isolated by preparative HPLC on a Waters Alliance 2695 Separations Module with a Waters 996 Photodiode Array Detector (Waters, Milford, MA, USA). The column was a Waters XTerra RP₁₈ 10 μm (250 \times 10 mm, 10 μm particle size) (Waters, Milford, MA, USA). The isocratic mobile phase consisted of nanopure water (A), acetonitrile (B) and methanol (C) [5:85:10] at 3.0 ml/min. After the column temperature reached 40 °C, 100 μl were injected and detection took place at 320 nm. Compounds **7** and **8** were collected separately. The mobile phase in each fraction was removed to give a white solid (2.0 and 1.5 mg respectively) for ¹H and ¹³C NMR analysis.

4.6. Fingerprint validation

The extraction method listed above used a polypropylene tube as the extraction vessel. Five points can be made to confirm polymer contamination was not responsible for the absorbances between 52 and 64 min. First, species from Eurasia, North America and South America had different profiles in the same region. Second, in both the ginkgo and ginseng extracts the peaks were not detected. At the same time the large scale isolation of the new compounds took place in the absence of polypropylene. Also, the small scale extraction was carried out in a glass vessel with acetone and a blank extraction with acetone was carried out in the polypropylene tube. In all three cases, the peaks shown in the *Ephedra* fingerprint were not detected.

4.7. Structure elucidation data

4.7.1. Compound **7**

4-(2-Eicosyloxycarbonyl-vinyl)-benzoic acid; Negative HRESIMS; m/z 471.3900 [M–H][–] (calc. for C₃₀H₄₈O₄ 472.3553); ¹H NMR data (500 MHz, in C₅D₅N); 7.90 (1H, *d*, $J=15.8$ Hz, H- β), 7.61 (2H, *d*, $J=7.5$ Hz, H-2 and H-6), 7.13 (2H, *d*, $J=7.5$ Hz, H-3 and H-5), 6.60 (1H, *d*, $J=15.8$ Hz, H- α), 4.25 (2H, *t*, $J=6.5$ Hz, H-1'), 1.65 (2H, *p*, $J=6.8, 13.9, 14.1$ Hz, H-2'), 1.23–1.25 [(CH₂)_{*n*}: H₂-3'→H₂-19'), and 0.82 (3H, *t*, $J=6.0$ Hz, H-20').

4.7.2. Compound **8**

4-(2-Docosyloxycarbonyl-vinyl)-benzoic acid; Negative HRESIMS; m/z 499.4210 [M–H][–] (calc. for C₃₂H₅₂O₄ 500.3866); ¹H NMR data (500 MHz, in C₅D₅N + DMSO-*d*₆); δ 9.95 (1H, *brs*, COOH), 7.59 (1H, *d*, $J=15.9$ Hz, H- β), 7.53 (2H, *d*, $J=8.4$ Hz, H-2 and H-6), 6.87 (2H, *d*, $J=8.4$ Hz, H-3 and H-5), 6.36 (1H, *d*, $J=15.9$ Hz, H- α), 4.14 (2H, *t*, $J=6.6$ Hz, H-1'), 1.67 (2H, *p*, $J=6.8, 13.9, 14.1$ Hz, H-2'), 1.28–1.30 [(CH₂)_{*n*}:

H₂-3'→H₂-21'), and 0.88 (3H, *t*, *J*=6.0 Hz, H-22'); ¹³C-NMR data (125 MHz, in C₅D₅N and DMSO-*d*₆): δ^{167.2} (*s*, COO), 162.0 (*s*, COOH), 145.1 (*d*, C-β), 130.5 (×3) (*s*, C-1; *d*, C-3 and C-5), 125.9 (*s*, C-4), 116.4 (×2) (*d*, C-2 and C-6), 114.8 (*d*, C-α), 64.2 (*t*, C-1'), 32.1 (*t*, C-21'), 29.1–30.2 [*t*, (CH₂)_n: C-3'→C-19'], 26.2 (*t*, C-2'), 22.8 (*t*, C-20'), 14.1 (*q*, C-22').

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References

- Bensky, D., Gamble, A., 1993. Chinese Herbal Medicine: Materia Medica. Eastland Press, Seattle.
- Betz, J.M., Gay, M.L., Mossoba, M.M., Adams, S., 1997. Chiral gas chromatographic determination of ephedrine-type alkaloids in dietary supplements containing *Ma huang*. *J. AOAC Int.* 80, 303–315.
- Cardellina, J.H., 2002. Challenges and opportunities confronting the botanical dietary supplement industry. *J. Nat. Prod.* 65, 1073–1084.
- Caveney, S., Charlet, D.A., Freitag, H., Maier-Stolte, M., Strarratt, A.N., 2001. New observations of the secondary chemistry of world *Ephedra* (Ephedraceae). *Am. J. Bot.* 88, 1199–1208.
- Chen, K.K., Schmidt, C.F., 1926. The action and clinical use of ephedrine. *J. Am. Pharm. Assoc.* 87, 836–842.
- Chinaka, S., Tanaka, S., Takayama, N., Komai, K., Ohshima, T., Ueda, K., 2000. Simultaneous chiral analysis of methamphetamine and related compounds by capillary electrophoresis. *J. Chrom. B* 749, 111–118.
- Fouad-Tarazi, F.M., Okabe, M., Goren, H., 1995. Alpha sympathomimetic treatment of autonomic insufficiency with orthostatic hypotension. *Am. J. Med.* 99, 604–610.
- Food and Drug Administration, 1997. Dietary supplements containing ephedrine alkaloids. Federal Register 62, 30677–30724.
- Department of Health and Human Services, 2000. Office of women's health; notice of public meeting on the safety of dietary supplements containing ephedrine alkaloids. Federal Register 65, 43021–43023.
- Fetrow, C.W., Avila, J.R., 1999. Complementary & Alternative Medicines. Springhouse, Pennsylvania.
- Gay, M.L., White, K.D., Obermeyer, W.R., Betz, J.M., Musser, S.M., 2001. Determination of ephedrine-type alkaloids in dietary supplements by LC-MS using stable-isotope labeled internal standard. *J. AOAC Int.* 84, 761–769.
- Gurley, B.J., Wang, P., Gardner, S.F., 1998. Ephedrine-type alkaloid content of nutritional supplements containing *Ephedra sinica* (ma-huang) as determined by high performance liquid chromatography. *J. Pharm. Sci.* 87, 1547–1553.
- Haller, C.A., Benowitz, N.L., 2000. Adverse cardiovascular and central nervous system events associated with dietary supplements containing *Ephedra* alkaloids. *N. Engl. J. Med.* 343, 1886–1887.
- Leung, A.Y., Foster, S., 1996. Encyclopedia of Common Natural Ingredients, 2nd Ed. John Wiley & Sons, New York.
- Price, R.A., 1996. Systematics of the *Gnetales*: a review of morphological and molecular evidence. *Int. J. Plant Sci.* 157, S40–S49.
- Ross, J.J., Pelders, M.G., De Smeet, P.A., 1999. A case of positive doping associated with a botanical food supplement. *Pharm. World Sci.* 21, 44–46.
- Skinner, H.F., 1990. Methamphetamine synthesis via hydriodic acid/red phosphorus reduction of ephedrine. *Forensic Sci. Int.* 48, 123–134.
- Tyler, V.E., 1999. Tyler's Honest Herbal. Haworth Herbal Press, New York.
- Walker, R.B., Dholakia, V.N., Brasfield, K.L., Bakhitiar, R., 1998. Effect of hydroxypropyl-β-cyclodextrin on the central stimulant activity of (–)-ephedrine and oxazolidine prodrug in rats. *Gen. Pharmacol.* 30, 725–731.
- Willaman, J. J., Scheubert, B. G., 1961. Alkaloid-bearing plants and their contained alkaloids. Agricultural Research Service, United States Department of Agriculture, Technical Bulletin 1234, Washington, DC, USA.
- Wink, M., 1998. A short history of alkaloids. In: Roberts, M.F., Wink, M. (Eds.), Alkaloids: Biochemistry, Ecology and Medicinal Applications. Plenum, New York, pp. 11–44.
- Zaaks, S.M., Klein, L., Tan, C.D., Rodriguez, E.R., Leikin, J.B., 1999. Hypersensitivity myocarditis associated with *Ephedra* use. *Clin. Toxicol.* 37, 485–489.