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GC-MS ANALYSIS OF THE SUPERCRITICAL CO_2 FLUID EXTRACTION OF *Ephedra sinica* ROOTS AND ITS ANTISUDORIFIC ACTIVITY

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Herba ephedrae (Mahuang in Chinese) has been used as an important medicinal herb in China for more than 5000 years. It is famous for its content of alkaloids of the ephedrine series, which are responsible for the perspiratory, antitussive, and anti-allergic effects of the crude drug. *Ephedra sinica* was thought to be of the best quality by Chinese traditional pharmacologists in *Ephedra* plants (Ephedraceae). Many studies on the chemical constituents of *E. sinica* and other *Ephedra* species, including alkaloids, flavonoids, lipids, and essential oils, have been previously reported [1–4]. The underground parts of *Ephedra* plants were said to have therapeutic effects opposite to those of "Mahuang" (aerial parts of *Ephedra* plants are usually used) and have been used as an antiperspirant in oriental medicine. Compared to Herba ephedrae, few studies on the chemistry of *Ephedra* roots have been carried out, and only a few surveys for the hypotensive principles of *Ephedra* roots have recently been reported, leading to the isolation of the macrocyclic spermine alkaloids ephedradines A, B, C, and D as active constituents [5, 6]. We now report on the determination of the main constituent of the supercritical CO_2 fluid extract of *E. sinica* roots by GC-MS of analysis. The antisudorific activities of these extracts are also reported, with the aim of giving an exploratory explanation about the opposite effects of Mahuang and its roots. To the best of our knowledge, this is the first report of the GC-MS analysis of *E. sinica* roots.

The roots of *E. sinica* were collected at the end of September, 2006 from Antu County, Jilin Province, dried, stored at room temperature, and subsequently cut into small pieces for extraction. These samples were identified and voucher specimens were deposited in the key Laboratory of Chinese Medicine, Chinese Academy of Sciences, Jilin Province.

The supercritical fluid extraction of *E. sinica* roots was achieved in the SFE system; 1.0 kg plant material (dried) was placed into the extraction cell and extracted with supercritical CO_2 modified by 10% ethanol. Supercritical conditions were: 25 Mpa as the extraction pressure, 50°C as the extraction temperature, 90 minutes as the extraction time, 8 Mpa the as separation pressure, and 35°C as the separation temperature [7–12]. The percentage yield of the extract was 1.35%, and its color was light yellow brown. The identified constituents of the extract by GC-MS are listed in Table 1.

The GC-MS analysis of the roots of *E. sinica* led to the identification and quantification of a total of 30 main compounds (Table 1), and these compounds accounted for 94.93% of the total components present. γ-Sitosterol (28.55%) and 9*Z*,12*Z*-octadecadienoic acid (17.71%) were the main components of the essential oil; the other components of the essential oil of *E. sinica* roots were 9*E*-octadecenoic acid (7.99%), ergost-5-en-3β-ol (7.51%), *n*-hexadecanoic acid (5.2%), 9*Z*-hexadecenoic acid methyl ester (3.37%), 1-eicosanol (4.72%), 9-octadecenoic acid methyl ester (2.33%), linoleic acid (2.11%), and ergost-4-en-3-one sitostenone (1.53%).

According to previous reports on the chemical components of the supercritical extract from *E. sinica* [12], the GC-MS analysis of the aerial parts of *E. sinica* showed 47 main compounds. *n*-Hexadecanoic acid (24.043%) and linolenic acid (21.287%) were the main components of the extract; the other components of the extract were linoleic acid (10.72%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (9.716%), cinnamic acid (6.121%), octadecanoic acid (4.421%), and eicosanoic acid (3.022%). The results showed that the components of the supercritical extracts of *E. sinica* aerial parts were different from those of the roots.

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TABLE 1. Identification of the Components of the Supercritical Extract of *Ephedra sinica* Roots

Compound	Relative content, %	Compound	Relative content, %
1-Tetradecanol	0.83	Methyl (*Z*)-5,11,14,17-eicosatetraenoate	0.95
11-Hexadecenoic acid methyl ester	0.15	1-Eicosanol	4.72
9-*Z*-Hexadecenoic acid methyl ester	0.21	Eicosanoic acid methyl ester	0.39
Palmitic acid methyl ester	3.37	Docosanol	2.74
n-Hexadecanoic acid	5.2	Docosanoic acid methyl ester	0.78
Palmitic acid ethyl ester	0.69	Docosanoic acid ethyl ester	0.11
n-Octadecanol	0.98	Tricosanoic acid methyl ester	0.33
Linoleic acid methyl ester	2.11	Tetracosanol	1.11
9-Octadecenoic acid methyl ester	2.33	Tetracosanoic acid methyl ester	0.51
11-*Z*-Octadecenoic acid methyl ester	1.81	Stigmast-5-en-3-ol, oleate	0.48
Phytol	0.19	Nonacosanol	5.07
Octadecanoic acid methyl ester	0.34	α-Tocopherol	0.51
9*Z*,12*Z*-Octadecadienoic acid	17.71	Ergost-5-en-3β-ol	7.51
9-*E*-Octadecenoic acid	7.99	γ-Sitosterol	28.55
Ethyl oleate	0.8	Ergost-4-en-3-one sitostenone	1.53

TABLE 2. The Components of the Supercritical Extract of *Ephedra sinica* Aerial Parts

Compound	Relative content, %	Compound	Relative content, %
Diethylsulfate	0.540	3,3,8,8-Tetramethyltricyclo[5,1,0,0,2,4]-oct-5-ene-5-propanoic acid	0.426
A-Limonene	0.271	Tetradecanoic acid	0.369
2-Pyrrolidinone	0.779	(1,5,5,8-Tetramethylbicyclo[4,2,1]-9-nonyl)-acetic acid	0.587
2,3,5,6-Tetramethylpyrazine	1.403	4-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one	0.474
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1.551	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	9.716
Benzoic acid	2.204	Hexahydrofarnesyl acetone	0.652
L-α-Terpineol	1.015	Butyloctylphthalate	0.230
γ-Terpineol	0.329	7,11-Hexadecadienal	0.766
5-(Hydroxymethyl)-2-furancarboxaldehyde	0.723	1-(1-Oxobutyl)-1,2-dihydropyridine	0.528
trans-2-*p*-Menthen-7-ol	0.356	1-Heptatriacotanol	0.982
(*E*)-Cinnamaldehyde	0.570	Nerolidyl acetate	0.512
4-(1-Methylethyl)-1-cyclohexene-1-carboxaldehyde	0.282	*n*-Hexadecanoic acid	24.043
Cumic alcohol	0.310	Palmitic acid ethyl ester	0.491
1,8-Terpin	1.018	Falcarinol-(*Z*)-1,9-heptadecadiene-4,6-diyn-3-ol	0.740
β-Terpineol	0.277	Phytol	0.378
3-Hydroxy-4-methoxymandelic acid	0.245	Linoleic acid	10.720
Cinnamic acid	6.121	Linolenic acid	21.287
2,3-Dimethyl-4-methoxyphenol	0.529	Octadecanoic acid	4.421
α-Curcumene	0.583	Oleic acid	0.601
β-Cedrene	0.343	Cyclopropane-butanoic acid,2[2,1]-2-pentylcyclopropyl)-methylcyclopropyl-cyclopropyl-methyl ester	0.841
Dihydroactinidiolide	0.426	α-Glyceryl-linolenate	0.949
Frambinone	0.915	Eicosanoic acid	3.022
Rhododendrol	1.205		
Eremanthin	0.400		
Syringaldehyde	0.238		

To observe the effect of the supercritical extract of *E. sinica* roots on mice anti-hydropoiesis function, 40 mice were divided randomly into three different dose groups and control groups after duplication of the corlin-induced kidney-yang-deficiency mouse model and given the extract for 6 successive days. We obtained sweat glands of the soles and count the sweat gland vacuole cells and normal sweat gland cells. The extract significantly reduced the number of sweat gland vacuole cells ($P<0.01$) compared with the control group. The results indicate that the supercritical extract of *E. sinica* roots reduced sweat excretion by alterating the form of the sweat gland cell.

In this report, using the supercritical extract of *E. sinica* roots as study subject, we investigate its pharmacodynamic effect; the results showed that the supercritical extract of *E. sinica* roots displayed strong antisudorific activities. These results show the difference in the chemical components of the supercritical extract of the aerial parts and the roots of *E. sinica*, which is perhaps concerned with the perspiratory function of Mahuang and the antiperspirant effect.

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