

COMPARISON OF VOLATILE COMPONENTS FROM *MARCHANTIA CONVOLUTA* OBTAINED BY MICROWAVE EXTRACTION AND PHYTOSOL EXTRACTION

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ABSTRACT

The volatile oils from *Marchantia convoluta* were obtained by microwave extraction (ME) and phytosol extraction (PE) methods. The attained extracts were analyzed by gas chromatography with mass spectrometric detector (GC-MS). The compounds were identified according to their retention indices and mass spectra (EI, 70 eV). A total of fourteen compounds were identified in ME extract accounting for 80.72% of total peak area. Isolongifolene (24.588%), 1, 2-benzenedicarboxylic acid, butyl cyclohexyl ester (10.768%), pyrene (9.328%) and hexadecanoic acid, ethyl ester (8.570%) were the major compounds identified from ME extract. The analytical results of ME extract were compared with PE method. The ME extract was found to be markedly different from that of PE extract. A total of seven compounds in PE extract were identified accounting for 74.275% total peak area. 1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10, 10a-octahydro-1, 4a-dimethyl-7-(1-methylethyl) methyl ester were the major compounds.

INTRODUCTION

Marchantiaceae plants are well-known traditional Chinese medicinal herbs and extensively used to treat tumefaction of skins, protect liver and treat hepatitis and used as antipyretic in countryside¹⁻³. There are a large number of Marchantiaceae plants in Guangxi Zhuang Autonomous District such as *Marchantia polymorpha*, *M. convoluta* and *M. paleacea*. These species grow together and it is difficult to distinguish one from the others because of their genetic similarity. *M. convoluta* was only found in China⁴.

The major identified constituents in *M. convoluta* were flavonoids, triterpenoids, and steroids⁵⁻⁹. The dried leaves were used in China to protect the liver and to treat tumefaction of skins. A high dosage of flavonoids from *M. convoluta* (20 and 40 µg/mL) can significantly reduce the activity of ALT (Alanine aminotransferase) and AST (Alanine aminotransferase) in the serum of mice with acute hepatic injury caused by CCl₄ and increase the contents of TP (Total protein) and ALP (Alkaline phosphatase), as well as inhibit the auricle tympanites of mice caused by dimethylbenzene¹. Flavonoids from *M. convoluta* strongly inhibited bacteria, anti-inflammation and diuresis in mice¹, as well as flavonoids from *M. convoluta* have anti-hepatitis B Virus activity². The ethyl acetate extract from *M. convoluta* strongly inhibited tumors in human liver and lung cancer cell lines^{3, 11}. Flavonoids from *M. convoluta* strongly inhibit *Staphylococcus aureus*^{1, 10}.

The microwave and phytosol extraction methods for extracting essential oils have attracted great interest among researchers¹²⁻¹⁴. The main virtue of the microwave technique is cost-effective and simple. In recent years, head space solid phase micro-extraction (HS-SPME) has gained wide acceptance as an effective extraction technique for a wide variety of samples¹⁵⁻¹⁶. HS-SPME is performed by exposing a fiber coated with single or multiple polymers to the headspace of a sample matrix until equilibrium is reached between the analyte partitioned in the fiber coating and the analyte in the sample matrix¹⁷⁻¹⁸.

Phytosol solvents are a new range of benign, non-CFC solvents, commercially available for the extraction of analytes from natural products, foods and environmental matrices¹⁹. Phytosol extraction (PE) is a new portable cold extraction technique without heat.

The aim of the present work was the investigation of the volatile components from *M. convoluta* obtained by Microwave-assisted solid phase micro-extraction (MPME) and Phytosol extraction (PE).

Materials and methods

Plant materials

The whole plants of *M. convoluta* were collected in Shangling City of Guangxi Zhuang Autonomous District in August 2003. The specimen (No. 20041364) was identified by Zhou Zi-jing, at the Biology Department of Guangxi Chinese Medical University. The dried leaves were stored in a dark place at 4 °C for 20 d. Immediately prior to the extraction process, the leaves were ground in a blender to produce a powder with an approximate size of 0.4 mm.

Reagents

Analytical grade methanol was purchased from Hanbon Company Limited (Jiangsu, China). Carbon dioxide (99.99% purity), contained in a cylinder with an educator tube, was obtained from CSU (Changsha, China). Phytol, cedrol, stigmasterol, 22, 23-dihydro-stigmasterol, and other organic compounds used to identify in GC/MS, were purchased from Sigma (USA). Phytosol solvent

(99.9% purity) was purchased from Hua Xue Company.

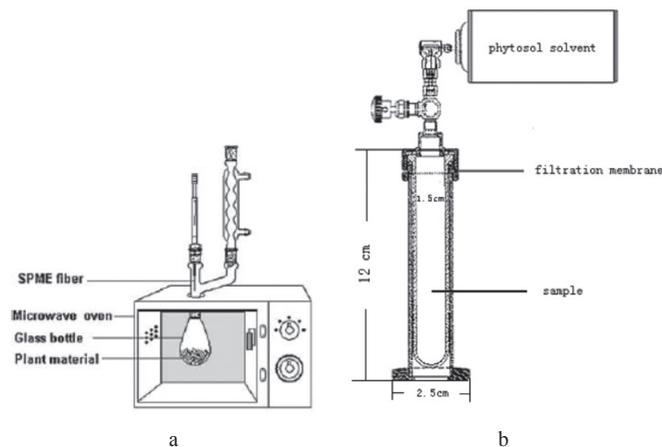


Fig. 1. The equipments of ME(a) and PE(b)

Apparatus

The home-made microwave extraction apparatus was illustrated in Fig. 1. The microwave oven (WD800CTL23-2H, maximum delivered power 650 W) was purchased from Galanz. The self-made phytosol solvent extraction apparatus was illustrated in Fig. 2.

Microwave extraction (ME)

Since various parameters potentially affected the extraction process, the optimization of the experimental conditions represents a critical step in the development of a microwave extraction method. The optimized condition was following: 10 g of dried materials was soaked in 200 ml acetic ester for 24 h, and irradiated in microwave oven for 120 s. The extraction was repeated for three times and the extracted solvent was collected in beaker after filtration

Phytosol extraction (PE)

A known quantity of powdered *M. convoluta* (approximately 10 g) was placed in the extraction vessel with a filter adapter, and then the phytosol solvent (10 ml) was introduced into the vessel. The assembled extraction cell was shaken periodically for 1 h at room temperature. Then the extraction cell was inverted and the filter adapter was attached. By careful opening of the tap on the filter adapter, phytosol solvent containing the volatile components was collected in the sealed container. This solvent can cause freezing the glass collector. After standing for a few minutes in an ambient laboratory temperature, the phytosol solvent was removed using a recycling device that preferentially used to remove the solvent leaving in the collector. The extraction procedure was repeated three times per sample. Then the sample residues were dissolved in methanol, quantitatively transferred into a volumetric flask (25 ml) and made up to volume using methanol. Prior to analysis the sample was diluted 10 times.

6. Gas chromatography-mass spectrometry

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a FID and a HP-5 fused silica column (60 m × 0.25 mm i.d., 0.25 mm film thickness) with a 5% (mole fraction) phenyl-substituted methylpolysiloxane phase. Oven temperature was programmed 60 °C for 5 min, and then increased to 250 °C at a rate of 5 °C/min. Injector and detector temperatures were 250 and 265 °C, respectively. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s. The sample (1 µL) was injected into GC/MS using the split mode with a split ratio of 1/60. The ionization energy was 70 eV with a scan time of 1 s and mass range of 40–540 amu. The percentages of compounds were calculated by the area normalization method without considering response factors. The components of the oil were identified by comparison of their mass spectra with those of the spectrometer database using the Wiley 275 L mass spectral database or with authentic compounds. The identifications were confirmed by comparison of the fragmentation pattern and their retention indices with those reported in the literature^{20, 21}.

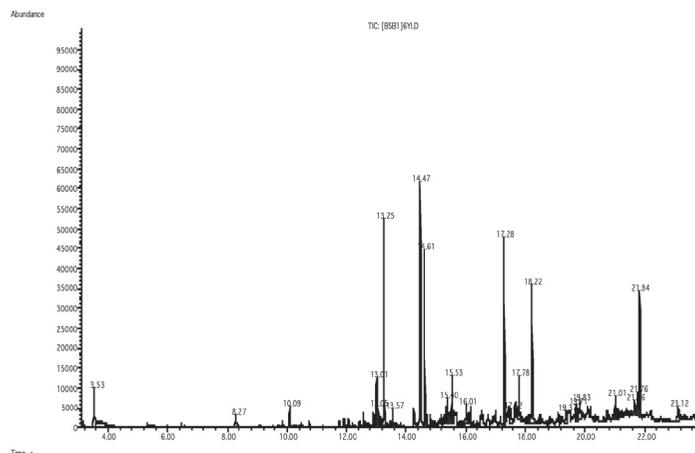


Fig. 2. GC-MS profiles of ME extract.

Table 1 GC-MS analytical results of *M. convoluta* oils obtained by ME

No.	Compound	t_R (min)	Match (%)	Relative amount (%)
1	2-Propenoic acid, butyl ester	3.53	91.2	3.343
2	2,6,11-trimethyl-Dodecane	10.09	85.1	1.773
3	Dodecanal	11.91	81.0	1.131
4	Eicosane	13.01	86.1	2.704
5	Nonadecane	13.05	75.2	1.424
6	2,4-bis(1,1-dimethylethyl)-Phenol	13.25	94.2	10.808
7	2,6,11,15-tetramethyl-Hexadecane	13.57	84.4	1.196
8	Hexadecane	14.25	78.5	1.138
9	Isolongifolene	14.47	70.9	24.588
10	Eicosane	16.01	83.1	2.653
11	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	17.28	90.6	10.768
12	2,6,10,15-tetramethyl-Heptadecane	17.52	82.6	1.296
13	Pyrene	18.22	90.9	9.328
14	Hexadecanoic acid, ethyl ester	21.84	89.6	8.570

Compounds were listed in order of elution time.

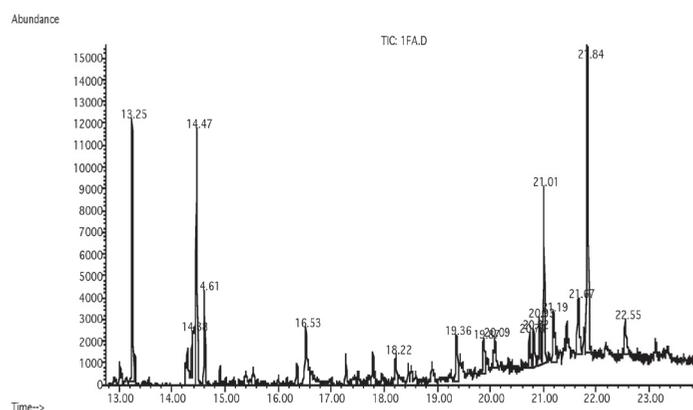


Fig. 3. GC-MS profiles of PE extract.

Table 2.- GC-MS analytical results of *M. convoluta* oils obtained by PE (compounds were listed in order of elution time)

No.	Compound	t_R (min)	Match (%)	Relative amount (%)
1	2,4-bis(1,1-dimethylethyl)-Phenol	13.25	93.3	9.856
2	Isolongifolene	14.47	61.8	11.74
3	9-methylene-9H-Fluorene	16.53	90.9	5.615
4	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	18.22	80.1	1.872
5	Pyrene	19.36	87.9	3.432
6	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5, 6,7,8,10,10a-dodecahydro-1,4a,7-trimethyl, methyl ester	21.01	73.7	7.552
7	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl), methyl ester	21.84	90.1	34.208

Compounds were listed in order of elution time.

RESULTS AND DISCUSSION

GC-MS analyses

The volatile components in *M. convoluta* were isolated, extracted, and concentrated by ME method and the analytes were then analyzed by GC-MS. The chemical components of the essential oil were identified from their retention indices and by mass spectral database search (Fig. 2). Twenty-three compounds were separated and fourteen compounds were identified by reference standards (Table 1). The peak area of compounds identified accounted for 80.72 % of total peak area. One of terpene, six of alkane, five of oxygen-containing compounds and one other compound were attained. The most abundant eight compounds were in the descending order: isolongifolene (24.588%), 2,4-bis(1,1-dimethylethyl)-phenol (10.808%), 1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester (10.768%), pyrene (9.328%), and hexadecanoic acid, ethyl ester (8.570%).

The components of essential oil obtained from *M. convoluta* by PE method were showed in Table 2. The chemical components of the essential oil were identified from their retention indices and by mass spectral database search (Fig. 3). It can be seen that, sixteen compounds were separated by HP-5 column and seven compounds were identified by reference standards. The peak area of compounds identified accounted for 74.275% of total peak area. The major compounds were 1-phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-methyl ester (34.208%), isolongifolene (11.74 %) and 2,4-bis(1,1-dimethylethyl)-phenol (9.856%).

M. convoluta was found only in China. There are few reports about the chemical components of its essential oils. Only the essential oils of *M. convoluta* obtained by petrol ether extraction and supercritical (carbon dioxide) extraction methods have been reported by Cao et al.^{6,10}. There are may be some novel compound essential oils of *M. convoluta*. Using only GC-MS, it has not been possible to identify all of the compounds in the essential oil of *M. convoluta*. Isolation and identification of chemical components from *M. convoluta* need further work.

Comparison of ME and PE methods

ME offers a rapid delivery of energy to the solvent and microwave radiation can be focused directly onto the sample, thus the heating is more efficient. The cell walls of the plants was disrupted mainly due to the mechanical effects of microwave cavitation, which can be observed legibly under the microscope. In this study, we present ME as an "environmentally friendly" extraction method suitable for sample preparation prior to essential oil analysis. ME is a very clean method, which avoids the use of large quantity of water and voluminous extraction vessels (HD). ME could also be used to produce larger quantities of essential oils by using existing big scale microwave extraction reactors. These microwave reactors are suitable for the extraction of 10, 20 or 100 kg of fresh plant material per time. These reactors could be easily modified and used for ME extractions.

Compared with ME, the virtue of PE was its low temperature. Moreover it is convenient for industrial application. An essential drawback in the use of phytosol is its low polarity, making the extraction of polar analytes difficult. Moreover the PE equipment is comparatively hard to control the reacting pressure and temperature. In the present work, the higher polar components such as isolongifolene (24.59%) and dodecanal (1.13%) were largely found in the ME extract. Because it is lower polarity for petrol ether, the lower

polar compounds were the main components in petrol ether extraction. This limitation can be overcome by means of addition of polar modifiers, such as methanol or ethanol to the phytosol solvent. In the present work, the higher polar components such as isolongifolene (24.588%) and dodecanal (1.131%) were largely found in the ME extract.

Comparison with Literature Results

Extraction of natural products by different methods may yield different chemical components²²⁻²⁷. There were few reports about the chemical components from *M. convoluta*. Cao et al extracted the volatiles from leaves of *M. convoluta* by petrol ether extraction (PEE) and supercritical carbon dioxide extraction (SFE). As shown in Table 3, the volatiles from *M. convoluta* by ME, PE, PEE and SFE are significantly different from each other. Higher levels of ester and organic acids were found in the PEE and the ME extracts. However, higher levels of terpenes (accounting for 24.59%), which weren't found previously in *M. convoluta* essential oils, were also found in the ME extracts.

PE appears to be a cost-effective technique in laboratory scale, but an accurate economic evaluation for large-scale units requires supplementary experiments. PE also can be considered as a kind subcritical fluid extraction. The advantages of PE over the PEE include: low operating temperature, hence no thermal degradation of most of the labile compounds; shorter extraction period; high selectivity in the extraction of compounds; no solvent residue with negative effects on the oils quality. The volatiles of plants have usually been isolated by either hydrodistillation or solvent extraction. The disadvantages of all these techniques are: low yield, loss of volatile compounds, long extraction time, toxic solvent residues and degradation of unsaturated compounds, giving undesirable off-flavour compounds, due to heat.

In the SFE-CO₂ technique, the extraction is controlled by molecular diffusion rates. The fluid density is proportional to the extraction pressure and directly related to the chemical composition of the volatile fraction obtained. At high pressure (more than 20 MPa), the fluid has a low selectivity leading to extraction of a large variety of relatively heavy compounds (stigmastrol, fatty acids). However, PE is a cold extraction technique without heat. The PE system used in this work is a low pressure extraction unit. The pressure of PE is only 3-4 MPa. So the extraction selectivity of PE increased and no relatively heavy compounds, such as fatty acids were extracted. The main virtue of the PE technique over SFE-CO₂ extraction is convenience, easy separation, and easy reclaim. The PE apparatus is also simpler than that of SFE-CO₂.

The ME method offers obvious advantages over petrol ether extraction, namely: shorter extraction time (120 s against 72 h for PEE); lower cost (energy cost is fairly higher for SFE-CO₂) and cleaner features (large volumes of organic solvents are avoided).

Table 3.- Analytical results of *M. convolute* oils obtained by SFE , PEE,ME and PE

No.	Compound	Relative content (%)			
		SFE	PEE	ME	PE
1	Benzoic acid	1.14	—	—	—
2	Methylsalicylate	1.41	0.77	—	—
3	Dodecamethylcyclohexasiloxane	1.23	—	—	—
4	Decamethylcycloheptasiloxane	1.35	—	—	—
5	Diethylphthalate	1.46	—	—	—
6	Cedrol	—	1.21	—	—
7	Eudesma-4,11-diene	—	0.88	—	—
8	Tributylphosphine oxide	4.61	—	—	—
9	Dibutylphthalate	0.61	1.19	—	—
10	n-Hexadecanoic acid	20.35	1.41	—	—
11	E-11-hexadecenoic acid ethyl Ester	—	9.77	—	—
12	Hexadecanoic acid ethyl ester	—	36.97	8.570	—
13	Phytol	—	7.74	—	—
14	Linoleic acid ethyl ester	—	4.63	—	—
15	Ethyloleate	5.75	10.47	—	—
16	Octadecanoic acid	4.55	—	—	—
17	Stigmasterol	31.26	—	—	—
18	22,23-dihydro-stigmasterol	—	—	—	—
19	2-Propenoic acid, butyl ester	—	—	3.343	—
20	2,6,11-trimethyl-Dodecane	—	—	1.773	—
21	Dodecanal	—	—	1.131	—
22	Eicosane	—	—	2.704	—
23	Nonadecane	—	—	1.424	—
24	2,4-bis(1,1-dimethylethyl)-Phenol	—	—	10.808	9.856
25	2,6,11,15-tetramethyl-Hexadecane	—	—	1.196	—
26	Hexadecane	—	—	1.138	—
27	Isolongifolene	—	—	24.588	11.74
28	Eicosane	—	—	2.653	—
29	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	—	—	10.768	1.872
30	2,6,10,15-tetramethyl-Heptadecane	—	—	1.296	—
31	Pyrene	—	—	9.328	3.432
32	9-methylene-9H-Fluorene	—	—	—	5.615
33	1-Phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a,7-trimethyl,methyl ester	—	36.97	—	7.552
34	1-Phenanthrenecarboxylic acid,1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl), methyl ester	—	—	—	34.208

CONCLUSIONS

The ME and PE of essential oil in *M. convolute* has been studied. The results showed that isolongifolene was the main component in the ME extract. The compounds in volatile oils of *M. convolute* obtained by ME and PSE were different from the literature. The ME method offers obvious advantages over petrol ether extraction, namely: shorter extraction time (4 h against 72 h for petrol ether extraction); lower cost (energy cost is fairly higher for performing petrol ether extraction than that required for reaching supercritical conditions) and cleaner features (large volumes of organic solvents are avoided). The main component found in the PSE essential oil was 1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester. The advantages of the PSE were convenience and simple..

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