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Essential Oil From *Marchantia Convoluta*

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ABSTRACT

Extracts from *Marchantia convoluta* were obtained by supercritical carbon dioxide extraction and petrol ether extraction methods. The extracts were analyzed by capillary gas chromatography with mass spectrometric detector (GC-MS). The compounds were identified according to their retention indices and mass spectra (EI, 70 eV). The effects of different parameters, such as pressure, temperature, modifier volume and extraction time, on the supercritical fluid extraction (SFE) of *Marchantia convoluta* were investigated. A total of eleven compounds accounting for 73.62% of SFE extracts were identified. 22,23-dihydrostigmasterol (31.26%), n-hexadecanoic acid (20.35%), stigmasterol (4.55%) and octadecanoic acid (5.75%) were the major compounds identified in *Marchantia convoluta* SFE extracts. The SFE method has been compared with petrol ether extraction (PEE) method. SFE products were found to be markedly different from the corresponding petrol ether extract. A total of ten compounds accounting for 74.04% of PEE extract were identified. Hexadecanoic acid ethyl ester (36.97%), ethyloleate (10.47%), E-11-hexadecenoic acid ethyl ester (9.77%) and linoleic acid ethyl ester (4.63%) were the major compounds identified.

KEYWORDS

Marchantia convoluta;
Extracts;
Supercritical fluid extraction;
GC-MS.

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 countryside[1-3]. There is 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[4]. *M. convoluta* is found only in China and is quite rare[5].

The major identified constituents in *M. convoluta* are flavonoids, triterpenoids and steroids[1-3, 6-8]. The flavonoids consist mainly of quercetin, luteolin, apigenin and their O- and C-glycosides[1-3,7]. The dried leaves are used in China to protect the liver and to treat tumefaction of skin. A high dosage of flavonoids from *M.convoluta* (20 and 40 μg/mL) can significantly reduce the activity of alanine aminotranferease (ALT) and aspartate aminotransferase (AST) in the serum of mice with acute hepatic injury caused by CCl_4 and increase the contents of total protein (TP) and alkaline phosphatase (ALP), as well as inhibit the auricle tympanites of mice caused by dimethyl benzene. Flavonoids from *M. convoluta* strongly inhibit *colibacillus*, *typhoid bacillus*, *Staphylococcus aureus*, *Bacillus enteritidis*, *hemolytic streptococci* type B and *diplococcus pneumoniae* and possess distinct effect of antibiosis, anti-inflammation and diuresis in mice[1]. Extracts from *M.convoluta* strongly inhibit tumors in human liver and lung cancer cell lines[2].

The extraction of essential oil components using solvent at high pressure, or supercritical fluids (SCF), has received much attention in the past several years, especially in food, pharmaceutical and cosmetic industries, because it presents an alternative to conventional processes such as organic solvent extraction and steam distillation[8-12]. Supercritical fluid extraction allows a continuous modification of solvent power and selectivity by changing the solvent density[13-16]. It has the density of a liquid and solubilizes solids like a liquid solvent, but has a diffusion power similar to a gas and permeates through solid materials very easily. The power of solubilization increases with the density of the fluid; high densities of a supercritical fluid are possible at high pressures and allow it to dissolve large quantities of organic compounds. The dissolved compounds can be recovered from the fluid by reduction of its density, made possible by decreasing the pressure or increasing the temperature. This low temperature separation process prevents the degradation of the chemical compounds of the extract due to heat, as in steam distillation[17-20].

The aim of the present work is the investigation of the effects of different parameters, such as pressure, temperature, modifier volume and dynamic extraction time, on the supercritical fluid carbon dioxide extraction of *Marchantia convoluta*. The essential oil obtained by petrol ether extraction was used for comparison. Several reports about compositions of the ethanol extracts from *Marchantia convoluta* were performed[3-7, 21]. The results were different from each other because of different methods dealing with the ethanol extracts. To the best of our knowledge, no report has yet appeared on the SFE of *Marchantia convoluta*. The composition of the extracts obtained was analyzed by GC-MS.

MATERIALS AND METHODS

Plant materials

The whole plants of *Marchantia 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 Biology Department of Guangxi Chinese Medical University. The dried leaves were stored in a dark place at 4°C for 20 days. 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

HPLC grade methanol and analytical grade petroleum ether were purchased from Hanbon Company Limited. Carbon dioxide (99.99% purity), contained in a cylinder with an eductor tube, was obtained from CSU Co. (Changsha, China).

Supercritical fluid extraction (SFE)

A Suprex MPS/225 system (Pittsburgh, PA) in the SFE mode was used for all the extractions. The extraction vessel was a 10-ml stainless steel vessel. Supercritical fluid extractions were conducted at pressures of 5, 10, 15 and 20 MPa and temperatures of 35, 45, 55 and 65 °C for a duration of 20 min, static, followed by 15, 25, 35, or 45 min, dynamic. A Durafow manual variable restrictor (Suprex) was used in the SFE system to collect the extracted analytes. In order to prevent sample plugging, the restrict point was warmed electrically. The supercritical CO_2 flow rate through the Durafow restrictor was approximately 0.3 - 0.4 ml/min (compressed). Plant powder (3.0 g) was well mixed with 2 mm diameter glass beads, and was then charged into the 10-ml extraction vessel. The essential oil was extracted from the plant using supercritical CO_2 under various conditions according to the Taguchi method. Table 1 shows the experimental conditions for each of the SFE runs. The extracted analytes were collected in dichloro methane in a 5.0 ml volumetric flask. The final volume of the extract was adjusted to 5.0 ml with dichloromethane at the end of the extraction. In order to improve the collection efficiency, the 5.0 ml volumetric flask was placed in an ice bath during the dynamic extraction stage. For all the modifier studies, methanol was spiked directly into the extraction vessel with charged sample prior to the extraction.

Four millilitres of solution were poured into a 20 ml beaker. Bubbling of the solution was done by using argon gas in order to evaporate the solution. Then the weight of essential oil was measured. Finally, the extraction yield was calculated.

Petrol ether extraction (PEE)

The sample (10 g of dried materials) was submitted to extract with petrol ether for 72 h, using Soxhlet extraction method. The volatile distillate was collected over anhydrous sodium sulphate and refrigerated until time of analysis. The yield of the oil was 2.7% v/w based on dry plant weight.

Gas chromatography-mass spectrometry (GC-MS)

GC analyses were performed using a Shimadzu GC- 9A gas chromatograph equipped with a FID and a HP-5 fused silica column (60 m x 0.25 mm i.d., 0.25 μm). 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 SFE samples (1 μl) were injected into GC/MS (without any further dilution) using the split mode with a split ratio of 1/60. Petrol ether extraction extract was diluted 30 times and 1 μl of diluted solution was injected into GC with the same split ratio. 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 a computer library or with authentic compounds. Data obtained were conformed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature[22].

RESULTS AND DISCUSSION

Optimization of the experimental conditions

Since various parameters potentially affect the extraction process, the optimization of the experimental conditions represents a critical step in the development of a SFE method. In fact, pressure and temperature of the fluid, percentage of the modifier and the extraction times are generally considered as the most important factors. The optimization of the method can be carried out step-by-step or by using an experimental design. TABLE 1 showed different conditions of experiments carried out with SFE for extraction according to the Taguchi experimental design. All the selected factors were examined using a four-level orthogonal array design with an $L_4 16$ (4^4) matrix. In general, a full evaluation of the effect of four factors from three levels on the yield needs 256 (4^4) experiments. In order to reduce the number of experiments, a L_4 (4^4) orthogonal design graph was used (TABLE 1). By this way, only sixteen experiments were needed. The yields obtained under or-

TABLE 1: The results of orthogonal test $L_{16}(4^4)$

NO.	Pressure (MPa)	Temperature (°C)	Dynamic time (min)	Modifier volume (ml)	Extraction yield (%, w/w)
1	5	35	25	30	0.99
2	5	45	15	40	0.87
3	5	55	45	10	1.34
4	5	65	35	20	2.12
5	10	35	15	10	2.68
6	10	45	25	20	3.27
7	10	55	35	30	3.81
8	10	65	45	40	4.01
9	15	35	35	40	4.69
10	15	45	45	30	4.23
11	15	55	15	20	3.56
12	15	65	25	10	1.38
13	20	35	45	20	2.79
14	20	45	35	10	2.41
15	20	55	25	40	2.00
16	20	65	15	30	1.16

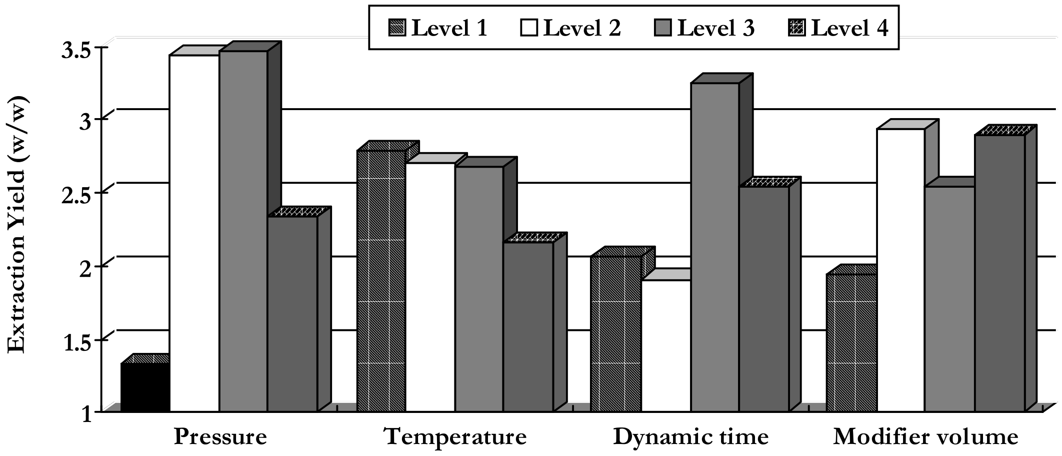

Figure 1: The yields of volatile oil under orthogonal conditions (for designations of factors pressure, temperature, dynamic time and modifier volume, see TABLE 1).

thogonal conditions are also shown in TABLE 1. The extraction yields were 0.87 – 4.69 %.

In this study, interactions among variables were not incorporated in the matrix and focus was placed on the main effects of the four most important factors. The results of the SFE experiments, based on extraction yields, are given in TABLE 1.

The mean values of the extraction yields for the corresponding factors at each level were calculated according to the assignment of the experiment (Figure 1). For example, the extraction yields of the four trials at 15 MPa were evaluated as mean values of the corresponding four runs. The mean values of the four levels of each factor (e.g., pressure) reveal how the extraction yield will change when the level of that factor is changed. Figure 1 shows the variations in extraction yield as a function of change in different levels of the factors studied. For the complete recovery of the main components of the plant, higher pressures are necessary. This is because raising the extraction pressure, at constant temperature, leads to higher fluid density, which increases the solubility of the analytes. To obtain quantitative recovery of analytes, they must be efficiently partitioned from the sample matrix into the supercritical fluid. The influence of temperature on the composition of the extracts was studied. Higher temperature resulted to lower extraction yield. For all the analytes, the volume of the modifier was found not to be significant as the main effect. The influence of the dynamic extraction time on the composition of the extracts was studied. Extraction was performed with supercritical carbon dioxide at the static extraction step of 20 min, followed by 15, 25, 35 and 45 min of dynamic extractions. Results showed that increasing dynamic extraction time to 35 min enhanced the extraction of most components.

Thus, the best conditions, obtained by preliminary test, for the extraction of oil were: extraction temperature, 35°C; dynamic time, 35 min; pressure, 15Mpa and modifier volume, 40 ml.

GC-MS analyses

The GC-MS profiles of SFE and PEE were shown in figure 2 and 3, respectively. The analytical results of GC-MS were recorded in TABLE 2.Twenty-nine compounds were separated by gas chromatographic analyses from SFE extract and eleven compounds were identified by reference standards. The peak area of compounds identified accounted for 73.62% of total peak area. Thirty-five compounds were separated by gas chromatographic analyses from PEE extract and ten compounds were identified by reference standards. The peak area of compounds identified accounted for 74.04% of total peak area.

The SFE and PEE extracts of *Marchantia convoluta* showed a relatively simple GC–MS chromatographic pattern. Detailed identification and quantitation of the compounds found in *Marchantia convoluta* oil, produced by SFE under NO. 9 orthogonal test condi-

TABLE 2: GC-MS analytical results of *Marchantia convoluta* oils obtained by SFE and PEE. The compounds were listed in order of elution time.

No.	Compound	SFE			PEE		
		RT /min	Reliability to standard MS (%)	Relative Content (%)	RT /min	Reliability to standard MS (%)	Relative Content (%)
1	Benzoic acid	5.15	91	1.14		-	
2	Methylsalicylate	5.70	97	1.41	5.70	97	0.77
3	Dodecamethylcyclohexasiloxane	7.18	91	1.23		-	
4	Decamethylcycloheptasiloxane	7.90	93	1.35		-	
5	Diethylphthalate	10.61	98	1.46		-	
6	Cedrol		-		10.86	96	1.21
7	Eudesma-4,11-diene				11.42	99	0.88
8	Tributylphosphine oxide	12.14	93	4.61		-	
9	Dibutylphthalate	13.90	96	0.61	13.89	98	1.19
10	n-Hexadecanoic acid	15.36	99	20.35	15.30	97	1.41
11	E-11-hexadecenoic acid ethyl ester		-		15.72	99	9.77
12	Hexadecanoic acid ethyl ester		-		15.89	99	36.97
13	Phytol		-		17.66	96	7.74
14	Linoleic acid ethyl ester		-		18.29	99	4.63
15	Ethyloleate		-		18.37	99	10.47
16	Octadecanoic acid	19.48	93	5.75		-	
17	Stigmasterol	28.47	98	4.55		-	
18	22,23-dihydro-stigmasterol	30.74	87	31.26		-	
Total content (%)			73.62			74.04	

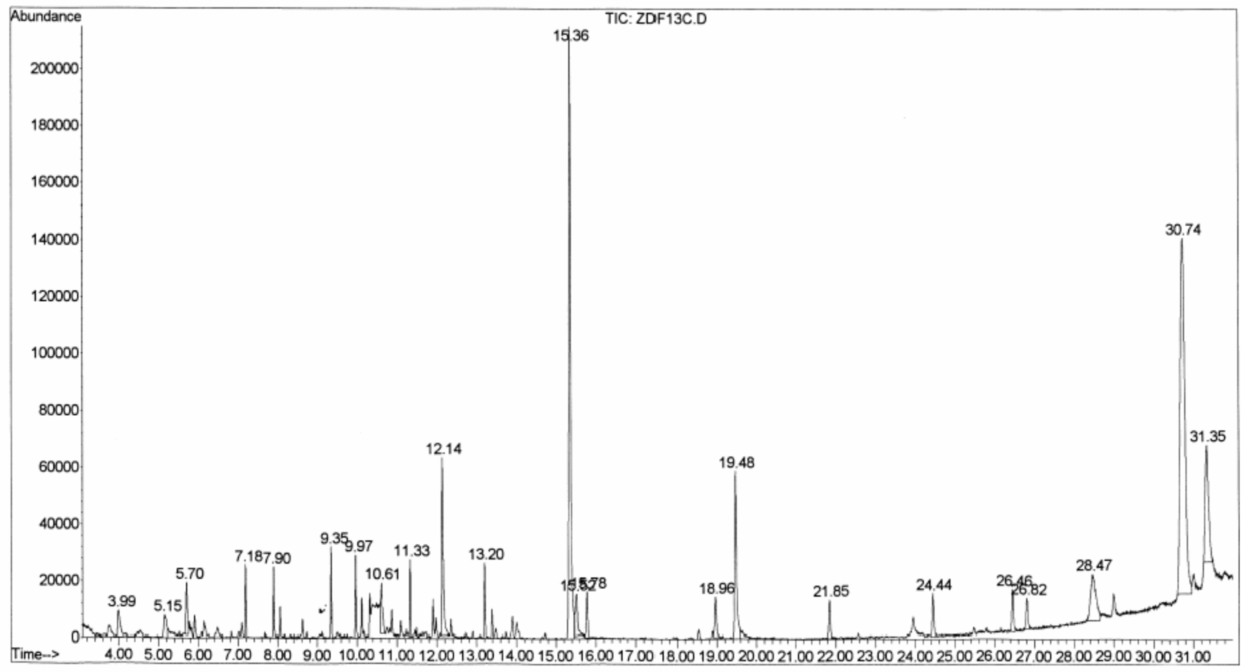

Figure 2: GC-MS profiles of SFE extract (NO. 9)

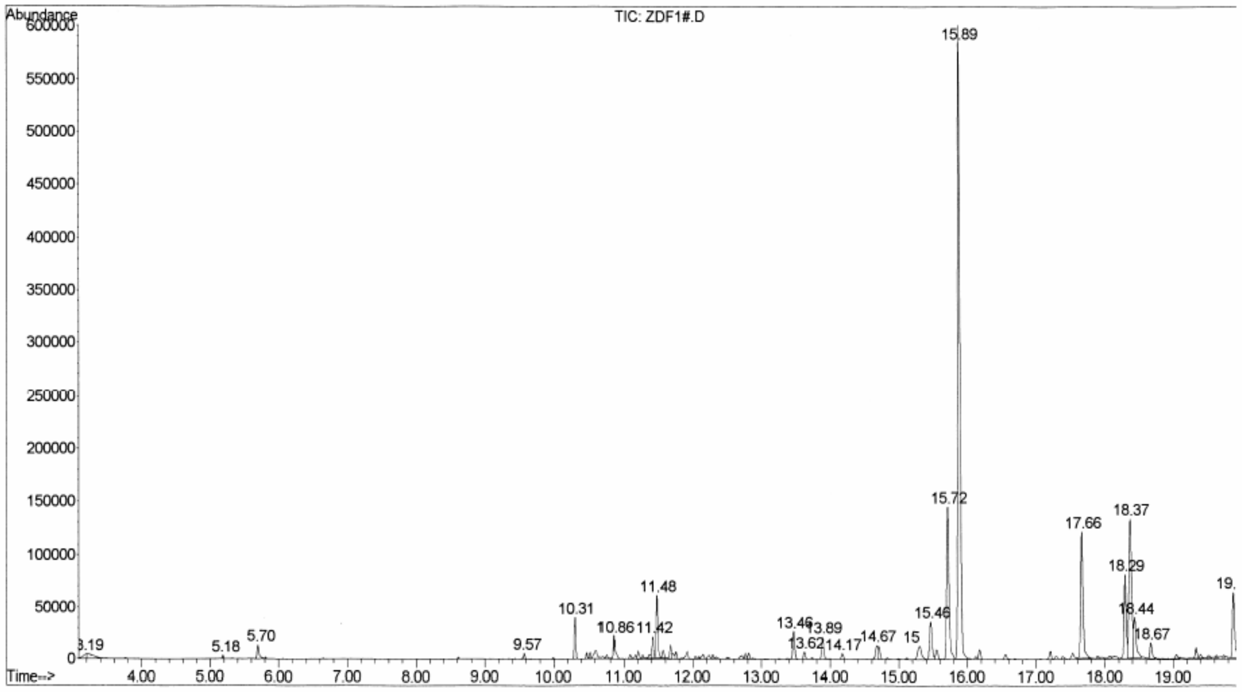

Figure 3: GC-MS profiles of PEE extract

tions, were performed by GC–MS, as shown in TABLE 2. Product obtained by petrol ether extraction was also analysed by GC–MS. The results are also shown in TABLE 2, for comparison.

The major compounds of *Marchantia convoluta* SFE extract (NO.9) were 22,23-dihydrostigmasterol (31.26%), n-hexadecanoic acid (20.35%), stigmasterol (4.55%) and octadecanoic acid (5.75%).

Marchantia convoluta was only found in China. There are few reports about the chemical components of the essential oil. Only two plant sterols, namely 22,23-dihydro-stigmasterol and stigmasterol were isolated from *Marchantia convoluta* by Zhu et al[5]. There are may be some novel essential oil of *Marchantia convoluta*. Only through GC-MS was not able to identify all compounds in the essential oil of *Marchantia convoluta*. Isolation and identification of chemical components from *Marchantia convoluta* need further work.

Comparison of SFE and PEE methods

Different extraction compositions could be obtained by different extraction methods applied to natural products[13, 23-26]. As shown by the results, the composition of the SFE products and the PEE essential oils of *Marchantia convoluta* were different. By comparing the composition of the SFE product with that of PEE, higher levels of ester (accounting for 57.21%) were found in the PEE oil and higher levels of acid (accounting for 27.27%) were found in the SFE oil. Also, TABLE 1 shows that the stigmasterol content in the SFE extract is considerable, however, this compound was not found in the PEE products. On the other hand, phytol was found in the PEE products, whereas it was not found in the SFE products.

22,23-dihydro-stigmasterol and stigmasterol were plant sterols. Phytosterol is one kind of active constituents, and can be obtained from some sorts of vegetable oil[27]. Phytosterol and its derivatives are widely applied in pharmaceutical, food, and cosmetic industry due to their special biological-activity, physical, and chemical properties[28]. Phytosterol obtained from vegetable oil usually consists of stigmasterol, β-sitosterol, campesterol, and brassicasterol. The molecular structures of these compounds are shown in figure 4. Zhu et al isolated stigmasterol from the Leafy Body of *Marchantia convoluta*[5].

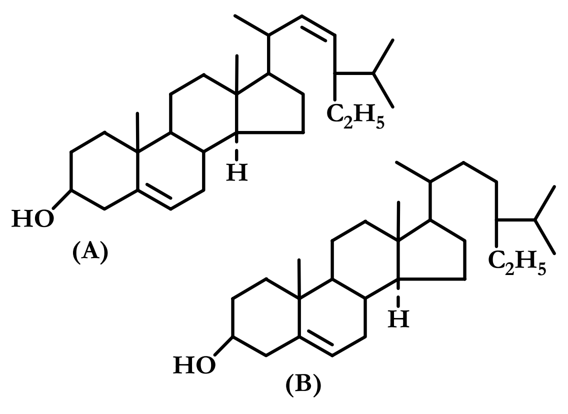

Figure 4: The molecular structures of plant sterols (A. stigmasterol B. campesterol, namely, 22,23-dihydrostigmasterol)

An essential drawback in the use of supercritical CO_2 is its low polarity, making the extraction of polar analytes difficult. Nevertheless, this limitation may be overcome by adding small amounts of polar modifiers, such as methanol or ethanol to the supercritical CO_2, in order to increase its solution power. In the present work, the modifier (methanol) enhanced the solubility of solutes in supercritical CO_2 and thus efficiency of extraction increased. The higher polar components such as n-hexadecanoic acid (20.35%) and octadecanoic acid (5.75%) were largely found in the SFE extract. Because it is lower polarity for petrol ether, the lower polar compounds were main components in petrol ether extraction.

The composition of the SFE products and PEE essential oils was significantly different. SFE appears to be a cost-effective technique at laboratory scale, but an accurate economic evaluation for large-scale units requires supplementary experiments. The advantages of SFE-CO_2 extraction over the petrol ether extraction 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 essential oils of plants have usually been isolated by either hydrodistillation or solvent extraction. The disadvantages of all these techniques are: low yield, losses of volatile compounds, long extraction times, toxic solvent residues and degradation of unsaturated compounds, giving undesirable off-flavor compounds, due to heat.

CONCLUSIONS

The supercritical fluid extraction of essential oil in *Marchantia convoluta* has been studied. The results showed that plant sterols were main components found in the SFE products. The oil obtained by SFE of *Marchantia convoluta* showed some differences in composition with the oil obtained by petrol ether extraction. The SFE 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 (as no great volume of organic solvent is involved). The method contributes to the automation of pharmaceutical industry.

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