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Component Analysis of Black Ant (*Polyrhachis lamellidens*) Extracts from Supercritical Fluid Extraction

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ABSTRACT

The Black ant *Polyrhachis lamellidens* Fr. Smith (Formicidae), found wild throughout China and Taiwan, has long been used as folk medicine for the treatment of rheumatism, rheumatoid arthritis, chronic hepatitis, sexual hypofunction and antiaging.

In this study, we extracted several components from this insect by using the supercritical fluid extraction (SFE) technique under different temperature, pressure, and extraction time. The extracts were analyzed by gas chromatographic-mass spectrometry (GC-MS) and identified by a mass library search. This paper presents a comparison of extraction quantity and composition by different extraction conditions.

The results show that thirty-nine known compounds were isolated from *P. lamelliden* for the first time. Pressure is the major factor in affecting the extraction yield in SFE. The results also indicated that (*Z*)-9-octadecenoic acid (oleic acid) is the major constituent in the extracts from all the SFE extraction and from 95% ethanolic extraction. In addition to oleic acid, the extracts are also rich in aliphatic hydrocarbons, aliphatic alcohols, aliphatic fatty acids, and steroids.

Key words: Black ant, *Polyrhachis lamellidens*, supercritical fluid extraction, GC-MS

INTRODUCTION

Ants have been consumed as a health food for hundreds of years. In his classic *Compendium of Materia Medica*, Li Shi-Cheng (1518-1593), the greatest Chinese herbalist and practitioner, introduced varieties of ants, properties and their living environment, and described how the ancient practice of eating ant's eggs and serving ants to tribal chiefs and noble lords. Li said that the ant is highly sensitive to the weather and able to forecast a rainy day.

The extract of the ant genus *Polyrhachis* contains over fifty nutritional elements, including 26 kinds of amino acids, of which eight are essential to the human body. The dry ant contains 30~70% protein, and also contains vitamins B_1, B_2, and B_{12} and trace elements such as Ca, Cu, Fe, Mg, Mn, Mo, P, Se, Zn[(1)]. Pharmacological tests and clinical trials have showed that certain components of the ant are active in combating arthritis, rheumatism, liver ailments, asthma, and even cancer[(2,3)]. Recent biological studies have indicated that ant extract is an immunopotentiator of broad-spectrum, which increased the amount of RNA and DNA as well as the number of immune cells[(4)]. Immunotherapy for ant hypersensitivity has also indicated that ant extract is effective in lowering the incidence of anaphylaxis during subsequent field stings; reducing specific immunoglobulin E by skin testing; and protecting from systemic reactions provoked by a sting challenge with a single ant[(5)]. *In vitro* tests have showed that the ant extract can efficiently prevent ferric-nitrilotriacetate (Fe-NTA) induced nephrotoxicity through quenching the free radicals mechanism[(6)]. It also inhibited the production of thiobarbituric acid-reactive substances, an index of lipid peroxidation, in rat brain homogenate[(7)]. Health food prepared from ant extract has been reported to be helpful for supplementing blood, building up physical strength and vital energy, increasing sexual desire, cleaning the lungs and liver, and prolonging life in general.

Although there have been reports on the composition analysis for ant genus *Polyrhachis* (Hymenoptera, Formicidae), there is no indication in the literature that the active components in genus *P. lamellidens* have been identified. In order to trace the active ingredients and to optimize the biological activities, a method for efficient fractionation of *P. lamellidens* has to be established. In this study, we used supercritical fluid extraction (SFE) to isolate the components of *P. lamellidens*. The efficiency of extraction in terms of extraction yield by different extraction conditions was compared. The components from the extracts was identified by GC/MS.

MATERIALS AND METHODS

I. *Materials*

P. lamelliden were collected in September 1999 in northeast China and identified by Dr. Wen-Jer Wu and Dr. Chung-Chi Lin, Department of Entomology, National Taiwan University, Taipei, Taiwan. A voucher specimen is deposited in the herbarium of the Graduate Institute of

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II. *Extraction Procedures and Sample Preparation*

(I) *Ultrasonics-assisted Solvent Extraction*

Ten grams of finely crushed *Polyrhachis lamellidens* was placed in a 100 mL Erlenmeyer flask with 50 mL of 95% ethanol. The flask was sonicated in a BRANSON 5210 OR-DTH ultrasonics (Branson Ultrasonics Co., CT, USA) at 40˚C for 30 minutes. The mixture was then filtered and concentrated to dryness. The extract (50 μL) was dissolved in 1 mL of $CHCl_3$ (GC grade, Merck).

(II) *Supercritical Fluid Extraction (SFE)*

Spe-ed SFE (Applied Separation, Inc., PA, USA) with supercritical CO_2 (50-60 g of dry ice was dropped into the chamber outside of the collector vial with septum assembly) was used to extract *P. lamellidens* (10 g). The extraction time varied from 7.5, 15, 30, 45 to 60 minutes for each run. The temperature was set at 35, 50, 65, 80 and 95˚C for each extraction. The pressure was set at 3000, 4000, 5000, 6000 and 7000 psi for each run. Fifty microliter of each extract was dissolved in 1 mL of $CHCl_3$ (GC grade, Merck) for chromatographic analysis.

III. *Chromatographic Analysis*

GC analysis was performed on a Hewlett-Packard (HP) 6890 Series gas chromatograph interfaced to an HP 5973 mass selective detector (MSD). An HP MS ChemStation Data system was used for the identification of the components. The column used was an HP-5 MS (Hewlett-Packard) cross-linked fused-silica capillary column (30 m, 0.25 mm i.d.) coated with 5%-phenylpoly(methylsiloxane) (0.25 μm phase thickness). The oven was programmed from 70˚C (held for 1 min) to 150˚C (held for 2 min) with a temperature increment of 10˚C/min, and then from 150˚C to 280˚C (held for 5 min) with a temperature increment of 3˚C/min. The pressure of the helium inlet was set at 8.75 psi, with linear velocity of 37 cm/sec (split flow 50 mL/min). The injector temperature was kept at 250˚C and the volume injected was 1 μL. The electron energy was set at 70 eV. Mass spectra and the reconstructed chromatograms were obtained by automatic scanning of the samples in the mass range of m/z 50-550 Da with a scanning rate of 2.94 scan/sec. Chromatographic peaks were checked for homogeneity with the aid of mass chromatograms with characteristic fragment ions. NIST 98 (NIST/EPA/NIH MASS SPECTRA LIBRARY) database was used for automatic identification of GC peaks.

RESULTS AND DISCUSSION

I. *Comparisons of Extraction Yield of Polyrhachis Lamellidens Extracted in Different SFE Conditions*

Time, temperature and pressure were varied in SFE for the extraction of *P. lamellidens*. The SFE conditions and the extraction yields are summarized in Table 1 and depicted in Figure 1. As indicated in Table 1, the extraction yield (weight by weight) increased with the extent of extraction time. The yield after 60 min. of extraction was 7.37% (TM5), which was about 177.07% to that of the extraction for 30 min. (2.66% for TM1). Temperature also had effect on the extraction yield. As the temperature increased from 35˚C (TP1) to 95˚C (TP5), the extract yield increased from 3.62% to 6.72%, which was increased for 85.64%. Pressure in SFE also had an effect on the extraction. The yield after extraction under 7000 psi of pressure (PS5) was 9.58%, which is about 787.04% to that of an extraction under 3000 psi of pressure (1.08% for PS1). Setting SFE at 30 min. 80˚C and 5000 psi as the starting basic condition (TM3=TP4=PS3 in Table 1), the extrac-

Table 1. The effect of different SFE conditions on the extraction yield of *P. lamellidens*

	Extraction parameters			
Conditions	Time (min.)	Temperature (˚C)	Pressure (psi)	Yield (%, wt/wt)
Time (*n*=3)				
TM1	7.5	80	5000	2.66
TM2	15	80	5000	4.83
TM3	30	80	5000	5.70
TM4	45	80	5000	6.14
TM5	60	80	5000	7.37
Temperature (*n*=3)				
TP1	30	35	5000	3.62
TP2	30	50	5000	4.70
TP3	30	65	5000	5.02
TP4	30	80	5000	5.70
TP5	30	95	5000	6.72
Pressure (*n*=3)				
PS1	30	80	3000	1.08
PS2	30	80	4000	3.26
PS3	30	80	5000	5.70
PS4	30	80	6000	8.61
PS5	30	80	7000	9.58

tion time, temperature and pressure was increased independently in different runs. The effectiveness of the three extraction parameters was compared. The extraction yields were increased by 29.30% (TM5 vs TM3), 17.90% (TP5 vs TP4) and 68.07% (PS5 vs PS3) respectively for time, temperature and pressure changes. The results suggested that pressure was the major factor in affecting the extraction yield in SFE.

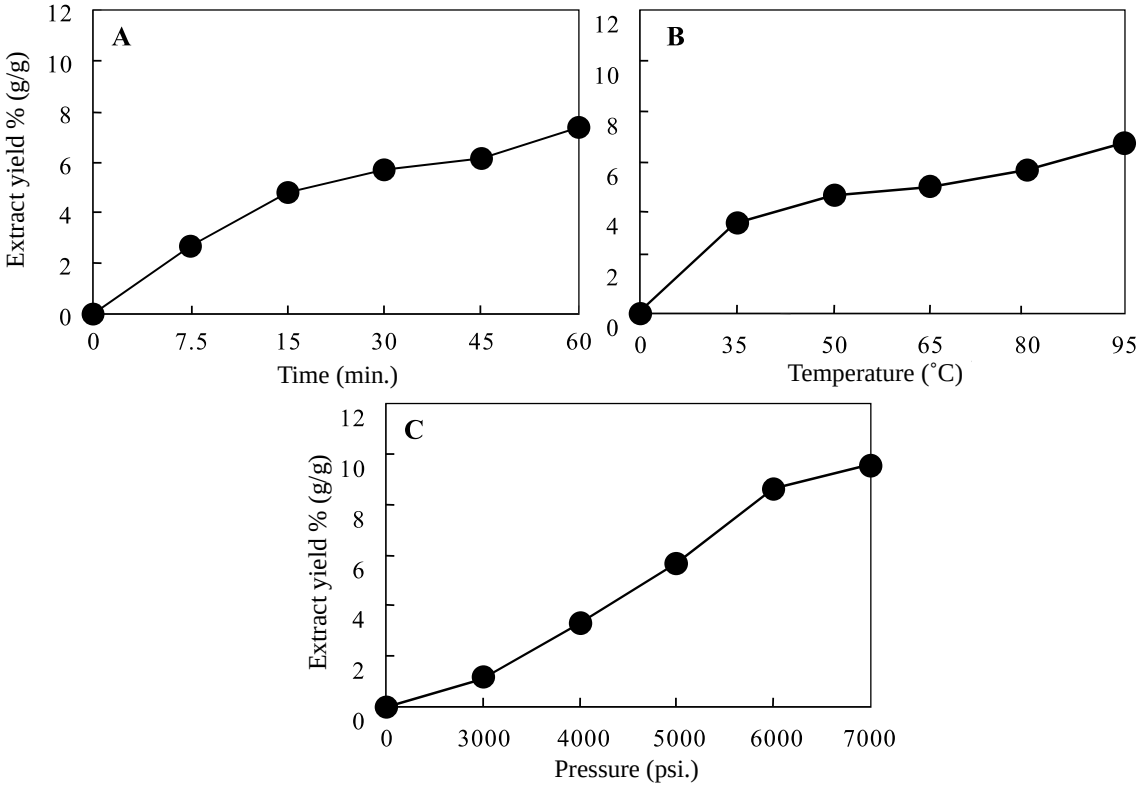

Figure 1. The effect of (A) collection time, (B) temperature and (C) pressure of SFE on the extraction yield of *P. lamellidens*.

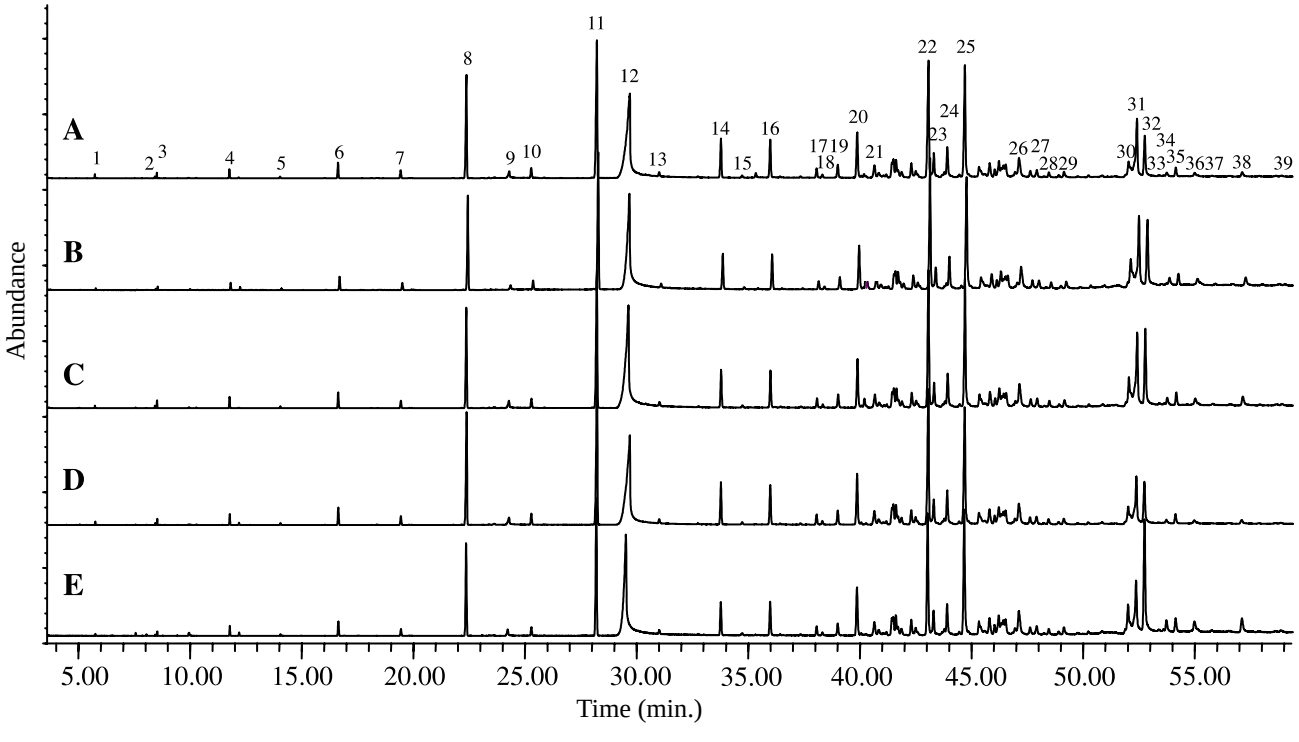

Figure 2. GC/MS chromatogram of *P. lamellidens* extracts obtained by SFE after (A) 7.5 min. (B) 15 min. (C) 30 min. (D) 45 min. and (E) 60 min. of collection time.

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II. *GC/MS Chromatogram of the Extracts from SFE and from 95% Ethanol Extractions*

The GC chromatograms of *P. lamellidens* extracts from SFE at different conditions and from 95% ethanol were shown in Figure 2, 3, 4, and 5 respectively. The GC chromatograms for SFE extracts from different extraction conditions were very similar except only differed in peak heights

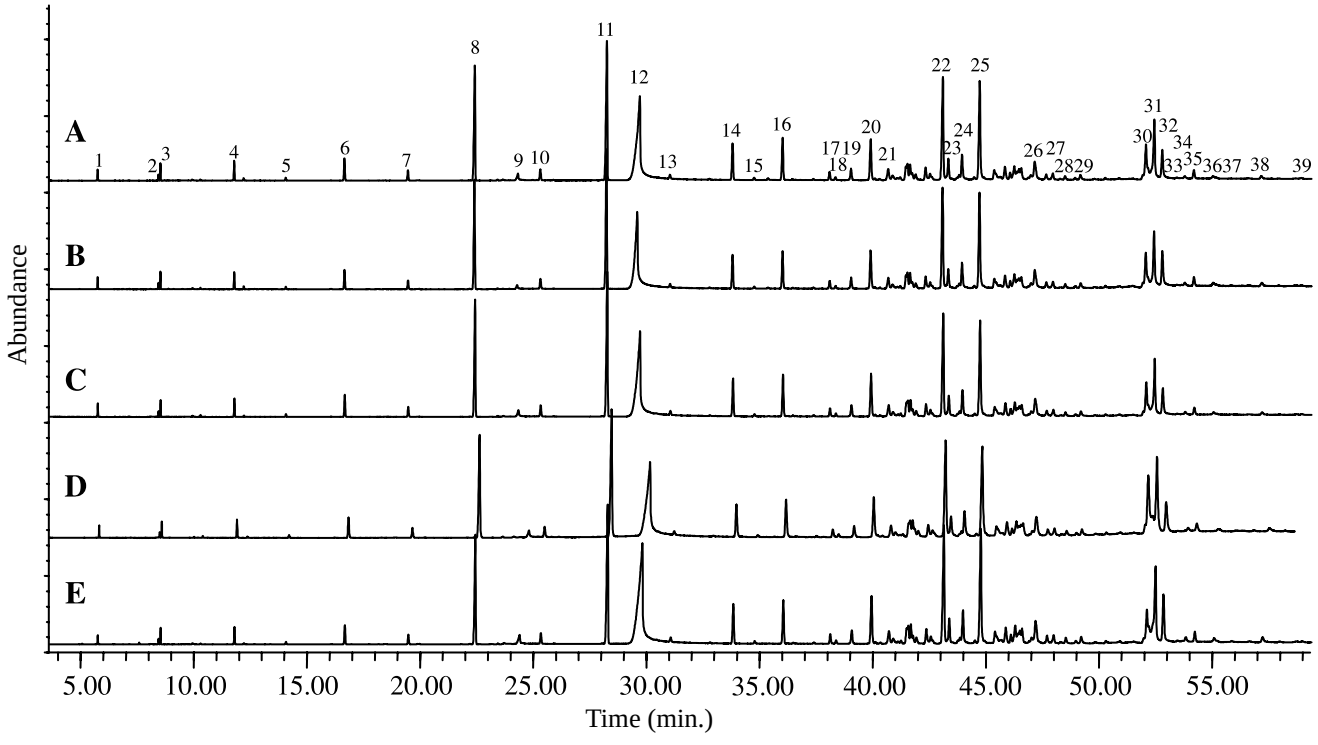

Figure 3. GC/MS chromatogram of *P. lamellidens* extracts obtained by SFE at (A) 35˚C, (B) 50˚C, (C) 65˚C, (D) 80˚C and (E) 95˚C of extraction temperature.

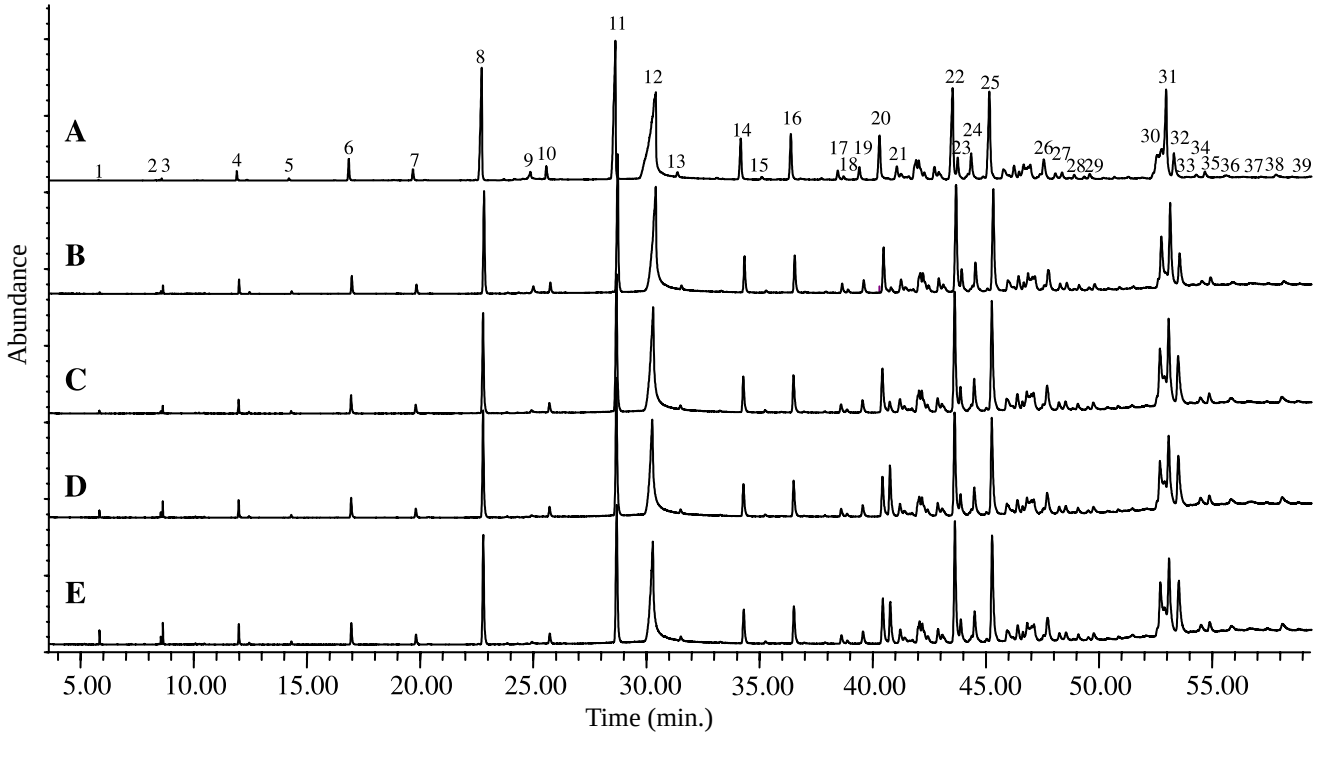

Figure 4. GC/MS chromatogram of *P. lamellidenes* extracts collected from SFE under (A) 3000 psi, (B) 4000 psi, (C) 5000 psi, (D) 6000 psi and (E) 7000 psi of pressure.

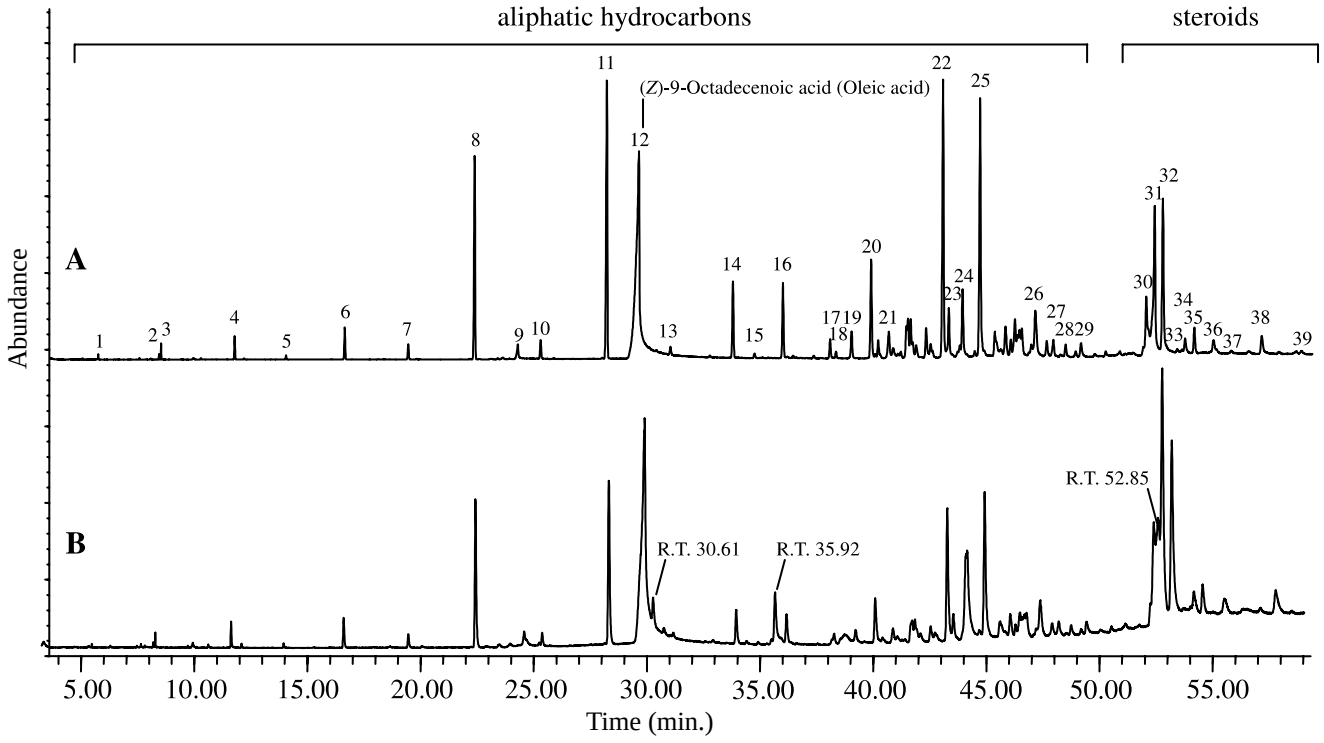

Figure 5. Comparison of the GC/MS chromatograms of *P. lamellidenes* extracts obtained from (A) 30 min. extraction by SFE at 80˚C and 5000 psi and from (B) 95% ethanolic extraction.

Table 2. Chemical compositions of *P. lamellidens* extracts obtained by SFE at different conditions. The constituents are reported as percentage in peak area of total extracts

		Time (*n*=5)					Temperature (*n*=5)					Pressure (*n*=5)				
Peak no.	Compound (M.W)[a]	TM1	TM2	TM3	TM4	TM5	TP1	TP2	TP3	TP4	TP5	PS1	PS2	PS3	PS4	PS5
1	Undecane (156)	0.08	0.05	0.06	0.07	0.03	0.22	0.27	0.27	0.26	0.18	0.01	0.03	0.04	0.11	0.22
2	(*Z*)-5-Tridecene (182)	0.04	0.01	0.08	0.06	0.01	0.13	0.15	0.13	0.13	0.11	0.01	0.07	0.04	0.10	0.12
3	Tridecane (184)	0.11	0.09	0.18	0.13	0.08	0.37	0.41	0.28	0.30	0.31	0.04	0.17	0.13	0.29	0.36
4	Pentadecane (212)	0.31	0.28	0.41	0.36	0.33	0.69	0.69	0.62	0.61	0.55	0.27	0.51	0.41	0.55	0.58
5	Hexadecane (226)	0.07	0.05	0.06	0.07	0.03	0.13	0.12	0.12	0.13	0.10	0.08	0.11	0.07	0.09	0.10
6	Heptadecane (240)	0.66	0.67	0.70	0.76	0.60	1.05	1.01	1.00	0.98	0.83	0.85	0.89	0.76	0.86	0.90
7	Octadecane (254)	0.38	0.38	0.32	0.42	0.32	0.54	0.52	0.70	0.51	0.44	0.51	0.46	0.41	0.45	0.47
8	Nonadecane (268)	5.30	5.31	5.01	5.71	4.35	6.72	6.51	6.55	6.30	5.51	6.60	6.10	5.29	5.60	5.67
9	*n*-Hexadecanoic acid (256)	0.60	0.38	0.55	0.48	0.43	0.56	0.31	0.56	0.60	0.66	0.08	0.60	0.41	0.48	0.48
10	Eicosane (282)	0.55	0.53	0.44	0.57	0.46	0.65	0.63	0.64	0.61	0.57	0.69	0.61	0.54	0.56	0.58
11	Heneicosane (296)	7.78	7.81	7.65	8.65	6.66	9.25	9.15	9.30	8.91	8.11	9.46	9.18	8.03	8.20	8.07
12	(*Z*)-9-Octadecenoic acid (282)[b]	16.98	13.57	16.42	16.37	15.10	14.93	13.25	14.43	13.14	15.86	18.25	18.04	14.26	12.28	12.47
13	Docosane (310)	0.27	0.24	0.25	0.18	0.18	0.25	0.25	0.26	0.25	0.24	0.28	0.24	0.24	0.27	0.20
14	Tricosane (324)	2.15	2.65	2.04	2.16	1.74	2.15	2.19	2.22	2.16	2.02	2.42	2.38	2.06	2.05	2.02
15	2-Methyl-tricosane (338)	0.14	0.15	0.14	0.15	0.12	0.16	0.15	0.15	0.15	0.14	0.16	0.13	0.13	0.13	0.14
16	2,6,10,14,18-Pentamethyl-2,6,10, 14,18-eicosapentaene (342)	2.23	2.15	1.89	2.30	1.86	2.65	2.50	2.54	2.54	2.30	2.87	2.60	2.31	2.33	2.32
17	Tetracosane (338)	0.56	0.56	0.48	0.54	0.49	0.55	0.57	0.57	0.57	0.55	0.61	0.54	0.55	0.51	0.53
18	(*Z*)-12-Pentacosane (350)	0.20	0.20	0.18	0.21	0.20	0.20	0.18	0.20	0.21	0.21	0.24	0.21	0.20	0.21	0.19
19	Pentacosane (352)	1.01	0.70	0.81	0.80	0.73	0.79	0.83	0.82	0.84	0.80	0.88	0.90	0.89	0.85	0.84
20	11-Butyl-docosane (366)	2.40	3.12	2.76	2.96	2.83	2.88	2.91	3.00	3.09	2.87	3.18	3.15	3.00	3.08	3.07
21	Hexacosane (366)	1.07	1.12	1.00	1.03	1.16	1.10	1.10	1.08	1.05	1.00	1.29	1.24	1.03	0.92	1.01
22	Heptacosane (380)	7.59	8.02	7.77	8.13	7.20	7.23	7.82	8.04	7.77	7.41	6.99	8.67	8.10	7.96	7.73
23	1-Heptacosanol (396)	1.75	1.81	1.68	1.65	1.60	1.66	1.75	1.57	1.76	1.70	1.55	1.50	1.79	1.82	1.68
24	Triacontane (380)	2.25	2.38	1.93	2.17	2.02	1.85	1.98	2.33	2.20	2.13	1.98	2.48	2.40	2.40	2.21
25	Octacosane (394)	7.51	7.57	7.51	7.52	7.93	7.15	7.67	7.72	7.51	7.35	6.75	7.28	7.99	7.73	7.71
26	Nonacosane (408)	2.22	2.46	2.04	2.12	2.49	2.04	2.22	2.04	2.13	2.08	1.88	2.32	2.35	2.28	2.20
27	Nonacosanol (424)	0.66	0.68	0.63	0.63	0.66	0.56	0.58	0.59	0.59	0.62	0.50	1.17	0.66	0.69	0.65
28	Hentriacontane (437)	0.39	0.41	0.43	0.37	0.49	0.42	0.36	0.34	0.39	0.37	0.35	0.38	0.34	0.52	0.38
29	Tetratriacontane (479)	0.48	0.57	0.55	0.47	0.60	0.45	0.52	0.48	0.52	0.52	0.39	0.50	0.57	0.50	0.57

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Table 2. Continued

30	NI[c] (464)	5.74	5.04	6.15	5.46	6.82	6.09	6.81	7.89	7.85	5.28	4.32	5.80	8.08	7.76	7.37
31	NI (464)	5.67	6.65	5.59	6.06	5.75	6.64	6.52	5.76	6.83	6.65	6.74	7.41	7.38	6.77	6.60
32	Cholesterol (386)	3.17	4.82	5.39	3.57	8.61	2.58	3.28	3.21	3.23	3.52	1.51	5.34	4.20	4.67	4.58
33	17-(1,5-Dimethyl-hex-4-enyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol (384)															
		0.10	0.12	0.17	0.15	0.10	0.17	0.10	0.10	0.10	0.10	0.05	ND[d]	ND	ND	ND
34	(3β, 2E, 24S)-Ergosta-5,22-dien-3-ol (398)															
		0.35	0.55	0.70	0.43	1.07	0.24	0.35	0.24	0.36	0.46	0.18	0.55	0.71	0.59	0.78
35	Cholestadiene (368)	0.65	0.80	0.78	0.65	0.95	0.67	0.56	0.55	0.71	0.55	0.23	0.31	0.78	0.73	0.72
36	Campesterol (400)	0.20	0.65	0.66	0.40	1.01	0.31	0.52	0.53	0.32	0.54	0.30	0.59	0.53	0.57	0.71
37	Ergosta-4,6,22-triene (380)	0.09	0.13	0.13	0.11	0.17	0.12	0.15	0.14	0.12	0.06	0.10	0.22	0.14	0.17	0.02
38	γ-Sitosterol (414)	0.37	0.43	0.91	0.59	1.44	0.28	0.47	0.14	0.39	0.45	0.20	0.26	0.65	0.78	0.55
39	Sigmastan-3,5-dien (396)	0.11	0.19	0.18	0.17	0.19	0.13	0.17	0.15	0.14	0.10	0.04	ND	ND	ND	ND

[a] molecular weight; [b] oleic acid; [c] NI: structurally unidentified component; [d] ND: not detected.

(Figure 2, 3, and 4). Thirty nine components were detected from the SFE extracts with concentration over 0.01% (GC peak area, %). In comparison with the GC chromatogram of SFE extract (Figure 5-A), three additional peaks appeared in that of the 95% ethanolic extract with retention times at 30.61, 35.92, and 52.85 minutes respectively (Figure 5-B).

III. *Chemical Compositions of Polyrhachis Lamellidens Extracts from SFE*

The chemical compositions of *P. lamellidens* extracts obtained from different SFE methods are listed in Table 2. The major components from *P. lamellidens* obtained by SFE were aliphatic hydrocarbons, aliphatic alcohols, aliphatic fatty acids and steroids. The main constituent after all the different SFE extractions was (*Z*)-9-octadecenoic acid (oleic acid), of which the structure was confirmed by comparison with the reference spectrum of NIST mass spectra library. Other compounds with high content were identified as nonadecane (4.35%-6.72%), heneicosane (6.66%-9.46%), tricosane (1.74%-2.65%), 2,6,10,14,18- pentamethyl-2,6,10,14,18,-eicosapentaene (1.86%-2.87%), 11-butyldocosane (2.40%-3.18%), heptacosane (6.99%-8.67%), 1-heptacosanol (1.50%-1.81%), triacontane (1.85%-2.48%), octacosane (6.75%-7.99%), nonacosane (1.88%-2.49%) and cholesterol (1.51%-8.61%). Besides cholesterol, other steroid compositions of steroids identified in SFE extracts were 17-(1,5-dimethyl-hex-4-enyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol, (3β,2E,24S)-ergosta-5,22-dien-3-ol, cholestadiene, campesterol, ergosta-4,6,22-triene, γ-sitosterol and stigmastan-3,5-dien. The content of total sterols was significantly increased by extensive extraction of this material at higher temperature under higher pressure.

CONCLUSION

In this study, a method for the extraction of *P. lamelliden* was established, and extraction parameters of SFE were optimized. Results from the present work indicated that (*Z*)-9-octadecenoic acid (oleic acid) was the major constituent in the extracts from all the SFE extraction and from 95% ethanolic extraction. In addition to oleic acid, the extracts were also rich in aliphatic hydrocarbons, aliphatic alcohols, aliphatic fatty acids, and steroids. The 39 isolated compounds were first reported present in *P. lamelliden*, although all are known. The three additional components isolated from 95% ethanolic extraction will be further investigated and identified in future research.

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黑螞蟻（*Polyrhachis lamellidens*）之超臨界二氧化碳抽取物的成分分析

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摘　要

蟻科昆蟲黑螞蟻（*Polyrhachis lamellidens* Fr. Smith）生長分布於中國大陸和臺灣等地，黑螞蟻在一般民間的藥用上是用來治療風濕病、類風濕關節炎、慢性肝炎、性功能低下和抗老化作用等。在本研究中我們利用超臨界二氧化碳萃取技術藉由設定各種不同溫度、壓力和萃取時間的實驗條件，由黑螞蟻昆蟲體中萃取出許多成分。而上述各種不同超臨界二氧化碳萃取條件之黑螞蟻抽取物，利用氣相層析質譜儀及質譜資料庫標準成分比對的方法進行黑螞蟻抽取物之成分分析研究。本篇文章將針對各種不同條件之超臨界二氧化碳萃取和95%酒精萃取的黑螞蟻抽取物進行抽取物間抽取量和抽取物成分組成之比較與探討。

研究結果顯示，三十九種已知的化合物首次在黑螞蟻抽取物中被分離出，而其中影響超臨界二氧化碳萃取率的主要因子就是萃取時之設定壓力。結果另外亦指出由超臨界二氧化碳萃取和95%酒精溶媒萃取出之抽取物的主要含量化合物是(*Z*)-9-octadecenoic acid (oleic acid)。上述抽取物中除了含oleic acid化合物外,另外亦含豐富的脂肪族碳氫類、醇類、脂肪酸類和類固醇類等化合物。

關鍵詞：黑螞蟻，*Polyrhachis lamellidens*，超臨界萃取，氣相層析質譜儀