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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF PETROLEUM ETHER EXTRACT OF *CANARIUM ALBUM*

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Chemical compositions and the relative yield of petroleum ether extract of *Canarium album* have been investigated by gas chromatography/mass spectrometry (GC/MS). Thirty components, representing 95.05% of petroleum ether extract, were identified. Among these, ethyl linoleate (27.94%), linoleic acid (22.14%), *n*-hexadecanoic acid (18.74%), and ethyl palmitate (8.63%) are the main constituents, and 12 components are first time reported for *C. album*. The antioxidant capacity of petroleum ether extract was determined by DPPH· and $ABTS^{+}$· assay. The results revealed that petroleum ether extract of *C. album* exhibited good antioxidant activity in both systems. It is the first report on the antioxidant activity of petroleum ether extract of *C. album*.

Keywords: *Canarium album*; petroleum ether extract; chemical composition; antioxidant activity.

1. INTRODUCTION

Canarium album (Lour.) Raeusch, called qingguo in China, is an evergreen tree in the Burseraceae family that grows above 30 m high [1]. It is mainly cultivated in the southeast area of China, and then has been introduced to other Asian tropical and semi-tropical regions. It is a hardy species reported to grow under various conditions, including saline or alkaline soils and rocky hillsides [2]. *C. album* has rich chemical composition, including polyphenols, flavones, triterpenes, etc., which imparts it with many unique pharmacological activities such as antibacterial, anti-inflammatory, anticancer, anti-hepatitis B, and antioxidant [3 – 5]. Accordingly, many *C. album*-related Chinese patent drugs have been made to treat various diseases [6].

Essential oils are natural liquid preparations comprising mixtures of volatile substances, which are produced from plant materials [7]. They are limpid and soluble in lipids and in organic solvents [8]. The chemical constituents of an essential oil basically include terpenes and molecules with the aromatic ring, which play the major role in biological action of essential oils. Besides, some minor components can strengthen the effect and/or increase additive functions [9]. Modern biomedical experiments confirm that essential oils have many pharmacological properties such as antibacterial, antifungal, antiviral, antidiabetic, antiparasitic, and insecticidal [10 – 12]. These effects are directly related to antioxidant ability [13].

Usually, essential oils are prepared by hydrodistillation or steam distillation. Extraction with a lipophilic solvent (petroleum, CS_2, CCl_4) is also feasible, which is often called solvent extraction at this time. In general, the olive oil that is widely used at present is mainly derived from common olive, but many other breeds of olive fruit like *C. album* also have a considerable amount of volatile oil. However, antioxidant capacity of essential oil from *C. album* has not been explored so far. Therefore, the aim of this study was to determine the composition and assess the antioxidant properties of petroleum ether extract of *C. album* with the purpose of providing theoretical basis for further study of the pharmacological activities of this extract.

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Fig. 1. Flowchart of obtaining petroleum ether extract from *C. album.*

2. EXPERIMENTAL

2.1. Plant Material, Chemicals and Instruments

The plant materials were collected in September 2014 from Jiangjin, Chongqing municipality and identified as the dried fruits of *Canarium album* (Lour.) Raeusch by Prof. Ren Shaoguang, College of Bioengineering, Chongqing University, China. A voucher specimen (No. 20140805) has been deposited in herbarium of the College of Environmental and Resources, Chongqing Technology and Business University, Chongqing, China.

All chemicals and reagents used were of analytical grade. The GC/MS analysis was carried out on an Agilent 7890A-5977A gas chromatograph – mass spectrometer system from Agilent Instrument Co. Ltd. (United States). The optical absorbance measurements were carried out on a T6 UV-Vis spectrophotometer from Purkinje General Instrument Co. Ltd. (Beijing, China).

2.2. Obtaining Petroleum Ether Extract

The ethanol extract from fruits of *C. album* was obtained by the heating reflux method with a Clevenger-type apparatus for 8 h using 80% ethanol as solvent. Then we carried out petroleum ether extraction from the ethanol extract, and the product was evaporated on a rotary evaporator until solvent was removed. The obtained light yellow essential oil (petroleum ether extract) was dried over anhydrous sodium sulfate and preserved in a sealed glass vial kept at 5°C until further analysis (Fig. 1).

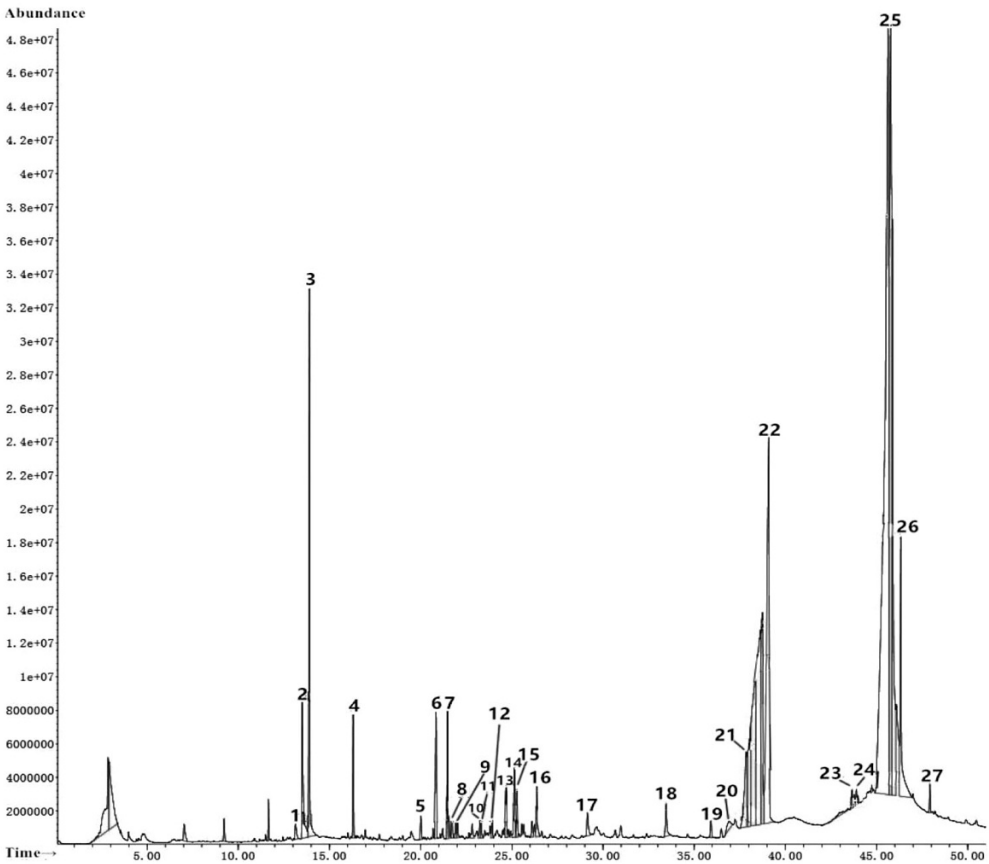

Fig. 2. Total GC/MS chromatogram of the petroleum ether extract of *C. album.*

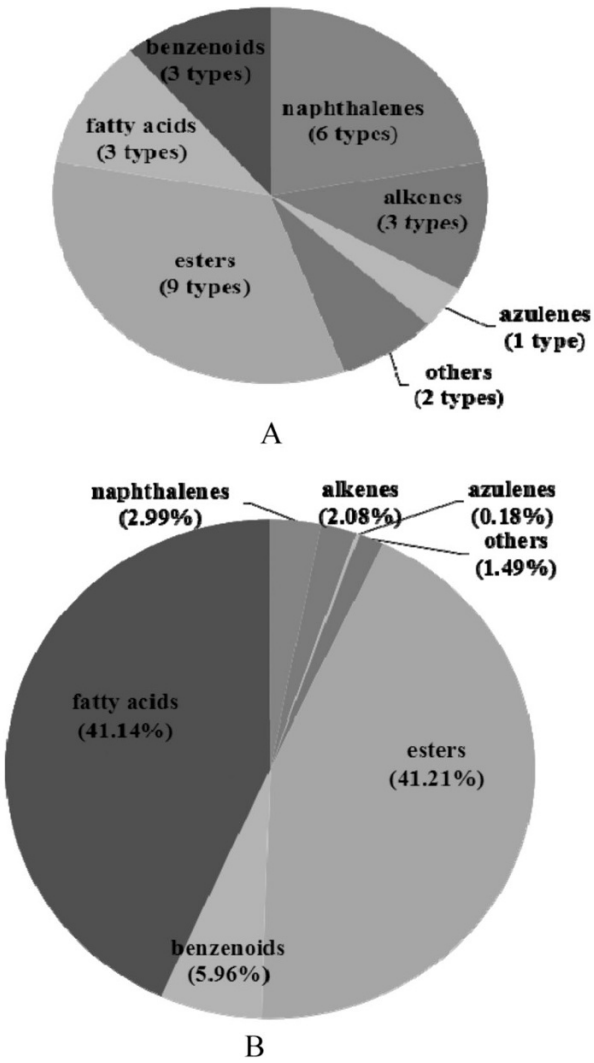

Fig. 3. Diagrams of (A) main families of compounds and (B) relative proportions (% total peak areas) of identified volatile compounds in the petroleum ether extract of *C. album*. The total number of identified volatile compounds was 27 and their relative amount accounted for 95.05% of the total petroleum ether extract yield.

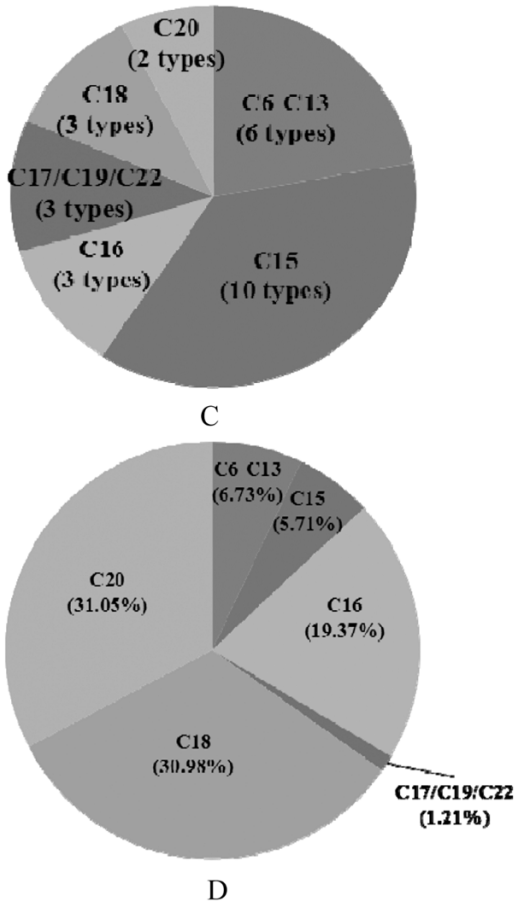

Fig. 4. Diagrams of (C) component fractions based on the carbon number and (D) relative proportions (% total peak areas) of identified compounds in the petroleum ether extract of *C. album*.

2.3. GC/MS Analysis

Analysis the petroleum ether extract was carried out using the Agilent GC/MS system equipped with HP-5 column of fused silica (30 mm × 0.25 mm; film thickness 0.25 μm) as described in the literature [14]; the injector and detector temperatures were 240 and 250°, respectively; the oven temperature was programmed as follows: 40° (2 min), 40 – 80° at a rate of 20°/min, 80° (1 min), 80 – 130° at a rate of 5°/min, 130° (5 minutes), 130 – 150° at a rate of 20°/min, 150° (1 min), 150 – 180° at a rate of 2°/min, 180° (5 min), 180 – 220° at a rate of 10°/min, and 220° (5 min). Helium (purity, above 99.99 %) was used as the carrier gas at a flow rate of 1 mL/min. The mass spectrometer was operating in the electron impact (EI) mode at 70 eV, the temperatures of the ion trap, ion trap mantle, and transfer line were 180°, 40°, and 250°, respectively, and the acquisition mass range was 50 – 700 amu.

Constituents of petroleum ether extract from *C. album* were identified individually based on their GC retention indices (RI) determined for all constituents using homologous series of n-alkanes on the HP-5 capillary column, by computer search using the libraries of NIST11.L [15] and Wiley Registry of Mass Spectral Data [16], as well as by comparison of fragmentation patterns in mass spectra with data published in the literature. The percentage composition of the petroleum ether extract was calculated using the electronic integration of peak areas.

2.4. Antioxidant Activity

Determination of antioxidant activity by the DPPH·assay. The antioxidant activity was evaluated by using the 2,2-diphenyl-β-picryl hydrazyl radical DPPH· as de-

scribed in the literature [17]. The DPPH· radical solution was dissolved in 95% ethanol with a final concentration of 0.1 mmol/L, the absorbance of the mixture solution with 2.7 mL DPPH· radical solution and 0.3 mL ethanol was measured as the negative control. The essential oil solutions in ethanol (0.3 mL) with various concentrations were pipetted into 2.7 mL DPPH· radical solution. Then, after a 30-minute incubation period at room temperature in the dark, the absorbance was read against the blank at 517 nm. The DPPH· scavenging activity was expressed according to the following equation:

$$S\% = [(A_{c(0)} - A_{c(t)})/A_{c(0)}] \times 100\%,$$

where $A_{c(0)}$ is the absorbance at 517 nm of the negative control reaction and $A_{c(t)}$ is the absorbance of 0.3 mL sample with 2.7 mL DPPH· solution. All samples were analyzed in triplicate. The DPPH· scavenging activity of the sample was expressed as a 50% scavenging concentration (SC_{50}), which represented the sample concentration (mg/mL) scavenging 50% of the DPPH· radical activity, with Trolox as the positive control.

Determination of antioxidant activity by the $ABTS^{+}$· assay. The $ABTS^{+}$ scavenging activity was measured as described [18] with some modification. First, the $ABTS^{+}$ working fluid was produced by mixing 0.2 mL $ABTS^{+}$· solution (7.4 mM) with 0.2 mL potassium persulfate (2.6 mM). The mixture was kept in the dark at room temperature for 12 h and then diluted about 50 times with 95% ethanol after sufficient reaction, To determine the scavenging activity, 8 mL aliquot of $ABTS^{+}$· reagent was mixed with 2 mL sample solutions of various concentrations, kept for 6 min in the dark after shaking for 10 sec immediately, and the absorbance at 734 nm was read against the blank. The $ABTS^{+}$· scavenging activity of samples was calculated as

$$I\% = [(A_{c(0)} - A_{c(t)})/A_{c(0)}] \times 100\%,$$

where $A_{c(0)}$ is the absorbance at 734 nm of 8 mL $ABTS^{+}$· working solution with 2 mL of 95% ethanol, while $A_{c(t)}$ is the absorbance of 8 mL $ABTS^{+}$· working solution with 2 mL sample. All samples were analyzed in triplicate. The $ABTS^{+}$· scavenging activity was also expressed as SC_{50}, with Trolox as the positive control.

3. RESULTS AND DISCUSSION

3.1. Analysis of Petroleum Ether Extract

Qualitative and quantitative analyses of the *C. album* petroleum ether extract composition was based on the total GC/MS flow chart (Fig. 2), which displayed the peaks of all components. A total of 27 components were isolated and identified as summarized in Table 1. These components were identified individually by search in library of the National Institute of Standards and Technology (NIST) database as well as by comparing their mass spectra to those reported in the literature.

The identified constituents account for 95.05% of the total oil. The volatile oil composition mainly included eight families of compounds (Fig. 3). There was a high percentage of esters (41.21%) and fatty acids (41.14%), followed by

TABLE 1. Composition of the Petroleum Ether Extract of *C. album*

No.	Retention index	Compound	Relative peak area (%)
1	1258	*p*-Anisaldehyde	0.18
2	1283	*trans*-Cinnamaldehyde	1.58
3	1289	Anethole	4.20
4	1377	Copaene	0.83
5	1475	1,2,4α,5,6,8α-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	0.24
6	1500	α-Muurolene	1.11
7	1523	1,2,3,5,6,8α-Hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1S-*cis*)-naphthalene	1.34
8	1532	1,2,3,4,4α,7-Hexahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene, 0.24	
9	1539	1,1,5-Trimethyl-1,2-dihydronaphthalene	0.20
10	1565	Isocaryophillene	0.14
11	1587	Gleenol	0.24
12	1602	Isolongifolol	0.18
13	1628	Naphthalene,1,2,3,4,4α,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-β-curcumene	0.57
14	1654	α-Cadinol	1.00
15	1695	1,2,3,4,4α,7,8α-octahydro-1,6-dimethyl-4-(1-methylethyl)-1-naphthalenol	0.40
16	1751	Cyclohexanol,2-[2-pyridyl]-1-(3-methyl-2-butenoxy)-4-(1-propenyl)benzene	0.49
17	1785	Ethyl *p*-methoxycinnamate	0.33
18	1855	Di-*sec*-butyl phthalate	0.37
19	1927	Hexadecanoic acid methyl ester	0.21
20	1944	Palmitoleic acid	0.26
21	1958	*n*-Hexadecanoic acid	18.74
22	1993	Ethyl palmitate	8.63
23	2118	Linoleic acid	22.14
24	2120	(E)-8-Octadecenoic acid methyl ester	0.17
25	2162	Ethyl linoleate	27.94
26	2197	Octadecanoic acid ethyl ester	3.11
27	2250	Tributyl citrate	0.21
Total			95.05

benzenoids (5.96%) and some other types of compounds such as naphthalenes (2.99%), alkenes (2.08%), azulenes (0.18%) etc. In particular, the major components were identified as ethyl linoleate (27.94%), (Z, Z)-9,12-octadecadienoic acid (22.14%), *n*-hexadecanoic acid (18.74%) and hexadecanoic acid ethyl ester (8.63%), which made up 77.45% of the gross volatile oil. Among these compounds, ethyl linoleate had maximum content, representing a pale yellow oily liquid with many pharmacological effects such as antibacterial, anti-inflammatory, and other [19].

According to previous studies, there is a large difference between the compositions of petroleum ether extract of *C. album* obtained by different extraction methods. According to Zhong [20], caryophyllene (33.83%) and D-germacrene (24.99%) were determined as the primary components of *C. album* by solid-phase micro-extraction. However, oleic acid (12.99%), ergostane (12.05%), and 3-ethyl -3-methylpentane (11.02%) were major components obtained by supercritical carbon dioxide extraction [21]. These results are significantly different, probably because of a strong influence of selected extraction pathways on the experimental results. In addition, Hiromu [22] determined α-pinene, camphene, β-pinene, etc. as the major constituents of *C. album* growing in Japan. At the same time, β-pinene (33.3%), α-terpinene (19.4%), γ-terpinene (14.1%) and terpinen-4-ol (11.9%) wore reported as the main components of petroleum ether extract obtained by hydrodistillation of *C. album* growing in Vietnam [23]. From these data, we can also see that constituents of petroleum ether extract from *C. album* can also vary depending on the material origin.

From another standpoint, the identified compounds fall into six overall categories on the basis of carbon number (Fig. 4). C-18 and C-20 compounds account for major ingredients of the total oil, with 30.98 and 31.05%, respectively, these compounds were mainly esters. Then, C-16 compounds amount to 19.37% and mainly include fatty acids. Besides, there were ten C-15 compounds, which accounted for 5.71% and primarily consisted of naphthalenes and alkenes.

It should be noted that 12 constituents of the petroleum ether extract of *C. album* were obtained and identified for the first time. These are benzaldehyde, 4-methoxy-cinnamaldehyde, anethole, α-muurolene, ethyl p-methoxy cinnamate, di-sec-butyl phthalate, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, (E)-8-octadecenoic acid methyl ester, ethyl linoleate, octadecanoic acid ethyl ester, and butyl citrate.

3.2. Antioxidant Activity

Essential oil is a strong active natural product, and antioxidant activity is one of its most important properties. There are many methods to evaluate the potential antioxidant activity due to different mechanisms. The DPPH· and $ABTS^+$ assays are both effective ways to test the antioxidant activity. In the DPPH· assay, the tested extract acts as a donor of hydrogen atoms or electrons in the transformation of DPPH· into PPH·-H. It can reduce the stability of DPPH· solution, which converts purple-colored radical DPPH· into yellow-colored DPPH·-H [24]. With Trolox used as the positive control, free-radical scavenging activities of the obtained essential oil and ethanol extract were tested by DPPH· assay. For both, in certain range of concentrations, there existed a good linear correlation between the antioxidant activity and concentration of essential oil solution. At high concentrations, the antioxidant activity tended to vary gently. The SC_{50} values were as follows: 0.038 mg/mL for Trolox, 3.700 mg/mL for petroleum ether extract of *C. album,* and 0.443 mg/mL for ethanol extract (Table 2). Based on the SC_{50} values, it is obvious that the petroleum ether extract of *C. album* expressed relatively weak DPPH· radical scavenging activity as compared to Trolox and ethanol extract. In order to evaluate petroleum ether extract of *C. album* more credibly, $ABTS^{+}$· assay system was also adopted for determi-

TABLE 2. DPPH· Scavenging Activities of Petroleum Ether Extract and Ethanol Extract of *C. album*

Sample	Regression equation	SC_{50} (mg/mL)	Linearity range (mg/mL)	R^2 (%)
Trolox	$Y = 1080.1X+8.39$	0.038	0.01 – 0.05	99.71
Petroleum ether extract	$Y = 10.864X+9.806$	3.700	1 – 6	99.33
Ethanol extract	$Y = 100.55X+5.465$	0.443	0.1 – 0.6	99.62

TABLE 3. $ABTS^{+}$· Scavenging Activities of Petroleum Ether Extract and Ethanol Extract of *C. album*

Sample	Regression equation	SC_{50} (mg/mL)	Linearity range (mg/mL)	R^2 (%)
Trolox	$Y = 1548.1X+7.273$	0.028	0.01 – 0.06	99.14
Petroleum ether extract	$Y = 14.218X+7.058$	2.991	1 – 6	99.65
Ethanol extract	$Y = 128.15X+4.1235$	0.358	0.2 – 0.7	99.59

nation of total antioxidant capacity. In the $ABTS^{+\cdot}$ assay, the SC_{50} values of Trolox, essential oil, and ethanol extract were 0.028 mg/mL, 2.991 mg/mL and 0.358 mg/mL respectively (Table 3). The tendency of results is almost the same as that in DPPH· system, namely, the radicals scavenging activity of petroleum ether extract is weaker than that of Trolox and ethanol extract.

It was reported in the literature that polyphenols possessed high potential to scavenge radicals, which also well explained good antioxidant capacity of the ethanol extract of *C. album* [25]. Obviously, phenolic compounds as the main contributors and the chemical components of petroleum ether extract only partly contributed to the antioxidant activity of *C. album*. The petroleum ether extract of *C. album* showed good radical scavenging activities in both DPPH· and $ABTS^{+}$ systems. Meanwhile, the radicals scavenging activity of petroleum ether extract was weaker than that of the ethanol extract. Therefore, it would provide a good direction to further explore other pharmacological activities of the petroleum ether extract of *C. album*. The chemical components possessing better radical scavenging activity should have larger polarity than that of the petroleum ether extract of *C. album*.

CONCLUSION

The petroleum ether extract of *C. album* was analyzed by GC-MS, and a total of 27 constituents of the extract were isolated and identified, including ethyl linoleate, (Z,Z)9,12-Octadecadienoic acid, *n*-hexadecanoic acid and hexadecanoic acid ethyl ester as the major compositions. Furthermore, the petroleum ether extract of *C. album* showed good potential antioxidant capacity. This study reports for the first time on the antioxidant activity of petroleum ether extract of *C. album* and provides some primary information for further exploration and use of *C. album* in the fields of food industry and medicine.

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