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FATTY ACID COMPOSITION AND ANTIOXIDANT ACTIVITY OF *CAMELLIA NINHII* SEED OIL COLLECTED IN LAM DONG PROVINCE

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Abstract

The seed oil of Camellia ninhii was studied for the first time on its fatty acid composition by gas chromatography-mass spectrometry (GC-MS) and antioxidant activity by the DPPH method. The results show that unsaturated fatty acids account for the largest amount, especially oleic acid with 45.43% of the total sample analyzed. In addition, other fatty acids, palmitic, linoleic, pentadecanoic, and two aromatic acids, benzoic and cinnamic, were present. The sample of C. ninhii seed oil exhibited mild antioxidant activity against DPPH free radicals with IC_{50} = 0.94 mg/mL.

Keywords: Antioxidant activity; *Camellia ninhii*; Fatty acid; GC-MS; Seed oil; Theaceae.

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1. INTRODUCTION

Camellia ninhii is a species of the genus *Camellia* (family Theaceae), which consists of a group of approximately 300 species (Luong & Le, 2016; Prince & Parks, 2000). Currently, a survey shows that there are about 3,000 cultivated varieties of Camellia grown worldwide, of which more than 2,500 are registered by the American Camellia Society (Mondal & Lanka, 2011). Camellias grow in the forests of mountainous areas of China and are grown only for oil; consequently, they do not affect arable land. Thus, they help reduce erosion in hilly areas, improve air quality, and generate income for local farmers (Liang et al., 2017).

Camellias, olive, palm, and coconut are the main tree oil crops in the world. Tea seed oil is also known as "oriental olive oil" because of its chemical composition, which is similar to olive oil in having high amounts of unsaturated fatty acids. Today, camellia oil is used as the primary cooking oil in the southern provinces of China. In addition to saturated and unsaturated fatty acids, the chemical composition of tea tree oil also contains polyphenols and squalene. The effects of this oil are known to be anti-tumor, lipid-lowering, hepatoprotective, heart-protective, antiseptic, anti-inflammatory, antioxidant, etc. (Li et al., 2011). Due to these effects, this oil is used in the production of drugs, cosmetics, soap, and hair oil in China. Additionally, seed pods are used as fuel, food for livestock, substances for cultivation – especially for growing mushrooms – and as absorbents (Liang et al., 2017).

C. ninhii is found in the evergreen broadleaf forest of Cat Tien district, Lam Dong Province, Vietnam. This species has a very short pedicel with undifferentiated bracts and sepals, androecium glabrous, styles 3, free, glabrous, capsule wall furfuraceous, larger leaves, young branches pubescent, and ovary pubescent (Luong & Le, 2016). Currently, research on fatty oils from camellia seeds in Vietnam is still limited. Therefore, we carried out this study to understand the chemical composition and antioxidant activity of *C. ninhii* seed fatty oil.

On that basis, *C. ninhii* seeds collected in the forest of Cat Tien district, Lam Dong Province, were selected as research objects. The *C. ninhii* seeds were extracted with n-hexane solvent to obtain tea seed oil. The chemical composition was determined by the gas chromatography-mass spectrometry (GC-MS) method, and antioxidant activity was measured by DPPH [2,2-diphenyl-1-picrylhydrazyl] assay. *C. ninhii* is a newly discovered species. It grows wild only in the forest, and there has been no in-depth study of its chemical composition. This is the first study on the fatty oil composition of *C. ninhii* seeds.

2. MATERIALS AND METHODS

2.1. Materials

The seeds of *C. ninhii* were collected in Cat Tien district (Lam Dong Province) in May 2021 and authenticated by our research team members of the Faculty of Biology,

Dalat University. A voucher specimen has been deposited at the Faculty of Biology, Dalat University.

Figure 1. The fruit of *C. ninhii*

Source: Photo by Hoang Thanh Truong.

Figure 2. The seeds of *C. ninhii*

Source: Photo by Hoang Thanh Truong.

2.2. Extraction

The seeds (180 g) were shelled to collect the kernels (96.5 g), which were then crushed. The moisture content (46.87%) of the crushed kernels was determined using a Ohaus MB120 moisture analyzer. The extraction of *C. ninhii* oil was accomplished in 6 hours using an automatic Soxhlet apparatus filled with 250 mL of n-hexane as the extraction solvent. After removal of the solvent under reduced pressure with a Buchi model R-300 rotary evaporator, a total mass of 3.98 g of *C. ninhii* oil was obtained.

2.3. Method for determining the fatty acid composition

The fatty acid composition of total *C. ninhii* oil was determined by GC-MS analysis at the Pharmaceutical Chemistry Research and Development Laboratory of the

Centre for Research and Technology Transfer, Vietnam Academy of Science and Technology.

The fatty oil sample was converted into a methyl ester form for the gas chromatography analysis. The GC instrument was a SCION SQ 456-GC (Rxi-5ms RESTEK capillary column 30 m/0.25 mm/0.25 µm). The carrier gas was helium, the constant flow rate was 1 mL/min, the injector temperature was 250°C, and the split ratio was 30. The oven temperature program started with an initial temperature of 50°C, which was held for 1 min. The oven temperature was then raised at rates that varied with the temperature range (50–150°C: 40°C/min; 150–220°C: 10°C/min; 220–230°C: 3°C/min; 230–280°C: 25°C/min). The final temperature was held constant for 5 min. For mass spectrometry, an electron impact (EI+) probe was used with an ionization energy of 70 eV, a full scan mode from 50 to 500 amu, a scan velocity of 1 s/scan, and an ionization source temperature of 250°C. The fatty acid methyl esters were identified and quantified by comparing with fatty acid methyl ester standards.

2.4. Antioxidant activity

The antioxidant activity of *C. ninhii* seed oil was measured by the DPPH method in the Laboratory of the Faculty of Biology, University of Natural Sciences, Vietnam National University Ho Chi Minh City.

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical compound. It has absorption maxima at a wavelength of 517 nm. The DPPH radical scavenging method was modified to evaluate the free radical scavenging effect of *C. ninhii* seed oil. The DPPH reagent was prepared by mixing 40 µg/mL DPPH with methanol (80%) solvent at room temperature. Then, to determine the scavenging activity, 0.75 mL DPPH reagent was mixed with 0.5 mL of the sample and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV spectrophotometer with methanol (80%) solvent used as a control. The DPPH scavenging effect was measured using the following formula: 100*[(A-B)/A], where A is the OD517 value of the control and B is the OD517 value of the sample (Le et al., 2020).

The IC_{50} DPPH value (the sample concentration required for inhibition of 50% of the DPPH radicals) was obtained by extrapolation from regression analysis. Subsequently, the antioxidant activity was evaluated based on this IC_{50} value.

3. RESULTS AND DISCUSSION

3.1. Oil content of *C. ninhii* seeds

A low amount of oil, approximately 7.76% in dried kernels and 4.16% in dried seeds, was extracted from *C. ninhii* seeds. The oil content of this seed is extremely low

compared to other seeds of genus *Camellia* (Table 1). This could be the result of harvesting the seeds from wild plants in the forest at a time when the oil content was not high.

Table 1. Oil content of some species of the genus *Camellia*

Species	Oil production		Source
	Oil content of dry kernels (w/w, %)	Oil content of dry seeds (w/w, %)	
C. ninhii	7.76	4.16	
C. sinensis[a]	17.60 ± 0.03		Phan et al. (2021)
C. vietnamensis	37.96 – 46.78	21.00 – 30.61	Liang et al. (2017)
C. oleifera	41.73 – 56.20	21.47 – 33.73	Liang et al. (2017)
C. meicarpa	34.02 – 46.52	20.50 – 31.60	Liang et al. (2017)
C. checkiangoleosa	50.40 – 56.60	27.00 – 34.10	Liang et al. (2017)
C. yuhsienenis	37.06 – 52.19	20.50 – 26.40	Liang et al. (2017)
C. semiserrata	56.15 – 64.19	20.50 – 29.40	Liang et al. (2017)
C. reticulata	54.25 – 58.94	25.00 – 32.00	Liang et al. (2017)

Note: a: Extraction with n-hexane.

3.2. The fatty acid composition of *C. ninhii* seed oil

The GC-MS analysis results of the chemical composition of *C. ninhii* seed oil are shown in Table 2. Oleic, palmitic, linoleic, and pentadecanoic fatty acids are the main components of *C. ninhii* seed oil, accounting for 79.06%. Oleic acid, which has the highest concentration, was found to have a cholesterol-lowering effect and other attributes, such as reducing blood pressure and the risk of strokes (Wang et al., 2011). The oil was found to contain 4.83% *trans*-cinnamic acid. It is a naturally occurring aromatic fatty acid that has been shown to reverse the growth of certain human cancer cells, such as glioblastoma, melanoma, and prostate and lung carcinoma cells (Liu et al., 1995).

Table 2. Composition of *C. ninhii* seed oil

No.	Name	Content (%)
1	Benzoic acid	3.60
2	trans-Cinnamic acid	4.83
3	Palmitic acid	27.83
4	Linoleic acid	3.78
5	Oleic acid	45.43
6	Pentadecanoic acid	2.02
7	Others	12.51

The oleic and linoleic acid content of *C. ninhii* seed oil was lower, and its palmitic acid content higher, than that of other species studied and analysed in the genus *Camellia* (Table 3).

Table 3. Fatty acid content of some *Camellia* seed oils

Species	Oleic acid (%)	Palmitic acid (%)	Linoleic acid (%)	Source
C. ninhii	45.43	27.83	3.78	
C. sinensis	52.90	17.70	24.20	Wang et al. (2011)
C. oleifera	77.84 ± 0.5	10.20 ± 0.5	8.30 ± 0.4	Yu et al. (2013)

3.3. Antioxidant activity

C. ninhii oil was found to have an antioxidant activity against DPPH free radicals with an IC_{50} value of 0.94 mg/mL. The IC_{50} value of vitamin C (positive control) was 0.005 mg/mL. The antioxidant activity of *C. ninhii* seed oil is much higher than that of other species in the same genus (Table 4).

Table 4. Antioxidant activity of *Camellia* seed oils

Species	Method	IC_{50} (mg/mL)	Solvent	Source
C. ninhii	DPPH	0.94	*n*-hexane	
Vitamin C (control)	DPPH	0.005		
C. sinensis O. Kuntze	DPPH	140.26 ± 0.01	*n*-hexane	Phan et al. (2021)
C. sinensis L.	DPPH	59.6	Petroleum ether	Wang et al. (2011)
C. oleifera	DPPH	3.31 ± 0.07	*n*-hexane	Yu et al. (2013)

The results show that *C. ninhii* seeds contain a relatively high amount of unsaturated fatty oils with mild antioxidant activity. Thus, it can be argued that *C. ninhii* is potentially a healthy food source and may become a profitable crop for farmers.

4. CONCLUSION

C. ninhii seeds collected in the forest of Cat Tien district, Lam Dong Province contained 7.76% fatty oil, calculated to dry materials. The main fatty acid components of *C. ninhii* seed oil are oleic acid (45.43%) and palmitic acid (27.83%). Other components are two fatty acids (linoleic and pentadecanoic) and two aromatic acids (benzoic and *trans*-cinnamic).

C. ninhii seed oil exhibited antioxidant activity against DPPH free radicals with an IC_{50} value of 0.94 mg/mL. The antioxidant activity of this seed oil was the highest among the seeds of the same genus investigated.

This is the first report of the chemical composition and antioxidant activity of the seed oil of this species.

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