Trop J Nat Prod Res, February 2023; 7(2):2426-2431 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oils Extracted from the Fresh and Dried Seeds of *Lanxangia tsao-ko* grown in Nghe An, Vietnam

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ARTICLE INFO

Article history:
Received 26 January 2023
Revised 07 February 2022
Accepted 08 February 2023
Published online 01 March 2023

ABSTRACT

Lanxangia tsao-ko has been used in traditional oriental medicine to treat malaria, abdominal pain, digestive disorders, and inflammation-related diseases. The aim of this study was to compare the volatile composition and evaluate the total phenolic content (TPC), antioxidant, and antimicrobial activities of *L. tsao-ko* fresh and dried seed essential oils (EOs) grown in Vietnam. The EOs of *L. tsao-ko* seeds were analyzed by gas chromatography-mass spectrometry. The results showed that the major constituents of the seed essential oils were 1,8-cineole, *α*-citral, tetracyclo[3.3.1.0.1(3,9)]decan-10-one, *α*-phellandrene, and *β*-citral. The TPC ranged from 1.47 mg GAE/g EO in EOs of fresh seeds to 1.70 mg GAE/g EO in EOs of dried seeds. The EOs extracted from fresh and dried seeds could scavenge DPPH with IC_{50} values of 68.79 and 56.44 mg/mL, respectively. Moreover, the EOs were shown to be active against *E. faecalis*, *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, *S. .enterica*, and *C. albicans* (with MIC = 32-128 µg/mL). The results generally indicated significant differences in chemical composition and bioactivities between EOs obtained from fresh and dried seeds. The findings of the study provide more information about the volatile profile and additional evidence of potential health-endorsing properties of *L. tsao-ko* EOs.

Keywords: 1,8-cineole, antimicrobial activity, essential oil; *Lanxangia tsao-ko*, total phenolic content.

Introduction

Lanxangia is a genus of the Zingiberaceae family with eight accepted species and is recorded in China South-Central, China Southeast, Laos, Myanmar, Thailand, and Vietnam.[1] Previously, the genus *Lanxangia* has been known as a synonym of the genus *Amomum* Roxb., however since 2018, *Lanxangia* has been considered a separate genus.[2] The most famous in the genus *Lanxangia* is *Lanxangia tsao-ko* (Crevost & Lemarie) M. F. Newman & Skornick, which is commonly found growing in the southwestern provinces of China, including Yunnan and Guangxi, Laos, and northern Vietnam.[3] Its dried fruit, often called "Căoguǒ" in Chinese and called "Thảo quả" in Vietnamese, is an important spice in Chinese cuisine.[4] In traditional Chinese medicine, it is often used to treat throat infections, malaria, cold dampness, abdominal pain, gastropathy, and dyspepsia.[5] Besides, it may support weight loss and have a positive effect on eliminating bad breath.[6] Research has revealed that *L. tsao-ko* possesses multiple bioactivities important to human health, such as antioxidant, anti-inflammatory, anticancer, antidiabetic, antimicrobial, and neuroprotective effects.[7-13]

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Citation: Tran HT, Le ND, Tran HTT, Tran THT, Ngo MT, Nguyen VTH, Nguyen KD, Vu D, Tran CV, Le GD. Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oils Extracted from the Fresh and Dried Seeds of *Lanxangia tsao-ko* grown in Nghe An, Vietnam. Trop J Nat Prod Res. 2023; 7(2):2426-2431 http://.www.doi.org/10.26538/tjnpr/v7i2.20

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The fruit of *L. tsao-ko* has great flavor and fragrance, and therefore, EOs from this plant part, also known as Chinese black cardamom oil, has attracted much attention. Studies on the chemical profile of EO showed the presence of major monoterpene hydrocarbons and oxygenated monoterpenes, including phellandrene, pinene, 1,8-cineole, geranial, and geraniol.[7] Numerous studies focused on therapeutic applications of 1,8-cineole to treat respiratory illness, inflammation, and pain in gout have been conducted.[14,15] Phellandrene has been known to exert a variety of bioactivities, such as antimicrobial, antioxidant, anti-inflammatory, insecticidal, and repellent properties.[16] Previous research has indicated the significant variations in volatile profiles of EOs from *Amomum cardamomum* fresh and dried seeds, also known as Indian cardamom oil.[17] Accordingly, this led to differences in odour and aroma quality characteristics of the resultant EOs, and perhaps, their bioactivities as well. In the present study, two EOs extracted from fresh and dried *L. tsao-ko* were investigated. To the best of our knowledge, this is the first study to compare the chemical composition, antimicrobial and antioxidant activities of the EOs of *L. tsao-ko* fresh and dried seed seeds collected in Vietnam. This will hopefully provide a better understanding of how volatile constituents contribute to aroma and flavor as well as potential health-endorsing properties of *L. tsao-ko* EOs.

Materials and Methods

Plant collection and identification

The seeds of *L. tsao-ko* were collected from Dong Van commune, Que Phong district, Nghe An province, Vietnam in December 2021 and were identified by Dr. Dang Van-Son, Institute of Tropical Biology, Vietnam Academy of Science and Technology. A voucher specimen

(HieuTT/1209) was deposited at the herbarium of the Department of Chemistry, College of Education, Vinh University, Nghe An Province, Vietnam.

Extraction of EOs

According to the Vietnamese Pharmacopoeia, the fresh and dried seeds (each 1.0 kg) of *L. tsao-ko* were chopped and hydro-distilled separately using Clevenger-type apparatus for 4h.[18] The distilled EOs were dehydrated over anhydrous sodium sulfate to remove traces of water and stored at 4ºC in the refrigerator before gas chromatography-mass spectrometry (GC/MS) analysis and bioactivity tests. ~~All the experiments were repeated three times~~.

Chemical identification of EO sample

GC/MS analysis was carried out using an Agilent GC-7980 coupled to an Agilent MS 5977C, with an HP-5MS UI column (30 m × 0.25 mm × 0.25 µm, Agilent). Helium was applied as carrier gas with a flow rate of 1 mL/min. An amount of 1 µL of EO sample (diluted in *n*-hexane, 1:100 ratio) was injected using a split ratio of 1:25. The column temperature program was initiated at 60ºC (held for 1 min), followed by an increase to 240ºC at the rate of 4°C/min and finally held for 4 min. The injector, MS Quad, and transfer line temperature were set at 300, 150, and 300ºC, respectively. The MS source was 230°C, the ionization voltage 70 eV, and the mass range m/z 50-550 (2.0 scan/s). The identification of the EO components was based on comparing of their mass spectra values to those in the literature (NIST17 and Adams book),[19] and then it was subsequently verified using a comparison of retention indices about to a homologous series of *n*-alkanes. For quantification, the relative peak area (%) was utilized.

Total phenolic content (TPC)

The TPC was determined using the published method.[20] Briefly, 0.1 mL of samples or standard (gallic acid solution from 0 - 100 µg/mL) was mixed with 0.5 mL of 10-fold diluted, 0.4 mL of 10% Na_2CO_3 solutions, and 1.5 mL of deionized water. The mixture was incubated for 2h and measured spectrophotometrically at 760 nm. The results were calculated on the calibration curve ($y = 0.2652x – 2.8283$, $R^2 = 0.9981$) and expressed in milligrams of gallic acid equivalents (GAE) per gram of dried extract.

DPPH scavenging assay

The scavenging activity of EOs against free radicals was determined using a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and vitamin C as a positive control.[20] The samples with concentrations ranging from 0 - 100 mg/mL or standards (0.1 mL) were mixed with 0.1 mL of 0.3 mM DPPH solution. The mixtures were incubated in dark for 30 min and measured absorbance at 517 nm. The scavenging activity of the standards or samples compared to the negative control was calculated as the following equation:

$$\text{Scavenging activity (\%)} = [(ANC- At)/ANC] \times 100$$

Where ANC denoted the absorbance of negative control and At represented the absorbance of the tested samples. The IC_{50} values were then estimated based on the fitted lines between concentrations and scavenging activity. The linear regression models for EOs were performed using Microsoft Excel (Microsoft Corporation, 2018). The smaller IC_{50} means the higher scavenging activity of samples.

Antimicrobial assay

The antimicrobial activity of the EOs was tested against *Enterococcus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 14579); *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 13076); and *Candida albicans* (ATCC 10231) which were obtained from the National Institute for Food Control (No. 65 Pham Than Duat Street, Mai Dich Ward, Cau Giay District, Ha Noi, Vietnam).

The antimicrobial activity of the EOs was evaluated by the microdilution broth susceptibility assay method according to the Clinical and Laboratory Standards Institute guidelines.[21] Luria Bertani Agar (LBA) and Sabouraud Dextrose Agar (SDA) were used to culture bacterial and yeast on 96-well plates in laboratory conditions, respectively. Streptomycin and cycloheximide were employed as reference compounds for bacteria and yeast, respectively, which were provided by the Institute of Drug Quality Control (Ho Chi Minh City, Vietnam). The experiments were conducted with the following steps. The EOs were diluted in 10% DMSO and prepared in eight different concentrations (from 2 to 256 µg/mL). The test bacterial and fungal samples were standardized on a 96-well plate with a concentration of approximately 2×10^5 CFU/mL. The mixtures of microbiological and testing EO samples were incubated at 37°C for 18 - 24h for bacteria and at 35 - 37°C for 36 - 48h for yeast. After 24h, the minimum inhibitory concentration (MIC) values were preliminarily determined as the lowest sample concentration, in which the samples showed complete inhibition of microbial growth after 24h. ~~The experiments were performed in triplicates~~.

Statistical analysis

All the experiments were conducted 3 times. The results were represented under Mean ± SD. The linear regression model and ANOVA one way were performed using Microsoft Excel (Microsoft, 2018). P value less than 0.5 was considered significant.

Results and Discussion

Chemical constituents of EOs

Two EO samples (fresh seed essential oil, HC-01; dried seed essential oil, HC-02) were pale yellow, lighter than water (d < 1). Steam hydrodistillation of fresh and dried seeds produced EOs in 1.07% and 1.19% (v/w) yields, respectively. The EOs of *L. tsao-ko* fresh and dried seeds of Vietnamese origin were analyzed by GC/MS. The results of the chemical composition of HC-01 and HC-02 samples are presented in Table 1, Figure 1, and Figure 2, respectively. As presented in Table 1, thirty-six components were identified in HC-01 oil and thirty-three components in HC-02 oil, representing 99.32 % and 98.13 % of the related essential oils, respectively, most of which were found to be oxygenated monoterpenoids (74.57% in HC-01 oil, 68.31% in HC-02 oil), followed by monoterpene hydrocarbons (12.42% in HC-01 oil, 21.10% in HC-02 oil), aldehyde compounds (8.31% in HC-01 oil, 6.66% in HC-02 oil), and phenolics compounds (4.02% in HC-01 oil, 2.06% in HC-02 oil). Specifically, the major constituents of the fresh seed essential oils were 1,8-cineole (40.75%), *α*-citral (13.67%), tetracyclo[3.3.1.0.1(3,9)]decan-10-one (10.31%), *α*-phellandrene (7.88%), and *β*-citral (6.07%), while the dried seed essential oils were dominated by 1,8-cineole (41.08%), *α*-phellandrene (12.84%), *α*-citral (12.01%), and tetracyclo[3.3.1.0.1(3,9)]decan-10-one (8.04%).

It is remarkable that the composition of the seed EOs collected in Vietnam is partly consistent with the previous report.[22] However, the components of the dried seed essential oil (collected in Lao Cai province, with 27 components) were lower than that of the seed essential oil (collected in Nghe An province, with 33 components). In comparison with the major EO components in previous studies, the EO samples in the present study shared some qualitative components, differing mainly in the quantitative components. These differences might be the result of variability associated with the origin of the herb and analytical techniques.[23,24] For example, by GC/MS analysis, thirty-two components (accounting for 96.35%) and thirty-three components (accounting for 96.93%) were identified in hydrodistillation and microwave-assisted hydrodistillation oils, respectively.[23] Furthermore, the main component of the EO, 1,8-cineole, was also found in *L. tsao-ko* EO, but the quantitative composition varied depending on the source of the sample collection (e.g., 28.9-40.89% in Chinese essential oil,[23,25] and 30.6% in Vietnamese essential oil).[22]

Total phenolic content (TPC)

The TPC of the EOs was tested with the results shown in Table 2. The TPC of HC-01 was slightly higher than that of HC-02: 1.70 and 1.47 mg GAE/g EO, respectively. The results indicated that the drying process could reduce the level of TPC in EOs extracted by 13%. In addition, other factors include solvent, extraction methods, extraction time, and temperature.[26]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Chemical composition of two EOs extracted from the fresh and dried seeds of *Lanxangia tsao-ko*

No.	Components	RI^{cal}	RI^{lit}	HC-01	HC-02	Method of identification
1	hexanal	-	800	0.06	0.07	MS
2	*α*-thujene	931	929	0.13	0.24	RI, MS
3	*α*-pinene	939	937	0.95	1.95	RI, MS
4	sabinene	978	974	0.17	0.34	RI, MS
5	*β*-Pinene	981	979	0.17	0.27	RI, MS
6	*β*-myrcene	992	991	0.42	0.7	RI, MS
7	octanal	1003	1003	0.66	0.53	RI, MS
8	*α*-phellandrene	1007	1005	**7.88**	**12.84**	RI, MS
9	3-carene	1013	1011	0.2	0.33	RI, MS
10	*α*-terpinene	1020	1017	0.16	0.22	RI, MS
11	*p*-cymene	1029	1025	0.84	0.94	RI, MS
12	limonene	1033	1030	1.64	2.66	RI, MS
13	1,8-cineole (= eucalyptol)	1037	1032	**40.75**	**41.08**	RI, MS
14	*cis*-*β*-ocimene	1041	1038	0.05	0.07	RI, MS
15	*trans*-*β*-ocimene	1052	1049	0.52	0.77	RI, MS
16	*trans*-2-octenal	1061	1060	3.63	2.44	RI, MS
17	*γ*-terpinene	1063	1060	-	0.52	RI, MS
18	terpinolene	1091	1088	0.13	0.19	RI, MS
19	linalool	1100	1099	0.22	0.16	RI, MS
20	*cis*-chrysanthenol	1168	1162	0.15	-	RI, MS
21	*δ*-terpineol	1170	1166	0.25	-	RI, MS
22	(*E*)-ocimenol	1172	1169	-	0.19	RI, MS
23	terpinen-4-ol	1180	1177	0.69	0.53	RI, MS
24	3,7-dimethyl-3,6-octadienal	1185	1184	0.25	0.22	RI, MS
25	*α*-terpineol	1192	1189	2.07	1.56	RI, MS
26	*cis*-4-decenal	1198	1193	0.09	0.05	RI, MS
27	*β*-citral	1245	1240	**6.07**	4.43	RI, MS
28	(*Z*)-2-decenal	1251	1252	0.08	0.06	RI, MS
29	linalyl acetate	1258	1257	0.14	0.09	RI, MS
30	2(*E*)-decenal	1265	1263	3.67	3.36	RI, MS
31	*α*-citral	1275	1270	**13.67**	**12.01**	RI, MS
32	tetracyclo[3.3.1.0.1(3,9)]decan-10-one	1290	-	**10.31**	**8.04**	MS
33	2-isopropylbenzaldehyde	1307	-	1.23	0.86	MS
34	indane-4-carboxaldehyde	1344	-	0.4	0.26	MS
35	hydrocoumarin	1358	1359	0.7	-	RI, MS
36	*α*-methylcinnamaldehyde	1375	-	0.4	-	MS
37	*α*-cyclopropylbenzyl alcohol	1380	-	0.45	-	MS
38	(*E*)-2-dodecenal	1468	1468	0.12	0.15	RI, MS
	monoterpene hydrocarbons			12.42	21.1	
	oxygenated monoterpenes			74.57	68.31	
	phenolic compounds			4.02	2.06	
	aldehyde compounds			8.31	6.66	
	Total identified %			99.32	98.13	

Notes: RI^{cal}: retention index calculated on HP-5MS Ultral Inert column; RI^{lit}: retention index reported in the literature; HC-01: EO sample extracted from the fresh seeds of *L. tsao-ko*; HC-02: EO sample extracted from the dried seeds of *L. tsao-ko*; RI, MS: Identified based on retention index (RI) matching and mass-spectra (MS) comparison of compounds on HP-5MS Ultral Inert column with those on the database NIST17 and literature

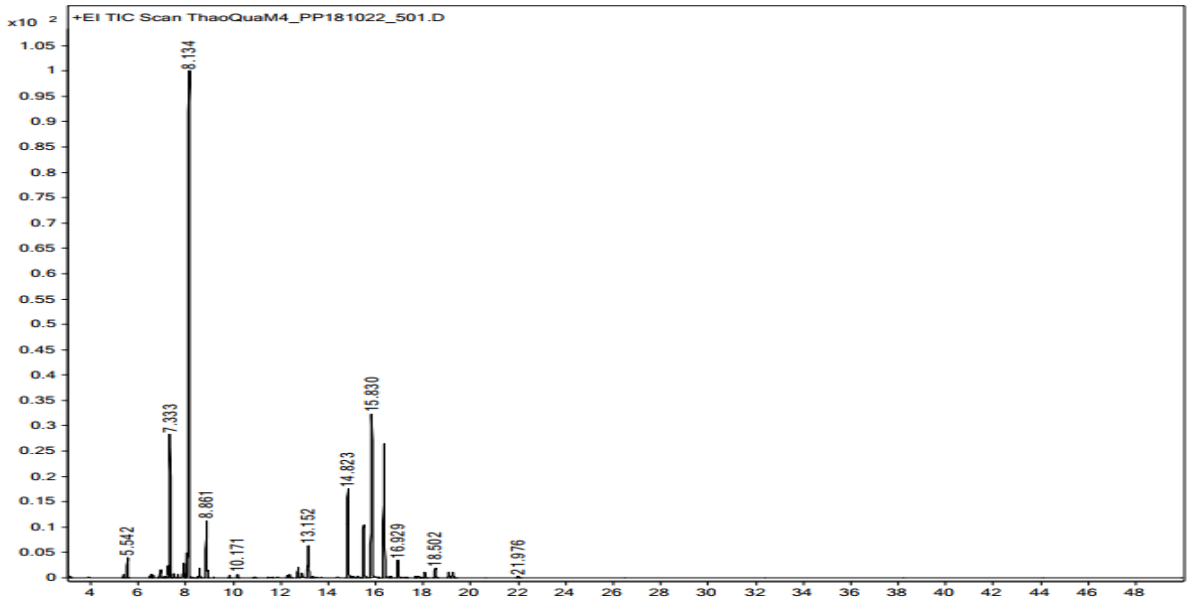

Figure 1: A representative gas chromatogram of HC-01

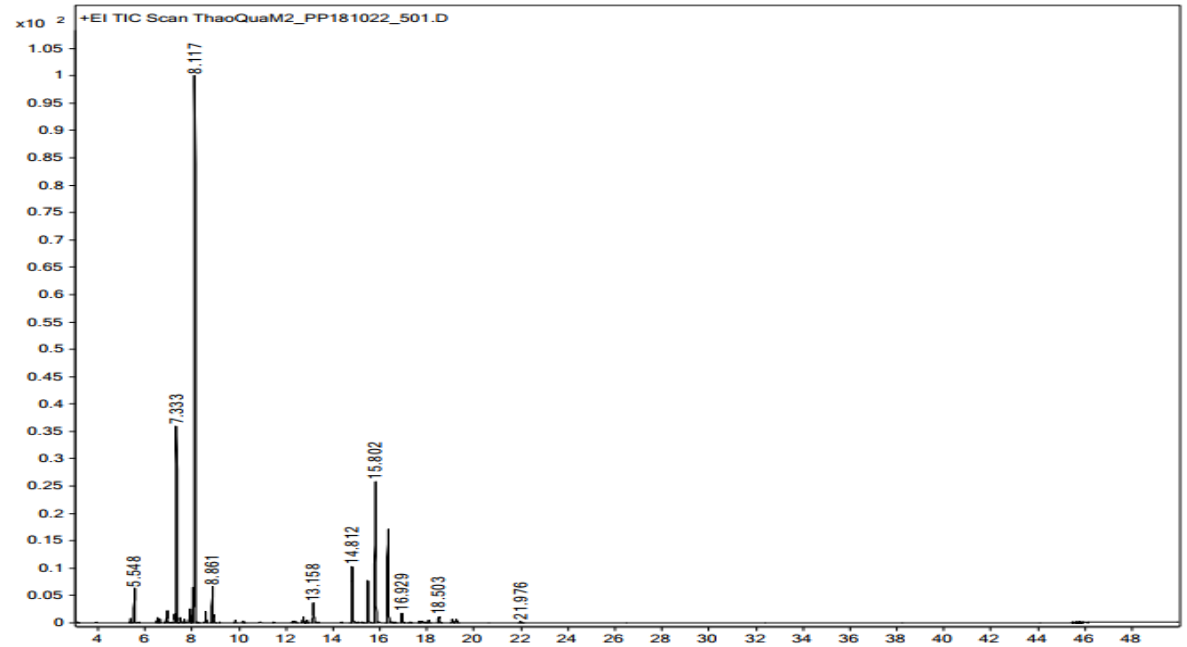

Figure 2: A representative gas chromatogram of HC-02

DPPH scavenging activity

The two EOs showed scavenging activities against DPPH. The IC_{50} values of HC-01 and HC-02 were 68.79 and 56.44 mg/mL, respectively (Table 3). Both EOs showed low antioxidant activities compared to ascorbic acid (IC_{50} = 14.48 µg/mL). In comparison to some other EOs, these scavenging activities were also lower but more comparable. IC_{50} values of EOs from *Haplophyllum tuberculatum* (Forsskal) A. Juss and *Calamintha incana* (Sm.) Helder (Lamiaceae) were 3.24 mg/mL and 15.38 mg/mL, respectively.[27,28] As TPC was reported to be the main component prescribed for antioxidant activities,[20,29] the small percentage of TPC (Table 2) in the two EOs could be one of the reasons for the low antioxidant activities tested. It should be noted that the EOs extracted from the dried seeds were more active than the fresh ones. However, this greater was not significant (P>0.05). This may be due to the similar volatile compositions of these two EOs.

Evaluation of in vitro antimicrobial activity

As shown in Table 4, HC-01 and HC-02 samples co-possessed a MIC value against the pathogenic microorganisms *S. aureus*, *E. coli*, *C. albicans* (MIC = 32 µg/mL), and *P. aeruginosa* (MIC = 128 µg /mL). Additionally, HC-02 had a lower MIC value than HC-01 (ca. 2 times) against the bacterium *E. faecalis*, *B. cereus*, and *S. enterica*. This result demonstrated that the antimicrobial activity of the dried seed essential oil was stronger than that of the fresh seed essential oil. Streptomycin (MIC = 32-256 µg/mL) and cycloheximide (MIC = 32 µg/mL) were used as the positive controls against the pathogenic microorganisms, respectively.

In a review of over 500 publications detailing the antimicrobial activity of plants, Van Vuuren (2008) proposed that EOs with MIC values of 200 µg/mL or lower could be considered noteworthy antibacterial.[30] This study showed that seed essential oil consisting mainly of oxygenated monoterpenoids, monoterpene hydrocarbons, aldehydes and phenolics was shown to be effective against pathogenic microorganisms (with MIC values of EO from less than 128 µg/mL).[30,31] Moreover, Li *et al.* (2022) indicated that 1,8-cineole, 2(*E*)-decenal, *α*-citral, *β*-citral, *α*-pinene, and *α*-terpineol are likely antibacterial compounds.[25] Previous research has shown that *L. tsao-ko* fruit EO possesses potential antimicrobial properties,[7,32] and that it is

extensively utilized as a flavoring and preservative in daily food.[33] It has been widely observed that Gram-positive bacteria are more sensitive to *L. tsao-ko* dried fruit EOs than gram-negative bacteria.[34,35] Gram-negative bacteria's less sensitivity to EOs can be explained by the presence of a hydrophilic outer membrane that prevents hydrophobic EOs from penetrating the target cell membrane. The antibacterial results of this study are consistent with those reported by previous studies.

Table 2: The TPC of two EO samples

EO samples	TPC (mg GAE/g EO)
HC-01	1.70 ± 0.08
HC-02	1.47 ± 0.05

Mean ± SD; n = 3.

Table 3: Antioxidant activity of two EOs as measured by DPPH assay

Concentration (mg/mL)	DPPH free radical scavenging (%)		
	HC-01	HC-02	Ascorbic acid
0	0.00 ± 5.54	0.00 ± 5.54	
10	6.05 ± 1.10	10.98 ± 10.49	
20	23.72 ± 0.97	27.06 ± 3.93	
40	34.78 ±1.59	39.55 ± 2.58	
60	44.20 ± 2.51	52.79 ± 4.42	
80	54.12 ± 3.57	63.38 ± 3.70	
100	68.92 ± 1.14	78.51 ± 2.90	
120	78.21 ± 1.19	82.13 ± 1.23	
140	82.02 ± 2.09	80.96 ± 2.40	
160	81.88 ± 1.28	81.38 ± 3.02	
IC_{50}	**68.79 (mg/mL)**	**56.44 (mg/mL)**	**14.48 (µg/mL)**

IC_{50}: the half maximal inhibitory concentration; Mean ± SD; n = 3.

Table 4: Antimicrobial activity of two EO samples, streptomycin, and cycloheximide.

Microorganisms	MIC (µg/mL)			
	HC-01	HC-02	Streptomycin	Cycloheximide
Enterococcus faecalis ATCC299212	64 ± 1.30	32 ± 0.51	256	-
Staphylococcus aureus ATCC25923	32 ± 1.34	32 ± 0.18	256	-
Bacillus cereus ATCC14579	64 ± 2.67	32 ± 0.48	128	-
Escherichia coli ATCC25922	32 ± 1.34	32 ± 1.12	32	-
Pseudomonas aeruginosa ATCC27853	128 ± 3.67	128 ± 2.41	256	-
Salmonella enterica ATCC13076	64 ± 1.89	32 ± 1.02	128	-
Candida albicans ATCC10231	32 ± 1.42	32 ± 1.07	-	32

MIC = Minimum inhibitory concentration; Mean ± SD; n = 3; (-) not tested.

Conclusion

This work was the first study to compare the compositions and bioactivities of EOs from *L. tsao-ko* fresh and dried seeds collected in Vietnam. A total of 36 and 33 compounds from fresh and dried seed essential oils were identified, respectively. The EO from dried seeds showed the strongest antioxidant activity with an IC_{50} value of 56.44 mg/mL. The EOs also showed antibacterial activity with MIC values of 32-128 µg/mL. These results suggested that EOs of *L. tsao-ko* seeds is beneficial for developing effective antioxidant and antibacterial herbal products or remedies.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. POWO. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. [Online]. 2022. [cited 2022 Dec 16]. Available from: http://www.plantsoftheworldonline.org/.
2. Boer HD, Newman M, Poulsen AD, Droop AJ, Fér T, Thu Hiền LT, Hlavatá K, Lamxay V, Richardson JE, Steffen K, Leong-Škorničková J. Convergent morphology in Alpinieae (Zingiberaceae): Recircumscribing *Amomum* as a monophyletic genus. Taxon. 2018; 67(1):6-36.

3. Zhang W, Chen JW, Shen Y, Yang ZQ, Xie SQ, Yang SC. Extraction and determination of essential oil in different cultivars of *Amomum tsao-ko*. Adv Mater Res. 2012; 549:474-477.
4. Ravindran PN. Tsao-ko cardamom – *Amomum tsao-ko*. In The encyclopedia of herbs and spices. 2. Wallingford, UK: CABI. 2017; 1003-1005.
5. Yu L, Shirai N, Suzuki H, Sugane N, Hosono T, Nakajima Y, Kajiwara M, Takatori K. The effect of methanol extracts of Tsao-ko (*Amomum tsao-ko* Crevost et Lemaire) on digestive enzyme and antioxidant activity in vitro, and plasma lipids and glucose and liver lipids in mice. J Nutr Sci Vitaminol. 2010; 56(3):171-176.
6. Starkenmann C, Mayenzet F, Brauchli R, Wunsche L, Vial C. Structure elucidation of a pungent compound in black cardamom: *Amomum tsao-ko* Crevost et Lemarié (Zingiberaceae). J Agric Food Chem. 2007; 55(26):10902-10907.
7. Cui Q, Wang LT, Liu JZ, Wang HM, Guo N, Gu CB, Fu YJ. Rapid extraction of *Amomum tsao-ko* essential oil and determination of its chemical composition, antioxidant and antimicrobial activities. J Chromatogr B. 2017; 1061:364-371.
8. Zhang XF, Tang YJ, Guan XX, Lu X, Li J, Chen XL, Deng JL, Fan JM. Flavonoid constituents of *Amomum tsao-ko* Crevost et Lemarie and their antioxidant and antidiabetic effects in diabetic rats–*in vitro* and *in vivo* studies. Food & Function. 2022; 13(1):437-450.
9. Xie L, Yu D, Li Y, Ju H, Chen J, Hu L, Yu L. Characterization, hypoglycemic activity, and antioxidant activity of methanol extracts from *Amomum tsao-ko*: *In vitro* and *in vivo* studies. Front Nutr. 2022; 9: 869749.
10. Xu XJ, Zhang ML, Hou YM, Zhang K, Yao DH, Li GY, Kou WB, Wang HY, Wang JH. The *Amomum tsao-ko* essential oils inhibited inflammation and apoptosis through p38/JNK MAPK signaling pathway and alleviated Gentamicin-induced acute kidney injury. Molecules. 2022; 27(20):7121.
11. Ming XG, Pei WJ, Min HL, Ping Y, Min D. Mechanism of apoptosis induced by essential oil from *Amomum tsaoko* in nasopharyngeal carcinoma 6-10B cells. Nat Prod Res Dev. 2021; 33(6):992-997.
12. He XF, Chen JJ, Huang XY, Hu J, Zhang XK, Guo YQ, Zhang XM, Geng CA. The antidiabetic potency of *Amomum tsao-ko* and its active flavanols, as PTP1B selective and α-glucosidase dual inhibitors. Ind Crops Prod. 2021; 160:112908.
13. Zhang TT, Lu CL, Jiang JG. Neuroprotective and anti-inflammatory effects of diphenylheptanes from the fruits of *Amomum tsaoko*, a Chinese spice. Plant Foods Hum Nutr. 2016; 71(4):450-453.
14. Juergens UR, Dethlefsen U, Steinkamp G, Gillissen A, Repges R, Vetter H. Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: a double-blind placebo-controlled trial. Respir Med. 2003; 97(3):250-256.
15. Yin C, Liu B, Wang P, Li X, Li Y, Zheng X, Tai Y, Wang C, Liu B. Eucalyptol alleviates inflammation and pain responses in a mouse model of gout arthritis. Br J Pharmacol. 2020; 177(9):2042-2057.
16. Thangaleela S, Sivamaruthi BS, Kesika P, Tiyajamorn T, Bharathi M, Chaiyasut C. A narrative review on the bioactivity and health benefits of alpha-phellandrene. Sci Pharm. 2022; 90(4):57.
17. Sim S, Tan SK, Kohlenberg B, Braun NA. *Amomum tsao-ko*—Chinese black cardamom: detailed oil composition and comparison with two other cardamom species. Nat Prod Commun. 2019; 14(7):1934578X19857675.
18. The Committee of Vietnamese Pharmacopoeia. Vietnamese Pharmacopoeia (5th ed.). Medical Publishing House: Vietnam; 2017, PL274.
19. Adams RP. Identification of essential oil components by gas chromatography/ quadrupolemass spectrometry, Allured Publ., Carol Stream, IL, USA; 2007.
20. Nguyen TH, Vu DC, Alvarez S, Nguyen KD, Nguyen TL, Tuan NN, Minh-Nguyet NT, Tam LN, Ho TL, Vo XT. Comparative examination of phytonutrients and antioxidant activity of commonly consumed nuts and seeds grown in Vietnam. Horticulturae. 2022; 8(6):521.
21. Clinical and Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing (30th ed.). CLSI supplement M100. Clinical and Laboratory Standards Institute, USA; 2020.
22. Dung NX, Bien LK, Leclercq PA. The essential oil of *Amomum tsao-ko* Crevost et Lemarie from Vietnam. J Essent Oil Res. 1992; 4(1):91-92.
23. Feng X, Jiang ZT, Wang Y, Li R. Composition comparison of essential oils extracted by hydrodistillation and microwave-assisted hydrodistillation from *Amomum tsao-ko* in China. J Essent Oil Res. 2010; 13(3):286-291.
24. Sabulal N, Baby S. Chemistry of *Amomum* essential oils. J Essent Oil Res, 2021; 33(5):427-441.
25. Li W, Li J, Qin Z, Wang Y, Zhao P, Gao H. Insights into the composition and antibacterial activity of *Amomum tsao-ko* essential oils from different regions based on GC-MS and GC-IMS. Foods. 2022; 11(10):1402.
26. Torres-Valenzuela LS, Ballesteros-Gomez A, Sanin A, Rubio S. Valorization of spent coffee grounds by supramolecular solvent extraction. Sep Purif Technol. 2019; 228:115759.
27. Althaher AR, Oran SA, Bustanji YK. Chemical composition, *in vitro* evaluation of antioxidant properties and cytotoxic activity of the essential oil from *Calamintha incana* (Sm.) Helder (Lamiaceae). Trop J Nat Prod Res. 2021; 5(8):1333-1339.
28. Agour A, Mssillou I, Saghrouchni H, Bari A, Lyoussi B, Derwich E. Chemical composition, antioxidant potential and antimicrobial properties of the essential oils of *Haplophyllum tuberculatum* (Forsskal) A. Juss from Morocco. Trop J Nat Prod Res. 2020; 4(12):1108-1115.
29. Dabetic N, Todorovic V, Malenovic A, Sobajic S, Markovic B. Optimization of extraction and HPLC–MS/MS profiling of phenolic compounds from Red Grape seed extracts using conventional and deep eutectic solvents. Antioxidants. 2022; 11(8):1595.
30. Van Vuuren SF. Antimicrobial activity of South African medicinal plants. J Ethnopharmacol. 2008; 119(3):462-472.
31. Huong LT, Sam LN, Dai DN, Ogunwande IA. Investigation into the chemical compositions and antimicrobial activity of essential oil from the rhizomes of *Boesenbergia quangngaiensis* NS Lý from Vietnam. J Essent Oil-Bear Plants. 2021; 24(5):1125-1133.
32. Yang Y, Yan RW, Cai XQ, Zheng ZL, Zou GL. Chemical composition and antimicrobial activity of the essential oil of *Amomum tsao-ko*. J Sci Food Agric. 2008; 88(12):2111-2116.
33. Pu ZH, Wang BS, Zhang SY, Sun FH, Dai M. A review on quality control, toxicity and clinical application of *Amomum tsao-ko* Crevost & Lemarié. Pharmacolog Resea-Moder Chin Medic. 2022; 100165.
34. Li W, Wang PJ, Shigematsu M, Lu ZG. Chemical composition and antimicrobial activity of essential oil from *Amomum tsao-ko* cultivated in Yunnan area. In Adv Mater Res. 2011; 183:910-914.
35. Lim TK. *Amomum tsao-ko*. Edible Medicinal And Non-Medicinal Plants. Fruits. 2013; 5:813-817.