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Chemical Profile and Biological Activities of The Essential Oil of Cinnamon (*Cinnamomum cassia* (L.) J. Presl) Twigs and Leaves

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ABSTRACT

The essential oil (EO) from twigs and leaves of *Cinnamomum cassia* (L.) J. Presl are isolated using the steam distillation method. This study aims to investigate physicochemical properties, such as freezing point, acid/saponification/ester value, relative/absolute density, and fragrance retention. Chemical profile of the oil was analysed by gas chromatography-mass spectrometry (GC-MS), and 21 major compounds were detected. The results showed that the highest main component is (E)-cinnamaldehyde (88.05%) with the predominance of phenylpropanoids (90.71%). The antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay with the half maximal inhibitory concentration (IC_{50}) at 435.961 mg/mL. In addition, the disk diffusion test is used to screen EO for antibacterial activity and the results revealed that the cinnamon EO also possesses excellent antibacterial activities (AA) against several foodborne bacteria (*Escherichia coli*, *Salmonella enteritidis*, *Bacillus cereus*, and *Staphylococcus aureus*). Strong bacterial inhibitory effects in the present study show the broad application potential of this material in the food industry in the future.

Keywords: Distillation, *Cinnamomum cassia*, herb, spice.

Introduction

Cinnamomum cassia (L.) J. Presl belongs to the family Lauraceae and comprises about 250 species. India, China, Uganda, Vietnam, Bangladesh, and Pakistan are common cultivating countries. This plant is used to treat different diseases; for instance, cardiovascular disease, chronic gastrointestinal disease, gynaecological disorders, and inflammatory disease.[1] Cinnamon is a tropical evergreen tree that can grow up to 7 m. Leaves are ovate-oblong in shape and 7-18 cm long. Flowers are arranged in panicles and are greenish with a distinct odor. The fruit is a purple, 1 cm long berry containing a single seed.[2] Currently, the cinnamon tree is grown in the provinces of Vietnam, such as Yen Bai, Quang Ninh, Thanh Hoa, Quang Nam, Quang Ngai, etc. The primary constituents of the essential oil (EO) are 65-80% cinnamaldehyde and less eugenol. The *C. cassia* EO is reported to have different pharmacological activities, such as anti- inflammatory, antioxidant, hepatoprotective activities, antibacterial activity, etc.[3] In addition, this oil also possess aphrodisiac, antihelminthic, insecticidal, antimutagenic activities, and tonic properties.[4] According to Kačániová *et al*. and El-Atki *et al*., they indicated that *C. cassia* EO inhibits strongly various microorganism, such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Stenotrophonomonas maltophilia*, *Bacillus subtilis*, *Penicillium citrinum*, *P. crustosum*, and *P. expansum*.[4,5] However, the antibacterial activity depends on the chemical components of oil. Therefore, evaluating the chemical components of material cultivated in different places is essential.

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In recent years, some studies have reported that *C. cassia* EO could be applied widely in food technology, for example, the combination of *C. cassia* EO and palm oil provided a higher antioxidant effect during the frying process when compared with the control group.[6] Also, using *C. cassia* EO can be used as novel natural substances for the shelf-life prolongation of wheat bread, etc.[7]

Nowadays, there are many related studies on *C. cassia* bark EO, but the studies on EOs produced from *C. cassia* twigs and leaves still need to be studied in depth. Moreover, these materials are by-products of the cinnamon harvesting process and contain many volatile compounds with high biological activities.[8] However, there are few published studies on these issues. In addition, cinnamon grown in Yen Bai (Vietnam) could have specific characteristics compared to other places. Thus, the primary purpose of this study was to determine the chemical profile and some biological properties of the oil from *C. cassia* twigs and leaves (CctlEO) in the Yen Bai province. Our study provides additional data to support using of EO from cinnamon as a natural additive used in food, cosmetics, and medicine.

Materials and Methods

Sample preparation

The twigs and leaves of *C. cassia* were collected at the tree's age of 3 to 20 years old in Yen Bai province in northern Vietnam (Coordinates: 21°54'0.79"N 104°34'0.08"E), in October 2022. The plant specimens (voucher number: CC031022VST) was deposited at Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Vietnam. The EO was isolated by steam distillation; on average, the per-batch yield was 200 kg twigs and leaves/batch and materials were distilled for 3 h at 100℃. The obtained EO has an efficiency of approximately 0.45% (v/w) and was kept at 4°C before analysis.

Chemicals

The chemicals used in this study included 2,2- diphenyl-1-picrylhydrazyl (DPPH) and dimethyl sulphoxide (DMSO, Sigma, USA). The culture and antibacterial media included Nutrient broth, Mueller-Hinton agar (MHA, HiMedia, India), and other chemicals meeting analytical standards.

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Bacterial strain
Foodborne bacteria strains were selected for this study, including *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 10376), *Bacillus cereus* (ATCC 11778), and *Staphylococcus aureus* (ATCC 25923).

Evaluation of the EO's physicochemical properties
The freezing point (FP), relative density (RD), and absolute density (AD) were determined according to ISO 1041 and 279.[9,10] In addition, the acid value (AV), saponification value (SV), and ester value (EV) were determined according to ISO 1242 and 7660, respectively.[11,12]

Evaluation of fragrance retention (FR) of the EO
According to Mahajan, the FR was determined by the concentration of the fragrance ingredients and their lasting duration.[13] First, the oil was dissolved in ethanol (96%, v/v) at different concentrations (5–25%, v/v). Next, three drops of the mixture were put on the odour test paper and allowed to penetrate the paper. Then, the time it took to lose the scent was recorded.

Evaluation of the antioxidant capacity (AC) of the EO
The DPPH free radical scavenging capacity ($DPPH_{RSC}$) was determined based on the assay of Gouder and Lingamallu with some minor adjustments.[14] The EO (0.3 mL) from *C. cassia* twigs and leaves in different ethanol concentrations (0–600 mg/mL) and 3.7 mL of 0.1 mM DPPH in 96% ethanol solution were mixed. The mixture was kept in the dark for 30 min at room temperature and the absorbance of mixture was recorded at 517 nm using a spectrophotometer (Genesys 20, Thermo Scientific, USA). Ascorbic acid was used as a standard to compare with the AC of the EO. The IC_{50} value (Half maximal inhibitory concentration) is estimated from the plot of serial EO dilutions versus the % inhibition. The AC was calculated as the following formula:

$$DPPH_{RSC}(\%) = \frac{A_0 - A_s}{A_0} \times 100\% \quad (1)$$

where A_0 and A_s are the absorbance of control and sample at 517 nm, respectively.

Evaluation of the antibacterial activity (AA) of the EO
AA was determined using the paper disc diffusion method for antibiotic susceptibility testing with some slight modifications.[15] Firstly, bacterial strains were cultured in Nutrient broth until their turbidity was equivalent to 0.5 McFarland (~ 1.5×10^8 CFU/mL) and a volume of 100 µL of bacteria suspension was spread on MHA media plates with a sterile spreader. Then, the sterile paper discs (6 mm in diameter) were impregnated by the selected EO (5 µL). The positive control was conducted with Gentamycin antibiotic disc (10 µg/disc) and the negative control was dimethyl sulfoxide (DMSO) solution (5%). Finally, all dishes were incubated for 24 h at 37°C and the AA was assessed by inhibitory zone with the paper disc.

Gas chromatography-mass spectrometry (GC-MS) analysis
The chemical profile of the EO was analyzed using GC-MS. The EO (1 µL) was injected into a gas chromatograph (Shimadzu Nexis GC-2030, Janpan) with a versatile capillary column (Rtx-5sil-MS, 30 m × 0.25 mm × 0.25 µm, Restek Technologies, USA) equipped with a quadrupole mass analyser (Shimadzu GC-MS-QP2020 NX, Japan). Helium was used as a carrier gas at a constant flow rate of 3.0 mL/min, and a split ratio of 10:1. The injection temperature was 250°C and the temperature program was set as follows: initial temperature of 50°C, held for 2 min; increased until 250°C at a rate of 10°C/min and held for 5 min; and increased until 280°C at a rate of 10°C/min and held for 3 min. Mass spectra were recorded at the ionisation energy of 70 eV in EI mode.

Statistical analysis
All analyses were performed in triplicate and data were expressed in form of mean ± standard deviation. Statgraphics Centurion XV software (USA) was used for the statistical analysis. ANOVA variance analysis and the Least Significant Difference (LSD) test were used to assess whether the differences in groups were statistically significant ($p < 0.05$).

Results and Discussion

Physicochemical properties of the EO of C. cassia twigs and leaves
CctlEO is a yellow liquid with a bitter and spicy taste and a characteristic odour. The pH value of the EO was roughly 3.853 (Table 1), which was similar to some EOs isolated from different plants, such as *Ceratonia siliqua* seeds (pH = 5.2 ± 0.3) and *Mentha arvensis* leaves (4.670 ± 0.006).[16,17] This prove that there will be many free volatile acids in the composition of the obtained EOs.
In general, most of the relative and absolute densities of EOs are lower than 1. CctlEO is very special when the relative density (RD) and absolute density (AD) values are higher than 1. The RD of the CctlEO is similar to that of the *C. zeylanicum* EO (1.050 ± 0.005).[18] However, this RD is higher than that of *M. arvensis* leaves EO (0.8987 ± 0.0003) and *M. cajuputi* leaves EO (0.9102 ± 0.0002).[17,19] In addition, the AD of CctlEO was significantly higher than the EO of *M. arvensis* leaves EO (0.8959 ± 0.0001 g/mL) and *M. cajuputi* leaves EO (0.9086 ± 0.0002 g/mL).[17,19] In this study, the FP of EO was determined to be approximately -38°C. It is lower than that of *M. arvensis* leaves EO (-7.33 ± 0.58°C) and *Eucalyptus camaldulensis* leaves EO (0-1°C).[17,20]
The acid (AV), saponification (SV), and ester values (EV) of the CctlEO were determined to be 4.675, 26.125, and 21.450 mg KOH/g, respectively (Table 1). They were similar to the EO from *C. burmannii* leaves (AV = 4.24 mg KOH/g, SV = 22.61 mg KOH/g, EV = 18.37 mg KOH/g).[21] Although the AV was higher than that of *M. arvensis* leaves EO (AV = 1.171 mg KOH/g),[17] the SV and EV were lower than *C. siliqua* seeds EO (SV = 37.2 mg KOH/g; EV = 33.22 mg KOH/g).[16] The AV, SV, and EV are the three main physical properties used to evaluate the quality of EOs; however, these parameters depend on distillation techniques, climatic conditions, varieties, regions, genotypes, harvest periods, etc. The fragrance retention (FR) of CctlEO was quite high and approximately 8 h with 25% EO, which agrees with the study of Ahmed *et al.* about perfume.[22] A mixture with 20-30% aromatic compounds will last 6-8 h. Meanwhile, the pure EO can last nearly 24 h, proving that this EO has a very passionate aroma that lasts a long time. Therefore, CctlEO is considered a high-potential application in cosmetics, as well as in food.

Table 1: Physicochemical properties of the EO of *C. cassias* twigs and leaves

No.	Physicochemical properties	Value
1	pH	3.853 ± 0.047
2	Freezing point (FP, °C)	-38.0 ± 0.000
3	Relative density (RD)	1.047 ± 0.002
4	Absolute density (AD, g/mL)	1.045 ± 0.002
5	Acid value (AV, mg KOH/g)	4.675 ± 0.000
6	Saponification value (SV, mg KOH/g)	26.125 ± 0.550
7	Ester value (EV, mg KOH/g)	21.450 ± 0.550
8	Fragrance retention (FR, h):	
	5% EO (v/v)	3
	10% EO (v/v)	4
	15% EO (v/v)	4.5
	20% EO (v/v)	5
	25% EO (v/v)	7.5
	100% EO	23.25

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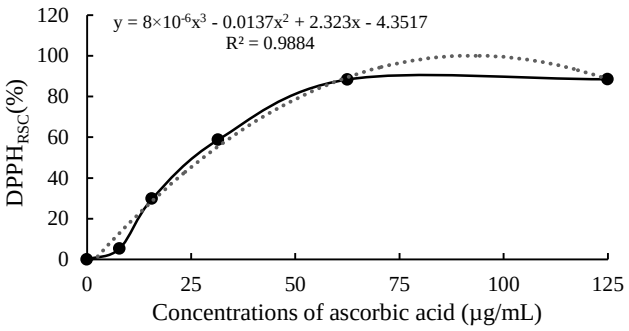

Figure 1: Antioxidant capacity of ascorbic acid

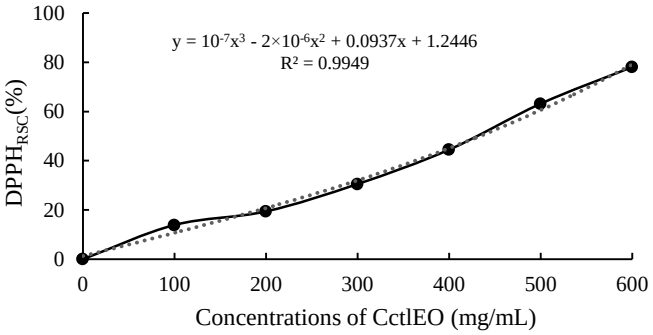

Figure 2: Antioxidant capacity of the EO of *C. cassia* twigs and leaves

Chemical profile of the EO of C. cassia twigs and leaves

The chemical profile of the CctlEO was analyzed by GC-MS. The obtained results reveal that there are 21 components in the EO (Table 2). All compounds were detected under retention times (RTs) ranging from 5.284 to 17.969 min. The CctlEO was found to contain (E)-cinnamaldehyde (88.05%), benzaldehyde (1.75%), and 2,5-hexanedione (1.55%) as major compounds. (E)-cinnamaldehyde is characteristic of EO in the study, accounting for the highest proportion, and its content also was higher than that in the EO of other *Cinnamomum* species, for example, *C. zeylanicum* bark EO (57.971%) and *C. verum* bark EO (52.26%).[23,24] According to the study by Usai and Sotto,[25] cinnamaldehyde has remarkable biological activities, such as antioxidant, antibacterial, anti-inflammatory, etc.

Notably, phenylethyl alcohol was detected. Although it accounts for only 0.22%, this compound is exciting and attractive due to its antibacterial properties. Due to its bactericidal effect, phenylethyl alcohol is often used in high concentrations to protect pharmaceuticals and cosmetics from damage. With a lower concentration, it has an inhibitory effect on bacteria.[26] In addition, the obtained results revealed three main groups in the EO, including terpenes and terpenoids, phenylpropanoids, and others. However, phenylpropanoids are the predominant group of compounds in the EO with typical components such as (E)-cinnamaldehyde (88.05%); 2-propenal, 3-phenyl- (0.23%); 3-phenylpropenal (1.05%); acetic acid, cinnamyl ester (1.02%); and ortho methoxy cinnamic aldehyde (0.36%). The findings of this study show that both the primary and minor ingredients have created positive values for cinnamon essential oil and contributed to the potential of the perfume, cosmetics, and food industries.

Table 2: Chemical profile of the EO of *C. cassias* twigs and leaves

No.	Compound	RT (min)	Content (%)
1	2,5-Hexanedione	5.284	1.55
2	β-Pinene	5.504	0.09
3	Benzaldehyde	5.969	1.75
4	4-Heptanone. 2.3:5.6 diepoxy-2.6-dimethyl	6.010	0.50
5	Cyclohexyl methyl ketone	6.045	0.65
6	L-Limonene	7.196	0.93
7	Phenylethyl alcohol	8.596	0.22
8	Benzenepropanal	9.433	0.55
9	2-Propenal, 3-phenyl-	9.699	0.23
10	Ethyl octanoate	9.946	0.19
11	Oxetane 2-propyl	10.053	0.06
12	3-Phenylpropenal	10.367	1.05
13	**(E)-Cinnamaldehyde**	**11.196**	**88.05**
14	2-Isopropyl-5-methyl-1-heptanol	11.735	0.21
15	11-Methyldodecanol	11.856	0.18
16	α-Ylangene	12.807	0.34
17	(E)-3-(2-hydroxyphenyl)-2-Propenoic acid	13.622	1.32
18	Acetic acid, cinnamyl ester	13.659	1.02
19	Ortho methoxy cinnamic aldehyde	14.805	0.36
20	Dodecyl nonyl ether	14.984	0.54
21	5-methyl-2-propan-2-ylheptan-1-ol	17.969	0.21
		Total	100
		Terpenes and terpenoids	1.36
		Phenylpropanoids	90.71
		Others	7.93

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Table 3: Antibacterial diameter of the EO of *C. cassia* twigs and leaves

No.	Tested bacteria	Antibacterial diameter of gentamicin (mm)	Antibacterial diameter of the EO (mm)
1	*E. coli*	$22.0^{Ab} \pm 2.0$	$43.3^{Bc} \pm 1.5$
2	*S. enterica*	$17.3^{Aa} \pm 2.5$	$27.0^{Ba} \pm 2.7$
3	*S. aureus*	$17.0^{Aa} \pm 1.0$	$27.7^{Ba} \pm 3.1$
4	*B. cereus*	$21.0^{Ab} \pm 1.0$	$33.7^{Bb} \pm 1.5$

Within a row (A-B) or a column (a-c), various letters indicate significant differences ($p < 0.05$)

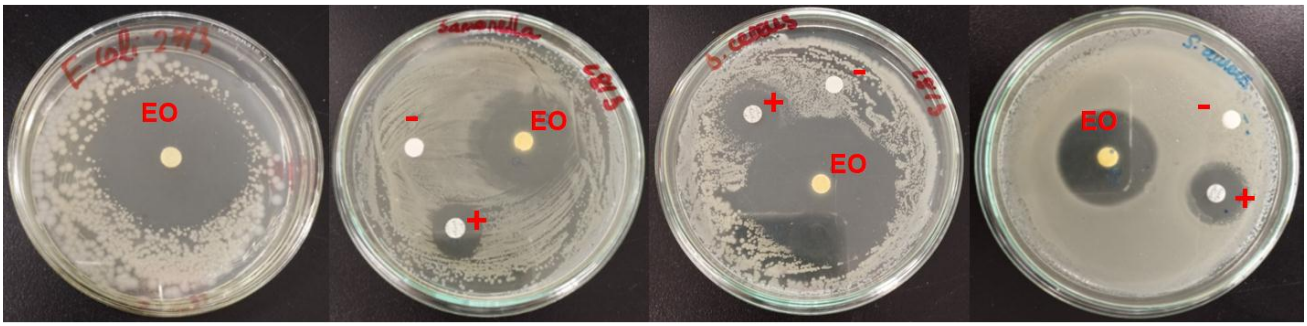

Figure 3: Antibacterial activity of *C. cassia* twigs and leaves oils ("EO": essential oil, "-": negative control, and "+": positive control)

Antioxidant capacity (AC) of the EO from C. cassia twigs and leaves

Figure 2 shows that the higher the EO concentration, the more effective the antioxidant capacity. The IC_{50} value of the EO was 435.961 mg/mL, while that of the control (acid ascorbic) was only 27.918 µg/mL (Figure 1). These results shows that the AC of this EO was very weak. Compared to other cinnamon EOs, the IC_{50} value of CctlEO in this study was much higher than that of the EO of *C. griffithii* leaves (IC_{50} = 82.4 µg/mL) and *C. macrocarpum* leaves (IC_{50} = 99.3 µg/mL).[27] In addition, it was also higher than that of some other material EOs, for example, the IC_{50} values of EOs of *M. arvensis* leaves and *Haplophyllum tuberculatum* aerial parts were 330 and 3.23 mg/mL, respectively.[17,28] These results indicated that the AC of CctlEO could be lower than desired. The difference in AC can be caused by various reasons, including chemical composition, soil, weather, climate, harvest time, plant variety, origin. etc.

Antibacterial activity (AA) of the EO from C. cassia twigs and leaves

The CctlEO indicates AA against four bacterial strains (Table 3). The AA of the CctlEO was very strong and stronger than that of the positive control (gentamicin) ($p < 0.05$). The AA of CctlEO, arranged in susceptible order, are *S. enterica* and *S. aureus* < *B. cereus* < *E. coli*, while those of gentamicin, arranged in susceptible order, are *S. aureus* and *S. enterica* < *B. cereus* and *E. coli*. All antibacterial diameter values of the EO are greater than 20 mm (Figure 3), which can be seen as the diameter of the extremely sensitive area for microorganisms. These results were superior to those of Ayoola *et al.*; they used *Psidium guajava* aerial parts EO to inhibit *S. aureus* (inhibition diameters of 23 mm) and *E. coli* (inhibition diameters of 29 mm).[29] Compared to cinnamon oils from Madagascar, our results are also better than those obtained by El-amrani *et al.*, who found an inhibition diameter of 37 mm for *E. coli*.[30] This proves that the EO is a potential material that can be applied in food to inhibit harmful bacteria.

The AA of CctlEO can be interpreted in many different ways. According to the research of Al-Harrasi *et al.*,[31] the antibacterial effects of the EO against pathogenic bacteria can occur through the degradation of a cell wall, disruption of the cytoplasmic membrane, the reduction of intracellular ATP synthesis, the change of osmotic pressure, etc. In this case, the reason why the EO has such a strong antibacterial effect is that the compound (E)-cinnamaldehyde, accounting for 88.05%, inhibits the growth of *E. coli* and *S. typhimurium* by depleting the intracellular ATP levels.[32] In addition, the AA of a given EO may depend on one or two major components that comprise the entire oil. Also, the presence of major constituents may not be the only factor affecting the inherent activity of essential oils, but the interaction between these and minor constituents in the EO could create a synergistic effect.[33]

Thereby, the CctlEO can be considered a natural preservative in food products. Overall, the EO in the study contributed to the bacteriostatic effect against several pathogens. Although CctlEO possesses some outstanding properties, the biggest challenge is low recovery yield compared to other EOs, and demand increases quickly, but harvest is seasonal.

Conclusion

In view of the results presented, our findings indicate that the Yen Bai province (Vietnam) has excellent potential for commercial production of high-quality oil from cinnamon twigs and leaves rich in (E)-cinnamaldehyde. It is noteworthy that the oil from the *Cinnamomum* plant native to the region under study obtained results in physical properties close to those of some studies using the same species of cinnamon. Additionally, the study also showed that the antioxidant capacity was not significantly effective compared to other oils, but the antibacterial activity of this oil was higher than that of other types of cinnamon, as well as other plants. It strongly inhibited four pathogenic bacteria, including *S. enterica*, *S. aureus*, *B. cereus*, and *E. coli*. Hence, the oil from cinnamon twigs and leaves is ideal for use in the pharmaceutical, food, and cosmetic industries.

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.

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