



Chemical Composition, Antibacterial and Antioxidant Activity of (*Cinnamomum cassia* (L) Presl) Essential Oil

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Abstract

Cinnamomum cassia is widely used to treat intestinal diseases, abdominal pain, helminth infections, digestion, stimulates digestive enzyme secretion, enhances appetite, reduces bloating, and indigestion. However, the chemical composition and biological properties of the essential oil of *Cinnamomum cassia* collected in Thanh Hoa, Vietnam, have not been investigated. Gas chromatography-mass spectrometry (GC-MS) was used to identify the chemical composition of *Cinnamomum cassia* essential oil. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the agar disc diffusion method were used to evaluate the antioxidant and antibacterial activities of the essential oil, respectively. Experimental data indicate that the leaf, flower, and bark portions of the tree all contain essential oils, with the bark exhibiting the highest content, ranging from 1-4%. Lower contents are found in the leaves and fruits, at 0.3-0.9%. The major components of the essential oils are as follows: in the bark, cinnamaldehyde (77.3%) and cinnamaldehyde (57.7%); in the branch, *trans*-cinnamyl acetate (27.6%); in the leaf, cinnamaldehyde (53.5%) and (Z)-9-octadecenamide (19.5%), and in the root, cinnamaldehyde (58.3%) and *p*-methoxy cinnamic aldehyde (12.0%). The strong antioxidant and antibacterial effects of *Cinnamomum cassia* essential oil were demonstrated with an IC₅₀ value of 6.25±0.3 µg/mL and with resistance of 89.70%, respectively. Our results indicate that the essential oil of *Cinnamomum cassia* collected in Thanh Hoa, Vietnam, has great potential in the pharmaceutical industry.

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Introduction

Cinnamon (*Cinnamomum cassia*) is an evergreen tree belonging to the Lauraceae family. It is a large woody plant growing to a height of 10–20 m. The trunk bark is smooth, gray-brown, with older bark becoming thick, sometimes up to 1.5 cm. Branches are nearly quadrangular and covered with brown hairs. The terminal buds are small, ovoid, and measure about 3–4 mm. It is widely cultivated in countries such as China, India, Vietnam, Indonesia, and several others [1]. The cinnamon is primarily used as a flavoring agent in food, perfumes, and pharmaceutical products. The bark contains 1 to 3% of volatile compounds, with cinnamaldehyde being the principal volatile component, contributing significantly to cinnamon's aroma and bioactivity [2]. This compound possesses anti-inflammatory, antibacterial, and hypotensive properties. Additionally, cinnamon also contains other compounds such as coumarins, cinnamic acid, cinnamyl acetate, hydroxycinnamaldehyde, and numerous other constituents [3, 4, 5].

In traditional medicine, parts such as the leaves, buds, and bark of the *Cinnamomum cassia* are considered to have high medicinal value and have been used for a long time in folk remedies. Several publications report the chemical composition of *Cinnamomum cassia*, especially that of the essential oil [6,7,8,9]. However, studies have also shown that the raw material from different regions and different extraction methods also greatly affect the content of *Cinnamomum cassia*. Therefore, when testing its chemical

activity, it is possible to discover different active constituents according to the above factors. This study aims to analyze and characterize the chemical composition and biological properties *Cinnamomum cassia* essential oil collected in Thanh Hoa, Vietnam, in support of its potential exploitation and effective utilization. Determination of the chemical composition of *Cinnamomum cassia* essential oil was conducted using gas chromatography-mass spectrometry (GC-MS). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the agar disc diffusion method were used to evaluate the antioxidant and antibacterial activities, respectively.

Material and Methods

Chemicals

Butylated hydroxytoluene (BHT), C7–C30 straight-chain hydrocarbons, reference chemicals for identification, Tween 80, and DPPH were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemical analytical grades, the culture media, and standard antibiotic discs were procured from Merck (Darmstadt, Germany) and Oxoid Ltd. (Basingstoke, Hampshire, UK), respectively.

Plant Material

The *Cinnamomum cassia* were collected from Thanh Hoa, Vietnam, in January 2024. A voucher specimen (No. QT-TH-01) was prepared and deposited at the Faculty of Natural Science and Technology, Hong Duc University, Thanh Hoa Province, Vietnam.



Fig 1: The fresh leaves of *Cinnamomum cassia* collected in Thanh Hoa Province.

Extraction of Essential Oil

Collected leaves were cleaned, cut into small pieces, and subjected to steam distillation for 4 hours by a Clevenger-type apparatus. The essential oil obtained was then dehydrated using anhydrous sodium sulfate and stored in a sealed vial at 10°C in the dark prior to subsequent experiments.

Analysis of Essential Oil by GC-MS

To analyze the composition of the essential oil from the leaves of *Cinnamomum cassia*, a Trace GC Ultra-ITQ900 system (Thermo Fisher Scientific, MA, USA) was used. Data were interpreted by MassFinder 4.0 software. The separation was performed on a fused silica capillary TG-SQC column (30 m × 0.25 mm i. d., 0.25 µm film thickness). The GC operational parameters included injector temperature 250 °C, detector temperature 260 °C, oven temperature program 60

to 260 °C at a heating rate of 4 °C/min, carrier gas helium at a flow rate of 1.0 mL/min, and sample injection volume 1 µL in split mode with a split ratio of 1:10. The mass spectrometer was operated in electron ionization (EI) mode with the following parameter values: ionization energy 70 eV, interface temperature 280 °C, ion source temperature 230 °C, MS quadrupole temperature 200 °C, and scan range 35–650 amu^[10]. The retention indices of the essential oil constituents were determined using an HP-5 MS column and standard C7–C30 straight-chain hydrocarbon reference standards (Sigma-Aldrich Chemical Company, USA). The mass spectra and retention indices of individual compounds were identified by comparing them with those in GC-MS libraries (National Institute of Standards and Technology-NIST 08 and Wiley 09th version) and/or with published data. The relative percentages of the identified compounds were calculated based on GC peak areas without applying correction factors.

Antioxidant Assay

The DPPH assay was used to evaluate the antioxidant activity of the essential oil from *Cinnamomum cassia* leaf and stems^[11]. The essential oil was dissolved in methanol to different concentrations (0.3125, 0.625, 1.25, 2.5, 5.0, and 10 µg/mL), and the positive control BHT was mixed with 200 µL of a methanolic solution (containing DPPH radicals at a concentration of 150 µmol/L). The mixture was then vigorously shaken and left in the dark for 30 minutes to complete the reaction. The absorbance solutions were measured using a Shimadzu UV1800 spectrophotometer (Shimadzu Corporation, Japan) at 517 nm. A blank sample (a control solution without extract or BHT) was used for comparison. The scavenging ability of the essential oil was calculated as in Equation 1.

$$\text{Scavenging ability (\%)} = \frac{A_{517 \text{ of control}} - A_{517 \text{ of sample}}}{A_{517 \text{ of control}}} \times 100 \quad (1)$$

Antimicrobial Activity

The agar disc diffusion method was used to evaluate the antibacterial activity of the essential oil against the Gram-negative bacterium *E. coli* (ATCC 25922). A liquid culture of *E. coli* (10⁷ CFU/mL) was evenly spread on solidified agar in Petri dishes. Circular filter paper discs (6 mm diameter) were placed at the center of each dish, and 40 µL of the essential oil (extracted by steam distillation and dissolved in 10% dimethyl sulfoxide, DMSO) was applied to the discs. DMSO (10%) served as a negative control. The plates were sealed and incubated at 37 °C, and the diameters of the inhibition zones around the discs were measured to assess antibacterial activity. All experiments were conducted in triplicate for accuracy^[12]. The minimum inhibitory concentration (MIC) of the essential oil was determined by the broth microdilution method, as described by Hanh *et al.* (2023). The essential oil was serially diluted two-fold with ethanol in a 96-well plate to achieve concentrations ranging from 1.0 to 10.0 mg/mL, and then 20 µL of bacterial suspension (pH 7.4–7.6) was added to each well. The plates were incubated at 37 °C for 24 h. MIC was defined as the lowest concentration of essential oil that visibly inhibited bacterial growth. Each assay was performed in triplicate to ensure result reliability.

Statistical Analysis

All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Statistica 5.5 software (StatSoft Inc., Tulsa, OK, USA) were utilized to analyze the results. The results are presented as the mean $\pm$ standard deviation (SD).

Results and Discussion

Essential oil was extracted from the leaves and stems of *Cinnamomum cassia* via hydrodistillation, with a 0.9% yield (w/w, based on fresh weight). The total ion chromatogram obtained from GC-MS analysis is shown in Figure 2.

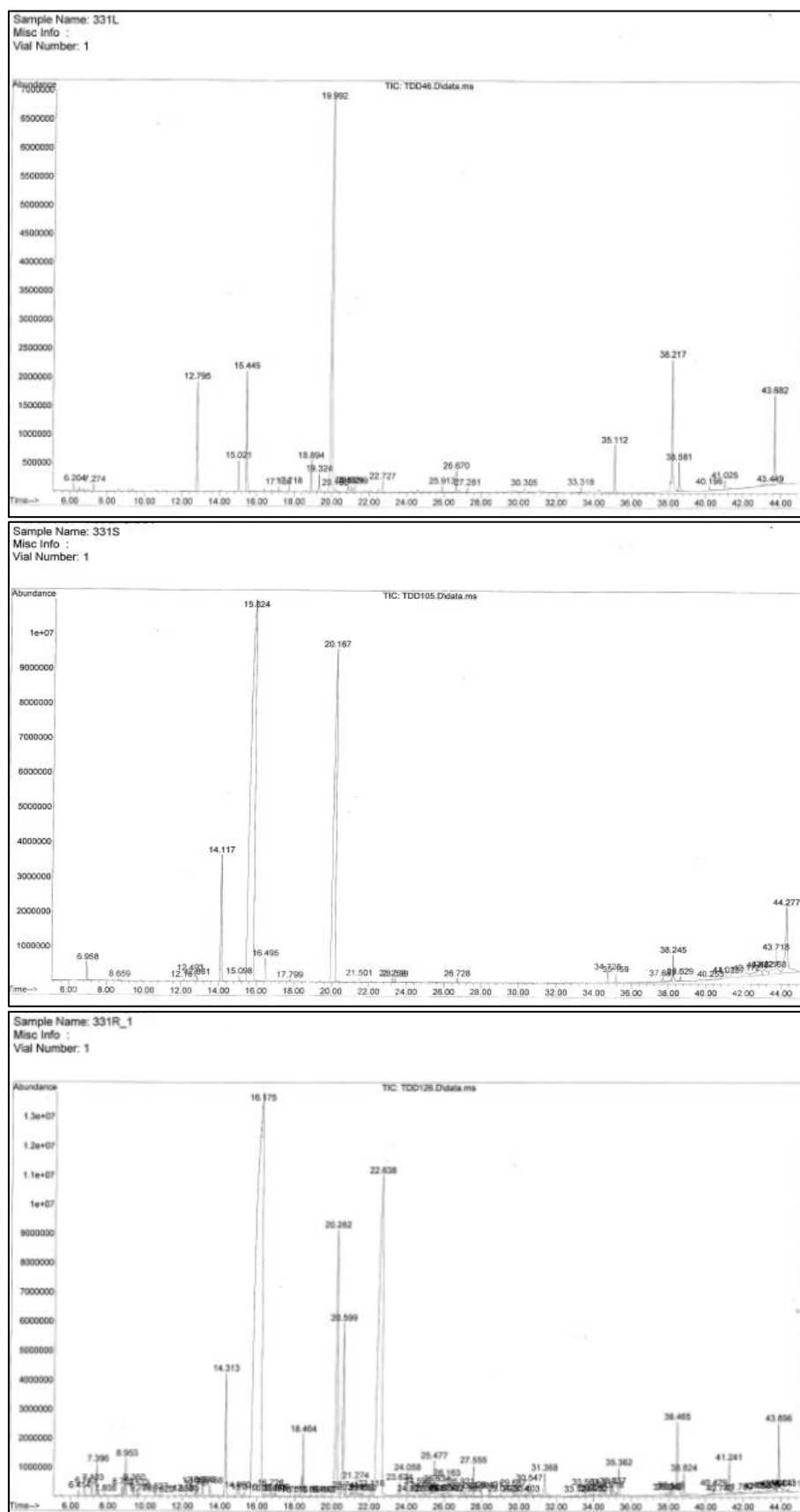


Fig 2: GC-MS total ion chromatogram of *Cinnamomum cassia* essential oil.

Essential oil was extracted from the of *Cinnamomum cassia* via hydrodistillation, with a 0.3–0.9% yield (w/w, based on fresh weight). All parts of the plant (leaf, flower, and bark) contain essential oils, with the bark exhibiting the highest content, ranging from 1–4%. Lower contents are found in the leaves and fruits, at 0.3–0.9%. The major components of the essential oils across the different parts are: Bark: Cinnamaldehyde (77.3%) and cinnamaldehyde (57.7%). Branch: trans-cinnamyl acetate (27.6%). Leaf: Cinnamaldehyde (53.5%) and (Z)-9-octadecenamide (19.5%). Root: Cinnamaldehyde (58.3%) and p-methoxycinnamaldehyde (12.0%). The total ion chromatogram obtained from GC-MS analysis is shown in Figure 2. These significant constituents contribute to the unique chemical and bioactive profiles of *Cinnamomum cassia* essential oil.

Based on the above comparisons and analyses, it can be affirmed that the essential oil extracted from *Cinnamomum cassia* chemical profile, reflecting its unique ecological signature. It is particularly rich in oxidized sesquiterpenoids and nitrogen-containing heterocyclic compounds components that are rarely reported in samples from other regions. These compounds not only contribute to the oil's promising biological activities, such as anti-inflammatory, antioxidant, and antibacterial effects, but also serve as important indicators for guiding the standardization of cultivation zones. This highlights the high application potential of raw materials from the Central Highlands in the development of herbal medicines and natural cosmetic products.

Antioxidant Activity of *Cinnamomum cassia* Essential Oil

The antioxidant activity was evaluated by the DPPH radical scavenging assay, with the results summarized in Table 1. The IC₅₀ value of *Cinnamomum cassia* essential oil was determined to be 6.25±0.2 µg/mL, compared to 6.1±0.1 µg/mL for BHT, a well-known synthetic antioxidant. These results indicate that the essential oil of *Cinnamomum cassia* collected in Thanh Hoa has strong antioxidant activity comparable to BHT, highlighting its potential as a natural antioxidant agent.

Table 1: Antioxidant activity of *Cinnamomum cassia* essential oil

Essential oil (mg/mL)	% Inhibition			IC ₅₀ (mg/mL)
	1	2	3	
30	91.23	95.14	95.64	6.25±0.3
15	67.27	68.34	64.57	
7.5	53.46	54.81	52.19	
3.75	49.15	44.06	45.12	
1.875	42.34	41.57	41.64	
BHT ^a				6.10±0.1

^aButylated hydroxytoluene was used as positive control

Antibacterial Activity of *Cinnamomum cassia* Essential Oil

The antimicrobial activity of *Cinnamomum cassia* essential oil against *E. coli* is summarized in Table 2. The essential oil demonstrated strong activity, with an inhibition zone of 28.0±0.1 mm and Resistance of 89.70%. Essential oil at a 2.00 mg/mL concentration. Antibacterial activity of *Cinnamomum cassia* essential oil against *E. coli* to similar Ciprofloxacin used as a positive control exhibited an inhibition zone of 34.0 mm and Resistance of 100%. This suggests that *Cinnamomum cassia* essential oil could serve as a natural antimicrobial agent, with promising applications in

combating bacterial infections.

Table 2: Antibacterial activity of *Cinnamomum cassia* essential oil against *E. coli*

Bacterial density	Essential oil (mg/mL)	IZD (mm)			Resistance (%)
		1	2	3	
10 ⁶ CFU	2.00	29	28.5	28	89.70
10 ⁶ CFU	1.75	23	23.5	22	72.45
10 ⁶ CFU	1.50	18	17	18	59.67
10 ⁶ CFU	1.25	12	11.5	12	44.92
10 ⁶ CFU	1.00	7	7	7.5	38.33
10 ⁶ CFU	0.75	3	2.5	3	13.14
10 ⁶ CFU	0.50	0	0	0	0.00
Ciprofloxacin ^a	1.00	34	34	34	100

^aCiprofloxacin: Positive control for antibacterial activity, IZD: inhibition zone diameters.

Conclusion

This study is the first to investigate the essential oil from the of *Cinnamomum cassia* collected in Thanh Hoa, Vietnam. GC-MS analysis identified a diverse chemical composition. The major constituent's Essential oil was extracted from the of *Cinnamomum cassia* via hydrodistillation, with a 0.3–0.9% yield (w/w, based on fresh weight). The major components of the essential oils are distributed as follows: Bark: Cinnamaldehyde (77.3%) and cinnamaldehyde (57.7%). Branch: trans-Cinnamyl acetate (27.6%). Leaf: Cinnamaldehyde (53.5%) and (Z)-9-octadecenamide (19.5%). Root: Cinnamaldehyde (58.3%) and p-Methoxycinnamaldehyde (12.0%). The essential oil exhibited notable biological activities, demonstrating strong antioxidant activity with an IC₅₀ value of 6.25±0.3 µg/mL, surpassing the reference compound, BHT (6.1±0.1 µg/mL). Additionally, the essential oil showed effective antibacterial activity against *E. coli*, with resistance of 89.70%. These findings underscore the potential of *Cinnamomum cassia* essential oil as a natural resource for pharmaceutical applications, particularly in antioxidant and antibacterial therapies.

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