

RESEARCH ON CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF CINNAMOMUM BURMANNII ESSENTIAL OIL IN BAO LAC, CAO BANG PROVINCE

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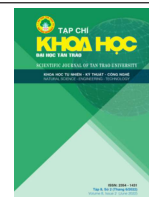
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Article info	Abstract:
<p>Received: 15/06/2022</p> <p>Revised: 15/07/2022</p> <p>Accepted: 01/08/2022</p>	<p>The objective of the study was to investigate the chemical composition and biological activity of Cinnamomum burmannii essential oil in Cao Bang province. Use the steam distillation method to extract essential oils. The chemical composition of essential oils was determined by Gas chromatography-mass spectrometry (GCMS). Evaluation of the antioxidant capacity of Cinnamomum burmannii essential oil by using DPPH free radical method. The results of this study have determined that Cinnamomum burmannii essential oil has 23 components with the main components including Citronellal (52.82%), Citronellol (25.13%), 1, 8-Cineole (5.04%). Cinnamomum burmannii essential oil has the antioxidant capacity with IC50 value = 12.03 µg/ml. These results created a base for further research, and development of functional products, care healthy products from the chemical components of this plant.</p>
<p>Keywords:</p> <p><i>Cinnamomum burmannii</i> Essential oil, GCMS, Cao Bang, DPPH</p>	



NGHIÊN CỨU THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH CHỐNG OXI HÓA CỦA TINH DẦU CINNAMOMUM BURMANII TẠI BẢO LẠC, TỈNH CAO BẰNG

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Thông tin bài viết	Tóm tắt
Ngày nhận bài: 15/06/2022	Mục tiêu của nghiên cứu nhằm khảo sát thành phần hóa học và hoạt tính sinh học của tinh dầu cây Quế trên ở tỉnh Cao Bằng. Sử dụng phương pháp chưng cất lôi cuốn hơi nước để trích ly tinh dầu. Thành phần hóa học của tinh dầu được xác định bằng phương pháp sắc ký khí khối phổ GCMS. Đánh giá khả năng kháng oxy hóa của tinh dầu Quế trên bằng phương pháp sử dụng gốc tự do DPPH. Kết quả của nghiên cứu đã xác định được tinh dầu Quế trên có 23 thành phần với thành phần chính gồm: Citronellal (52.82%), Citronellol (25.13%), 1, 8-Cineole (5.04%). Tinh dầu Quế trên có hoạt tính kháng oxy hóa $IC_{50} = 12,03 \mu\text{g/ml}$. Những kết quả nghiên cứu này tạo cơ sở cho các nghiên cứu tiếp theo và phát triển các sản phẩm chức năng, sản phẩm chăm sóc sức khỏe từ thành phần hóa học của loại cây này.
Ngày sửa bài: 15/07/2022	
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Từ khóa:	
<i>Cinnamomum burmanii</i> Essential oil, GCMS, Cao Bằng, DPPH	

1. Introduction

Cinnamomum burmannii is one of several species of plants in the genus *Cinnamomum*, the family Lauraceae. They are native to Southeast Asia to have China, Indonesia, Vietnam... It is a woody plant, growing on rocky mountains over 1000 meters above sea level in Bao Lac district, Cao Bang province or known as Phjac Chac, Que Tren, or Tren Tren.

Cinnamomum burmannii is a traditional medicinal plant that has long been used as a spice, food preservative, and food flavoring [1].

The pharmacological studies have shown high antioxidant, anti-bacterial, anti-fungal, anti-thrombotic, anti-inflammatory, anti-tumor, dental plaque formation and periodontal disease inhibitory, glycosylation

inhibitory, and radical scavenging activities of essential oil of *Cinnamomum burmannii* [2, 3].

In Vietnam, the tree grows in green forests at altitudes between 500 m and 1500 m from Ha Tay, Ninh Binh, Thanh Hoa through Nghe An, Quang Tri, Thua Thien-Hue, to Khanh Hoa, Lam Dong. *Cinnamomum burmannii* is a woody plant, 6-8 meters high, and the branches and leaves have the smell of lemongrass. Peduncle 8-12 mm long, rounded, slightly rough, leaf blade oval to ovate, 9-12 cm long, 3-4.5 cm wide, the base of blade wedge-shaped, tip-shaped, 10-12 mm long, hairless, dark green on both sides, upper side concave veins, arc-shaped, starting from the base of the leaf blade to the end of the leaf blade, the veins are light brown when dry. Inflorescences panicle, short, weak,

flower axis has slit longitudinally when dry, with short soft hairs, bracts spoon-shaped, 6-8 mm long, directed

upwards. Flowers pale yellow cream, flower stalks as long as bracts, 6-8 mm long, lower part wide funnel-shaped, 1-1.5 mm high, soft hairs, upper split into 6 lobes, divided into 2 rings, each ring 3 lobes, lobes oblong, 7-8 mm long.

Both bark and leaves of *Cinnamomum burmannii* are fragrant, this aroma also varies depending on the distribution area of the tree. Root bark, stem bark, leaves, and branches are spicy, slightly sweet, and warm. It affects fighting colds, headaches, rheumatism, joint pain, and stomach pain. In addition, *Cinnamomum burmannii* has been cultivated for everyday requirements (cinnamon spice in food) and illness treatment. Its bioactive components have potential for application as natural food preservatives [4].

The chemical components of extracts were identified by GC-MS, HPLC-MS, LC-MS [2, 4, 5] in their studies on *Cinnamomum burmannii* essential oil such as the study on the essential oil of *Cinnamomum burmannii* leaves analyzed by GC-MS showing the presence of 40 volatile components, accounting for 99.4% of the total oil quantity. The main components found were D-borneol (78.6%), Bornyl acetate (3.26%), (-)-spathulenol (2.60%) and eucalyptol (1.92%) [5]. In another effort, Deng et al. (2010) investigated 61 components in *C. burmannii* essential oil in Guangxi, the main components were identified as caryophyllene (21.71%), eucalyptol (18.22%), guaialol (7.52%), (+)- α -terpineol (7.06%), (-)- β -pinene (3.57%), γ -eudesmol (3.33%), bulnesol (3.16%); and investigated the oxidizing activity of essential oils from *Cinnamomum burmannii* leaves and found that the maximum removal rate on the DPPH radical was 21.71% [6]. According to research by Nguyen Thi Thu Thao et al. (2021), studying the chemical composition of cinnamon essential oil from leaves and young branches in Phu Tho, the obtained results show that there are 31 compounds identified, of which the main component E-cinnamaldehyde (75.25%), E-o-methoxycinnamaldehyde (9.31%), benzaldehyde (3.54%) [7].

When studying four important *Cinnamomum* species in China including *C. cassia*, *C. loureiroi*, *C. wilsonii*, and *C. burmannii* the results showed 47 compounds identified in *n*-butane extracts and 11 compounds in ethanol extracts totally [8].

Following Zhang, et al, 2009 studied the effects of temperature, light, and pH on the anthocyanin's radical scavenging activity which was extracted from the fruit extract of *C. burmannii* using semi-preparative HPLC. The IC₅₀ of the anthocyanin was 4.6 μ g/ml. and its antioxidant activity was shown to be drastically reduced after heating it for 5 hours at 100°C or 30 minutes at 130°C. The DPPH radical scavenging activity was not altered by increasing the pH. However, exposure

to fluorescence radiation and sunlight intensity also influenced the anthocyanin's DPPH radical scavenging activity [9]. According to Harlinda, Kuspradini et al. (2016) the highest rate of DPPH radical scavenging activity (98%) was expressed in the 100 ppm μ g/ml essential oil of *Cinnamomum burmannii*. Their values at different concentrations (25-100 ppm) were higher than those of ascorbic acid (97%) [10];

There have not been many studies on *Cinnamomum burmannii* essential oil grown in the country, this study aims to provide more information on the chemical composition as well as antioxidant capacity of *C. burmannii* essential oil grown in Bao Lac, Cao Bang.

2. Material, chemical, and method

2.1. Material and chemical

Cinnamomum burmannii were collected in Bao Lac, Cao Bang province, Vietnam, identified by Mr. Nguyen Quoc Binh, Vietnam Academy of Science and Technology identify the scientific name was *Cinnamomum burmannii* (Nees.) Blume, 1826, Lauraceae family.

Chemicals: Ethanol, *n*-hexan, Natrisulfat, DPPH (2,2-diphenyl-1-picrylhydrazyl), Ascorbic acid.

2.2. Essential oil extraction method

Cinnamomum burmannii essential oil is extracted by direct steam distillation. The essential oil was evaporated with water at 150°C for 50 minutes. After steam distillation, the essential oil will be collected and separated by a separating funnel used to separate the immiscible liquids of the two layers of essential oil and water. Wait for the water and essential oil to separate to form two separate layers and obtain the essential oil. The oil after separation is anhydrous with Na₂SO₄.

2.3. Analysis of the chemical composition by GC-MS method

The chemical composition of *Cinnamomum burmannii* essential oil was analyzed by Gas chromatography-mass spectrometry (GC/MS): Agilent 7890A gas chromatograph paired with Agilent 5975C Mass Selective Detector, HP-5MS column size (30m, 0.25 mm, 0.25 μ m). Gradient program with 60°C conditions increases temperature by 4°C /min to 240°C. Components were identified based on their retention coefficients (calculated according to the *n*-alkane homologous sequence) and compared their mass spectra with standard mass spectrometric data stored in the spectrometric library (HPCH1607, NIST08, Wiley 09). The relative concentrations of the components were calculated based on the peak areas obtained from the chromatogram. The mass spectrometry software is Mass Finder 4.0.

2.4. Antioxidant assay

Investigation of the antioxidant capacity of *Cinnamomum burmannii* essential oils was tested using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique by Radical Scavenging Activity method (Goldschmidt, S., & Renn, K., 1922)

DPPH is a free radical used to perform a screening reaction for the antioxidant activity of the studied substances. The antioxidant activity was demonstrated by reducing the color of DPPH free radicals, as determined by measuring the optical absorbance at 517 nm.

Dilute 0.1 mM DPPH solution in ethanol by dissolving 4 mg of DPPH with a sufficient amount of ethanol to dissolve DPPH. Then put in a volumetric flask and add enough ethanol to 100 ml, in a colored glass bottle.

The extract of *Cinnamomum burmannii* essential oil with concentration of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml was used in this test. From each concentration, 1 ml was taken and reacted with 3 ml of DPPH. Samples were kept in the dark, at room temperature. After 30 minutes, measure the absorbance at 517 nm. The experiment was performed in 3 replicates.

The percentage of scavenged DPPH of the extract was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \frac{A_c - A_e}{A_c} \times 100$$

In there:

Ac: Absorbance of control reaction

Ae: Absorbance in presence of test or standard sample

The IC₅₀ value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated from sample concentration and DPPH(%), using Excel software, make a regression equation of the form $y = ax + b$ showing the correlation between DPPH (%) (y) and concentration (x). The lower absorbance of the reaction mixture indicated higher free radical activity.

2.5. Some physicochemical of *Cinnamomum burmannii* essential oils

2.5.1 Sensory evaluation

Preliminary sensory examination of essential oils is based on the observation of exterior indications such

as odor, taste, color, and transparency. This allows for a preliminary assessment of the essential oil's quality as well as the planned usage of the essential oil. Sensory assessment based on TCVN 8460: 2010.

2.5.2 Determination of the acid index

Determination of acid value based on TCVN 8450:2010.

The acid index is defined as the number of milligrams of potassium hydroxide (KOH) required to neutralize free acids in 1 gram of essential oil. The acid number may be used to calculate the quantity of free acid in the essential oil.

The acid value of essential oil is determined by its freshness and shelf life. The acid index of the essential oil will grow with time owing to oxidation, and the ester in the essential oil will be broken down.

2.5.3 Determination of the saponification index

Determination of saponification index based on TCVN 6126:2015.

The saponification index is the number of milligrams of KOH required to neutralize all the free and conjugated acids are present in 1 gram of essential oil.

2.5.4 Data statistical analysis methods

All of the tests were carried out in triplicate. The results are provided as means with standard deviations from three separate studies. Analysis of variance (ANOVA) was used to find significant differences, which were then tested using the Duncan test at a P < 0.05 level. Data were analyzed by using SPSS Statistics software, version 20.0.

3. Result and discussion

3.1 The chemical component of *Cinnamomum burmannii* essential oil

By means of gas chromatography-mass spectrometry (GC-MS), the chemical components of essential oils were determined and recorded in Table 1.

Table 1: Chemical composition of *Cinnamomum burmannii* essential oil

No.	Time	RI	Hit %	Chemical name	Integral	% FID
1	8.15	851	4	<i>cis</i> -3-Hexen-1-ol	4902364	0.25
2	10.49	939	84	α-Pinene	67504510	2.40
3	11.69	978	86	Sabinene	5467875	0.20
4	11.88	984	91	β-Pinene	28894820	1.11
5	12.09	991	88	Myrcene	32932788	1.38
6	13.51	1033	86	Limonene	10917944	0.39
7	13.65	1038	37	1,8-Cineole	152638894	5.04
8	14.02	1048	76	<i>trans</i> -β-ocimene	13103478	0.49

No.	Time	RI	Hit %	Chemical name	Integral	% FID
9	14.23	1054	34	Bergamal	5428014	0.21
10	15.82	1101	83	Linalool	51484263	1.96
11	17.66	1153	83	Isopulegol	15263894	0.50
12	17.82	1158	83	Citronellal	1425494830	52.82
13	18.11	1166	35	Iso-Isopulegol	4909378	0.18
14	20.36	1230	90	Citronellol	659199518	25.13
15	20.41	1232	83	Geraniol	2200193	0.18
16	20.88	1246	70	Neral	2895885	0.12
17	21.85	1274	83	Geranial	3117577	0.13
18	24.53	1354	61	Citronellyl acetate	100485397	2.96
19	24.90	1365	85	Neryl acetate	2297443	0.11
20	25.52	1384	91	Geranyl acetate	5060096	0.18
21	26.16	1403	65	<i>cis</i> - β -Elemene	3516694	0.11
22	26.28	1407	53	Methyl eugenol	137680708	3.78
23	29.23	1501	71	Methyl isoeugenol	6336838	0.29
				Total		99.92

The analysis uses Gas chromatography-mass spectrometry and GC/FID flame ionization detectors to determine the composition of volatiles in the sample.

From the above results, the chemical composition of *Cinnamomum burmannii* essential oil obtained include 23 compounds, of which the highest content was Citronellal (52.82%), Citronellol (25.13%), 1,8-Cineole (5.04%). The results of the study are different from the results of previous studies on the composition of *C. burmannii* essential oil. The cause of this difference may be due to differences in climate, soil or experimental conditions, so the composition of essential oils is different.

3.2. Antioxidant capacity of *Cinnamomum burmannii* essential oil

3.1.1 Investigation of DPPH free radical scavenging ability of Ascorbic acid

Formulate an Ascorbic acid standard curve based on the percentage of free inhibition and the concentration of Ascorbic acid

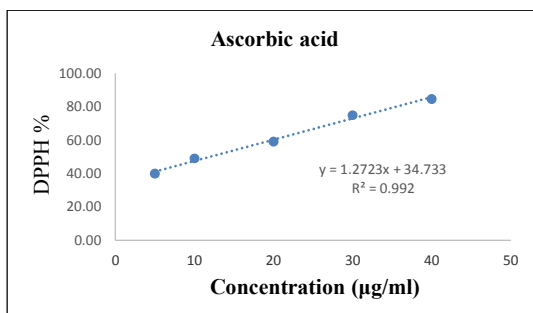


Figure 1. Standard curve of antioxidant capacity of Ascorbic acid

From the equation deduced the IC₅₀ value of ascorbic acid is: IC₅₀ = 12.00 (µg/ml)

3.2.2. Investigation of DPPH free radical scavenging ability of essential oil

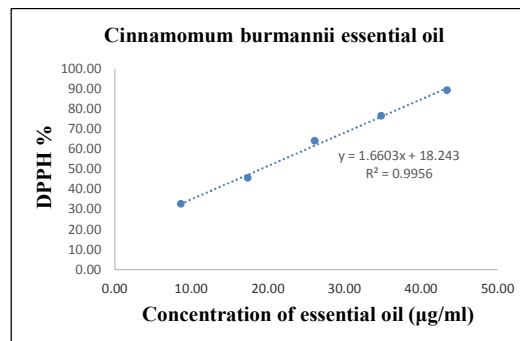


Figure 2. Correlation between free radical inhibitory activity and concentration of *Cinnamomum burmannii* essential oil

From the equation deduced that *Cinnamomum burmannii* essential oil has an IC₅₀ value = 41.10 µg/ml, 3.5 times higher than the IC₅₀ value of Ascorbic acid (12.00 µg/ml). Thus, compared with Ascorbic acid, the antioxidant activity of essential oil is lower than that of Ascorbic acid. This study has results consistent with the study of Harlinda, Kuspradini *et al.* (2016) and Deng *et al.* (2010) about investigated the oxidizing activity of essential oils from *Cinnamomum burmannii* leaves. The antioxidant activity of *Cinnamomum burmannii* essential oil compared with cinnamon is often similar.

3.3 Result for determining some physicochemical of essential oils

The physicochemical characteristics of *Cinnamomum burmannii* essential oils are determined and presented in Table 2.

Table 2: Some physicochemical of Cinnamomum burmannii essential oils

Features	Result
Color	Light yellow
Odor	Specific smell of essential oil
Taste	Bitter, warm nature
Solubility	Insoluble in water, soluble in organic solvents such as methanol, diethyl ether, chloroform...
Density	0.867g/ml
Acid index (I_A)	4.24
Saponification index (I_S)	22.61
Ester index (I_E)	18.37

4. Conclusion

Based on the results and discussion of the study, the chemical composition of *Cinnamomum burmannii* essential oil collected in Bao Lac, Cao Bang was determined to include 23 components with the main components being Citronellal (52.82%), Citronellol (25.13%), 1, 8-Cineole (5.04%). *Cinnamomum burmannii* essential oil has antioxidant capacity with IC_{50} value = 41.10 μ g/ml.

This study contributes to the direction of research on antioxidant capacity from essential oil-rich plants such as *Cinnamomum burmannii*. Besides, it is possible to continue researching the antimicrobial and anti-inflammatory ability of this plant. However, it should be noted the difference in the chemical composition of *Cinnamomum burmannii* essential oil under different ecological conditions.

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