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Research Article
Phytochemical Composition and Bioactivity Assessment of Essential Oil of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet from Xuan Son National Park, Vietnam

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Abstract

Background and Objective: *Cinnamomum bejolghota* (Buch.-Ham.) Sweet, belonging to the *Cinnamomum* genus, was cited as a medicinal plant due to its essential oils. This study aimed to discover the chemical constituents and biological properties of the essential oil from the bark of *C. bejolghota* collected from Xuan Son National Park, Phu Tho Province, Vietnam. **Materials and Methods:** The bark essential oils of *C. bejolghota* were extracted by the hydrodistillation method and investigated by Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography-Flame Ionization Detection (GC-FID). The antimicrobial feature of the essential oil was estimated using disc diffusion and microdilution broth assays. **Results:** The bark essential oils of *C. bejolghota* grown in Xuan Son National Park, Vietnam were obtained in yields of 1.1% (v/w, ±0.01). Major components of the essential oil were geraniol (26.2%), 1,8-cineole (24.73%), α-terpineol (9.30%) and terpinene-4-ol (3.90%). The bark essential oils of *C. bejolghota* displayed antimicrobial activity against the yeast *S. cerevisiae* and filamentous fungi *F. oxysporum* with minimum inhibitory concentrations of 500 ppm. **Conclusion:** The present study first represents phytochemical and bioactivity analysis of the essential oil of the *C. bejolghota* from Northern of Vietnam.

Key words: *Cinnamomum bejolghota*, essential oil, chemical composition, antimicrobial activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

By using methods such as steam distillation, extrusion, cold-soaking, or solvent extraction, essential oils, the volatile, fragrant compounds generated spontaneously by plants, are extracted from many organs including flowers, leaves, stems, roots, or fruits. Approximately 3000 essential oils have been extricated from at least 2000 plant species, but only 300 of them are economically significant[1]. Essential oils are useful due to their antibacterial, antifungal, insecticidal, antiviral, anticancer, antioxidant and many other properties[1-4].

The genus *Cinnamomum* L. (Lauraceae) contains 250 species found in subtropical and tropical parts of Asia, Oceania, North America and Central America[4]. Belonging to the *Cinnamomum* genus, *Cinnamomum bejolghota* (Buch.-Ham.) Sweet is a healing herb[5]. The bark and its infusions are used to cure coughs, toothaches, liver problems, diabetes, gallstones, bone fracture and wounds[6]. This species is widely distributed in China, Thailand, Laos, Myanmar, Nepal, Sri Lanka, Bangladesh, India and Vietnam[7]. Linalool has been reported as a prominent compound in the essential oil from leaf and panicle, whereas E-nerolidol and α-terpineol as the substantial components in the essential oil from the bark of *C. bejolghota* cultivated in India[8,9]. However, 1,8-cineole has been identified as the primary component of the essential oil from the bark of *C. bejolghota* cultivated in Jorhat (India)[9] and Thailand[10]. To date, only one previous study reported that *C. bejolghota* bark essential oil possessed potent antifungal and antibacterial activities[10]. Herein, the chemical composition as well as its antimicrobial activities of the essential oil from the bark of *C. bejolghota* grown in Xuan Son National Park, Vietnam, was reported for the first time.

MATERIALS AND METHODS

Study area: This work was carried out from July, 2021 to July, 2022 at the Faculty of Natural Sciences, Hung Vuong University and the Institute of Natural Products Chemistry (INPC), Vietnam Academy of Science and Technology (VAST). Plant materials: Stem bark of *C. bejolghota* (Buch.-Ham.) Sweet was collected from Xuan Son National Park, Phu Tho Province, Vietnam (21°9'N, 104°56'E) in July, 2021.

Research procedure

Isolation of essential oil and chemical component investigation: A total of 500 g dried bark of *C. bejolghota* (Buch.-Ham.) Sweet was used for hydrodistillation for 3 hrs by a Clevenger-type apparatus. Essential oils were separated, dehydrated and investigated as described by Nguyen *et al.*[11].

Microbial strains: Seven microorganism strains from American Type Culture Collection (ATCC, Manassas, VA, USA) (*Bacillus subtilis* subsp. *spizizenii* (ATCC 6633), *Staphylococcus aureus* subsp. *aureus* (ATCC 25923) *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Aspergillus niger* (ATCC 6275), *Fusarium oxysporum* (ATCC 7601) and *Candida albicans* (ATCC 10231)) and two strains, *Saccharomyces cerevisiae* (VTCC-Y-62) and *Salmonella enterica* (VTCC 12277), from the Vietnam Type Culture Collection (Institute of Microbiology and Biotechnology, Vietnam National University, Ha Noi, Vietnam) were used for antimicrobial activity of *C. bejolghota* bark oils.

The antibacterial property of essential oil was tested using the agar disk diffusion technique[12-14]. Prior to testing, microorganisms kept at -80°C were switched on by culture medium to a concentration of 1.0×10^6 CFU mL^{-1}. A total of 100 µL of inoculum solution was brought and applied uniformly to the agar surface. The positive (*B. subtilis* subsp., *spizizenii* (ATCC 6633) and *S. aureus* subsp., *aureus* (ATCC 25923)) and negative Gram bacteria (*E. coli* (ATCC 25922), *Salmonella enterica* (VTCC 12277)) were cultured in the Trypcase Soy Broth medium (TSB-Sigma). Using an aseptic approach, three holes (approximately 6 mm in diameter each) were created on agar plates. A pipette was used to place 50 µL of essential oil into each hole. The Petri dishes were kept at 37°C for 18-24 hrs after being incubated at room temperature for 2-4 hrs. On each plate culture, the presence or absence of growth surrounding each hole carrying an antibacterial agent was noted. The ruler with millimeter graduations was used to measure the diameters of the inhibiting growth zones.

The standard liquid dilution method[15] was performed to determine the *in vitro* antimicrobial activity experiments on a 96-well microtiter plate as previous description[16]. The fungi were grown in the Sabouraud Dextrose Broth medium (SDB-Sigma). After that, the agar plates were kept at 30°C or 37°C for 24 or 48 hrs depending to the fungal species. The MIC (Minimum inhibitory concentration) value was calculated. For respective Gram (+) and Gram (-) bacteria, streptomycin and tetracycline were used as positive controls whereas nystatin was used for fungi and yeasts.

Statistical analysis: Statistical investigation of all data was carried out using the ANOVA and means sorted out by Duncan's Multiple Range Test at the 5% level of significance ($p = 0.05$).

RESULTS AND DISCUSSION

Composition analysis of essential oil of *C. bejolghota* (Buch.-Ham.) Sweet: The essential oil obtained by hydrodistillation of

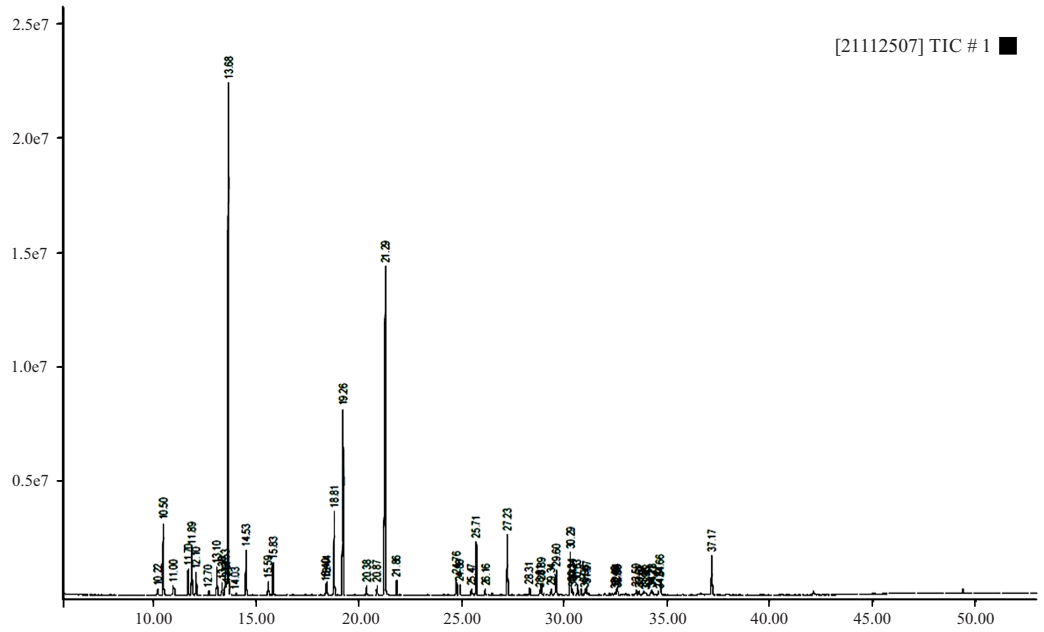

Fig 1: GC/MS total ion chromatogram of the bark essential oil of *C. bejolghota*

C. bejolghota bark with a refractive index of 1.4752 (20°C), was a pale-yellow color liquid with a pleasant odor. The extraction yield was 1.1% (v/w), quantified on a dry weight basis.

The chromatogram of the essential oil from *C. bejolghota* bark was presented in Fig. 1. A total of 53 compounds, accounting for 99.45 % of the essential oil composition were identified (Table 1). The main classes of compounds found in the essential oil were oxygenated monoterpenes (68.43%), followed by monoterpene hydrocarbons (14.78%), sesquiterpene hydrocarbons (11.32%), oxygenated sesquiterpene (2.42%) and non-terpenes (2.54%). Major components of the essential oil were geraniol (26.2%), 1,8-cineole (24.73%), followed by α-terpineol (9.30%), terpinene-4-ol (3.90%), α-pinene (3.34%), β-pinene (2.40), β-caryophyllene (2.69%), α-copaene (2.11%), respectively.

In comparison to the essential oil components of *C. bejolghota* reported in the literature, there was a slight difference between the essential oil compositions of *C. bejolghota* growing in northern of Vietnam and in other regions, including India[8,9] and Thailand[10] (Table 2). The content of monoterpene hydrocarbons of Vietnamese essential oil samples was lower than in other samples. However, the content oxygenated monoterpene of Vietnamese essential oil samples was quite high, almost equivalent to the Thailand oil sample and was significantly superior to the Indian samples. There were a few interesting points such as α-Terpineol, the chemical composition was detected in lower quantity in this study in comparison with the previous studies[8,9]. The content of cineole-1,8 (eucalyptol) of *C. bejolghota* grown in Phu Tho, Vietnam, was significantly higher than *C. bejolghota* grown in India, but lower than sample collected in Thailand. Especially, the high content of Geraniol was discovered for the first time in our study. Similarities and differences between the study results could be explicated by a variety of factors including the nature and age of the plants as well as depending on growth area and other conditions[17].

Bioactivity analysis of essential oil of *Cinnamomum bejolghota* (Buch.-Ham.) Sweet: The biological activities of *C. bejolghota* bark essential oil were determined (Fig. 2 and Table 3) with regard to inhibitory zone diameter and MIC. The essential oil from the bark of *C. bejolghota* inhibited *E. coli* and *S. enterica* growth, with inhibitory zone diameters of 6.55±1.10 mm and 4.75±1.20 mm, respectively. The MIC value of the essential oil from the bark of *C. bejolghota* was 500 µg/mL for *S. cerevisiae* and *F. oxysporum*. These MIC values for both fungi were higher for six other examined microorganisms (Table 3).

Although the antibacterial effects of essential oils from *Cinnamomum* spp., have been extensively documented, the efficiency of *C. bejolghota* bark oil against pathogenic species has been poorly investigated. Atiphasaworn *et al.*[10], found that the essential oil of *C. bejolghota* bark from Thailand was efficient against bacterial and fungal infections.

Table 1: Compositions of the essential oil from the bark of *C. bejolghota* grown in Xuan Son National Park, Vietnam

Rt (min)	Class/compound	RI	FID (%)
Monoterpene hydrocarbons			**14.78**
10.22	α-Thujene	930	0.23
10.50	α-Pinene	939	3.34
11.00	Camphene	956	0.41
11.70	Sabinene	978	1.16
11.89	β-Pinene	984	2.40
12.10	Myrcene	991	1.12
12.70	α-Phellandrene	1010	0.16
13.10	α-Terpinene	1022	1.14
13.36	o-Cymene	1029	0.49
13.53	Limonene	1034	1.06
13.57	β-Phellandrene	1035	0.61
14.03	Z- β-Ocimene	1049	0.10
14.53	γ-Terpinene	1063	1.95
15.59	Terpinolene	1094	0.61
Oxygenated monoterpene			**68.43**
13.68	1,8-Cineole (Eucalyptol)	1038	24.73
15.83	Linalool	1101	1.47
18.40	δ-Terpineol	1174	0.49
18.44	Borneol (endo-Borneol)	1175	0.58
18.81	Terpinen-4-ol	1186	3.90
19.26	α-Terpineol	1199	9.30
20.38	Nerol	1231	0.50
20.87	Neral	1245	0.51
21.29	Geraniol	1258	26.20
21.86	Geranial	1274	0.75
Sesquiterpene hydrocarbons			**11.32**
24.76	α-Cubebene	1361	0.64
25.47	Cyclosativene	1382	0.25
25.71	α-Copaene	1390	2.11
26.16	*cis*-β-Elemene	1404	0.28
27.23	E-Caryophyllene (β-Caryophyllene)	1437	2.69
28.31	α-Humulene (α-Caryophyllene)	1472	0.33
28.81	*trans*-Cadina-1(6),4-diene	1488	0.17
28.89	γ-Muurolene	1490	0.48
29.34	β-Selinene	1505	0.28
29.60	α-Muurolene	1514	1.34
30.29	δ-Cadinene	1537	1.62
30.34	*trans*-Calamenene	1538	0.27
30.42	Zonarene	1541	0.24
30.63	*trans*-Cadina-1,4-diene	1548	0.37
30.98	α-Calacorene	1560	0.25
Oxygenated sesquiterpene			**2.42**
31.07	Elemol	1563	0.30
32.54	Guaiol (Champacol)	1613	0.19
33.50	1-epi-Cubenol	1647	0.19
33.61	γ-Eudesmol	1651	0.20
33.86	epi-α-Muurolol	1660	0.20
33.96	α-Muurolol (δ-Cadinol)	1663	0.16
34.21	β-Eudesmol	1672	0.17
34.28	α-Eudesmol	1675	0.24
34.61	Bulnesol	1686	0.15
34.66	Patchoulol	1688	0.62
Non-terpenes			**2.54**
24.88	Chavibetol (*m*-Eugenol)	1365	0.42
32.46	4-Allyl-2,6-dimethoxyphenol	1610	0.21
32.60	Tetradecanal	1615	0.27
37.17	Benzyl benzoate	1781	1.64
Total			99.49

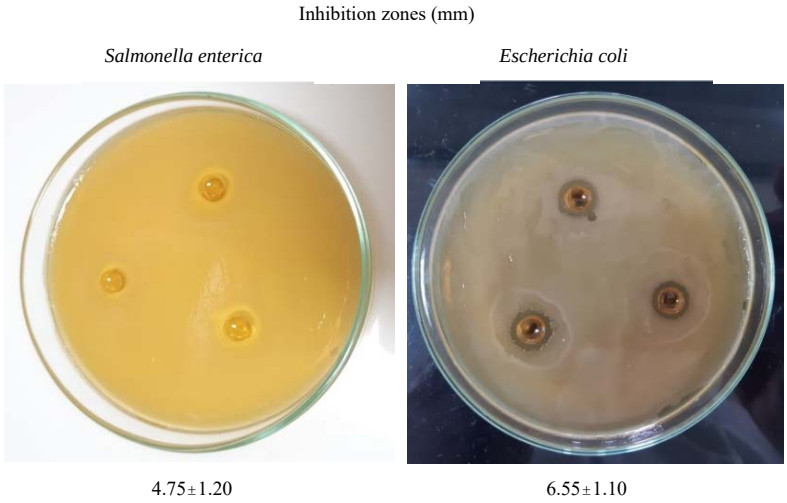

Fig. 2: Antibacterial property of essential oils from barks of *C. bejolghota* (mean±standard deviation and n = 3)

Table 2: Majority compounds in bark essential oil of *C. bejolghota* collected from Vietnam, Thailand and India: A comparative study with literature

Class/compound	*C. bejolghota* bark oil in Vietnam (this study)	*C. bejolghota* bark oil in Jorhat, Assam India[8]	*C. bejolghota* bark oil in Sibsagar, Assam India[9]	*C. bejolghota* bark oil in Jorhat, Assam India[9]	*C. bejolghota* bark oil in Thailand[10]
Extraction yield	1,1%	0,1%	0,08%	0,08%	1.01%
Total compounds (representing % total oil)	53 (99.49%)	18 (61%)	54 (99.7)	32 (92%)	36 (97.96)
Monoterpene hydrocarbons	**14.78**	**-**	**17.8**	**5.7**	**16.44**
α-Pinene	3.34		2.9	0.7	6.58
α-Phellandrene	0.16		0.3	-	-
p-Cymene	-		2.4	1.3	0.09
Oxygenated monoterpene	**68.43**	**37.9**	**49.1**	**76.5**	**73.41**
Cineole-1,8 (Eucalyptol)	24.73	1.07	7.2	31.3	40.24
Linalool	1.47	8.20	19.9	20.0	0.11
α-Terpineol	9.30	18.20	12.7	21.3	-
γ-Terpineol	-		-	-	15.41
Terpinen-4-ol	3.90	1.8	8.3	3.2	7.55
Geraniol	26.20		-	-	-
Sesquiterpene hydrocarbons	**11.32**		**9.5**		**2.15**
α-Copaene	2.11		2.5	0.6	-
E-Caryophyllene (β-Caryophyllene)	2.69		0.4	0.2	-
Oxygenated sesquiterpene	**2.42**	**15.3**	**21.0**	**11.2**	**3.23**
epi-α-Cadinol	-		4.6	3.3	0.48
Caryophyllene oxide	-		0.7	-	-
Patchoulol	0.62		-	-	-
(E)-Nerolidol		15.30	-	-	

Table 3: MIC values of bark essential oil of *C. bejolghota*

	Microorganisms	MIC (μg mL^{-1})
Gram-positive bacteria	*B. subtilis* subsp., *spizizenii* (ATCC 6633)	>500
	S. aureus subsp., *aureus* (ATCC 25923)	>500
Gram-negative bacteria	*E. coli* (ATCC 25922)	>500
	P. aeruginosa (ATCC 10145)	>500
Yeast	*C. albicans* (ATCC 10231)	>500
	S. cerevisiae (VTCC–Y–62)	500
Fungus	*A. niger* (ATCC 6275)	>500
	F. oxysporum (ATCC 7601)	500

The antibacterial and antifungal effects of *C. bejolghota* bark essential oil might be attributed to the range of its bioactive compounds. Geraniol (26.20%) and 1,8-cineole (24.73%), the two principal components of *C. bejolghota* bark essential oils, exhibit many biological activities, including antibacteria[18], fungicidal[18,19], anti-inflammatory,

anticarcinogenic and antioxidant[20]. The antimicrobial activity (lipophilic character) of Geraniol may be explained by its capacity to cling to the microorganism's cell membrane lipids, join with its components, making it more permeable and adhering important intracellular spots, thereby destroying their structures.

CONCLUSION

This work surveyed the components and biological activities of the essential oil from the bark of *C. bejolghota* grown in Xuan Son National Park in Vietnam. The main chemical classes of components were oxygenated monoterpene (68.43%), monoterpene hydrocarbons (14.78%), sesquiterpene hydrocarbons (11.32%), oxygenated sesquiterpene (2.42%) and non-terpenes (2.54%), respectively. The dominant compounds of the essential oil were geraniol (26.2%) and 1,8-cineole (24.73%). Biological activity analysis demonstrated that *C. bejolghota* bark essential oil has potent antibacterial and antifungal properties. From the foregoing, the essential oil of *C. bejolghota* might be a source of potential antimicrobial agents.

SIGNIFICANCE STATEMENT

The phytochemical compositions and antibacterial activity of the essential oil from the bark of *C. bejolghota* grown in Northern Vietnam were first investigated in this work. Some components, such as geraniol and other chemicals in trace amounts, were discovered in *C. bejolghota* essential oil for the first time. As a result, the essential oil of *C. bejolghota* might be considered a bioactive natural product which had many potential applications in aesthetic or postharvest conservation. This work helps to reveal the components and biological activities of the essential oil of *C. bejolghota* grown in northern Vietnam that many other researchers did not explore.

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