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Chemical composition, antibacterial and antifungal activities of *Cinamomum bejolghota* bark oil from Thailand

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ABSTRACT

The volatile constituents of *Cinnamomum bejolghota* bark essential oil were investigated by using gas chromatography-mass spectrometry (GC-MS). Thirty-six volatile constituents were identified with the major components being 1,8-cineole, γ-terpineol, borneol and terpinen-4-ol. Essential oil of *C. bejolghota* bark was firstly screened for their antibacterial and antifungal activities against Gram-positive and Gram-negative bacteria, as well as, *Colletotrichum* sp. fungi using disc diffusion method. Minimal inhibitory concentration (MIC) of *C. bejolghota* bark oil was further analyzed by microdilution. Essential oil of *C. bejolghota* bark was most effective against bacteria with MIC ranging from 31.25-62.50 µg/mL, whereas inhibition against fungal pathogens was moderate, with MIC of 125- 500 µg/mL. The strong antimicrobial activity of *C. bejolghota* bark oil was correlated mainly to 1,8-cineole, γ-terpineol, borneol, terpenen-4-ol and linalool.

INTRODUCTION

Volatile components of essential oils are mainly represented by terpenoids, phenylpropanoids or benzenoids, fatty acid derivatives and amino acid derivatives (Dudareva *et al*., 2006). Volatile components of essential oils possess potential antimicrobial and insecticidal activities against pathogens including those causing human pathogenic diseases and crop spoilage in agriculture (Singh & Maurya, 2005). Use of essential oils as antimicrobial agents is environmentally safe and economical. In addition, essential oils from various parts of plants are widely used for gargles in throat infection, skin care (Gutiérrez *et al*., 2008), beauty treatments (Price, 2003), herbal medicines (Schultz *et al*., 2001), aromatherapy (Price, 2003), cosmetics (Tisserand & Young, 2013) and perfumery applications (Nielsen & Rios, 2000). Essential oil of *Cinnamomum* plant, belonging to the Lauraceae family, is obtained from its leaves and barks and is widely used as a flavoring agent in food, as well as for cosmetic and pharmaceutical applications (Sudmoon *et al*., 2014). The essential oil of *Cinnamomum* genus plants contained the great antimicrobial (Ooi *et al*., 2006), antifungal (Giordani *et al*., 2006), anti-inflammatory (Miguel, 2010) and antixidant (Jayaprakasha *et al*., 2003) properties. *Cinamomum bejolghota* (Buch.-Ham.) is a medicinal plant, apply as the treatment of a cough, cold, toothache, liver complaints (Rao, 1979). The plant is widely distributed in China, Vietnam, Sri Lanka, Madagascar, India and East of Thailand (Li *et al*., 2013). Baruah *et al*., 1997 reported linalool as a major volatile in essential oil of *C. bejolghota* leaf and panicle cultivated in India, whereas α-terpineol and *E*-nerolidol were found as the main components in its stem bark oil. Conversely, high amounts of 1,8-cineole and α-terpineol were detected in essential oil of *C. bejolghota* bark collected from different areas in India (Choudhury *et al*., 1998). Only few studies have identified volatile profiles of *C. bejolghota* essential oil, though there is no previous study reporting the antimicrobial and antifungal properties of *C. bejolghota* oil. The aim of this study was to investigate the chemical composition of *C. bejolghota* oil from Thailand, to provide baseline data on its antibacterial and antifungal properties, and to predict its usefulness as a natural antimicrobial and antifungal agent in postharvest processing.

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EXPERIMENTAL

Plant material

Stem bark of *C. bejolghota* (Buch.-Ham.) was collected in April 2015 from Trat province, Eastern Thailand and air dried for 7 days. Voucher herbarium specimen (MFLU No. 10000) of the 1-year old plant was identified and deposited at the Mae Fah Luang University Botanical Garden, Chiang Rai, Thailand.

Extraction of essential oil and chemical composition analysis

One hundred grams of *C. bejolghota* dried bark were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The essential oils were dried using anhydrous sodium sulfate. The chemical composition of *C. bejolghota* essential oil was carried out on a Hewlett Packard model HP6890 gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5MS (5% phenylpolymethylsiloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies, USA) employed with an HP model 5973 mass selective detector (MS). The oven temperature was programmed at an initial temperature of 60 °C prior ramping at a 3 °C/min until a maximum of 200 °C was reached. The temperatures of the injection and detection steps were set at 250 and 280 °C, respectively. Helium was used as the carrier gas with a flow rate of 1 mL/min. The EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 29-300. The electron multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. One microliter of *C. bejolghota* essential oil was dissolved in n-hexane (1:100 v/v) prior to injection into the GC-MS system with a split ratio of 1:200. Identification of essential oil composition was accomplished by comparison between their relative retention indices (RI) to C_8-C_{16} *n*-alkanes, and using a comparison of the mass spectra of individual components with the reference mass spectra in the W8N08 and NIST08 databases, and published literature. Quantification of all identified components was investigated by using a percent relative peak area.

Antibacterial activity assay

Antibacterial activities of *C. bejolghota* bark oil were investigated against 6 bacterial pathogens representing three Gram-negative bacteria (*Salmonella typhimurium* TISTR292, *Pseudomonas aeruginosa* TISTR781 and *Escherichia coli* TISTR780) and three Gram-positive bacteria (*Staphylococcus aureus* TISTR1466, *Bacillus subtilis* TISTR008 and *B. cereus* TISTR687). All bacterial pathogens were obtained from the Thailand Institute of Scientific and Technological Research, Thailand. The antibacterial activities of *C. bejolghota* essential oil were determined by using a disc diffusion assay (Ross *et al*., 2013). Each bacterial strain was cultured in tryptic soy agar medium at 37 °C which the single colony was collected and further adjusted to 0.5 McFarland standard. Subsequently, the bacteria were swabbed on a Mueller Hinton agar medium plate by using sterilized cotton. Essential oil of *C. bejolghota* bark was diluted by two-fold dilution method with dichloromethane to perform the final concentrations of 1000, 500, 250, 125, 62.50 31.25, 7.81 and 3.91 μg/mL, respectively. Twenty microliters of *C. bejolghota* bark oil with different concentrations were loaded into a 6 mm-diameter sterile paper disc (Whatman™, USA) and then placed on Mueller Hinton agar medium plate. All plates were incubated at 37 °C for 24 h. The inhibition zone diameter of different *C. bejolghota* oil concentrations was measured in millimeters. Minimum inhibitory concentration (MIC) values inhibiting bacterial growth were also determined. Penicillin was used as positive control in this study. All experiments were performed in triplicate.

Antifungal activity assay

The plant pathogenic fungi used in this study were obtained from the Institute of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand including *Collectotrichum asianum* MFULCC10-0286, *C. fruticola* MFLUCC10-0288, *C. tropica* MFLUCC11-0114, and *C. magna* MFLUCC12-0713. The antifungal activities of *C. bejolghota* essential oil were determined by using the disc diffusion method (Murray *et al*., 1995). Essential oil of *C. bejolghota* bark was prepared by using two-fold dilution method at final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 7.81 and 3.91 μg/mL. Initially, all pathogenic fungi were cultured on potato dextrose agar (PDA) media and incubated at 30 °C for 1 week. A plug of 1-week old fungal culture (6 mm diameter) of each strain was placed on the center of PDA medium plates. Ten microliters of different essential oil concentrations were individually loaded into 6 mm-diameter sterile paper disc (Whatman™, USA) and then placed on plates containing a plug of fungal culture. The plates were incubated at 30 °C for a week. The mycelial fungal growth inhibition of each fungal strain was calculated according to the following equation: Percentage of inhibition (%) = 100 [(1-radical growth of treatment (mm)/radical growth of control (mm)]. All experiments were performed in triplicate. In addition, MIC values inhibiting mycelial fungal growth were also determined.

Data analysis

The experiments were performed in triplicate and are reported as mean ± standard deviation. Quantitative variations were analyzed by one-way ANOVA (at $P<0.05$). Duncan's Multiple Range test combined with the Statistical Analysis System (Sas, 1990) was used to study the differences among samples.

RESULTS AND DISCUSSION

The extraction yield of *C. bejolghota* bark oil was 1.01%v/v with pale yellow color. Thirty-six volatile components were detected in the essential oil of *C. bejolghota* bark, accounting for 97.96% of the total oil composition. Oxygenated monoterpene and monoterpene were considered as the major compounds as shown in Table 1. The major constituent was 1,8-cineole (40.24%), followed by γ-terpineol (15.41%), borneol (7.86%),

terpinen-4-ol (7.55%) and α-pinene (6.58%), respectively (Adams, 1995; König *et al*., 1999). Volatile compounds represented aromatic profile of Cinnamomum plant were also detected such as *Z*-cinnamaldehyde, α-amyl cinnamyl alcohol, *E*-isoamyl cinnamate, *E*-2-hexyl cinnamaldehyde, benzyl cinnamate and phenethyl cinnamate. The volatile profiles in this study differed from the study of Baruah *et al*., 1997 and Choudhury *et al*., 1998 whereby α-terpineol and *E*-nerolidol were identified as the principle components in bark oil of *C. bejolghota*. The high variation of essential oil components between locations could be due to differences in the time of harvesting and extraction method (Heywood, 2002). Extrinsic variables based on geographic origin include climatic and soil-growth conditions, both of which may cause environmental stress and variability of chemical composition (Vokou *et al*., 1993).

Table 1: Chemical composition of *C. bejolghota* bark oil with the percentage of content obtained by hydrodistillation.

No.	Compound	RI	%Peak area
1	α-Thujene	930	0.07
2	α-Pinene	932	6.58
3	Camphene	946	3.38
4	Sabinene	969	0.08
5	β-Pinene	971	3.23
6	Myrcene	981	0.78
7	δ-3-Carene	1005	0.09
8	α-Terpinene	1014	0.69
9	1,8-Cineole	1026	40.24
10	*E*-β-Ocimene	1044	0.07
11	γ-Terpinene	1049	0.81
12	cis-Sabinene hydrate	1064	0.08
13	*ρ*-Mentha-2,4(8)-diene	1079	0.34
14	*ρ*-Cymenene	1089	0.09
15	Linalool	1095	0.11
16	endo-Fenchol	1114	0.13
17	*cis*-ρ-Mentha-2-en-l-ol	1118	0.08
18	Camphor	1141	1.17
19	Camphene hydrate	1140	0.19
20	Isoborneol	1150	0.05
21	Borneol	1163	7.86
22	Terpinen-4-ol	1174	7.55
23	γ-Terpineol	1191	15.41
24	Verbenone	1204	0.17
25	*Z*-Cinnamaldehyde	1217	0.26
26	Thymol methyl ester	1232	0.05
27	Chavicol	1247	0.06
28	*trans*-Piperitone epoxide	1253	0.05
29	Geraial	1262	0.05
30	Dihydro-linalool acetate	1275	0.11
31	Isobornyl acetate	1280	0.11
32	Safrole	1288	0.09
33	Geranyl formate	1299	0.09
34	Dihydro-carveol acetate	1306	0.09
35	Limonene aldehyde	1327	0.05
36	δ-Elemene	1333	0.05
37	α-Cubebene	1339	0.05
38	Eugenol	1359	0.05
39	α-Ylangene	1373	0.11
40	β-Elemene	1383	0.05
41	α-Chamipinene	1396	0.09
42	α-Gurjunene	1409	0.06
43	α-trans-Bergamotene	1433	0.08
44	Prezizaene	1444	0.08
45	α-Humulene	1452	0.05
46	α-Zingiberene	1491	0.07
47	Gernacrene A	1506	0.21
48	7-epi-α-Selinene	1520	0.22
49	δ-Cadinene	1522	0.11
50	Zonarene	1528	0.55
51	α-Cadinene	1537	0.07
52	Selina-3,7(11)-diene	1545	0.19
53	trans-Cadinene ether	1559	0.16
54	Himachalene epoxide	1579	0.06
55	Neryl isovalerate	1582	0.14
56	Guaiol	1602	0.11
57	β-Cedrene epoxide	1622	0.64
58	epi-α-Cadinol	1637	0.48
59	Isoamyl geranate	1651	0.55
60	5-iso-Cedranol	1671	0.47
61	α-Amyl cinnamyl alcohol	1682	0.27
62	*Z*-α-trans-Bergamotol	1690	0.07
63	Eumesm-7(11)-en-4-ol	1697	0.09
64	Longifolol	1715	0.11
65	iso-Longifolol	1732	0.08
66	*E*-Isoamyl cinnamate	1740	0.09
67	*E*-2-Hexyl cinnamaldehyde	1750	0.05
68	Benzyl benzoate	1762	0.06
69	epi-Cyclocolorenone	1777	0.11
70	*Z*-α-trans-Bergamotol acetate	1793	0.13
71	α-Bisabolol acetate	1801	0.05
72	β-Chenopodiol	1810	0.06
73	Acorone	1824	0.08
74	7-Hydroxy coumarin	1841	0.06
75	*Z,Z*-Farnesyl acetone	1858	0.07
76	*Z*-Spiroether	1874	0.10
77	8S,14-Cedranediol	1886	0.10
78	Cataponone	1896	0.05
79	11,12-Dihydroxy valencene	1918	0.05
80	Carissone	1924	0.05
81	Isohibaene	1935	0.05
82	Phytol	1948	0.08
83	Columellarin	1954	0.09
84	3*Z*-Cembrene A	1964	0.07
85	4-Methoxy stilbene	1991	0.09
86	*Z,E*-Geranyl linalool	1998	0.09
87	13-epi-Manool oxide	2007	0.08
88	Juvibione	2016	0.12
89	Bergaptene	2050	0.09
90	Sclareolide	2075	0.09
91	Benzyl cinnamate	2098	0.09
92	Laurenan-2-one	2117	0.09
93	*E*-Isoeugenyl benzyl ether	2128	0.09
94	Abienol	2149	0.07
95	Phenethyl cinnamate	2179	0.08

RI, linear temperature program retention index on DB-5 column.

The antibacterial activities of *C. bejolghota* bark oil in terms of inhibition zone diameter and MIC are demonstrated in Table 2. The most sensitive bacterial strain was *E. coli* TISTR780 followed by *P. aeruginosa* TISTR781, *S. aureus* TISTR1466, *B. subtilis* TISTR008, *S. typhimurium* TISTR292 and *B. cereus* TISTR687.

Table 2: Antibacterial activities of essential oils of *C. bejolghota* bark oil and penicillin.

Bacteria	inhibition Diameter (mm)		MIC (µg/mL)	
	Essential oil	Penicillin	Essential oil	Penicillin
Gram-positive				
B. cereus	7.87±1.94	5.08±0.91	62.50	3.91
B. subtilis	7.47±2.05	3.75±1.22	31.25	3.91
S. aureus	7.57±1.53	10.08±0.29	31.25	7.81
Gram-negative				
E. coli	10.43±1.11	6.41±0.56	31.25	7.81
P. aeruginosa	7.83±1.95	3.75±1.20	31.25	3.91
S. typhimurium	9.73±1.25	4.55±1.10	62.50	7.81

The MIC of *C. bejolghota* bark oil against various bacterial species ranged between 31.25 and 62.25 µg/mL. The antifungal properties of *C. bejolghota* bark oil against four postharvest pathogenic fungi and MIC values are shown in Table 3. The *C. bejolghota* bark oil displayed the strongest antifungal activity against *C. asianum*, with MIC of 125 µg/mL, while the MIC values against other postharvest pathogenic fungi ranged between 250 and 500 µg/mL. Although antimicrobial activities of *Cinnamomum* spp. essential oils have been widely reported, the effectiveness of *C. bejolghota* bark oil on pathogenic species has been less studied.

Table 3: Percentage of growth inhibition of *Colletotrichum* sp. fungi by *C. bejolghota* bark oil.

Fungi	Radical growth inhibition (%)	MIC (µg/mL)
C. asianum	30.86±2.14	125
C. fruticola	13.79±3.45	250
C. magna	24.24±2.62	500
C. tropica	17.33±2.31	250

The mechanisms of the antimicrobial action of essential oil from plants are still not clearly understood. Terpenoids are major components of essential oil possessing hydrophobic and hydrophilic parts with different functional groups. This enables terpenoids to simply transport across bacterial or fungal cell walls and interact with the microbes (Burt, 2004; Koroch *et al*., 2007). The antimicrobial activity of *C. bejolghota* bark oil may be correlated to the diversity of its bioactive compounds. These include 1,8-cineole (comprising 40.24% of the oil) and γ-terpineol (15.41%) in the essential oil, both of which have potent antibacterial and fungicidal activities (Carson *et al*., 2002; Hendry, Worthington *et al*., 2009; Wang *et al*., 2012). Mahboubi and Kazempour, 2009 reported that antibacterial activity of whole essential oil was greater than that obtained from major components alone. The great antimicrobial activity of *C. bejolghota* bark oil could be also contributed to a combination of minor components including linalool, borneol, isoborneol, α-pinene, β-pinene and camphor (Koutsoudaki *et al*., 2005; Santoyo *et al*., 2005; Sivropoulou *et al*., 1997). The strong antimicrobial activity against *E. coli* and *B. cereus* is particularly interesting, because both microbes are classified as human pathogens. According to the Advisory Committee on Dangerous Pathogens, both bacteria belong to the second hazard group of biological agents which pose risk to human health. Moreover, growth inhibition of these bacteria is important because of their role in food contamination. In addition, strong antimicrobial activity was significant against *C. asianum* with 30.86% growth inhibition. Therefore, essential oil of *C. bejolghota* bark is a potential antibacterial and antifungal agent that may find wider applications in food industry and postharvest processing.

CONCLUSIONS

The present study indicated that essential oil obtained from the stem bark of *C. bejolghota* is rich in oxygenated monoterpenes, mainly 1,8-cineole, which constitutes 40.24% of the total oil composition. Biological evaluation revealed that the *C. bejolghota* bark oil possesses strong antibacterial and antifungal activities. *C. bejolghota* oil may be viewed as a bioactive natural product with cosmetic or postharvest production applications.

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