Chemical composition, antibacterial and antifungal activities of \textit{Cinamomum bejolghota} bark oil from Thailand

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### ABSTRACT

The volatile constituents of \textit{Cinamomum 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, \( \gamma \)-terpinene, borneol and terpinen-4-ol. Essential oil of \textit{C. bejolghota} bark was firstly screened for their antibacterial and antifungal activities against Gram-positive and Gram-negative bacteria, as well as, \textit{Colletotrichum} sp. fungi using disc diffusion method. Minimal inhibitory concentration (MIC) of \textit{C. bejolghota} bark oil was further analyzed by microdilution. Essential oil of \textit{C. bejolghota} bark was most effective against bacteria with MIC ranging from 31.25-62.50 \( \mu \)g/mL, whereas inhibition against fungal pathogens was moderate, with MIC of 125-500 \( \mu \)g/mL. The strong antimicrobial activity of \textit{C. bejolghota} bark oil was correlated mainly to 1,8-cineole, \( \gamma \)-terpinene, 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 \textit{Cinamomum} genus plants contained the great antimicrobial and pharmaceutical applications (Sudmoon et al., 2014). The essential oil of \textit{Cinamomum} genus plants contained the great antimicrobial (Ooi et al., 2006), antifungal (Giordani et al., 2006), anti-inflammatory (Miguel, 2010) and antioxidant (Jayaprakasha et al., 2003) properties. \textit{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 \textit{C. bejolghota} leaf and panicle cultivated in India, whereas \( \alpha \)-terpinene and \textit{E}-nerolidol were found as the main components in its stem bark oil. Conversely, high amounts of 1,8-cineole and \( \alpha \)-terpinene were detected in essential oil of \textit{C. bejolghota} bark collected from different areas in India (Choudhury et al., 1998). Only few studies have identified volatile profiles of \textit{C. bejolghota} essential oil, though there is no previous study reporting the antimicrobial and antifungal properties of \textit{C. bejolghota} oil. The aim of this study was to investigate the chemical composition of \textit{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 to 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 C₅-C₇₆ 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 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 asiaticum MFLUCC10-0286, 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. Penicillin was used as positive control in this study. All experiments were performed in triplicate.

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 monoterpenes were considered as the major compounds as shown in Table 1. The major constituent was 1,8-cineole (40.24%), followed by β-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-amy1 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 Barua 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-Thuene                      | 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-Terpineol                   | 1014 | 0.69        |
| 9   | 1,8-Cineole                   | 1026 | 40.24       |
| 10  | E-β-Ocimene                   | 1044 | 0.07        |
| 11  | γ-Terpineol                   | 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-1-ol        | 1118 | 0.08        |
| 18  | Camphor                       | 1141 | 1.17        |
| 19  | Camphene hydrate              | 1140 | 0.19        |
| 20  | Isoeugenol                    | 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  | Geralal                       | 1262 | 0.05        |
| 30  | Dihydro-linalool acetate      | 1275 | 0.11        |
| 31  | Isoeugenyl acetate            | 1280 | 0.11        |
| 32  | Safrole                       | 1288 | 0.09        |
| 33  | Geranial 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-Cubebe                      | 1339 | 0.05        |
| 38  | Eugenol                       | 1359 | 0.05        |
| 39  | α-Ylangene                    | 1373 | 0.11        |
| 40  | β-Elemene                     | 1383 | 0.05        |
| 41  | α-Chamipine                   | 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        |

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. asiunum*, 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.

| Fungi       | Radical growth inhibition (%) | MIC (µg/mL) |
|-------------|-------------------------------|-------------|
| *C. asiunum* | 30.86±2.14                    | 125         |
| *C. fraticola* | 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-terpinelol (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 bark oil may be contributed to a combination of minor components alone. The great antimicrobial activity of *C. bejolghota* bark oil could be also attributed 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. asiunum* 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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**Conflict of Interests:** There are no conflicts of interest.

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