Journal of Medicinal Plants Research Vol. 6(18), pp. 3487-3494, 16 May, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.157
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Composition and biological activities of the essential oil extracted from a novel plant of *Cinnamomum camphora* Chvar. Borneol

Jianyu Su[1,2,3]*, Jianping Chen[1], Shengmei Liao[3], Lin Li[1], Liang Zhu[1] and Lei Chen[4]

[1]College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, Guangdong, China.
[2]State Key Laboratory of Food Safety Technology for Meat Products, Xiamen 361100, Fujian, China.
[3]Guangdong Jiaying Pharmaceutical Co., Ltd, Meizhou 514021, China.
[4]Institute for Agro-product Processing, Anhui Academy of Agricultural Sciences, Hefei 230031, People Republic of China.

Accepted 22 March, 2012

This study analyzed the chemical composition, *in vitro* antioxidant and antimicrobial activities of the hydrodistillated essential oil obtained from fresh leaves of a novel plant of *Cinnamomum camphora* Chvar. Borneol. Gas chromatography–mass spectrometry (GC-MS) analysis of the oil resulted in determination of 27 compounds, composing 98.14% of the oil. *d*-Borneol (81.58%), camphor (2.96%), and α-pinene (2.03%) were determined as the major components. Antioxidant activities of the essential oil and *d*-Borneol were analyzed in two aspects, namely β- carotene/linoleic acid and reducing power. The essential oil exhibited the highest antioxidant activity in β-carotene/linoleic acid, slightly weaker than that of butylated hydroxytoluene (BHT), the standard commercial synthetic antioxidant among the experiments examined. Moreover, the essential oil exhibited moderate reducing power which was evaluated in terms of the total phenolic and flavonoid contents. The essential oil and *d*-Borneol exhibited antimicrobial effect as a diameter of zones of inhibition (9.21±0.6 to 22.12±1.3 and 7.32±0.5 to 20.42±1.4 mm), respectively, along with their MIC values (31.25 to 125 and 62.5 to 250 µg/ml) against bacteria, yeasts and moulds.

Key words: *Cinnamomum camphora* Chvar. Borneol, essential oil, *d*-Borneol, antioxidant activity, antimicrobial activity.

INTRODUCTION

Borneol ($C_{10}H_{18}O$) is a classical traditional Chinese medicine (TCM), which is composed of many TCM prescriptions and used as the adjuvant. It has been used as a therapeutic agent in China for more than 1500 years (Jiang et al., 2008). According to the pharmacopoeia of People's Republic of China (State Pharmacopoeia Committee, 2010), borneol is an ingredient in about 65herbal products. There are two different kinds of borneol:

The first one is synthetic borneol. It is a mixture of *d*-Borneol and isoborneol, in which the *d*-Borneol contents should be no less than 55.0%; the second one is natural borneol. The main component of natural borneol is *d*-Borneol, which should be >95.0% of natural borneol. Because of the rare resources and expensive price of natural borneol, synthetic borneol was widely used in Chinese formulas in recent years. However, synthetic borneol can be transformed into camphor during storage over a long period of time and causes safety problems which have already arouse wide concern (Zeng and He, 2004). Therefore, a management strategy to avoid the toxic effects of camphor is to use natural borneol instead of synthetic one in different borneol products. Natural borneol is mainly extracted from the essential oils of

*Corresponding author. E-mail: jysu@scut.edu.cn. zhuliang@scut.edu.cn. Tel: +86 20 87113252. Fax:+86 20 87113252.

numerous medicinal plants of the families *Dipterocarpaceae* (e.g. *Dipterocarpus turbinatus* tree), Lamiaceae (e.g. *Rosmarinus officinalis* or *Salvia officinalis*), Valerianaceae (e.g. *Valeriana officinalis*) and Asteraceae (e.g. *Matricaria chamomilla*), etc. (Tabanca et al., 2001). Since there was a shortage of natural source, the price of natural borneol was gradually increased. Our previous studies had shown that natural borneol could also be extracted from the leaves of a *Cinnamomun burmannii* physiological type grown in Guangdong province, China, and it was safe for consumption (Chen et al., 2011). Therefore, the newly discovered sources of the *C. burmannii* physiological type in China would provide a great source of natural borneol. Additionally, we recently found a novel plant of *C. camphora* Chvar. Borneol (*C. camphora* Chvar. Borneol) growing wildly in Southern China. The essential oil from the fresh leaves of this species was very rich in *d*-Borneol.

As far as our literature survey could ascertain, we could reach no report on the biological activities of the essential oil of this plant, especially in antioxidant systems and antimicrobial activities. Therefore, the aims of this work were: (i) To characterize essential oil composition of *C. camphora* Chvar. Borneol, obtained by using a Clevenger distillation apparatus, with gas chromatography–mass spectroscopy (GC–MS) method; (ii) To evaluate the *in vitro* antioxidant properties of the essential oil, by using two complementary assays, namely β-carotene/linoleic acid and reducing power assays; (iii) To evaluate the antimicrobial properties of the essential oil, by disc diffusion method and minimum inhibitory concentrations (MICs). Our results demonstrate that *C. camphora* Chvar. Borneol would have new sources of natural borneol and its essential oil would be further applied in pharmaceutical and food industries as natural valuable products.

MATERIALS AND METHODS

Leave samples and reagents

Fresh leaves of *C. camphora* Chvar. Borneol were obtained from South China Botanical Garden, Chinese Academy of Sciences (Guangzhou, China). Taxonomic identification of the plant material was confirmed by professor Liangfeng Zhu, South China Botanical Garden, Chinese Academy of Sciences. Standard *d*-Borneol (*d*-borneol content >99.90%, molecular weight 154.24) was purchased from the Natural Institute for the Control of Pharmaceuticals and Biological Products, Beijing, China. All other chemicals and solvents were of analytical grade and purchased from China National Medicine Co., Ltd.

Essential oil extraction

Fresh leaves of *C. camphora* Chvar. Borneol were submitted for 2 h to water- distillation using a Clevenger distillation apparatus (Clevenger-type). The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, and stored at +4℃ until tested and analyzed.

Gas-chromatography–mass spectrometry (GC-MS) analysis

Quantitative and qualitative analysis of the essential oil was performed using a GC-MS (Model 6890-5975 GC-MS, Aglient, USA) equipped with a HP-5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. Injector and mass transfer line temperature were set at 250 and 280℃, respectively. Essential oil solution (1 µl) in hexane was injected and analyzed with the column held initially at 40℃ for 1 min and then increased to 250 ℃ with a 3℃/min heating ramp and subsequently kept at 250℃ for 20 min. The Kovat s indices were calculated for all volatile constituents using a homologous series of n-alkanes C_8–C_{25} on HP-5 MS column. The major components of oils were identified by co-injection with standards (wherever possible), confirmed with Kovats indices using the Wiley (V.7.0) and National Institute of Standards and Technology (NIST) V.2.0 GC–MS library. The relative concentration of each compound in essential oil was quantified based on the peak area integrated by the analysis program.

Antioxidant activity

Total antioxidant activity by β-carotene–linoleic acid method

In this assay, antioxidant capacity was determined by indirectly measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade), 25 µl linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml distilled water saturated with oxygen (30 min, 100 ml/min) was added with a vigorous shaking. 2.5 ml of this reaction mixture was dispersed to test tubes and 350 µl of various concentrations (0.4 to 2.0 mg/ml) of the essential oil were added. Emulsion system was incubated for 2 h at 50℃. The same procedure was repeated with the po sitive control BHT and a blank. After this incubation period, absorbance of the mixture was measured at 490 nm. Antioxidative capacities of the essential oil were compared with BHT at 2.0 mg/ml concentration.

The reducing power

The reducing power of samples were determined according to the method of Oyaizu (1986) with little modification. 2 ml of various concentrations (0.2, 0.4 and 1.0 mg/ml) of the essential oil and *d*-Borneol in ethanol were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide.

After the mixture was incubated at 50℃ for 20 min, 2 .5 ml of 10% trichloroacetic acid, 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride were added and then the absorbance was measured at 700 nm against a blank. The blank consist of all the reagents without the test sample. High absorbance of the reaction mixture indicates strong reducing power. BHT was used as a control with the same concentrations.

Assay for total flavonoids

The total flavonoid content was determined using the method of Meda et al. (2005) with minor modifications. In brief, 5 ml of 2% aluminium trichloride ($AlCl_3$) was mixed with the same volume of essential oil. Absorbance readings at 415 nm (UV-2802, Unico Co.

Ltd., Shanghai, China) were taken after 10 min against a blank sample consisting of 5 ml of sample solution and 5 ml of ethanol without aluminium trichloride. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

Absorbance = 0.0274 quercetin (µg) + 0.0012 (R^2:0.9991).

Assay for total phenolics

Total phenolic constituent of the essential oil were determined by employing the methods given in the literature (Slinkard and Singleton, 1977). In this method, Folin–Ciocalteu reagent and gallic acid were used as standard agents according to procedure described by Wettasinghe and Shahidi (1999) using catechin as a standard. Suitable aliquots of the fractions were taken in a test tube. Then, 1 ml of Folin-Ciocalteau reagent and 3 ml of Na_2CO_3 (2%) solution were added sequentially in each tube. The total volume of the system was adjusted to 45 ml with distilled water. The mixture was allowed to stand for 2 h by intermittent shaking. The absorbance was immediately measured at 760 nm. The concentrations of phenolic compounds were calculated according to the following equation obtained from the standard gallic acid graph:

A = 0.0183 gallic acid (µg) - 0.0324 (R^2: 0.9908)

Antimicrobial activity

The *in vitro* antimicrobial activities of the essential oil was evaluated against panel which included laboratory control strains obtained from the China Center for Type Culture Collection (CCTCC): Two Gram-negative bacteria (*Pseudomonas aeruginosa* CCTCC AB93066 and *Escherichia coli* CCTCC AB91112), two Gram-positive bacteria (*Bacillus subtilis* CCTCC AB92068 and *Staphylococcus aureus* CCTCC AB91053), two yeasts (*Hansenula anomala* CCTCC AY92046 and *Saccharomy cescerevisiae* CCTCC AY92042) and two moulds (*Aspergillus niger* CCTCC AF91004, *Chaetomium globosum* CCTCC AF 200039). They were maintained on an agar slant at 4℃. Bacterial strains were culture d on Muller-Hinton Broth (MHB) at 37℃ for 24 h, yeasts were cult ured on Sabouraud dextrose agar (SDA) at 28℃ for 48 h and fun gal strains were cultured on SDA at 28℃ for 120 h before testing.

Inhibitory effect by disc diffusion method

Disc diffusion method was employed for the determination of antimicrobial activities of the essential oils (NCCLS, 2007). Petri plates were prepared by pouring 20 ml of MBH or SDA and allowed to solidify. Plates were dried and 0.1 ml of standardized inoculum containing 10^6 to 10^6 CFU/ml of bacterial suspension was poured and uniformly spread, and the inoculum was allowed to dry for 5 min. A Whatman No. 1 sterile filter paper disc (6 mm diameter) was impregnated with 1000 µg/disc of essential oils. Negative controls were prepared using the same solvent DMSO and aqua sterilisata employed to dissolve the samples. Standard reference antibiotics, gentamicin (10 µg/disc) were used as positive controls for the tested bacteria. The plates were incubated for bacteria at 37℃ for 24 h, incubated for yeasts at 28℃ for 48 h and incu bated for fungi at 28℃ for 120 h. Antimicrobial activity was evaluat ed by measuring the diameter of the zones of inhibition against the tested organisms. The experiments were repeated in triplicate and the results were expressed as average values.

Determination of minimum inhibitory concentration (MIC)

MICs of the essential oils against the test microorganisms were determined by broth microdilution method (NCCLS, 2007). Stock solutions of essential oils and compounds were prepared in DMSO. Serial dilutions were prepared in sterile distilled water from dilutions of the essential oil prepared in MHB or SDA in a 96-well microtiter plate ranging from 2000 to 1.94 µg/ml for the essential oils and from 1000 to 0.97 µg/ml for the pure compounds. Exactly 0.5 MacFarland standard suspensions of the test microorganisms were inoculated in the tubes. A control test was also performed containing inoculated broth or agar supplemented with only DMSO under identical conditions with gentamicin as reference. The tubes were incubated for bacteria at 37℃ for 24 h, incuba ted for yeasts at 28℃ for 48 h and incubated for fungi at 28℃ for 96 h. The lowest concentrations of the test samples, which did not show any visual growth of test organisms after incubation time, were determined as MICs, which were expressed in µl/ml.

Statistical analyses

Tests were carried out in triplicates and the results were calculated as mean ± SD.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The essential oil extracted by hydrodistillation from the fresh leaves of *C. camphora* Chvar. Borneol was quantitatively analyzed by GC–MS analysis. Twenty-seven components were identified as constituents of the essential oil, comprising about 98.14% of the total oil (Table 1). The oil was dominated by the *d*-Borneol (81.58%), followed by camphor (2.96%), *α*-pinene (2.03%), *d*-limonene (1.64%), 1,8- cineole (1.60%), and camphene(1.54%).

The genus *Cinnamomum* belonging to the Family *Lauraceae* comprises about 250 species which are distributed in India, China, Srilanka and Australia. It has been reported that natural borneol existed in the essential oils of the Families Dipterocarpaceae (e.g. *D. turbinatus* tree) (30 to 65%), *Lamiaceae* (e.g. *Rosmarinus officinalis* or *Salvia officinalis*) (27-39%), etc., (Tabanca et al., 2001). Tabanca et al. (2006) also pointed out that the essential oil of *S. macrochlamys* was characterized with 1,8-cineole (27%), borneol (13%), and camphor (11%) as major constituents. Together with 1,8-cineole, borneol and camphor had been also reported in many *Salvia* essential oils, for instance in *Salvia fruticosa* Miller, *Salvia tomentosa* Miller, *Salvia pomifera* L., *Salvia willeana* (Holmboe) Hedge, and *Salvia officinalis* L. In a study, the essential oils extracted from leaves of *Blumeariparia* (bl) Dc were found to contain the highest borneol (80 to 90%) that had ever been reported. However, more than 85% of the borneol was *l*-bornel (Ma et al., 2009). In our previous report, the essential oil of a *Cinnamomum burmannii* physiological type showed the essential oils yield accounting about 0.60% dry weight, and the abundance of *d*-Borneol (78.46%) in the oil. Our results in this research showed that the yield of the essential oil was 1.23%, and the components with *d*-Borneol were up to

Table 1. Essential oil composition of fresh leaves of *C. camphora* Chvar. Borneol.

No.	RI[a]	Constituent	Percent (%)	Identification[b]
1	928	α-Thujene	0.19	MS, RI
2	936	α-Pinene	2.03	MS, RI
3	949	Camphene	1.54	MS, RI
4	973	Sabinene	0.32	MS, RI
5	986	β-Myrcene	0.95	MS, RI, Co
6	998	α-Phellandrene	0.08	MS, RI
7	1017	α-Terpinene	0.16	MS, RI
8	1020	p-Cymene	0.19	MS, RI
9	1030	d-Limonene	1.64	MS, RI, Co
10	1038	1,8- Cineole	1.60	MS, RI
11	1040	(Z)-β-Ocimene	0.06	MS, RI
12	1146	Camphor	2.96	MS, RI
13	1099	Linalool	0.93	MS, RI
14	1191	α-Terpineol	0.27	MS, RI
15	1286	Bornyl acetate	0.52	MS, RI, Co
16	1375	α-Copaene	0.71	MS, RI
17	1402	Methyl eugenol	0.05	MS, RI
18	1418	β-Caryophyllene	0.91	MS, RI
19	1454	α-Humulene	0.11	MS, RI
20	1508	β-Bisabolene	0.64	MS, RI
21	1165	Borneol	81.58	MS, RI
22	1293	Safrole	0.26	MS, RI
23	1562	(trans)-Nerolidol	0.09	MS, RI
24	1608	Guaiol	0.14	MS, RI
25	1650	γ-Eudesmol	0.12	MS, RI, Co
26	1905	(Z)-7-Hexadecenoic acid, methyl ester	0.05	MS, RI
27	1948	Isophytol	0.04	MS, RI, Co
		Total identified (%)	98.14	
		Monoterpene hydrocarbons	7.16	
		Oxygenated monoterpenes	6.28	
		Sesquiterpene hydrocarbons	2.37	
		Oxygenated sesquiterpenes	81.93	
		Others	0.4	
		Oil yield (%) (v/w)	1.23	

Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector. [a], Retention index relative to n-alkanes C8-C25 on HP-5 MS capillary column. [b], RI is the retention index; MS is mass spectrum; Co is co-injection with authentic compound.

81.58%. As to our knowledge, this plant could be assumed as containing the highest *d*-Borneol.

Antioxidant activity

The importance of accurate quantitative assessment of antioxidant status/oxidant stress, whether in patient tissue determination from the standpoint of nutritional decency: Disease pathogenesis, or in the identification of novel compounds with therapeutic potential as antioxidants, has been outlined in the introduction. A major finding in the present investigation is the apparent variation in antioxidant capacity of various plantoil extracts depending on the particular assay method employed to determine antioxidant status. The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metalion catalysts (Mao et al., 2006). It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods and different substrates are used. The methods chosen

Tabel 2. Antioxidant activity (%) of essential oil from leaves of *C. camphora* Chvar. Borneol at different concentrations, measured by β-carotene-linoleic acid method.[a]

Sample	Sample concentration (mg/ml)		
	0.4	1.0	2.0
Essential oil	88.65±1.15	90.21±0.85	92.15 ± 0.12
BHT	-	-	97.26±0.48

[a], Values expressed are means ± S.D. of three parallel measurements.

are the most commonly used for the determination of antioxidant activities of plant extracts.

In β-carotene-linoleic acid system, β-carotene undergoes a rapid discoloration in the absence of an antioxidant. The presence of an antioxidant such as phenolics can hinder the extent of β-carotene destruction by ‘‘neutralizing” the linoleate free radical and any other free radicals formed within the system (Kamath and Rajini, 2007). Table 2 depicts the inhibition of β-carotene bleaching by the essential oil of *C. camphora* Chvar. Borneol, and by the positive control (BHT). The scavenging ability of the all samples showed a concentration-dependent activity profile in Table 2. It was possible that the content of antioxidant compounds in the essential oil increased as the concentration increased from 0.4 to 2.0 mg/ml. The strongest free radical scavenging activity was exhibited by essential oil (9.15±0.12%) at 2.0 mg/ml. Synthetic antioxidants BHT exhibited 97.26% activity in this system. The antioxidative activity of the essential oils may be attributed to the presence of borneol (81.58%) and camphor (2.96%). Our results are in accordance with those of Farag et al. (1989) who reported that there is a relationship between inhibition of the hydroperoxide formation and the presence of some phenolic nucleus in some essential oils (carvenone, camphor, borneol, eugenol, thymol). Moreover, the antioxidative effectiveness in natural sources was reported to be mostly due to phenolic compounds (Hayase and Kato, 1984). Ramarathnam et al. (1986) also discovered that phenolic compounds play an important role in inhibiting autoxidation of the oils.

Reducing power is generally associated with the presence of reductones, which exert antioxidant action by breaking the free radical chain through donating a hydrogen atom (Duan et al., 2007). In this assay, Fe^{3+}/ferricyanide complex is reduced to the ferrous form by antioxidants and can be monitored by measuring the formation of navy blue color at 700 nm (Gupta and Prakash, 2009). Reducing power of the samples is presented in Figure 1. Reducing power of the essential oil showed a concentration-dependent activity profile. The essential oil exhibited moderate reducing power which was weaker than that of synthetic antioxidant BHT at the same concentration. *d*-Borneol showed the weakest reducing power in the concentration parameters examined. It is extremely important to point out that, reductive potentials of the essential oil are strictly related with the polarities of the phytochemical. The reducing power of the essential oil is probably due to the presence of hydroxyl group in phenolic compounds which might act as electron donors. This could be confirmed by Table 3. As can be seen from the Table 3, the essential oil was found to have some phenolics (124.95 ±2.70 µg/mg) and flavonoid content (2.7384± 0.01µg/mg).

Antimicrobial activity

The disc diameters of the zones of inhibition and the MICs of the essential oils from *C. camphora* Chvar. Borneol are shown in Tables 4 and 5. These results indicate that the oils have moderate antimicrobial activity against all test microorganisms. In every case, gentamicin showed highest antimicrobial effect, while the essential oil exhibited higher antimicrobial activity than that of standard *d*-borneol in every case. The essential oil and *d*-borneol exhibited antimicrobial effect as a diameter of zones of inhibition 9.21±0.6 to 22.12±1.3 and 7.32±0.5 to 20.42±1.4 mm, respectively, along with their MIC values 31.25 to 125 µg/ml and 62.5 to 250 µg/ml against bacteria, yeasts and moulds. As negative control, DMSO and aqua sterilisata did not affect the growth of the sample strains at the concentration used in this study.

Earlier papers on the analysis and antibacterial properties of the essential oils of *C. camphora* species have shown that it has varying degree of growth inhibitory effects against some bacterial due to its chemical compositions such as limonene, β-phellandrene, α-phellandrene, γ-terpinene, β-caryophyllene and α-pinene (Jiang et al., 2008; Nirmal et al., 2005; Koheil, 2000).

In our opinion, the antimicrobial activity of the essential oil from *C. camphora* Chvar. Borneol also could, in part, be associated with major constituents such as α-pinene, 1,8-cineole, limonene, camphor, and borneol. Pinene, one of the major components of the oils, has been previously shown to be active against many organisms (Bakkali et al., 2008; Wu et al., 2011). Limonene, another major components of the oil, has been demonstrated to have bacteriostatic activity against several microorganisms (Donsì et al., 2011). 1, 8-Cineole and

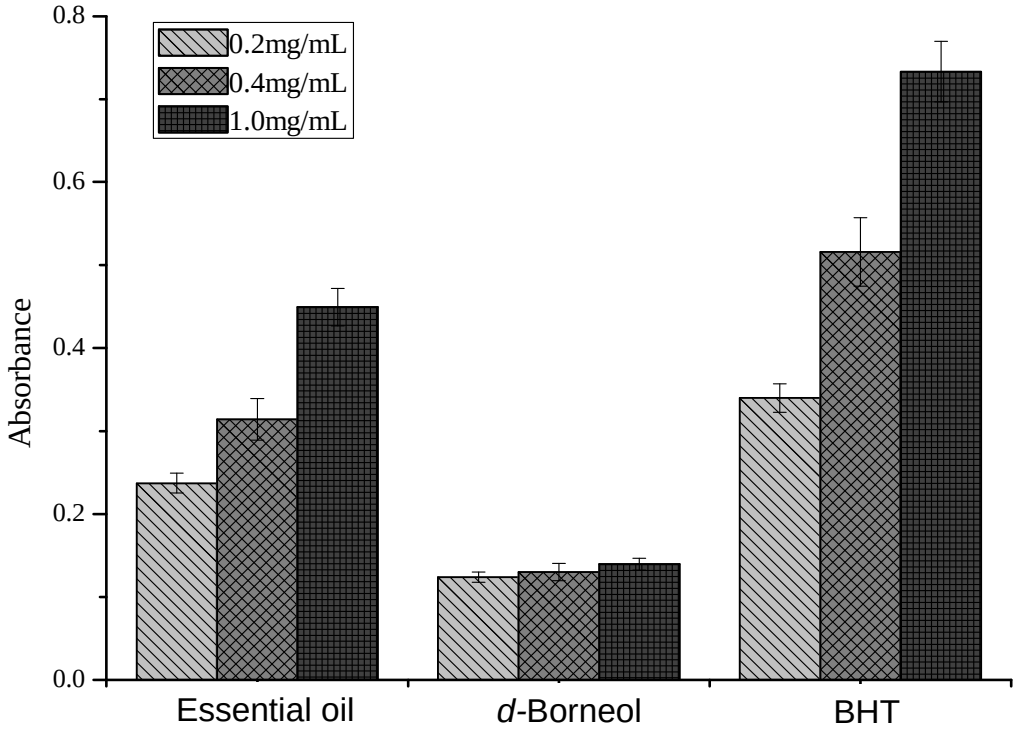

Figure 1. Reducing power (absorbance at 700 nm) of essential oil from *C. camphora* Chvar. Borneol, standard *d*-Borneol and BHT at different concentrations.

Table 3. Total phenolics and flavonoids content of essential oil from leaves of *C. camphora* Chvar. Borneol [a].

Sample	Phenolic content (l µg GAEs/mg extract)[b]	Flavonoid content (1 µg QEs/mg extract)[c]
Essential oil	124.95 ±2.70	2.7384± 0.01

[a] Values expressed are means ± S.D. of three parallel measurements. [b] GAEs, gallic acid equivalents. [c], QEs, quercetin equivalents.

camphor are well-known for their antibacterial activities of some medicinal plants, have significant antibacterial and antifungal activities (Simic et al., 2002).

In addition, the components in lower amount such as β-caryophyllene, α-humulene, bornyl acetate, safrole, β-bisabolene, linalool and α-terpineol could also contribute to the antimicrobial activity of the oil. It is also possible that the minor components might be involved in some type of synergism with the other active compounds (Giles et al., 2010). In fact, the synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into consideration to account for their biological activity (Burt, 2004).

The mechanism of action of this class of compounds has not been completely elucidated, but it is speculated that these chemical components exert their toxic effects against these microorganisms through the disruption of bacteria or fungal membrane integrity (Filipowicz et al., 2003). α-Pinene and borneol are able to destroy cellular (Vardar-Unlu et al., 2003). Borneol, the main constituents integrity, and thereby, inhibit respiration and ion transport processes. They also increase the membrane permeability in yeast cells and isolated mitochondria (Andrews et al., 1980; Uribe et al., 1985). Conner and Beuchat (1984) suggested that the antimicrobial activity of essential oils of herbs and spices or their constituents could be the result of damage to disturbance in several enzymatic cell systems, including involvement in energy production and structural components synthesis.

Conclusion

In conclusion, the essential oil from leaves of *C. camphora* Chvar. Borneol is an outstanding new source of *d*-borneol, a compound highly appreciated in the pharmaceutical and food industry. In addition, it shows interesting antimicrobial and antioxidant activities which

Table 4. Antimicrobial activity of essential oils of *C. camphora* Chvar. Borneol against the growth of microbes.

Microorganism	Diameter of zones of inhibition				
	Essential oil[a]	*d*-Borneol	GM	DMSO	Aqua sterilisata
Pseudomonas aeruginosa	15.12±0.8	14.02±0.2	18.98±0.9	-	-
Escherichia coli	16.33±1.0	15.04±0.4	19.95±0.9	-	-
Bacillus subtilis	14.62±0.5	9.43±0.5	21.31±0.9	-	-
Staphylococcus aureus	14.68±1.1	13.60±1.1	25.55±1.5	-	-
Hansenula anomala	13.57±0.8	12.60±0.7	20.42±1.1	-	-
Saccharomy cescerevisiae	9.21±0.6	7.32±0.5	19.57±1.2	-	-
Aspergillus niger	10.82 ± 0.5	9.41 ± 0.8	21.6 ± 0.6	-	-
Chaetomium globosum	22.12±1.3	20.42±1.4	22.5 ± 0.6	-	-

Diameter of inhibition zones (mm) including the diameter of disc (6 mm), values are given as mean ± SD of triplicate experiment. [a] Diameter of inhibition zones of essential oil (tested volume 1000 µg/disc). [b], Standard antibiotics: GM, gentamicin (tested volume 10 µg/disc).

Table 5. Minimum inhibitory concentrations of essential oils from *C. camphora* Chvar. Borneol against the growth of microorganisms.

Microorganism	MICs[a]		
	Essential oil	*d*-Borneol	GM
P. aeruginosa	125	125	125
E. coli	62.5	250	62.5
B. subtilis	125	125	125
S. aureus	125	125	15.6
H. anomala	125	250	62.5
S. cescerevisiae	62.5	125	125
A. niger	125	250	62.5
C. globosum	31.25	62.5	31.25

[a] MIC, Minimum inhibitory concentration (values in µg/ml).

make this essential oil a potential industrial resource of new products. In future, these plants may be under the designation of protected origin, more detailed investigations of biological activities, due to their unique properties.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 31101278 and 31130042), the Fundamental Research Funds for the Central Universities (2011ZM0099), the National Science and Technology Support Program (2012BAD37B01), and the Science and Research Program of Guangdong Province (No. 2009A090100041, and 2010B090500033).

REFERENCES

Andrews RE, Parks LW, Spence KD (1980). Some effects of Douglas fir terpenes on certain microorganisms. Appl. Environ. Microb., 40: 301.
Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effects of essential oils – A review. Food Chem. Toxicol., 46: 446.
Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. Int. J. Food Microbiol., 94: 223.
Chen L, Su JY, Li L, Li B, Wang L (2011). A new source of natural D-borneol and its characteristic. J. Med. Plants Res., 5(15): 3440-3447.
Conner DE, Beuchat LR (1984). Effects of essential oils from plants on growth of food spoilage yeasts. J. Food Sci., 49: 429.
Dapkevicius A, Venskutonis R, Beek TA, Linssen PH (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J. Sci. Food Agric., 77: 140-146.
Duan X, Wu G, Jiang Y (2007). Evaluation of antioxidant properties of phenolics from litchi fruit in relation to pericarp browning prevention. Molecule, 12: 759-771.
Donsì F, Annunziata M, Sessa M, Ferrari G (2011). Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. LWT-Food Sci. Technol., 44: 1908-1914.
Farag RS, Badei AZMA, El-Baroty GSA (1989). Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. J. Am. Oil Chem. Soc., 66: 800-804.
Filipowicz N, Kaminski M, Kurlenda J, Asztemborska M (2003). Antibacterial and antifungal activity of juniper berry oil and its selected components. Phytother. Res., 17: 227.
Giles M, Zhao J, An M, Agboola S (2010). Chemical composition and antimicrobial properties of essential oil of three *Australian Eucalyptus* species. Food Chem., 119: 731.
Gupta S, Prakash J (2009). Studies on Indian green leafy vegetables for their antioxidant activity. Plant Food. Hum. Nutr., 64: 39-45.
Hayase F, Kato H (1984). Antioxidative components of sweet potatoes.

J. Nutr. Sci. Vitaminol., 30: 37-46.
Jiang XF, Zou JL, Yuan YM, Francis CL, Qiao YJ, Yao MC (2008). Preliminary study: biotransformation of borneol to camphor in mice, rats, and rabbits. Mode Tradit. Chin. Med. Mater. Med., 10(3): 27-36.
Jiang GB, Chen S, Zeng RS (2008). Identification and fungitoxicity of volatiles of invasive plant *Wedelia trilobata* L. Chin. J. Eco-Agrc., 16: 905.
Kamath V, Rajini PS (2007). The efficacy of cashew nut (Anacardium occidentale L.)skin extract as a free radical scavenger. Food Chem., 103: 428-433.
Koheil MA (2000). Study of the essential oil of flower-heads of *Wedelia* (L.) Hitch. Al-Azhar. J. Pharm. Sci., 26: 288.
Ma ZY, Lin CW, Han KJ (2009). Analysis of volatile components from the fresh stems and leaves of Blumea riparia(Bl.) DC. by headspace solid phase microextraction-gas chromatography-mass spectrometry. Acta Sci. Natur. Univ. Sunyatseni, 48(1): 46-50.
Mao LC, Pan X, Que F, Fang XH (2006). Antioxidant properties of water and ethanol extracts from hot air-dried and freeze dried daylily flowers. Eur. Food. Res. Technol., 222: 236-241.
Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem., 91: 571-577.
NCCLS (National Committee for Clinical Laboratory Standards) (2007). Performance standards for antimicrobial susceptibility testing; 17th International Supplement, Wayne Pa. M100-S17.
Nirmal SA, Chavan MJ, Tambe VD, Jadhav RS, Ghogare PB, Bhalke RD, Girme AS (2005). Chemical composition and antimicrobial activity of essential oil of *Wedelia trilobata* leaves. Indian J. Nat. Prod., 21(3): 33.
Oyaizu M (1986). Studies on products of browning reactions:antioxidative activities of browning reaction prepared from glucosamine. Jpn. J. Nutr., 44: 307-315.
Ramarathnam N, Osawa T, Namiki M, Tashiro T (1986). Studies on the relationship between antioxidative activity of rice hull and germination ability of rice seeds. J. Sci. Food Agric., 37: 719-726.
Simic N, Palic R, Vajs V, Milosavljevic S, Djkovic D (2002). Composition and antibacterial activity of *Achillea asplenifolia* essential oil. J. Essent. Oil Res., 14(1): 76.
Slinkard K, Singleton VL (1977). Total phenol analyses: automation andcomparison with manual methods. Am. J. Enol. Viticult., 28: 49-55.
State Pharmacopoeia Committee (2010). The Pharmacopoeia of People's Republic of China. First edition, Chem. Ind. Press. pp. 98-99.
Tabanca N, Kirimer N, Demirci B, Demirci F, Baser KHC (2001). Composition and Antimicrobial Activity of the Essential Oils of Micromeria cristata subsp. phrygia and the Enantiomeric Distribution of Borneol. J. Agric. Food Chem., 49(9): 4300-4303.
Tabanca N, Demirci B, Baser KHC, Aytac Z, Ekici M, Khan SI, Jacob MR, Wedge DE (2006). Chemical composition and antifungal activity of Salvia macrochlamys and Salvia recognita essential oils. J. Agric. Food Chem., 54(18): 6593-6597.
Uribe S, Ramirez J, Peña A (1985). Effects beta-pinene on yeast membrane functions. J. Bacteriol., 161: 1195.
Vardar-Unlu G, Candan F, Sokmen A, Daferera D, Polissiou M, Sokmen M, Donmez E, Tepe B (2003). Antibacterial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey var. pectinatus (Lamiaceae). J. Agric. Food Chem., 51: 63.
Wettasinghe M, Shahidi F (1999). Evening primrose meal: A source of natural antioxidants and scavenger of hydrogenperoxide and oxygen-derived free radicals. J. Agric. Food. Chem., 47: 1801-1812.
Wu N, Fu YJ, Kong Y, Zu YG, Liu XL (2011). Chemical composition and antimicrobial activity of the essential oil of Rosemary. Environ. Toxicol. Pharm., 32: 63-68.
Zeng CQ, He GF (2004). Analysis of ten batches of borneol on market by GC-MS. J. Chin. Med. Mat., 27: 347.