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A new source of natural D-borneol and its characteristic

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The essential oils from leaves of Mei Pian tree (a *Cinnamomun burmannii* physiological type), were obtained by hydro-distillation and analyzed by gas chromatography and mass spectrometry (GC/MS). Forty compounds representing 99.61% of the total oil containing D-borneol (78.6%) as the major component were obtained. D-borneol was purified by sublimation, and the characteristic of the refined D-borneol was compared with standard D-borneol and synthetic borneol by infrared spectra analysis and optical activity analysis. The chemical composition and the optical rotation of refined D-borneol from leaves of Mei Pian tree were extremely similar to the standard D-borneol and materially surpass synthetic borneol. Antioxidant activities of the refined D-borneol and standard D-borneol were determined by testing their DPPH and hydroxyl radicals scavenging activities and the reducing power. The refined D-borneol and standard D-borneol had the same antioxidant activity. They exhibited higher hydroxyl radicals scavenging activity, but weaker reducing power, compared with the synthetic borneol.

Key words: Borneol, D-borneol, synthetic borneol, gas chromatography and mass spectrometry (GC-MS), antioxidant activity.

INTRODUCTION

Borneol ($C_{10}H_{18}O$), a bi-cyclic mono-terpenoid alcohol, one of the valuable medical material, senior aromatic spices, chemical materials, has been used in food and also folk medicine in China and India. According to the Pharmacopoeia of People's Republic of China (2005), borneol is an important ingredient among about 63 herbal products. There are two different kinds of borneol; (a) synthetic borneol which is a mixture of DL-borneol and isoborneol, in which the DL-borneol content should be no less than 55.0%, and; (b) natural borneol whose main component is D-borneol, which should be >95.0% of natural borneol. Previous studies showed that synthetic borneol degraded slowly during storage and noxious camphor level might be as high as 45 to 97% with concomitant lower levels of synthetic borneol (Zeng and He, 2004). These results indicated that camphor was a degradation product of synthetic borneol. It is widely known that camphor is toxic, whereas natural borneol is nontoxic. Therefore, a management strategy to avoid the toxic effects of camphor is to use natural borneol instead of synthetic borneol in different borneol products. Natural borneol is mainly extracted from the essential oils of numerous medicinal plants of the families *Dipterocarpaceae* (for example, *Dipterocarpus turbinatus* tree), *Lamiaceae* (for example, *Rosmarinus officinalis* or *Salvia officinalis*), *Valerianaceae* (for example, *Valeriana officinalis*) or *Asteraceae* (for example, *Matricaria chamomilla*), etc., (Tabanca et al., 2001). Since there was a shortage of natural source, the price of natural borneol was gradually increased. New sources of natural borneol would be urgently needed. Luckily, the newly discovered source of natural borneol in China has provided this opportunity.

Recent studies showed that natural borneol can also be extracted from the leaves of *Cinnamomum camphora* (L.) Presl. plants grown in Jiangxi province and Hunan province in China (Li, 1992). Wang et al. (2003) and Hu et al. (2006) had evaluated the potential toxicity of natural borneol from the plants and found that it was safe for consumption since it neither induced red blood cell micronuclei nor inhibit the bone marrow of mice. Thus, it can be safely used to replace synthetic borneol. Additionally, we recently found a plant of *Cinnamomum*

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burmannii physiological type called Mei Pian tree growing wildly in the Guangdong province of China. The essential oil from the leaves of this species was rich in D-borneol. The purposes of the present studies were; (i) to obtain the essential oil from Mei Pian tree by using a Clevenger distillation apparatus and the chemical composition of the essential oil was evaluated by using gas chromatography-mass spectroscopy (GC-MS); (ii) to separate and purify D-borneol from the essential oil by freezing centrifugation, then sublimation, and then, evaluate the in vitro antioxidant properties of D-borneol by determining its 2,2-diphenyl-1- picrylhydrazyl (DPPH), hydroxyl radicals scavenging activities and the reducing power. As to our knowledge, there were few reports on the essential oil composition and the characteristic of D-borneol of Mei Pian tree. Therefore, our study would be the first to report this new natural source for producing D-borneol and evaluate its characteristic and antioxidant activity.

MATERIALS AND METHODS

Chemicals and reagents

Synthetic borneol (chemical purity >98.0%) were purchased from Huangpu Chemical Plant, Guangzhou, China. Standard D-Borneol (batch number：110743-200504) was obtained from the Natural Institute for the Control of Pharmaceuticals and Biological Products, Beijing, China. Chromatographic Grade hexane was purchased from SK Chemicals, Korea. Ethanol and sodium sulphate were analytical grades, which were purchased locally.

Plant material

Fresh leaves of Mei Pian tree 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.

Extraction of the essential oil

Fresh leaves of the plants were submitted for 2 h to water distillation using a Clevenger distillation apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored at + 4°C until tested and analyzed. The extraction yield was determined by the method of Zhang, Bi, and Liu (2007). The dried extract was weighed, and the extract yield was then calculated and expressed as the percentage of the weight of the crude extract to the raw material.

Purification of D-borneol

The D-borneol was extracted by freezing centrifugation from the essential oil and then sublimated by the transpiration method as was described by Zielenkiewicz et al. (1999).

GC/MS analysis

The analysis of the essential oil was performed with a Thermofinnigan Trace GC/Trace DSQ/6890 (E.I.Quadrapole) equipped with a HP-5MS fused silica capillary column (30 mm × 0.25 mm, film thickness 0.25 µm). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 2 ml/min. Injector and MS transfer line temperatures were set at 250 and 300°C respectively. The oven temperature was programmed from 80 to 250°C at 20°C /min, then held isothermal for 5min. Diluted samples (1/100, v/v, in methylene chloride) of 0.5 µl were injected manually in the splitless mode. The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on HP-5 capillary column, and by matching of their mass spectra of peaks with those obtained from authentic samples and, or the Wiley 7N and TRLIB libraries spectra and published data (Adams, 2007).

FT-IR analysis

All analyses were carried out using a Varian (formerly BioRad) FTS 6000 FT-IR spectrometer. Spectral collection was conducted under ambient conditions. The operating range was from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} using a DTGS detector. In all cases, 64 interferograms per sample were co-added and averaged for each spectrum. Following the pellet preparation, KBr pellets were immediately placed into the sample compartment (refined D-borneol，synthetic borneol，standard D-borneol) of the spectrometer, left to purge with dry air for 15 min to remove ambient water vapor and FT-IR spectra were subsequently recorded. All spectra were measured with a blank KBr pellet as the background and were ratioed against the background.

Optical rotation analysis

0.1 g samples (refined D-borneol, synthetic borneol, standard D-borneol) were dissolved in 1ml dehydrated alcohol respectively, configured as the solution of 0.1g/ml, and the specific rotation of the solutions were determined by Digit automatic polarimeter.

Antioxidant activity of refined D-borneol and standard D-borneol

Scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

The hydrogen atoms or electron donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses stable radical DPPH as a reagent (Cuendet et al., 1997; Burits and Bucar, 2000). The effect of methanolic extracts on DPPH radical was estimated according to Hatano et al. (1988). 2 ml of various concentrations (0.02 to 0.50 mg/ml) of refined D-borneol and standard D-borneol in ethanol were added to 2 ml of DPPH radical solution in ethanol (the concentration of DPPH was 0.2 mM). After 30 min incubation at 20°C, the absorbance was read against a blank at 517 nm. BHT at 0.2 mg/ml were used as the control.

Hydroxyl radical scavenging activity assay

The assay of scavenging activity for hydroxyl radical was based on Fenton reaction (Yu et al., 2004). After incubation at 37°C for 45 min, the absorbance at 536 nm was measured. BHT at 0.4 g/ml was used as the control.

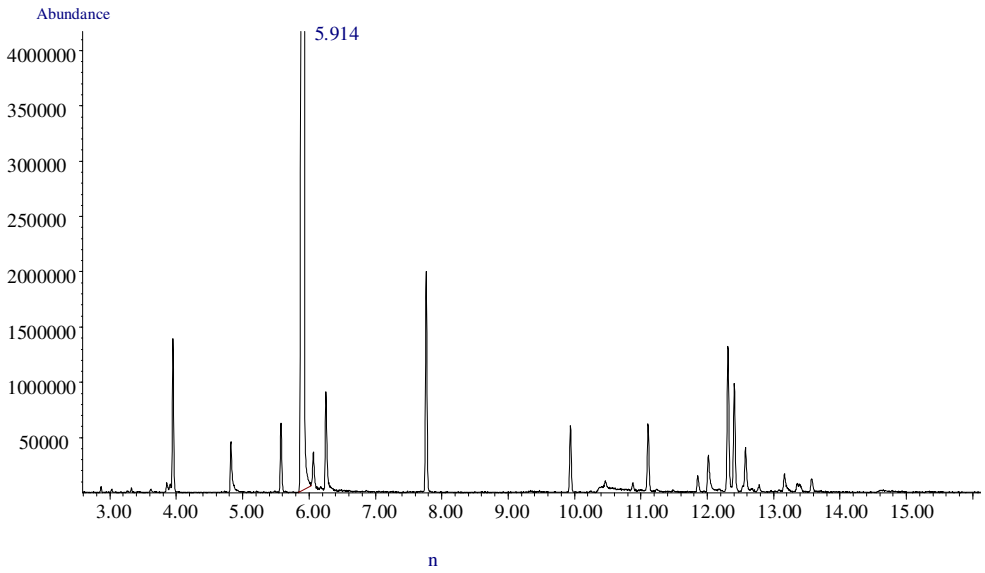


Figure 1. GC-MS chromatogram of essential oil.

The reducing power

The reducing power of samples was determined according to the method of Oyaizu (1986) as described by Siddhuraju et al. (2002) with a little modification. 2 ml of various concentrations (0.2 to 1.0mg ml^{-1}) of D-borneol and standard 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 ferricynide. After the mixture was incubated at 50°C 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. BHT at 1.0 mg/ml was used as the control.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The essential oil extracted by hydro distillation from the fresh leaves of Mei Pian tree was quantitatively analyzed by GC–MS analysis (Figure 1). Forty components were identified in the oil sample analyzed amounting to 99.4% of the total oil, which are listed in Table 1. The oil was dominated by the D-borneol (78.6%), and the Bornyl acetate, Spathulenol and Eucalyptol were determined as the major compounds of the oil (3.26, 2.60 and 1.92%, respectively).

It has been reported that natural borneol existed in the essential oils of the families *Dipterocarpaceae* (for example, *Dipterocarpus turbinatus* tree) (30 to 65%), *Lamiaceae* (for example, *Rosmarinus officinalis* or *Salvia officinalis*) (27 to 39%), etc., (Tabanca et al., 2001). Tabanca（2006）pointed out that the 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 also been reported in many *Salvia* oils, for instance in *S. fruticosa* Miller, *S. tomentosa* Miller, *S. pomifera* L., *S. willeana* (Holmboe) Hedge, and *S. officinalis* L. In a study of the essential oils extracted from leaves of *Blumeariparia* (bl), Dc was 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). D-borneol was represented in a high quantity (85%) in the essential oil from leaves of *Cinnamomum camphora* (L.) Presl plants, which grow in Jiangxi province and Fujian province, China. However, Safroles was found to be concomitant with D-borneol, which may affect the quality.

Data revealed from this study showed the essential oils from leaves of Mei Pian tree accounting about 0.60% dry weight, and the abundance of D-bornol (78.46%) in the oil. However, there were very few previous reports regarding to the essential oil composition of this plant. Therefore, data presented here could be assumed as the first report on this *Cinnamomum burmannii* physiological type variety.

Composition of refined D-borneol and synthetic borneol

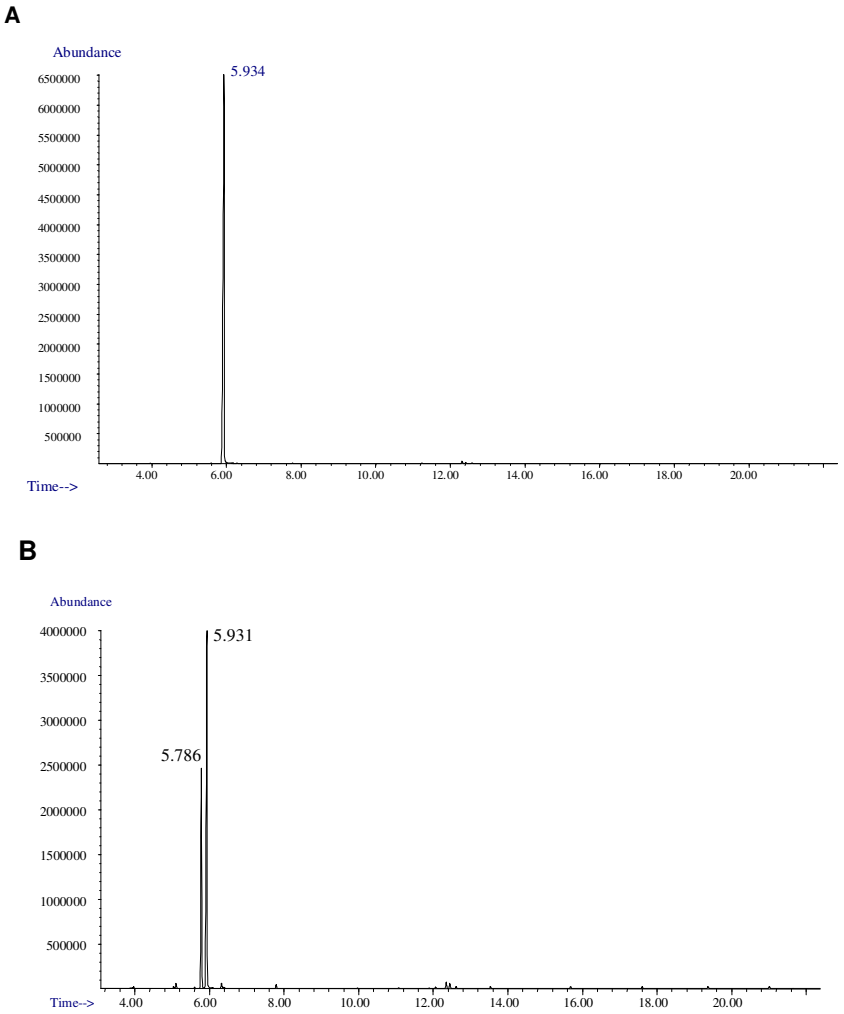
The D-borneol was extracted from the essential oil by freezing centrifugation and then sublimated by the transpiration method. Figure 2 showed the chromatograms of refined D-borneol (Figure 2a), synthetic borneol (Figure 2b), and standard D-borneol (Figure 2c) used in the present study. The refined D-borneol was analyzed amounting to 99.30%, with 0.15% Eucalyptol as the main component, while 18 components were identified

Table 1. Volatile compounds in the leaves of Mei Pian tree identified.

No	RT(min)	Components	Matching (%)	Percent (%)	Identification methods
1	2.872	1*R*-.alpha.-Pinene	70	0.06	GC,MS
2	3.032	1,4-Pentadiene	93	0.03	GC,MS
3	3.322	5,5-dimethyl-7-Oxabicyclo	87	0.05	GC,MS
4	3.617	7,7-dimethyl-2-methylene-1,4-Cyclohexadiene	85	0.06	GC,MS
5	3.859	Benzene	84	0.17	GC,MS
6	3.912	Limonene Cyclohexene	86	0.12	GC,MS
7	3.951	Eucalyptol	97	1.92	GC,MS
8	4.821	2-aminobenzoate	70	0.99	GC,MS
9	5.581	1,7,7-trimethyl-	96	1.00	GC,MS
10	5.914	*D-borneol*	98	78.46	GC,MS
11	6.064	3-Cyclohexen-1-o	70	0.86	GC,MS
12	6.176	Oxime	82	0.13	GC,MS
13	6.253	Bicyclo[2.2.1]hept-2-ene	89	1.81	GC,MS
14	7.767	Bornyl acetate	93	3.26	GC,MS
15	9.474	Ethylene oxide	79	0.02	GC,MS
16	9.938	Caryophyllene	95	1.04	GC,MS
17	10.412	1-Phenyl-2-nitro-1-propene	60	0.17	GC,MS
18	10.466	3-Carene	78	0.25	GC,MS
19	10.529	2,3-Dimethylamphetamine	79	0.02	GC,MS
20	10.553	acetate	64	0.02	GC,MS
21	10.582	Benzeneethanamine	80	0.02	GC,MS
22	10.877	Benzyl isocyanate	72	0.14	GC,MS
23	11.109	gamma.-Elemene	82	1.11	GC,MS
24	11.486	8-Quinolinol	81	0.04	GC,MS
25	11.859	Cyclohexanol	90	0.28	GC,MS
26	12.018	3,7,11-Trimethyldodeca-2,6,10-trienylacetat	66	0.86	GC,MS
27	12.120	2(5*H*)-Furanone	80	0.07	GC,MS
28	12.183	Acetamide	62	0.05	GC,MS
29	12.313	(-)-Spathulenol	92	2.60	GC,MS
30	12.410	Caryophyllene oxide	65	1.88	GC,MS
31	12.574	Guaiol	91	0.95	GC,MS
32	12.666	Cyclohexane	62	0.11	GC,MS
33	12.782	3-Heptyne	85	0.16	GC,MS
34	13.082	Cyclohexane methanol	99	0.06	GC,MS
35	13.165	Docosahexaenoic acid methyl ester	90	0.48	GC,MS
36	13.363	Cyclohexane methanol	90	0.17	GC,MS

Table 1. Contd.

37	13.392	Patchoulene	83	0.19	GC,MS
38	13.576	Caryophyllene-(I1)	78	0.26	GC,MS
39	14.625	Cinnamaldehyde	64	0.05	GC,MS
40	14.654	Phenyl 2-propynyl ether	88	0.02	GC,MS



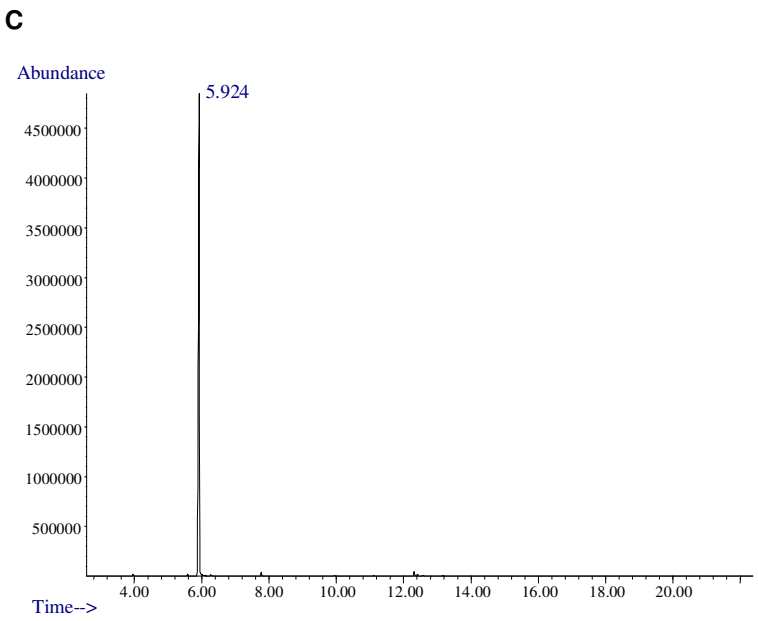


Figure 2. (A)GC-MS chromatogram of refined D-borneol; (B) GC-MS chromatogram of synthetic borneol; (C) GC-MS chromatogram of standard D-borneol.

in the synthetic borneol, dominated by the D-borneol (58.53%), and followed by isoborneol (33.26%). There was no camphor peak in any of these chromatograms. The chemical composition of refined D-borneol extracted from Mei Pian tree was extremely similar to the standard D-borneol, materially surpass synthetic borneol.

Infrared spectrogram

Generally, Infrared spectrogram of the refined D-borneol was basically the same as standard D-borneol from 4000 to 400 cm^{-1}. The result also indicated that the chemical composition of refined D-borneol extracted from Mei Pian tree was extremely similar to the standard D-borneol (Figure 3).

The Optical rotation analysis

As shown in Table 2, the optical activity of natural D-borneol extracted from Mei Pian tree was almost the same as the standard D-borneol. The optical rotation of synthetic borneol was minus. It was because synthetic borneol was composed of D-borneol (58.53%) and isoborneol (33.26%).

Antioxidant activity of refined D-borneol and standard D-borneol

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

The scavenging activity of DPPH radical was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The activity of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). As can be seen from Table 3, refined D-borneol showed almost the same scavenging activity as that of standard D-borneol in DPPH radical scavenging activity assay, but obviously weaker than that of synthetic antioxidant BHT(88.36 ± 0.76%) at 0.2 mg/ml. Hydroxyl radicals generated in the Fe^{2+}/H_2O_2 system were assayed by a spectrophotometric method. As can be seen from Table 4, the scavenging ability on hydroxyl free radical of the samples showed a dose-dependent manner from 0.2 to 1.0 mg/ml. The scavenging ability of refined D-borneol was almost the same as standard D-borneol, and obviously stronger than that of synthetic antioxidant BHT when the volume was from 0.6 to 1.0 ml.

The reductive potential measures the ability of a sample to act as electron donor and reacts with free radicals converting them to more stable products and

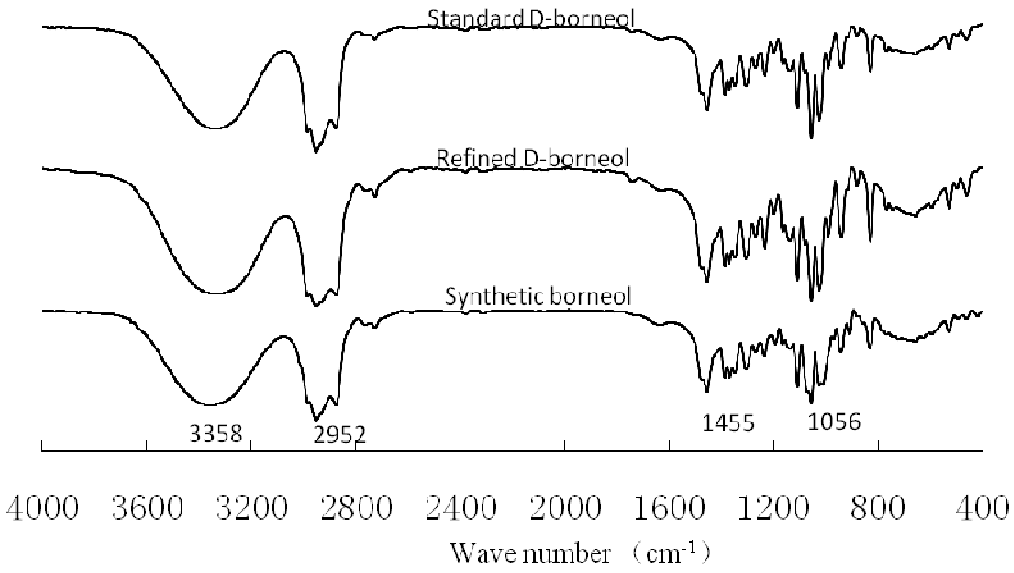


Figure 3. Infrared spectrogram of standard borneol, refined D-borneol, synthetic borneol.

Table 2. Optical activity analysis of refined D-borneol, standard D-borneol and synthetic borneol.

Compounds	Refined D-borneol	Standard D-borneol	Synthetic borneol
Optical rotation	+36.4±0.1º	+37.5±0.1º	-22.4±0.1º

Table 3. Scavenging activity (%) on DPPH radical of refined D-borneol from Mei Pian tree and standard D-borneol at different concentrations.[a]

Samples	Sample concentration(mg/ml)			
	0.02	0.1	0.2	0.5
Refined D-borneol	32.76±1.05	30.90±0.45	37.56±0.48	33.53± 1.25
Standard D-borneol	33.58±1.45	31.68±0.54	38.79±0.78	34.65± 1.64
BHT	-	-	86.20±1.45	

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

Table 4. Scavenging effect (%) on hydroxyl free radical of refined D-borneol from Mei Pian tree and standard D-borneol at different volumes.[a]

Samples	Sample concentration(mg/ml)				
	0.2	**0.4**	**0.6**	**0.8**	**1.0**
Refined D-borneol	13.27±1.05	70.15±0.76	92.49±0.48	92.94±0.81	93.86±1.31
Standard D-borneol	15.76±1.43	75.89±1.12	95.37±0.68	94.65±1.21	97.54±1.57
BHT	5.68±0.76	22.59±1.31	40.13± 0.32	41.45± 0.23	52.24 ±1.36

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

thereby terminates radical chain reactions. The reducing power of the samples is presented in Table 5. 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

Table 5. Reducing power (absorbance at 700 nm) of refined D-borneol from Mei Pian tree and standard D-borneol at different concentrations.[a]

Samples	Sample concentration(mg/ml)		
	0.2	0.4	1.0
Refined D-borneol	0.1240± 1.25	0.1302 ± 1.65	0.1396 ± 0.56
Standard D-borneol	0.1353± 1.57	0.1403 ± 1.87	0.1432± 0.91
BHT	-	-	0.7223±0.35

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

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). Refined D-borneol and standard D-borneol exhibited weaker reducing power than that of synthetic antioxidant BHT at the same concentration. Reductive potentials of the samples are highly related with the polarities of their phytochemicals, while D-borneol contains the non-polar secondary metabolites (terpenoids), remained almost inactive.

Conclusion

Results showed that the predominant compounds were D-borneol (78.6%), Bornyl acetate (3.26%), (-)-Spathulenol (2.60%), and Eucalyptol (1.92%) among the essential oil extract from the leaves of *Cinnamomun burmannii B1* physiological type grown in Guangdong province, China. The molecular composition and physical property of refined natural D-borneol was extremely similar to the standard D-borneol, materially surpass synthetic borneol. The refined D-borneol as well as standard b D-borneol exhibited moderate antioxidant activity compared with synthetic antioxidant among the antioxidant activity assays. Therefore, the refined D-borneol extracted from leaves of Mei Pian tree could be used to replace synthetic borneol and could avoid the toxic effects of camphor.

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