Chemical Composition and Antifungal Activity of Extracts from the Xylem of *Cinnamomum camphora*

Quan Li,[a] Xiao-Xian Wang,[a] Jin-Guo Lin,[a,]* Jing Liu,[a] Mao-Sheng Jiang,[b] and Lei-Xia Chu [b]

Cinnamomum camphora (L.) Presl. is one of the most important hardwood species indigenous to China that possesses significant antifungal activity. The chemical composition of the extracts from the xylem parts of *C. camphora* was examined by various solvent extractions. Thirty different components accounting for 79.8% of the total methanol extracts from the xylem of *C. camphora* were identified by gas chromatography-mass (GC/MS) spectrometry. The major chemical components of methanol extracts are camphor (14.3%), *α*-terpineol (9.9%), and *trans*-linalool oxide (furanoid) (7.7%). The chemical composition of chloroform extracts are mainly camphor (17.6%), *α*-terpineol (11.8%), tetradecanal (5.6%), and (-)-g-cadinene (7.4%). The extracts of *C. camphora* were tested for resistance to two wood-decaying fungus with hyphal growth. All the *C. camphora* extracts showed some antifungal activity against the test fungus. The 50% effective concentration of chloroform extracts for *Coriolus versicolor* (*C. versicolor*) was 7.8 mg/mL, which was highly toxic, followed by acetone extracts. The methanol extracts with 8 mg/mL concentration had the best suppression effect for *Gloeophyllum trabeum* (*G. trabeum*) with an EC_{50} of 0.3 mg/mL. The results indicated that the major components of the extracts had antifungal activities; thus *C. camphora* could provide a renewable source for wood preservatives.

Keywords: Antifungal activity; Cinnamomum camphora; EC_{50}; GC/MS; Wood protection

*Contact information: a: College of Material Engineering, Fujian Agriculture and Forestry University, Fuzhou 350002, China; b: College of Life Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China; * Corresponding author: fjlinjg@126.com*

INTRODUCTION

Wood preservatives include water-borne and oil-borne preservatives. Two examples are chromated copper arsenate (CCA), which is widely used in residential, commercial and industrial building, and pentachlorophenol, which is usually used for treating utility poles and cross arms (Hingston *et al.* 2001; Schultz *et al.* 2007). The environmental hazards associated with the use of both chromium and arsenic salts limit their future application. Developing novel low-toxic formula, environmentally compatible products is desirable for wood preservation (De Groot and Woodward 1999). There is considerable interest in the extraction of wood preservatives from natural biomass sources. In recent years there has been increasing research related to the biological control of wood decay fungi, and this approach holds potential to decrease the need of chemical preservatives for the wood preservation industry (Onuorah 2000, 2002).

Plant extracts, which are naturally occurring substances, generally have a broad spectrum of bioactivity because of the presence of several active ingredients that work

through several modes of action (Cheng *et al.* 2003). Plant secondary metabolites result from interactions between plants and environments during the long-term evolution process. They are produced by the various parts of plants, which have formed the basis of many applications in wood preservation industries. Extracts of wood are an important class of plant secondary metabolite (Schultz and Nicholas 2000, 2002). Several wood species appear to owe their rot-resistant nature to the presence of "extractives", *i.e.* extractable chemical compounds (Bhardwaj *et al.* 2012; Wu and Yang 2005); these extractives also help the tree become resistant to insect attacks. For example, Kamdem (1994) used methanol extracts from heartwood of black locust (*Robinia pseudoacacia*) to increase the decay resistance of aspen (*Populus tremuloides*) blocks. High fungal resistance of cypress pine (*Callitris glaucophylla*) was attributed to the presence of extractives in the heartwood (Minnick *et al.* 1987).

Cinnamomum camphora (L.) Presl. is one of the most important and frequently used evergreen species distributed and cultivated in China and in many tropical and subtropical areas, including Southeast Asia and East Asia. It plays an important role in protecting regional environments. The volatile gas of camphor can kill many types of harmful bacteria. Studies have reported on the chemical components of extractives, as well as their antimicrobial and insecticidal effects (Kim *et al.* 2005; Wu *et al.* 2000). The plant has historically been used for herbal medicine and furniture in China. Recently, the extractives from *C. camphora* have been more commonly used in the form of the essential oil, which can be obtained from the trunk, leaves, and twigs by the method of steam distillation, or by using various solvents (Chua *et al.* 2008). The extract is used not only in traditional medicine for the treatment of diseases, such as rheumatism, sprains, bronchitis, and muscle pains (Singh *et al.* 2008), but also as an antibacterial agent for animals and plants against pathogenic bacteria, such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilis*, *etc*. *C. camphora* has certain preservative, bacteriostatic, and insecticidal activities that could restrain the growth of microbes in water and air, which can be used for food flavourings or preservatives (Liu *et al.* 2006; Walch *et al.* 2011). *C. camphora* extracts contains large amounts of camphor, *α*-terpineol, linalool, eucalyptol, safrole, *etc.* (Pandey *et al.* 1997).

Natural (+)-(1R, 4R)-camphor is usually produced during the steam distillation of *C. camphora*, and the different components can be used to develop high value-added products, such as essences and flavors, biomedicine, food, cosmetics, and special industrial materials. Despite camphor being utilized as an important antimicrobial (Alvarez-Castellanos *et al.* 2001), only few studies have reported on its effect against white-rot fungus and brown-rot fungus. To evaluate the extracts of *C. camphora* antifungal bioactivity, in this study, we analyze the antifungal compounds of extracts and the potential applications in wood preservation for development and utilization of resources of *C. camphora*.

EXPERIMENTAL

Materials and Methods

C. camphora (45 years old) was collected from Fuzhou, China. The voucher specimen (No. 978) has been deposited in the herbarium of our laboratory. All samples were taken from the stem of the three *C. camphora* trees, which consisted of sapwood and heartwood. The *C. camphora* xylem was chopped and milled to 40 ~ 60 mesh

particles. The formula for making Malt agar solid medium is 50 g malt extract, 30 g agar, and 1000 mL distilled water. Malt agar solid medium was obtained from the College of Plant Protection, Fujian Agriculture and Forestry University (Fuzhou, China). Methanol, acetone, ethyl acetate, and chloroform were purchased from Shanghai Chemical Reagent Factory (Shanghai, China). Ammoniacal copper quats (ACQ) and camphor powder were purchased from two factories in Fujian province.

Coriolus versicolor (*C. versicolor*) and *Gloeophyllum trabeum* (*G. trabeum*) were kindly provided by Prof. Jiang in the College of Plant Protection, Fujian Agriculture and Forestry University (Fuzhou, China). For the wood decay fungi, the related standard method of Chinese standard (GB/T 13942.1-2009) was used and one strain of white-rot fungi, *C. versicolor*, and one strain of brown-rot fungi, *G. trabeum*, were tested. The fungal strain cultures were maintained on a malt agar solid medium at 4 °C.

Extraction of Wood Samples

The xylem of *C. camphora* was pulverized (40 ~ 60 mesh) after air drying. The *C. camphora* powder (100 g) was placed in a heat reflux extraction. Hot water, methanol, acetone, ethyl acetate, and chloroform were used as solvents, respectively. The solvents were heated to their respective boiling point temperature. The row materials were extracted for two times, first heated for 5 h with a ratio of gardenia to solvent of 1:10 (g/mL), then heated for 3 h with a ratio of gardenia to solvent of 1:10 (g/mL), then the extractives of two times were mixed and filtered to obtain the extracted liquid. The next step was to retrieve solvents by reduced pressure distillation, and the *C. camphora* extracts were obtained at the same time. The obtained extracts were analyzed by GC/MS and used in antifungal activity tests.

Antifungal Assay

The antifungal activity of *C. camphora* extracts was tested against two wood-rot fungi by the growth rate of poison medium culture method (Bajpai *et al.* 2008). The extracts of *C. camphora* with various concentrations were interfused into the maltose liquid medium. The toxic compounds were effective in inhibiting radial growth of *C. versicolor* and *G. trabeum*. In detail, a certain amount of the selected extract was added separately to each 9 cm petri dish containing solvent. Malt agar solid medium then was mixed with the *C. camphora* extracts and solvents to obtain the poison malt agar solid medium. The total volume of malt agar solid medium, the *C. camphora* extracts, and the solvents was 10 mL. The final concentrations of 8 mg/mL, 4 mg/mL, 2 mg/mL, 1 mg/mL, 0.5 mg/mL, and 0 mg/mL (control plates, the volume of malt agar solid medium is 9 mL and that of corresponding solvent is 1 mL) were obtained. Every solvent may affect the fungal hyphae growth, so in the control plates, the volume of the solvent was also 1 mL as well. The point should be reinforced that the antifungal effect of the methanol solvent also needs to be considered. Camphor was configured to concentrations of 0.2 mg/mL, 0.1 mg/mL, 0.05mg/mL, 0.025 mg/mL, 0.0125 mg/mL, and 0 mg/mL with chloroform. ACQ was configured to concentrations of 0.05 mg/mL, 0.04 mg/mL, 0.03 mg/mL, 0.02 mg/mL, 0.01 mg/mL, and 0 mg/mL with water. After transferring the hyphae of white-rot fungus and brown-rot fungus strains (7 mm), cultures of the fungi were maintained at the center of a petri dish and incubated at 28 °C ± 2 °C and 75% to 85% relative humidity for several days. When the control plates' fungal hyphae reached the edges of the control dishes, the antifungal indices were calculated (Cheng *et al.* 2006). All tests were performed in triplicate. The EC_{50} (effective dose for 50% inhibition)

was calculated by probit analysis. The antifungal index was calculated as follows (Wang *et al.* 2005),

$$\text{Antifungal index (\%)} = \frac{X - Y}{X} \times 100\% \quad (1)$$

where X is the diameter of growth zone in the experimental dish with an average of 3 replicates (mm), and Y is the diameter of growth zone in the control dish with an average of 3 replicates (mm).

GC/MS Analysis Conditions

The analyses of the extractives were carried out on an Agilent GC/MS system (Agilent 5975C/7890N). The fused-silica column DB-17MS (30 m × 0.25 mm × 0.25 μm) for quantitative analysis was directly coupled to the mass spectrometer. An injection port temperature of 280 °C was used. Ionization of the sample components was performed in the E.I. mode (70 eV). The carrier gas was helium with a flow of 1.0 mL/min. The GC oven temperature program was kept at 50 °C and held isothermally for 3 min. It was then heated at a program of 20 °C/min up to 80 °C, retained 3 min, followed by an increase to 120 °C at a rate of 2°C/min. The system was held isothermally for 5 min, then increased up to 130 °C at a rate of 1.5 °C/min, retained 5 min, and then increased to 180 °C at a rate of 2 °C/min, held isothermally for 5 min, finally heated at a rate of 5 °C/min up to 250 °C and held for 5 min. The identification of extracts was achieved using computer matching software of the mass spectra from the NIST library. For those mass spectra of extracts that were not found in the NIST library, reference compounds were analyzed by GC/MS for comparison.

RESULTS AND DISCUSSION

Antifungal Activity

The antifungal activity of extracts was evaluated by the growth rate of poison medium culture method. The white-rot fungus and brown-rot fungus used in the fungicidal bioassay were *C. versicolor* and *G. trabeum*, respectively. As indicated in Figs. 1 and 2, all the extracts showed certain antifungal activity against the tested fungi. This growth inhibition ratio of chloroform extracts was calculated in the ranges of 0.5 mg/mL to 11.4%, 1 mg/mL to 13.4%, 2 mg/mL to 20.7%, 4 mg/mL to 32.8%, and 8 mg/mL to 57.5% for *C. versicolor*, respectively. The extracts of hot water, methanol, acetone, ethyl acetate and chloroform at 8 mg/mL inhibited the growth of white-rot fungus at 17.8%, 27.8%, 49.2%, 37.1%, and 57.5%, respectively. This indicates that chloroform extracts had the best antifungal effect on *C. versicolor* among these five extracts. The growth inhibition ratios for methanol extracts were 0.5 mg/mL to 51.7%, 1 mg/mL to 56.3%, 2 mg/mL to 58.3%, 4 mg/mL to 61.3%, and 8 mg/mL to 64.9% for *G. trabeum*, respectively. The fungicide test results presented in Fig. 2 revealed that 8 mg/mL methanol extracts possessed the greatest inhibition of hyphal growth of *G. Trabeum* among these five extracts. The results of the current tests were consistent with those of Roszaini *et al.* (2013), who found that methanol extracts from *C. camphora* xylem presented strong antifungal, antibacterial, and antiviral activities. The extracts of hot water, methanol, acetone, ethyl acetate, and chloroform at 8 mg/mL inhibited the

growth of *G. trabeum* at 44.4%, 64.9%, 53.7%, 59.0%, and 55.7%, respectively, and of *C. versicolor* at 17.8%, 27.8%, 49.2%, 37.1%, and 57.5%, respectively. As shown in Fig. 1, the percentage of growth inhibition ratio of hot water extracts was higher than that of the other extracts in 0.5 mg/mL concentration for *C. versicolor*. The overall variation trend showed that growth inhibition ratio of chloroform extracts at an 8 mg/mL concentration was visibly higher than that of others.

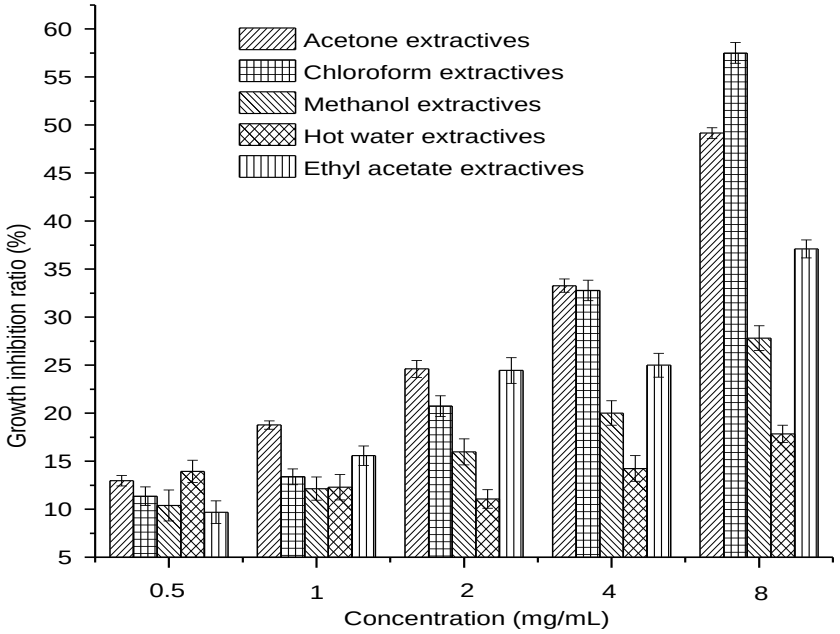

Fig. 1. Antifungal activity of different solvent extracts against a white-rot fungus, *C. versicolor*

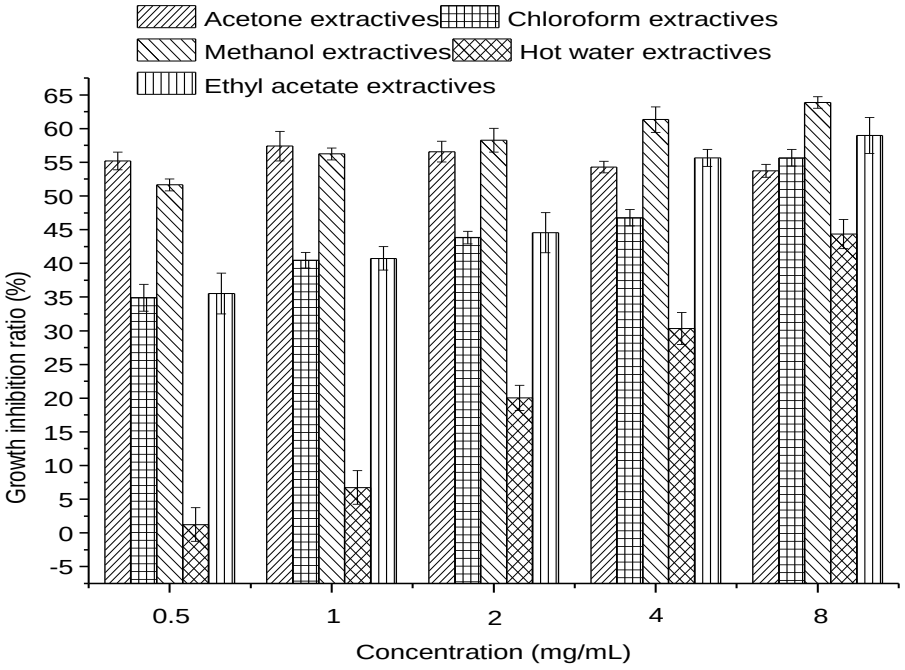

Fig. 2. Antifungal activity of different solvent extracts against a brow-rot fungus, *G. trabeum*

Further bioassays of the extracts revealed some inhibitory effect on the two wood-decaying fungi, with the chloroform and the methanol extracts showing the best results. The EC_{50} of chloroform extracts on white-rot fungus and brown-rot fungus were 7.8 mg/mL and 4.53 mg/mL, respectively. EC_{50} values of methanol, acetone, ethyl acetate, and chloroform extracts were calculated as 112.2 mg/mL, 10.2 mg/mL, 22.9 mg/mL, and 7.80 mg/mL for *C. versicolor*, respectively. EC_{50} values of hot water, methanol, ethyl acetate, and chloroform extracts were calculated as 8.1 mg/mL, 0.3 mg/mL, 2.8 mg/mL, and 4.5 mg/mL for *G. trabeum*, respectively. These results are possibly due to acetone extracts and hot water extracts volatile partly in the process of test section. Another possible reason is that the range of the five different concentrations of the poison culture medium is too small to reflect the growth inhibition regular, therefore the hot water extracts and acetone extracts of *C. camphora* did not show any regular pattern on the EC_{50} value (Table 1). Pure camphor showed good antifungal activity against wood-decaying fungus; the EC_{50} values were 1.5 and 0.123 for *C. versicolor* and *G. trabeum*, respectively. Therefore, *C. camphora* extracts showed high antifungal activity against *C. versicolor* and *G. trabeum*, respectively.

Table 1. Toxicity of Extracts and Preservatives on Wood-decaying Fungi

Extracts	Fungi	[a] Toxic regression equation	R^2	[a] EC_{50} mg/mL
Hot water extracts	*C. versicolor*	/	/	/
	G. trabeum	y=1.7325x + 3.4277	0.9667	8.1
Methanol extracts	*C. versicolor*	y=0.5544x + 3.8623	0.9741	112.2
	G. trabeum	y=0.2516x + 5.1345	0.9869	0.3
Acetone extracts	*C. versicolor*	y=0.8858x + 4.1032	0.9790	10.2
	G. trabeum	/	/	/
Ethyl acetate extracts	*C. versicolor*	y =0.756x + 3.971	0.9554	22.9
	G. trabeum	y=0.5224x + 4.7683	0.9675	2.8
Chloroform extracts	*C. versicolor*	y=1.1466x + 3.9776	0.9248	7.8
	G. trabeum	y=0.4062x + 4.7335	0.9609	4.5
Camphor	*C. versicolor*	y = 0.9151x + 4.8352	0.9353	1.5
Camphor	*G. trabeum*	y=0.7251x + 5.6594	0.9842	0.123
ACQ	*C. versicolor*	y=0.7117x + 5.7286	0.9543	0.095
ACQ	*G. trabeum*	y=1.229x + 7.2931	0.9567	0.0138

[a] Average of three replicates.

Antifungal experiments showed that methanol, ethyl acetate, and chloroform extracts had fungicidal activity against *C. versicolor* and *G. trabeum* at all test concentrations. *C. camphora* extracts can effectively inhibit the fungus. Although the inhibitive effect of *C. camphora* extracts is weaker than that of ACQ, the *C. camphora* extracts still has the further development and utilization value because of its environmentally friendly and renewable characteristics. Antifungal results reported by Lee *et al.* (2006) and Yu *et al.* (2012) suggested that these extracts have a potential use for developing new natural wood preservatives.

Chemical Composition of Extracts of *C. camphora* Xylem

The chemical composition of the extracts of *C. camphora* xylem was quantified from GC peak areas. By comparing the ion fragments obtained by electron impact (EI) ion source, the match factor between the observed compounds and the references listed by NIST are > 80% (80~99%). Thirty different chemical components were identified in the methanol extracts. The identified components accounted for approximately 79.8 wt% of the total extracts. The amount of each chemical components identified in the extracts were given in Table 2. The components are listed in order of their GC retention time.

Table 2. Chemical Constituents (%) Identified in Methanol Extracts of the *C. camphora* Xylem

No	Retention time (min)	Compound	Molecular formula	Composition (%)	Matching rate (%)
1	8.682	Cineole	$C_{10}H_{18}O$	0.79	99
2	11.315	Linalool	$C_{10}H_{18}O$	2.90	97
3	16.102	(-)-4-Terpineol	$C_{10}H_{18}O$	1.97	91
4	16.387	Camphor	$C_{10}H_{16}O$	14.29	98
5	17.409	*α*-Terpineol	$C_{10}H_{18}O$	9.88	86
6	18.036	(E)-2,6-Dimethyl-3,7-octadiene-2,6-diol	$C_{10}H_{18}O_2$	1.60	83
7	20.74	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol	$C_{10}H_{18}O_2$	0.89	97
8	27.674	Camphane-2,5-dione	$C_{10}H_{14}O_2$	0.69	81
9	28.645	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	1.20	90
10	33.179	2-(5-methyl-5-vinyltetrahydrofuran) ethyl	$C_{13}H_{22}O_4$	3.67	80
11	33.6	*trans*-Linalool oxide (furanoid)	$C_{10}H_{18}O_2$	7.66	81
12	40.178	DL-Isoborneol	$C_{10}H_{18}O$	1.19	85
13	42.164	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	$C_{16}H_{30}O_4$	1.06	86
14	42.714	Tetradecanal	$C_{14}H_{28}O$	7.72	99
15	48.587	1-Methyl-6-methylene-4-(1-methylethyl)- 1,2,3,5,6,7,8,8a-octahydro-naphthalene	$C_{15}H_{24}$	1.03	93
16	50.082	(-)-g-Cadinene	$C_{15}H_{24}$	5.49	80
17	53.769	Myristic acid	$C_{14}H_{28}O_2$	2.07	99
18	54.021	2,6-dimethoxy-4-(2-propen-1-yl)-Pheno	$C_{11}H_{14}O_3$	0.64	86
19	59.545	Pentadecanoic acid	$C_{15}H_{30}O_2$	1.77	99
20	64.74	Palmitic acid	$C_{16}H_{32}O_2$	1.06	94
21	65.264	Palmitic acid vinyl ester	$C_{18}H_{34}O_2$	0.81	87
22	70.723	Cyclopentadecane	$C_{15}H_{30}$	1.05	94
23	74.43	1-Heptadecanol	$C_{17}H_{36}O$	0.75	85
24	75.827	Linoleic acid	$C_{18}H_{32}O_2$	0.67	99
25	76.926	3,6-Dihydro-4-methoxy-2H-pyran	$C_6H_{10}O_2$	3.89	81
26	77.231	6-(Methylamino)- 4(3H)-pyrimidinone	$C_5H_7N_3O$	1.76	84
27	83.667	*cis*- Decahydro-4a-methyl-naphthalene	$C_{11}H_{20}$	0.37	80
28	86.882	Dioctyl phthalate	$C_{24}H_{38}O_4$	0.96	99
29	90.763	2-Methylphenanthro[3,4-d][1,3]oxazol-10-ol	$C_{16}H_{11}NO_2$	0.84	83
30	94.845	2,4,7,9-Tetramethyl-5-decyne-4,7-diol	$C_{14}H_{26}O_2$	1.16	81

Identification was achieved using computer matching of the mass spectra with the NIST library.

These constituents of *C. camphora* varied from those listed in earlier research reports (Cardullo and Gilroy 1973; Yeh *et al*. 2009). The major constituents of the methanol extract, as listed in Table 2, were linalool (2.9%), (-)-4-terpineol (2.0%), camphor (14.3%), *α*-terpineol (9.9%), 2-(5-methyl-5-vinyltetrahydrofuran) ethyl (3.7%), *trans*-linalool oxide (furanoid) (7.7%), (-)-g-cadinene(5.5%), 2H-pyran,3,6-dihydro-4-methoxy- (3.9%), *etc*. Camphor was the major component, representing 14.3% of total extracts in *C. camphora* xylem, followed by *α*-terpineol (9.9%).

The GC/MS analyses showed that the *C. camphora* extracts had profound antifungal effects on the growth of some bacteria and fungus. Thus, *C. camphora* extracts may be recommended as a plant-based preservative for wood decay control.

Table 3. Chemical Constituents (%) Identified in Chloroform Extracts of *C. camphora* Xylem

No	Retention time (min)	Compound	Molecular formula	Composition (%)	Matching rate (%)
1	8.689	Cineole	$C_{10}H_{18}O$	2.00	99
2	11.334	Linalool	$C_{10}H_{18}O$	5.93	97
3	16.121	(-)-4-Terpineol	$C_{10}H_{18}O$	1.60	95
4	16.412	Camphor	$C_{10}H_{16}O$	17.58	98
5	17.441	*α*-Terpineol	$C_{10}H_{18}O$	11.79	80
6	18.049	(E)-2,6-Dimethyl-3,7-octadiene-2,6-diol	$C_{10}H_{18}O_2$	1.34	82
7	20.746	2,3-pinanediol	$C_{10}H_{18}O_2$	0.73	97
8	25.831	1,7-Dimethyl-7-(4-methyl-3-pentenyl)-tricyclo[2.2.1.0(2,6)]-heptane	$C_{15}H_{24}$	0.77	99
9	25.992	Safrole	$C_{10}H_{10}O_2$	1.93	98
10	29.369	(1S,2R,4R)-2-Methyl-3-methylene-2-(4-methyl-3-penten-1-yl)-bicyclo[2.2.1]heptane	$C_{15}H_{24}$	1.04	95
11	33.205	cis-Linaloloxide	$C_{10}H_{18}O_2$	3.95	80
12	33.619	(2R,5R)-rel-5-Ethenyltetrahydro-*α*,*α*,5-trimethyl-2-furanmethanol	$C_{10}H_{18}O_2$	6.22	83
13	36.058	d-Cadinene	$C_{15}H_{24}$	1.09	98
14	42.714	Tetradecanal	$C_{14}H_{28}O$	5.59	99
15	50.101	(-)-g-Cadinene	$C_{15}H_{24}$	7.40	83
16	53.717	Myristic acid	$C_{14}H_{28}O_2$	1.24	99
17	59.532	Pentadecanoic acid	$C_{15}H_{30}O_2$	1.18	98
18	86.752	1-Octacosanol,1-acetate	$C_{30}H_{60}O_2$	0.80	99
19	86.888	Dioctyl phthalate	$C_{24}H_{38}O_4$	4.06	98
20	89.708	(3b,24S)-Stigmast-5-en-3-ol	$C_{29}H_{50}O$	2.67	95
21	91.772	1-Acetate-1-octacosanol	$C_{30}H_{60}O_2$	1.85	95

Identification was achieved using computer matching of the mass spectra with the NIST library.

The major constituents of the chloroform extracts of *C. camphora* are shown in Table 3. A total of 21 compounds were identified, and the major constituents were: cineole (2.0%), linalool (5.9%), (-)-4-terpineol (1.6%), camphor (17.6%), *α*-terpineol (11.8%), cis-linaloloxide (4.0%), tetradecanal (5.6%), (-)-g-cadinene (7.4%), myristic acid (1.2%), pentadecanoic acid (1.2%), dioctyl phthalate (4.1%), stigmast-5-en-3-ol, (3b, 24S)- (2.7%), 1-octacosanol,1-acetate (1.85%), *etc*.

Through comparisons with other studies about *Cinnamomum camphora* (Sun *et al*. 2007), the present investigation found the type and content of the *C. camphor* extractives' components is changing because of the difference of the varieties, processing technology, geographical environment, season, cultivation and management measures. In this paper, the results showed that the content of α-terpineol was higher than the other studies and the content of camphor was lower (Baruah *et al*. 1975; Yu *et al*. 2010). Some new

chemical components had been found by the authors: myristic acid and linoleic acid *etc*. Camphor and *α*-terpineol were the major constituents in chloroform extracts of *C. camphora* xylem. Camphor is one of the world's first natural organic ingredients be used. It is not only effective against the fungus growth, but it also has many pharmacological effects, such as stimulation, cardiac, anti-inflammatory, analgesic, antibacterial, cough, penetration, reducing mitochondrial respiration, regulating liver enzyme, mites, *etc* (Ding *et al.* 2012). Besides the industrial importance of the extracts, the *C. camphora* have been used for many centuries in the traditional Chinese medicine for expelling parasite and rheumatism beriberi therapy. For example: Tiger Balm (Chinese herbal remedy) based on camphor and menthol, is great for easing bronchial congestion (Schattner and Randerson 1996). A number of studies have concluded that some minor components are critical to the activity and may have a synergistic effect or potential influence to the extracts (Skočibušić *et al.* 2006). Pattnaik *et al.* (1997) found that linalool exhibits strong antimicrobial activity, which could destroy 17 bacteria and 10 fungi. Linalool and terpene compounds exhibit good antibacterial activity to *Escherichia coli*, *Staphylococcus*, *Saccharomycetes*, *Aspergillus niger*, *etc*. *α*-Terpineol is a fragrance ingredient used in decorative cosmetics, fragrances, shampoos, toilet soaps, and other toiletries, as well as in non-cosmetic products, such as household cleaners and detergents (Bhatia *et al.* 2008). *α*-Terpineol also has significant antibacterial effect on *E. coli* (Zhu *et al.* 2005). Through further experimentation, the author found that the α-terpineol and camphor can inhibit the wood-decaying fungus. Linalool has an antifungal effect against *Escherichia coli*, while it has no antifungal effect on wood-decay fungus.

Although the antibacterial and antifungal activities of extracts from many plant species has been extensively surveyed, their antimicrobial mechanism has not been reported in great detail. Most scholars think that some compounds possessing antibacterial properties include chemically diverse structure types such as terpenoids, alkaloids, flavones, glycosides, saponins, quinines, coumarins, stilbenes, esters, phenols, aldehydes, alcohols, sterids, organic acids, *etc*. Studies have shown that phenolic compounds influence the durability of natural wood, and that resin acids have a restraining effect on fungi (Ekeberg *et al.* 2006). The mechanism of antifungal activity of extracts from the xylem of *C. camphora* could be explained as some phenolic compounds attacking the cell wall and cell membrane, altering their function and structure, causing swelling and permeability augmentation (Mishra *et al.* 1991).

CONCLUSIONS

The chemical constituents of the extracts from the xylem of *C. camphora* by various solvent extractions were investigated. The toxicity of *C. camphora* extracts on wood-decaying fungi showed that chloroform extracts had the best antifungal effect on *C. versicolor* and methanol extracts possessed the greatest inhibition of hyphal growth *G. Trabeum* among these five extracts. GC/MS technology was used to analyze the chemical constituents of *C. camphora* xylem; the extracts contained abundant levels of camphor, *α*-terpineol, linalool, cineole, *etc*. Plants extracts meet consumer demand for safe products, and there is a potential market for using plant extracts as wood preservatives. In this paper, the authors found that methanol and chloroform can effectively extract natural biocides from *C. camphora* and these extracts can be impregnated into perishable wood,

rendering it resistant against wood-decaying fungus. *C. camphora* is a kind of biomass resource which could provide a renewable source for wood preservatives.

ACKNOWLEDGEMENTS

This research was supported by the National Spark Plan Project (No. 2012GA720001) and the State Forestry Administration Research and Public Service Industry of China (No. 200704010). The authors thank Dr. Hui Wu for his assistance with the English translation of this paper.

REFERENCES CITED

Alvarez-Castellanos, P. P., Bishop C. D., and Pascual-Villalobos M. J. (2001). "Antifungal activity of the essential oil of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens," *Phytochem.* 57(1), 99-102.

Bajpai, V. K., Shukla, S., and Kang, S. C. (2008). "Chemical composition and antifungal activity of essential oil and various extract of *Silene armeria* L," *Bioresource Technol.* 99(18), 8903-8908.

Baruah, A. K. S., Bhagat, S. D., Hazarika, J. N., and Saikia, B. K. (1975). "Examination of volatile oil of *Cinnamomum camphora* grown at Jorhat, Assam," *Indian Journal of Pharmacy* 37(2), 39-41+1 pl.

Bhardwaj, S., Singla, S., and Bhardwaj, R. (2012). "Evaluation of plant extracts as antifungal agents against wood rotting fungi *Coriolus versicolor* (L.: Fr.) Quelet," *J. Indian Acad. Wood Sci.* 9(1), 62-65.

Bhatia, S. P., Letizia, C. S., and Api, A. M. (2008). "Fragrance material review on alpha-terpineol," *Food Chem. Toxicol.* 46(11, Supplement), S280-S285.

Cardullo, M. A., and Gilroy, J. J. (1973). "Growth inhibition of *Escherichia coli* strain 82/r by d-camphor," *Can. J. Microbiol.* 19(8), 1015-1019.

Cheng, S.-S., Liu, J.-Y., Hsui, Y.-R., and Chang S.-T. (2006). "Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*)," *Bioresource Technol*. 97(2), 306-312.

Cheng, S.-S., Chang, H.-T., Chang, S.-T., Tsai, K.-H., and Chen, W.-J. (2003). "Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae," *Bioresource Technol*. 89(1), 99-102.

Chua, M.-T., Tung, Y.-T., and Chang, S.-T. (2008). "Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*," *Bioresource Technol*. 99(6), 1918-1925.

De Groot, R. C., and Woodward, B. (1999). "Using copper-tolerant fungi to biodegrade wood treated with copper-based preservatives," *Int. Biodeter. Biodegr.* 44(1), 17-27.

Ding, Y.-G., Ma, H.-M, and Zhang, B.-L. (2012). "Research on pharmacology, toxicology and safety of camphor: Review and prospect," *Chin. J. Pharmacovigilance* 9(1), 38-42.

Ekeberg, D., Flæte, P.-O., Eikenes, M., Fongen, M., and Naess-Andresen, C. F. (2006). "Qualitative and quantitative determination of extractives in heartwood of Scots pine (*Pinus sylvestris* L.) by gas chromatography," *J. Chromatogr. A* 1109(2), 267-272.

Hingston, J. A., Collins C. D., Murphy R. J., and Lester J. N. (2001). "Leaching of chromated copper arsenate wood preservatives: A review," *Environ. Pollut.* 111(1), 53-66.

Kamdem, D. P. (1994). "Fungal decay resistance of aspen blocks treated with heartwood extractives," *For. Prod. J.* 44(1), 30-32.

Kim, D.-I., Park J.-D., Kim S.-G., Kuk H., Jang M.-S., and Kim S.-S. (2005). "Screening of some crude plant extracts for their acaricidal and insecticidal efficacies," *J. Asia-Pac. Entomol.* 8(1), 93-100.

Lee, H. J., Hyun E.-A., Yoon W. J., Kim B. H., Rhee M. H., Kang H. K., Cho J. Y., and Yoo E. S. (2006). "In vitro anti-inflammatory and anti-oxidative effects of *Cinnamomum camphor*a extracts," *J. Ethnopharmacol.* 103(2), 208-216.

Liu, C. H., Mishra, A. K., Tan, R. X., Tang, C., Yang, H., and Shen, Y. F. (2006). "Repellent and insecticidal activities of essential oils from *Artemisia princeps* and Cinn*amomum camphora* and their effect on seed germination of wheat and broad bean," *Bioresource Technol.* 97(15), 1969-1973.

Minnick, P. D., Brown, C. M., Braselton, W. E., Meerdink, G. L., and Slanker, M. R. (1987). "The induction of equine laminitis with an aqueous extract of the heartwood of black walnut (*Juglans nigra*)," *Vet. Hum. Toxicol.* 29(3), 230-233.

Mishra, A. K., Dwivedi, S. K., Kishore, N., and Dubey, N. K. (1991). "Fungistatic properties of essential oil of *Cinnamomum camphora*," *Pharm. Biol.*29(4), 259-262.

Onuorah, E. (2000). "The wood preservative potentials of heartwood extracts of *Milicia excelsa* and *Erythrophleum suaveolens*," *Bioresource Technol.* 75(2), 171-173.

Onuorah, E. (2002). "Relative efficacy of heartwood extracts and proprietory wood preservatives as wood protectants," *J. Forest Resour.* 13(3), 183-190.

Pandey, A. K., Bora, H. R., Deka, S. C., Rastaji, R. C., and Banch, A. K. S. (1997). "Composition of the essential oil of the bark of *Cinnamomum camphora*," *J. Med. Aromatic Plant Sci.* 19(2), 408-409.

Pattnaik, S., Subramanyam, V. R., Bapaji, M., and Kole, C. R. (1997). "Antibacterial and antifungal activity of aromatic constituents of essential oils," *Microbios.* 89(358), 39-46.

Roszaini, K., Nor Azah, M. A., Mailina, J., Zaini, S., and Mohammad Faridz, Z. (2013). "Toxicity and antitermite activity of the essential oils from *Cinnamomum camphora, Cymbopogon nardus, Melaleuca cajuputi* and *Dipterocarpus* sp. against *Coptotermes curvignathus*," *Wood Sci. Technol.* 47(6), 1273-1284.

Schattner, P., and Randerson, D. (1996). "Tiger Balm as a treatment of tension headache. A clinical trial in general practice," *Aust. Fam. Physician* 25(2), 216, 218, 220.

Schultz, T. P., and Nicholas, D. D. (2000). "Naturally durable heartwood: Evidence for a proposed dual defensive function of the extractives," *Phytochem.* 54(1), 47-52.

Schultz, T. P. (2002). "Development of environmentally-benign wood preservatives based on the combination of organic biocides with antioxidants and metal chelators," *Phytochem.* 61(5), 555-560.

Schultz, T. P., Nicholas, D. D., and Preston, A. F. (2007). "A brief review of the past, present and future of wood preservation," *Pest Manag. Sci.* 63(8), 784-788.

Sun, C., Huang, K., Chen, C., and Zang, Y. (2007). "Analysis of chemical composition of volatile oil in *Cinnamomum camphora* leaves and branches by GC-MS," *Flavour Fragrance Cosmetics*, 1, 002.

Singh, P., Srivastava, B., Kumar, A., and Dubey, N. K. (2008). "Fungal contamination of raw materials of some herbal drugs and recommendation of *Cinnamomum camphora* oil as herbal fungitoxicant," *Microbial Ecol.* 56(3), 555-560.

Skočibušić, M., Bezić, N., and Dunkić, V. (2006). "Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis. growing in Croatia," *Food Chem.* 96(1), 20-28.

Walch, S. G., Kuballa, T., Stühlinger, W., and Lachenmeier, D. W. (2011). "Determination of the biologically active flavour substances thujone and camphor in foods and medicines containing sage (*Salvia officinalis* L.)," *CHEM. CENT J.* 5(1), 1-10.

Wang, S.-Y., Chen, P.-F., and Chang, S.-T. (2005). "Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi," *Bioresource Technol.* 96(7), 813-818.

Wu, C., Wu, Z., and Wu, Y. (2000). "Study on antibacterial activity of ethanol extract of *Cinamomum camphora*," *Amino Acids Biotic Resour.* 22(2), 41-42.

Wu, G. X., and Yang, X. L. (2005). "Evaluation for antifungal activity of exrtacts of 64 species of plants against phytopathogenic fungi in vitro," *J. Yangtze Univ. (Nat. Sci. Ed.)* 1(2), 77-82.

Yeh, R.-Y., Shiu, Y.-L., Shei, S.-C., Cheng, S.-C., Huang, S.-Y., Lin, J.-C., and Liu, C.-H. (2009). "Evaluation of the antibacterial activity of leaf and twig extracts of stout camphor tree, *Cinnamomum kanehirae*, and the effects on immunity and disease resistance of white shrimp, *Litopenaeus vannamei*," *Fish Shellfish Immun.* 27(1), 26-32.

Yu, T., Lee, S., Yang, W. S., Jang, H.-J., Lee, Y. J., Kim, T. W., Kim, S. Y., Lee, J., and Cho, J. Y. (2012). "The ability of an ethanol *Cinnamomum cassia* extract to inhibit Src and spleen tyrosine kinase activity contributes to its anti-inflammatory action," *J. Ethnopharmacol.* 139(2), 566-573.

Yu, H. Z., Sun, Y. L., Liao, X. Y., and Wang, H. Y. (2010). "Chemical composition, antibacterial and antifungal activities of ethanol extracts of *Cinnamomum camphora* (Linn.) presl seeds from Northwestern Hubei Province," *Southwest China Journal of Agricultural Sciences* 23(4), 1094-1098.

Zhu, S.,Yang, Y., Yu H., Ying Y., and Zou G. (2005). "Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*," *J. Ethnopharmacol.* 96(1-2), 151-158.

Article submitted: November 19, 2013; Peer review completed: January 19, 2014; Revised version received: February 26, 2013; Second revision received accepted: March 12, 2014; Published: March 24, 2014.