European Food Research and Technology (2018) 244:881–891
https://doi.org/10.1007/s00217-017-3006-z

ORIGINAL PAPER

Antimicrobial activities of bamboo (*Phyllostachys heterocycla cv. Pubescens*) leaf essential oil and its major components

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Received: 25 June 2017 / Revised: 18 October 2017 / Accepted: 4 November 2017 / Published online: 17 November 2017
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Abstract

This research aimed to investigate the seasonal variations and antibacterial activities of essential oils (EOs) and their major components from bamboo leaves. The *Phyllostachys heterocycla* cv. *Pubescens* leaves have undergone steam distillation to prepare EOs; leaves collected in autumn had the highest amount of EOs (0.33%). Chemical characterization by gas chromatography/mass spectrometry (GC/MS) allowed the identification of 36 compounds. The major compounds (%) were ketones (52.25), alcohols (17.79), carboxylic acids (9.41) and alkane hydrocarbons (7.68), which included 6, 5, 7 and 9 distinct compounds, respectively. Antibacterial activities were registered against Gram-negative bacteria [*Escherichia coli* (minimum inhibitory concentration [MIC] = 2.25 mg/mL)], Gram-positive bacteria [*Bacillus subtilis* (MIC = 2.25 mg/mL)] and *Saccharomyces cerevisiae*. The antibacterial effect of the three major monomeric compounds (tricosane, cedrol and hexadecanoic acid) in bamboo leaf EOs and their mixtures was studied. We showed for the first time that tricosane had antimicrobial activity. We found that cedrol had the strongest antibacterial effect, followed by hexadecanoic acid. Cedrol at 5 mg/mL had the largest inhibition zones of up to 14.93 mm against *Flavobacterium SHL45*. The antimicrobial effect of the equal mixture ratio of the three monomers was higher than that when tricosane and hexadecanoic acid were tested alone, and was also higher than that of the combination of any two of them.

Keywords Antibacterial activity · *Phyllostachys heterocycla* cv. *Pubescens* leaves · Essential oils · Chemical components

Introduction

Bamboo is one of the most valuable naturally occurring plants worldwide due to its different edible parts that contain important nutrients [19]. Bamboo leaves have been used in Traditional Chinese Medicine for fever treatment and detoxification for over 1000 years [21]. Many studies have also indicated that bamboo leaves have multiple biological effects, such as anti-free radical, antioxidation, anti-aging, and prevention of cardiovascular diseases [21, 22]. There are more than 1250 bamboo species all over the world and China is a country with one of the most abundant bamboo resources [10]. *Phyllostachys heterocycla* cv. *Pubescens* accounts for about 70% of the bamboo in South China.

One of the main functional components in bamboo leaves is EOs [7]. Numerous studies have demonstrated that EOs obtained from bamboo leaves have antioxidant [16, 17, 21], antimicrobial [26, 28, 33] and antibacterial function [12]. EOs are used in different fields, such as the food beverage, perfumery, industries, to enhance the bouquet flavor, fragrance compositions and pharmaceutical industries [26].

Different seasonal environmental parameters, such as temperature, rainfall, relative humidity, and total sun exposure as well as the different stages of plant metabolism, have a significant effect on the leaf surface, especially of species that have histological structures for the storage of EOs [11]. Meanwhile, due to the variation tendency of EOs' yield, it is important to optimize the harvesting time of bamboo leaves. In addition, the antimicrobial activities of EOs from plants have been proven to be dependent on the chemical constitutions of the oils [3]. The antimicrobial activity of EOs from *P. heterocycla* cv. *Pubescens* leaves against Gram-positive

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(*Staphylococcus aureus*) and Gram-negative model bacteria (*Escherichia coli*) has been studied [12], but antibacterial activities of EOs and their major components from *P. heterocycla* cv. *Pubescens* leaves have not been tested against a wide range of food-related microorganisms, such as *Flavobacterium, Pseudomonas fluorescens, E. coli, Bacillus subtilis* and *Saccharomyces cerevisiae* [2, 15, 23, 27, 30]. The combination of essential components from EOs may lead to an additive or synergistic effect on the antimicrobial activity [5, 24]. However, research on the interaction between major components in bamboo leaf EOs is still scarce.

EOs from the *P. heterocycla* cv. *Pubescens* leaves were obtained by steam distillation with a modified device [18, 21, 25]. In this study, we determined the effect of seasonal variations on yield, the chemical composition of *P. heterocycla* cv. *Pubescens* leaf EOs using GC/MS and evaluated the antimicrobial activities of EOs and their dominant components against three types of microorganisms [Gram positive: *B. subtilis; Flavobacterium* (*SHL45*). Gram negative: *E. coli; P. fluorescens* (*SHL5, SHL7*). Yeast: *S. cerevisiae* (*Ja64, Tokay, Y-8*)].

Materials and methods

Samples and sample preparation

Materials

The fresh *P. heterocycla* cv. *Pubescens* leaves from *Dendrocalamopsis oldhamii* were collected during different seasons (September and December 2009, March, and June 2010) in Nanping City, Fujian Province, China. The leaf samples of this species were sent to the College of Biological Sciences and Biotechnology of Beijing Forestry University for precise identification. The fresh bamboo leaves were dried in the shade at room temperature, cut into small pieces in 40 mesh, and stored at 4 °C until extraction.

Chemicals

All reagents used in the experiment were of either analytical grade or chromatographic grade. Nonyl acetate (purity 99%, CAS: 143-13-5) was bought from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Tricosane (purity 99%, CAS: 638-67-5), hexadecanoic acid (purity 95%, CAS: 57-10-3) and cedrol (purity 99%, CAS: 77-53-2) were purchased from Sigma Chemical Co. (St. Louis, MO., USA).

Isolation of EOs

The bamboo leaves from different seasons were ground and extracted in a modified EO extraction device described by Lv et al [20]. The bamboo leaf powder (20 g) was subjected to hydrodistillation (liquid:solid = 35:1) for 4.5 h. During the heating, the water steam containing essential oil was condensed. Then the water passed through the *n*-hexane layer and the essential oil existing in the water was extracted by *n*-hexane. The water with no oil returned back to flask. At last, *n*-hexane were collected and dried over anhydrous sodium sulfate. Then the solution was removed using a rotary evaporator ($T < 40$ °C) under vacuum, leaving behind the EOs. To accurately calculate the yield of essential oils, the extraction process was repeated three times and the results were averaged. The obtained EOs were stored at 4 °C. EO yield was calculated using following equation:

$$\text{Yield of EOs } (\%) = M_{\text{EOs}}(\text{g}) \big/ M_{\text{bl}}(\text{g}) \times 100\%, \quad (1)$$

where M_{EOs} is the mass of obtained EOs, M_{bl} is the mass of dry powder of bamboo leaves. Further analysis was performed only on EOs from the season with the highest EO yield.

Analysis of EOs by GC–MS

GC–MS

The components of the EO samples were identified by GC–MS analyses (GC-MS-QP5050A, Shimadzu, Japan). The GC was equipped with a DB-5MS capillary column (0.25 mm × 30 m × 0.25 µm). The injector temperature was set at 250 °C, and the injection volume was 1.5 µL in the split mode with a 10:1 split. The oven temperature for GC analysis was initially held at 50 °C, and raised to 250 °C at 6 ℃/min, and finally to 280 ℃ at 15 ℃/min with a 10 min final hold period. The total run time was 55 min. The interface temperature was at 250 °C. The MS was scanned from m/z 30 to 500 and started its run from 10 min after the injection to the end. The election impact ionization (EI) was at 70 eV. The components of the EO were identified by comparing their mass spectral fragmentation patterns with those of similar compounds from databases (NIST11 and Wiley Mass Spectral Libraries). For each compound on the GC/MS, the percentage of peak area relative to the total peak area of all compounds was determined and reported as relative amount of that compound.

Internal standard quantity analysis of seven main compounds

The response factors were determined using quantitative standards with 0.4 µL of nonyl acetate as internal standard

in 1 mg/mL EO solution. Nonyl acetate was added based on common concentration of major compounds that occur in EO.

Antimicrobial activity assays

Microbial strains

The tests were performed against two Gram-positive, three Gram-negative bacteria and three Yeast. Gram positive: *B. subtilis* CICC 20034; *Flavobacterium SHL45* CICC 51823. Gram negative: *E. coli* CICC 23845; *P. fluorescens* (*SHL5* CICC 20066, *SHL7* CICC 21620). Yeast: *S. cerevisiae* (*Ja64* CICC 1346; *Tokay* CICC 1388; *Y-8* CICC 1390). All strains were obtained from the lab of College of Biological Sciences and Biotechnology of Beijing Forestry University. The cultures were maintained on slants of nutrient agar (NA) for bacteria and yeast peptone dextrose agar (YPDA) for yeast (Table 1). All microorganisms were stocked at − 6 °C in appropriate conditions and regenerated twice before use in the manipulations.

Double-plate punching method for antimicrobial tests

Double-plate punching method was used for the antimicrobial activity of the EO from the highest yield season and different concentrations (2.5, 5, 10 and 20 mg/mL) of the selected components (cedrol, tricosane and hexadecanoic acid) and the other mixed solutions. Cultures were prepared as follows. In brief, about 10–15 mL aseptic agar solutions were first evenly poured into sterile petri dishes (90 mm in diameter) and after its solidification, 20 mL mixed solutions of the bacteria suspension with the concentration of 10^8–10^9 CFU/mL and the respective culture medium were perfused on the second floor. A plastic straw with the diameter of 5 mm was then inserted without piercing the agar surface for the tested samples. After sterilized under UV radiation at 300 nm for 5 min, filter paper discs (9 mm in diameter) individually impregnated with 30 μL of EO and other sample solutions were placed on the inoculated plates for 2 h at 4 °C and then incubated at the conditions described in Table 1. The diameter of inhibition zones was measured in millimeters. This antimicrobial test was carried out duplicate.

Determination of minimum inhibitory concentration (MIC)

36 mg/mL bamboo leaves' EO was diluted for 2, 4, 8, 16 times with half dilution method. Serial dilutions of EO were prepared with liquid nutrient media and spread in petri dishes. The dish was left to cool down and to solidify at room temperature for 30 min. The plates were spotted, and then inoculated with 2 μL of bacterial strains (8 × 106 cells/mL) or yeast (5 × 105 spores/mL). *N*-Hexane was set as the blank solution. These plates were incubated at 30 °C for 24 h for bacteria and for 48 h for yeast. The MIC is defined as the lowest concentration of the EO at which the microorganism does not demonstrate visible growth. Tests were carried out in duplicate.

Statistical analysis

The OriginPro system (v8.5 SR6, OriginLab Corporation, Northampton, MA, USA) was used for statistical analysis. Statgraphics Plus 5.1 and multiple comparisons were used to estimate the significant differences among the mean values at a 5% probability level. Results are expressed as the average ± standard deviation (SD).

Results and discussion

Content of EOs from bamboo leaves in different seasons

The modified EOs extraction device was used to extract the *P. heterocycla* cv. *Pubescens* leaves obtained from four seasons. Figure 1 shows the levels of EOs from *P. heterocycla* cv. *Pubescens* leaves in four seasons. The yields of autumn, winter, spring, and summer analysis were, respectively, as follows: 0.33, 0.25, 0.29, and 0.25%. Therefore, it can be inferred that the *P. heterocycla* cv. *Pubescens* leaves are relatively mature in autumn. EOs' yields of *P. heterocycla* cv.

Table 1 Culture media and maintenance of yeast and bacteria species

Microorganism	Culture media	Incubation temperature ± 2 (℃)	Duration of incubation (h)
Yeast			
Saccharomyces cerevisiae (*Ja64, Tokay, Y-8*)	YPDA	28	48
Bacteria			
Escherichia coli	NA	37	24
Bacillus subtilis	NA	37	24
Pseudomonas fluorescens (*SHL5, SHL7*)	NA	25	48
Flavobacterium (*SHL45*)	NA	25	48

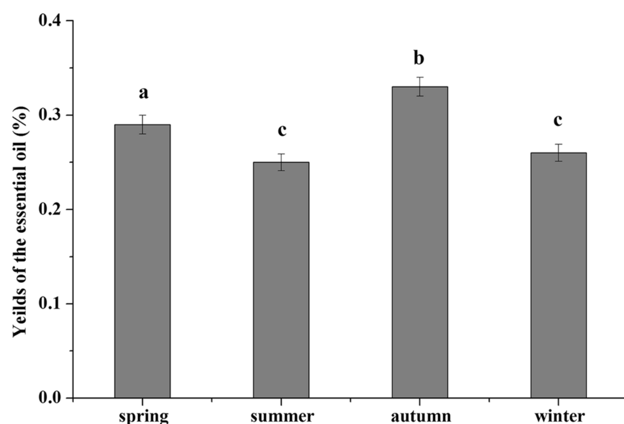

Fig. 1 Yields of EOs from *P. heterocycla* cv. *Pubescens leaves* in four seasons

Pubescens leaves were affected by seasonal variation, which was consistent with the previous results [4, 31].

Chemical composition of EOs

GC/MS was used to analyze the chemical components of EO obtained from *P. heterocycla* cv. *Pubescens* leaves in the season (autumn) of the highest EO yield. After each peak of the total ion current mapping was scanned, the mass spectrum was obtained and the relative contents of the constituents of the EO were determined by the area normalization method. Information of the 36 detected constituents from EO of *P. heterocycla* cv. *Pubescens* leaves is listed in Table 1.

The compositions of EO (in percentage) were obtained by the GC/MS analysis method. The classifications of the 36 identified components in EO are summarized in Table 2. EO consisted mainly of ketones (52.25%), followed by alcohols (17.79%), carboxylic acids (9.41%) and alkane hydrocarbons (7.68%). Among the 36 identified compounds, six were ketones, five were alcohols, seven were carboxylic acids, and nine were alkanes. Besides the four major compounds, EO also contained a few other compounds (3%, aldehyde, ester, etc.). Among the six identified ketones, 6,10-dimethyl-5,9-laurylene-2-ketone, 6,10,14-trimethyl-2-pentadecanone and 6-heptyltetrahydro-2H-pyran-2-ketone constituted 53% of the EO. The contents of 6,10,14-trimethyl-2-pentadecanone were relatively abundant (33.65%) and the contents of 6,10-dimethyl-5,9-laurylene-2-ketone and 6-heptyltetrahydro-2H-Pyran-2-ketone were 15.57 and 3.78%, respectively. Of the five identified alcohols, cedrol and α-eudesmol were relatively abundant compared with other alcohols. Among the seven carboxylic acids and nine alkanes, hexadecanoic acid and tricosane were relatively abundant. The ketone, carboxylic acid, alkane, aldehyde, and ester components have been reported to show great contribution to the antioxidant activities of the EOs [9, 16, 17].

Quantification of the seven main compounds' content

The activities of EOs are dependent on the constituent purified compounds that can inhibit the growth of a wide variety of microorganisms [4, 31]. We selected the seven main compounds for the further study based on the highest concentration, including 6,10-dimethyl-5,9-laurylene-2-ketone, 6,10,14-trimethyl-2-pentadecanone, 6-heptyltetrahydro-2H-pyran-2-ketone, cedrol, α-eudesmol, hexadecanoic acid and tricosane.

Figure 2 showed the seasonal tendency of the seven components of EOs from the *P. heterocycla* cv. *Pubescens*. As indicated, 6,10-dimethyl-5,9-laurylene-2-ketone and cedrol followed the yield variation tendency of EOs in different seasons; their contents reached the highest in autumn and the lowest in summer and winter, while the rest did not follow the variation tendency.

Different patterns of variation were observed in these seven main components from each season. Ketone compound 6,10,14-trimethyl-2-pentadecanone had the highest concentration among the seven compounds. It reached the highest in spring and decreased gradually from spring to winter (from 440 to 250 mg/g). However, the other six components were all below 150 mg/g and did not vary as much as 6,10,14-trimethyl-2-pentadecanone. Additionally, it has been reported as an active substance in the study of constituents of bamboo leaf EOs [8, 29, 32] and plays a significant role in regulating the growth of the *P. heterocycla* cv. *Pubescens* [29]. Ketone compound 6,10-dimethyl-5,9-laurylene-2-ketone reached its highest concentration in autumn, up to 140 mg/g, while ketone compound 6-heptyltetrahydro-2H-pyran-2-ketone reached the highest concentration in summer (40 mg/g) because it is predominant during breeding or high metabolic activities such as flowering and fructification.

Figure 2 also shows the trends of the two alcohols, cedrol and α-eudesmol. The concentrations of cedrol in spring and autumn were both 54 mg/g which is twofold higher than that in summer and winter, since the plants needed more cedrol in spring and autumn. However, α-eudesmol content increased gradually from spring (82 mg/g) to winter (106 mg/g).

The concentration of hexadecanoic acid was higher in autumn and winter, approximately 65 mg/g, about twice as much as spring's and summer's. We speculated that hexadecanoic acid is detrimental to plant growth, due to its decreased concentration in spring and summer. Tricosane reached the highest concentration in spring (57 mg/g), about a twofold increase to its summer concentration. Many pathways must be considered to explain seasonal variations for volatile compounds, such as metabolism, catabolism, and biosynthesis. Some variations may be due to their importance in flowering and resistance to environmental stresses

Table 2 Chemical composition of *P. heterocycla* cv. *Pubescens* leaf EOs from the season of autumn

Compounds species	Total content (%)	Amt.	Name of components	Molecular formula	Molecular weight	Area (%)	Retention index (RI)
Ketone	54.42	6	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	$C_{13}H_{20}O$	192	0.49	1457
			6,10-Dimethyl-5,9-Undecadien-2-one	$C_{13}H_{22}O$	194	15.57	1420
			1,1a,5,6,7,8-Hexahydro-4a,8,8-trimethyl-cyclopropa[d]naphthalen-2(4aH)-one	$C_{14}H_{20}O$	204	0.40	1497
			6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268	33.65	1754
			6,10,14-Trimethyl-5,9,13-pentadecatrien-2-one	$C_{18}H_{30}O$	262	0.53	1902
			6-Heptyltetrahydro-2H-pyran-2-one	$C_{12}H_{22}O_2$	198	3.78	1602
Alcohol	18.54	5	Cedrol	$C_{15}H_{26}O$	222	5.15	1543
			β-Eudesmol	$C_{15}H_{26}O$	222	10.54	1593
			α-Eudesmol	$C_{15}H_{26}O$	222	0.41	1598
			2-Ethyl-1-dodecyl alcohol	$C_{14}H_{30}O$	214	2.04	1591
			1-Octadecanol	$C_{18}H_{38}O$	270	0.40	2053
Carboxylic acid	9.79	7	Octanoic acid	$C_8H_{16}O_2$	144	0.59	1173
			n-Nonylic acid	$C_9H_{18}O_2$	158	0.62	1272
			Myristic acid	$C_{14}H_{28}O_2$	228	0.61	1769
			Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.30	1615
			Heptadecanoic acid	$C_{17}H_{34}O_2$	270	0.49	2067
			Oleic acid	$C_{18}H_{34}O_2$	282	0.42	2175
			Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.76	1104
Alkane	8	9	Eicosane	$C_{20}H_{42}$	282	0.49	2009
			10-Methyl-eicosane	$C_{21}H_{44}$	296	0.55	2045
			Tricosane	$C_{23}H_{48}$	324	2.67	2307
			Hexacosane	$C_{26}H_{54}$	366	0.42	2606
			Heptacosane	$C_{27}H_{56}$	380	0.35	2705
			Octacosane	$C_{28}H_{58}$	394	0.34	2804
			Triacontane	$C_{30}H_{62}$	422	2.01	3003
			Pentatriacontane	$C_{35}H_{72}$	492	0.41	3500
			Hexatriacontane	$C_{36}H_{74}$	506	0.76	3600
Aldehyde	0.81	1	*n*-Nonyl aldehyde	$C_9H_{18}O$	142	0.81	1104
Ester	0.94	1	Terpinyl acetate	$C_{22}H_{32}O_2$	328	0.94	1348
Others	7.50	7	4-Hydroxy-2-methyl acetophenone	$C_9H_{10}O_2$	150	1.71	1363
			4,5,7,7a-Tetrahydro-4,4,7a-trimethyl-2(6H)-benzofuranone	$C_{11}H_{16}O_2$	180	0.98	1426
			1-Octadecyne	$C_{18}H_{34}$	250	0.68	1828
			Decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methyl-2,4–pentadienyl)-naphthalene	$C_{20}H_{32}$	272	0.94	1909
			9-Eicosyne	$C_{20}H_{38}$	278	1.06	2027
			9-Octadecenal	$C_{18}H_{34}O$	266	1.73	2007
			1-Docosene	$C_{22}H_{44}$	308	0.40	2198

Area = percentage of total peak area

such as heat or drought. Furthermore, during the development stage of plants, the generation of secondary metabolites can be affected by radiation, temperature, humidity, and other factors that regulate seasonal variation [4].

Since EOs mainly contained ketones, alcohols, carboxylic acids, and alkane hydrocarbons, we speculated that these compounds were responsible for the antimicrobial activity of EOs. Therefore, we chose cedrol, hexadecanoic acid and

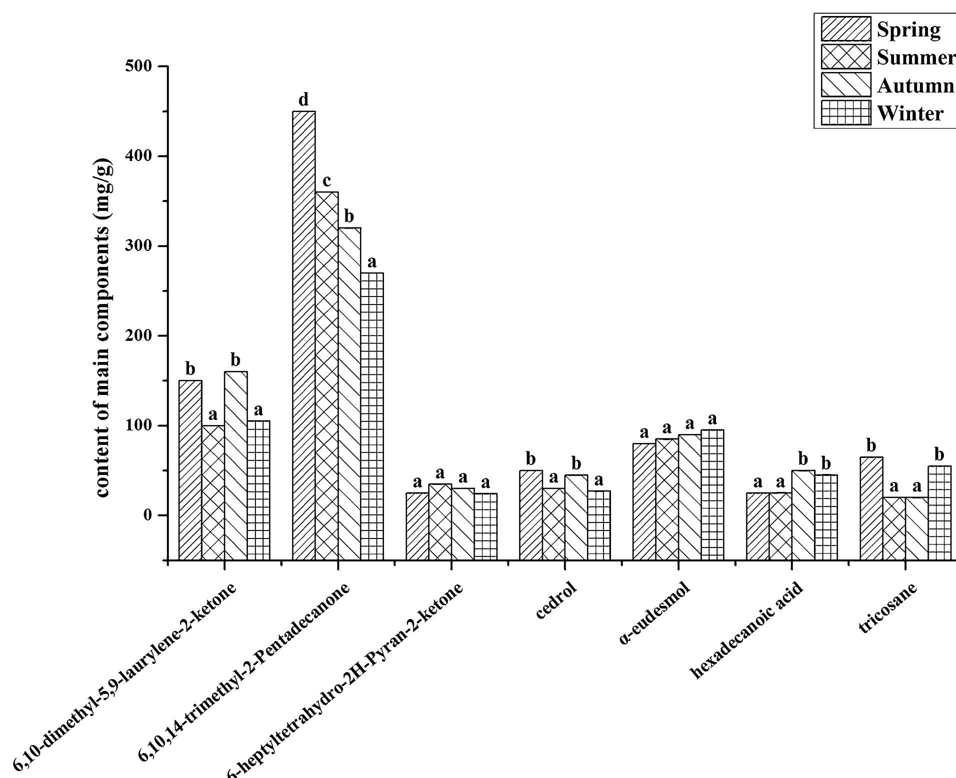

Fig. 2 Contents of seven main components from *P. heterocycla* cv. *Pubescens* leaves in four seasons

tricosane, major components of EOs, to test against three types of microorganisms.

Antimicrobial activity

Antimicrobial activity of EOs

The primary screening of antimicrobial activity by the double-plate punching method showed that bamboo leaf EOs were active against all microorganisms with inhibition zones ranging from 6.98 to 20.14 mm. The results are shown in Table 3, indicating that the EOs displayed a variable degree of antimicrobial activities against the eight tested strains.

EOs of bamboo leaves had different antimicrobial activity against different bacteria (Table 3). It had the strongest bacteriostatic effect on *Flavobacterium SHL45* ($p < 0.05$), followed by *P. fluorescens SHL5, SHL7* and *B. subtilis* ($p < 0.05$). Compared to the strains above, EOs had a significantly weaker inhibitory effect against *E. coli* and *S. cerevisiae Tokay* and the weakest antimicrobial activity on *S. cerevisiae Y-8* and *Ja64* ($p < 0.05$).

The MIC values of EO ranged from 2.25 to 36.0 mg/mL. Bamboo leaf EOs had a strong inhibitory effect on *E. coli* and *B. subtilis* and responded to increasing concentrations of EOs (Table 4). Thus, this indicates that the bamboo leaf EOs in relatively low concentrations have an apparent bacteriostatic action. The determination of the relationship between EOs' chemical composition and the registered MICs from the obtained results highlighted that MICs of different harvest time of bamboo leaves were correlated with the presence of various components [4]. Further investigations are required to identify the mechanisms of how each active component acts on the identified sensitive strains.

Table 3 Antimicrobial activity (diameter of inhibition) of *P. heterocycla* cv. *Pubescens* leaf EOs

Experiment bacterium	Diameter of inhibition ± SD
Gram-negative bacteria	
Escherichia coli	9.4 ± 0.05^{c}
Pseudomonas fluorescens SHL5	13.0 ± 0.06^{b}
Pseudomonas fluorescens SHL7	13.3 ± 0.05^{b}
Gram-positive bacteria	
Flavobacterium SHL45	20.0 ± 0.14^{a}
Bacillus subtilis	13.0 ± 0.51^{b}
Saccharomyces cerevisiae	
Tokay	$8.3 \pm 0.06^{c,d}$
Y-8	7.0 ± 0.02^{d}
Ja64	7.6 ± 0.06^{d}

^{a,b,c,d}indicates statistically significant difference between the means ($p < 0.05$)

Table 4 Minimal inhibitory concentration (MIC, mg/mL) of *P. heterocycla* cv. *Pubescens* leaf EOs against *E. coli* and *Bacillus subtilis*

Concentration of EO (mg/mL)	Diameter of inhibition ± SD (mm)	
	E. coli	*Bacillus subtilis*
36.00	12.0 ± 0.40	12.3 ± 0.14
18.00	9.25 ± 0.17	10.5 ± 0.10
9.00	6.75 ± 0.05	8.0 ± 0.06
4.50	6.0 ± 0.08	6.3 ± 0.12
2.25	5.0	5.0

Antimicrobial activity of three major components of bamboo essential oil

With the double-plate punching method, the three tested compounds (tricosane, cedrol and hexadecanoic acid) showed various degrees of inhibition against the eight bacterial strains (Fig. 3). The zone of inhibition was significantly different ($p < 0.05$) with different concentrations of each of these compounds.

At a concentration of 20 mg/mL, tricosane was active against all microorganisms, with inhibition zones ranging from 8.03 to 15.97 mm. Tricosane showed a concentration-related increase in inhibition zones against *P. fluorescens SHL7, E. coli, B. subtilis, S. cerevisiae Ja-64 and Y-8. Bacillus subtilis* was the most sensitive strain with the largest inhibition zones (7.20–15.97 mm; Fig. 3). Alkane compounds account for 15.46% of all the compounds in bamboo leaf EOs. While the antimicrobial effect of tricosane has not been reported in the past, the results of this experiment showed that tricosane has antimicrobial effects within a certain range of concentration, which is significant for the adequate development and utilization of waste bamboo leave resources.

Cedrol showed weak activity against *S. cerevisiae Y-8*, and in fact, no inhibition zone was detected (Fig. 3). Among the three compounds, cedrol was the most effective against *Flavobacterium SHL45, P. fluorescens SHL5, SHL7, E. coli* and *S. cerevisiae Ja-64*. In the antimicrobial tests, bamboo leaf EOs had the strongest bacteriostatic effect on *Flavobacterium SHL45* ($p < 0.05$), so it was likely that cedrol was the largest contributor to antibacterial activity of bamboo leaf EOs. Moreover, except *Flavobacterium SHL45* and *P. fluorescens SHL7*, cedrol showed a concentration-related increase in inhibition zone against other kinds of bacteria. The results were consistent with

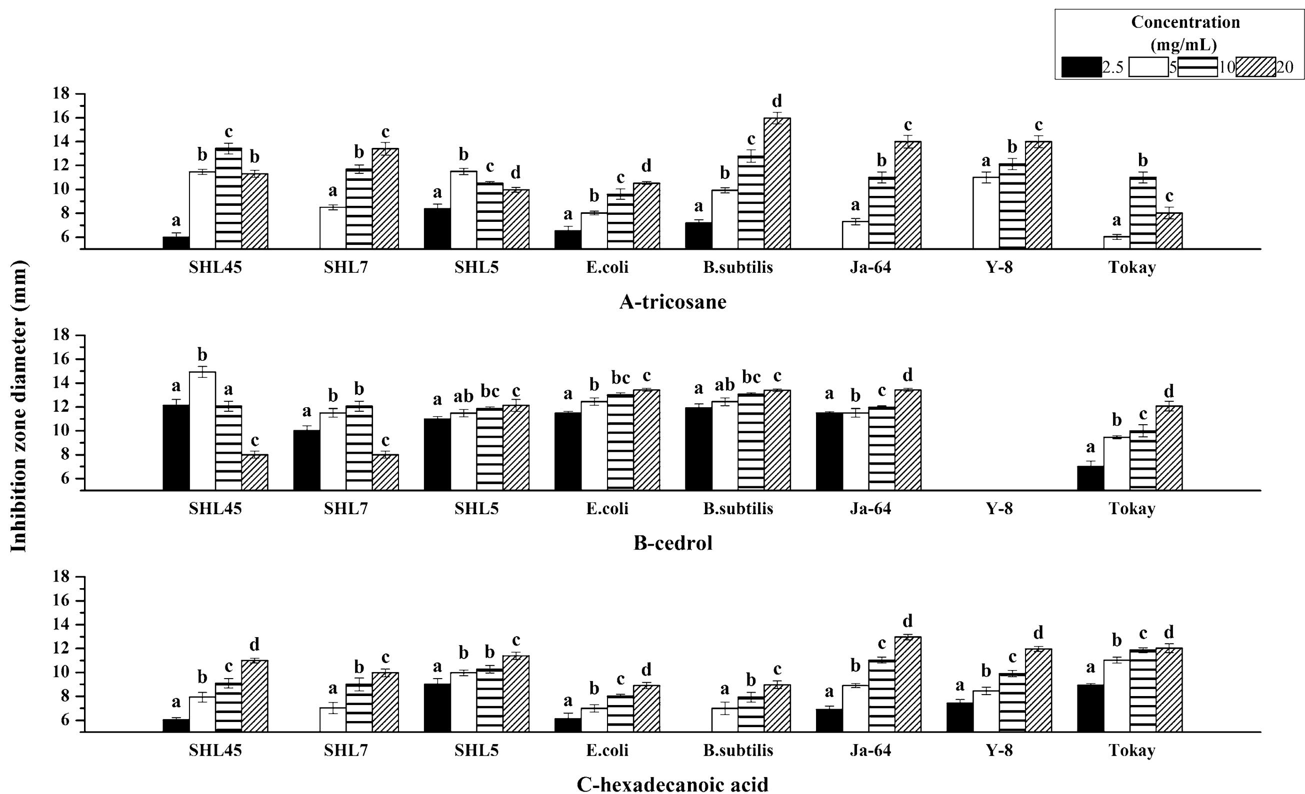

Fig. 3 Antibacterial effect of tricosane, cedrol and hexadecanoic acid against eight tested strains (SHL45—*Flavobacterium SHL45*, SHL7—*Pseudomonas fluorescens SHL7*, SHL5—*Pseudomonas fluorescens SHL5, E. coli—Escherichia coli, B. subtilis—Bacillus subtilis, Ja-64—Saccharomyces cerevisiae Ja-64, Y-8—Saccharomyces cerevisiae Y-8, Tokay—Saccharomyces cerevisiae Tokay*)

previous reports [1, 31] that demonstrated that terpene alcohols in plants had promising antibacterial activity.

Hexadecanoic acid showed a concentration-related increase in inhibition zone against all eight tested strains. Compared with the bacteria, it revealed stronger antibacterial activities on the yeast and *S. cerevisiae Ja-64* was the most sensitive strain. Antibacterial activity of compounds was related to the amount and type of unsaturated bonds, so compounds which contained unsaturated double bonds, like alcohols, aldehydes, ketones, acids, and terpenes, might play an important role in antibacterial effects. Hexadecanoic acid is a typical carboxylic acid and its structure and strong antimicrobial activity have also been reported by previous studies [13].

Combined antimicrobial activity of three major components

As shown in Fig. 4, the mixed tricosane and cedrol was more effective against *Flavobacterium SHL45, P. fluorescens SHL5, SHL7, B. subtilis* and *S. cerevisiae Ja-64*. Among them, *Flavobacterium SHL45, P. fluorescens SHL5, SHL7* were especially sensitive to the mixed solution, with inhibition zones in the range of 8.93–13.00 mm. The mixed tricosane and cedrol showed relatively weak activity against *E. coli, S. cerevisiae Y-8 and Tokay*, with inhibition zones in the range of 6.03–11.57 mm. The antibacterial effect of the mixed solution was distinctly better than that of tricosane alone, and slightly less than that of cedrol alone. It can tentatively be inferred that cedrol may play a synergistic role in the antibacterial effect of tricosane, whereas tricosane did not show synergistic effects on that of cedrol. In addition, the content of tricosane and cedrol reached the highest in spring, so it is possible that the EOs in spring was the most potent inhibitor against eight tested bacteria, especially for *Flavobacterium SHL45* and *P. fluorescens SHL5, SHL7*.

Flavobacterium SHL45, P. fluorescens SHL7 and *S. cerevisiae Ja-64* were more susceptible to the mixed tricosane and hexadecanoic acid, the maximal inhibition zones reached 12.07 mm. The antibacterial effect of mixed solution of two monomers was better than that of tricosane alone and slightly less than hexadecanoic acid alone. Therefore, we speculate that hexadecanoic acid may play a synergistic role in the antibacterial effect of tricosane, while tricosane

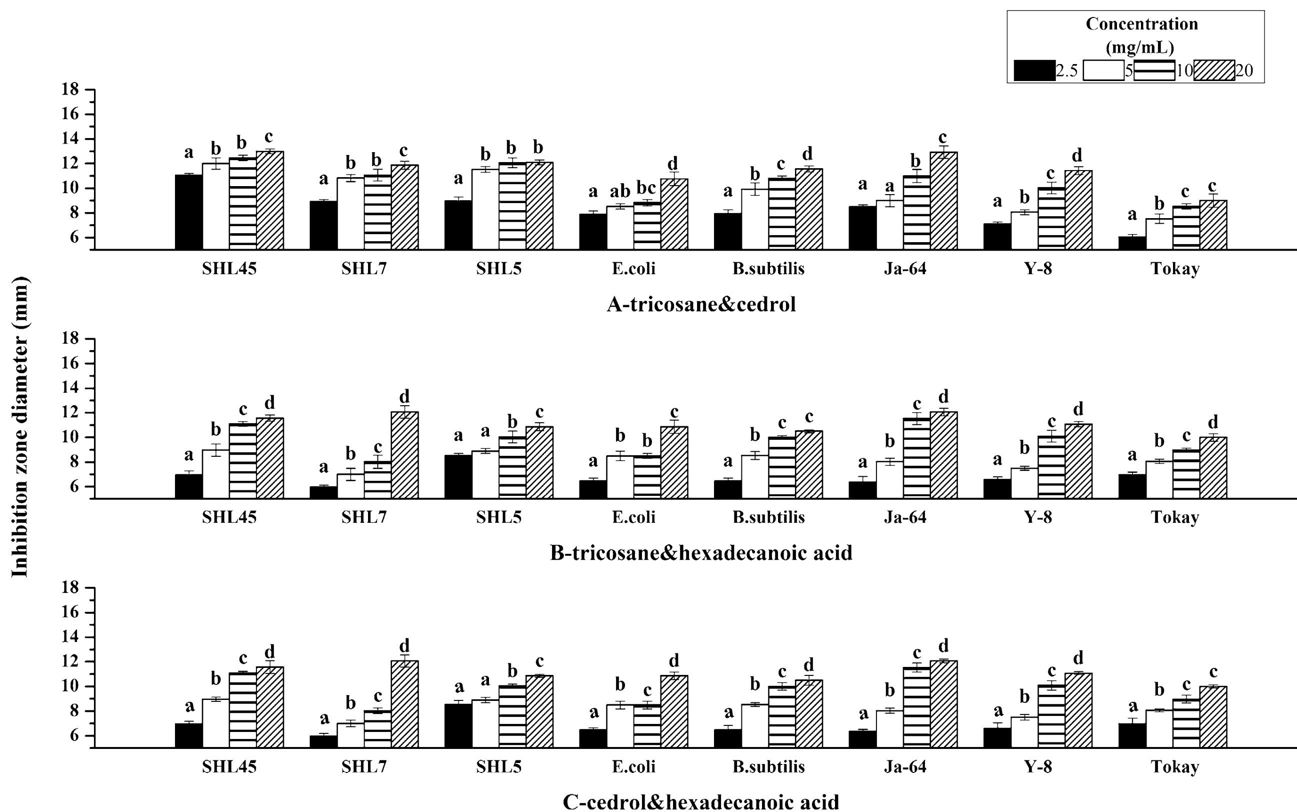

Fig. 4 Antibacterial effect of mixed tricosane and cedrol, mixed tricosane and hexadecanoic acid, mixed cedrol and hexadecanoic acid on eight tested strains (SHL45—*Flavobacterium SHL45*, SHL7—*Pseudomonas fluorescens SHL7*, SHL5—*Pseudomonas fluorescens SHL5, E. coli—Escherichia coli, B. subtilis—Bacillus subtilis, Ja-64—Saccharomyces cerevisiae Ja-64, Y-8—Saccharomyces cerevisiae Y-8, Tokay—Saccharomyces cerevisiae Tokay*)

did not show synergistic effects on antibacterial activity of hexadecanoic acid. In addition, the contents of tricosane and hexadecanoic acid were higher in winter, so it is likely that antibacterial activity of EOs in winter was strong against *Flavobacterium SHL45, P. fluorescens SHL7* and *S. cerevisiae Ja-64*.

Although less activity of mixed cedrol and hexadecanoic acid was observed against *S. cerevisiae Y-8* and *Tokay*, with inhibition zones in the range of 5.93–11.50 mm, the antibacterial effect of the two monomers combined was higher than hexadecanoic acid alone and slightly less than cedrol alone. We speculate that cedrol may play a synergistic role in the antibacterial effect of hexadecanoic acid, whereas hexadecanoic acid did not show synergistic effects on antibacterial activity of cedrol. In addition, the content of cedrol and hexadecanoic acid was higher in autumn, so it can be inferred that antibacterial activity of autumn's EOs was greater against most strains except *S. cerevisiae Y-8*.

The mixed solution of three compounds showed a concentration-related increase in inhibition zone against all eight tested strains (Fig. 5). The mixed solution of tricosane, cedrol and hexadecanoic acid showed strong antibacterial activity against *Flavobacterium SHL45, P. fluorescens, SHL7, SHL5, E. coli, B. subtilis and S. cerevisiae Ja-64*, while the effect against *Y-8* and *Tokay* was weak. The antimicrobial spectrum of mixed solution was broader than cedrol. The antibacterial efficacy of the mixed solution was better than not only that of the three compounds alone, but also better than the mixtures of any two of them. Therefore, it can be concluded that the combination of cedrol, tricosane and hexadecanoic acid may have an additive or synergistic effect.Previous studies have found that the bacteriostatic effect of one volatile oil varies from microorganism to microorganism [14]. Similarly, in this experiment, the three compounds we tested showed different antibacterial efficacy on different microbial strains. In addition, we also found that the antibacterial activity of different compounds within EOs from bamboo leaves on the same strain also varies. The result is consistent with previous studies which were mentioned in the research by He [7]. Some single components alone and the mixture of them in EOs from bamboo leaves present antibacterial activity on microorganism. Thus, it can be inferred that the antimicrobial activity of EOs is the result of all antibacterial ingredients and the additive or synergistic effects therein [6, 34].

Conclusion

This study demonstrates that seasonal variations induced the different EO chemical compositions that may lead to differing antimicrobial activities. The EOs obtained from the *P. heterocycla* cv. *Pubescens* leaves were significantly different in four seasons. The amount was the highest in autumn, which achieved 0.33% yield, and the lowest in summer and winter with 0.25%. A total of 36 compounds were identified, the major components of *P. heterocycla* cv. *Pubescens* leaf EOs were tricosane, cedrol and hexadecanoic acid. EO had strong antimicrobial activity against the Gram-positive bacteria and Gram-negative bacteria [Gram positive: *B. subtilis*. Gram negative: *E. coli; P. fluorescens* (*SHL5, SHL7*); *Flavobacterium* (*SHL45*)], but weak inhibition against yeast [Yeast: *S. cerevisiae* (*Ja64, Tokay, Y-8*)]. Meanwhile, the three major monomeric compounds (tricosane, cedrol and

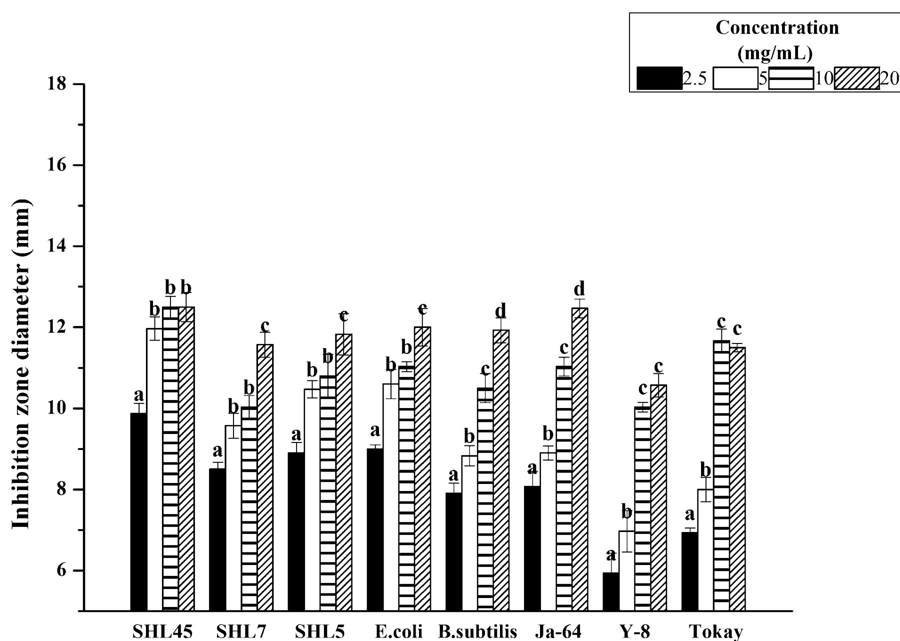

Fig. 5 Antibacterial effect of mixed tricosane, cedrol and hexadecanoic acid on eight tested strains (SHL45—*Flavobacterium* SHL45, SHL7—*Pseudomonas fluorescens* SHL7, SHL5—*Pseudomonas fluorescens* SHL5, *E. coli—Escherichia coli, B. subtilis—Bacillus subtilis, Ja-64—Saccharomyces cerevisiae Ja-64, Y-8—Saccharomyces cerevisiae Y-8, Tokay—Saccharomyces cerevisiae Tokay*)

hexadecanoic acid) were also effective against the tested bacteria strains and had additive or synergistic effects when combined. The results we have obtained confirmed the possibility of using bamboo leaf EOs as a potent antibacterial source and lay the foundation for further research on their utilization in the field of food safety.

Acknowledgements The authors wish to acknowledge the support from the Science and technology research project of Beijing Forestry University (2015-01) and "National Science-technology Support Plan Projects, research and development of botanical novel green preservative" (Project number: 2015BAD16B01).

Author contributions J. Wu analyzed data, drafted the manuscript and revised it critically for important intellectual content. ZL. Lv designed the study and supervised the research group. Y. Liu collected test data. M. Liu carried out experiments. C. Tao interpreted the results.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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