竹叶挥发油化学成分及其抗氧化特性*

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摘 要:采用水蒸气蒸馏法从黄金间碧竹、孝顺竹、毛竹和麻竹4种竹叶中提取挥发油,用气相色谱-质谱联用技术对4种竹叶挥发油化学成分进行分析和鉴定,共获得168个色谱峰,鉴定其中132种化学成分,并运用气相色谱面积归一化法确定各组分的相对含量;以TBHQ为对照品,采用DPPH法研究4种竹叶挥发油对自由基的清除作用。结果表明:采用水蒸气蒸馏法黄金间碧竹竹叶挥发油的得率最高(0.827%),而毛竹竹叶挥发油的得率仅为0.391%。4种竹叶挥发油的化学成分在含量和组成上不同,竹叶挥发油主要化学成分是3-甲基-2-丁醇,麻竹竹叶挥发油含量最高达到46.25%;其他主要化学成分有4-乙烯基-2-甲氧基-苯酚、己-2-烯醛、橙花叔醇、植物醇、苯乙醛、天竺葵醛、植酮、二氢猕猴桃内酯和异植物醇。4种竹叶挥发油均有较强的抗氧化活性,竹叶挥发油的抗氧化活性与挥发油的浓度呈正相关($r=0.91$),其中黄金间碧竹竹叶挥发油的抗氧化活性最强(IC_{50} = 2.705 mg·mL^{-1}),孝顺竹竹叶挥发油抗氧化活性较低(IC_{50} = 3.442 mg·mL^{-1})。综合研究结果表明,竹叶挥发油具有较高的应用价值,可作为食品和药品的功能性组分进一步开发和利用。

关键词:竹叶;挥发油;化学成分;抗氧化活性
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Chemical Compositions and Antioxidant Capacity of Essential Oils from Different Species of the Bamboo Leaves

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Abstract: The antioxidant capacity of essential oils obtained by steam distillation from four bamboo species of the *Bambusa vulgaris*, *Bambusa multiplex*, *Phyllostachys pubescens*, and *Dendrocalamus latiflorus*, were evaluated using the DPPH assays. The yield of oils from the leaves of the four species was variable with the greater amount obtained from *Bambusa vulgaris* (0.827%), and the least from *Phyllostachys pubescens* (0.391%). The chemical compositions in bamboo leaves were analyzed by GC-MS. The results showed that 168 chromatographic humps were gained. 132 kinds of composition were identified. The major volatile components detected and identified by GC-MS were also variable. A major volatile was 3-methyl-2-butanol, detected in four bamboo species (maximum in *Dendrocalamus latiflorus* at 46.25%). Other major components detected were 2-methoxy-4-vinylphenol, 2-hexenal, 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, phytol, benzeneacetaldehyde, nonanal, 6,10,14-trimethyl-2-pentadecanone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H) benzofuranone and isophytol. In the DPPH assays, strong antioxidant capacity was evident in all the oils but the greater antioxidant capacity was shown by that obtained from *Bambusa vulgaris* (IC_{50} = 2.705 mg·mL^{-1}) compared to *Bambusa multiplex* (IC_{50} = 3.442 mg·mL^{-1}). Antioxidant capacity was positively correlated (r = 0.91) with the concentration of essential oils. The data indicated that essential oils obtained from various bamboo leaves may play an important role in functional foods and in the preservation of pharmacologic products.

Key words: bamboo leaves; essential oil; chemical compositions; antioxidant activity

Reactive oxygen species (ROS) or free radicals are generated as byproducts or intermediates of aerobic metabolism and through reactions with drugs and environmental toxins. The elevated cellular levels of

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free radicals cause damage to nucleic acid, proteins, and membrane lipids and have associated with many aging related problems including carcinogenesis and heart diseases (Halliwell *et al*., 1992; Halliwell, 1996; Wang *et al*., 2000). The balance between the production and scavenging of ROS can therefore determine the susceptibility of the body to oxidative damage. Although almost all organisms possess antioxidant defense and repair systems, which quench or minimize the production of oxygen-derived species, thus protecting organisms against oxidative damage, these protective systems are insufficient to entirely prevent the damage (Simic, 1988) caused by endogenous or exogenous (Sun, 1990).

Moreover, ROS are predominant cause of qualitative decay of foods, which lead to rancidity, toxicity and destruction of biomolecules important in physiologic metabolism. However, with safety concerns identified for these synthetic antioxidant (Kitts, 1996; Wichi *et al*., 1998), considerable interest has arisen in finding alternative sources of antioxidants for use in food systems and increased in researches regarding natural antioxidants. The most widely used synthetic antioxidants used historically in the preservation of foodstuffs such as BHA (butylated hydroxyanisol), BHT (butylatedhydroxytoluene) and TBHQ (tert-butyl hydroquinone) are suspected to cause or promote negative health effects (Namiki, 1991). Indeed, they have been replaced in Japan since 1996 by the natural secondary plant metabolite ellagic acid. For this reason, there is a growing interest in replacing synthetic compounds with natural secondary plant metabolites as potential antioxidants. The use of natural antioxidants has the advantage that the consumer, considered to be safe because of no chemical contamination, readily accepts them and no safety tests are required by the legislation if the food component is Generally Recognized As Safe (GRAS) (Pokorny, 1991). A range of plants has been studied in recent years as potential sources of antioxidants. Among these many essential oils of aromatic plants and spices have been shown to be effectives in retarding the process of lipid peroxidation in oils and fatty food and have gained the interest of many research groups. Therefore, a systematic examination of antioxidant properties of various plant extracts is extremely important to validate the use of, essential oils as preservatives in both the food and pharmaceutical industries. Over the past several decades, a number of studies on the antioxidant activities of essential oils from various aromatic plants have already been shown. **摇摇**

Bamboo is one of the most important forest resources. More than 1 250 species belonging to 75 genera, are being reported worldwide, which are mainly distributed in the tropical and sub-tropical zone, and a few in the temperate and frigid zone. China is one of the bamboo distribution centers of the world with the most abundant bamboo resources, a high economic value and the largest bamboo area. China boasts a long history of utilizing bamboo both as edible food and medicine, but the research on chemical composition of bamboo extracts did not start until the 1950s in China. Antioxidant of bamboo leaves (AOB), a pale brown powder extracted from bamboo leaves, was capable of blocking chain reactions of lipid auto oxidation, chelating metal ions of transient state, scavenging nitrite compounds and blocking the synthetic reaction of nitrosamine reported by previous study (Lou *et al*., 2004). Moreover, AOB was testified to be a strong antioxidant activity and inhibitory effect on transition metal ion and free radical induced deterioration of macromolecules *in vitro* (Hu *et al*., 2000). The particular interest has focused on the potential applications of essential oil that have low toxicity and a strong antioxidant activity as alternative chemical control measures. There are some reports about studies on analysis of essential oil composition from *Phyllostachys pubescens*, *Pleioblastus amarus*, *Sinocalamus affinis*, *Indocalamus latifolius* and *Indocalamus tessellatus* leaves (Mao *et al*., 2001; Wang *et al*., 2001; 2002; Yang *et al*., 2002; Li *et al*., 2007). However, the studies on antioxidant capacity of essential oils from the bamboo leaves were not reported. The objectives of this study were to compare the antioxidant activity of the essential oils from the bamboo leaves, detecting the main components of the extracts by gas chromatography mass spectrometry (GC-MS), in an attempt to contribute to the use of these as alternative products for food preservation.

1摇Materials and methods

1.1摇Chemical

Ethyl ether, Ethanol, Hexane were obtained from Beijing Chemical Factory; Sodium sulfate, anhydrous from Beijing Yili Chemical Company; Tert-butyl hydroquinone (TBHQ) and 2, 2-diphenyl-1-picrylhydrazil (DPPH) from Sigma Chemie. All chemicals used were of analytical grade. All solutions were made up in double-distilled water.

1.2摇Plant material

Leaves from adult plant of four species of the bamboo were collected during the autumn (September) from the Jiangxi Academy of Forestry in China, and sample authenticated by professor Peng Jiusheng. The dried samples were ground into fine powder. The ground samples were kept in an air-tight container and stored in a freezer (-20 ℃)until further analysis.

1.3摇Steam distillation

Essential oils were extracted by using extracted device of essential oil. The leaves of the bamboo species were mixture with distilled water (1∶8). The essential oils were extracted (6 h) by steam distillation using hexane as the collecting solvent. The solvent was separated throughout an auto-oil/water separator. The water fraction was extracted using ethyl ether as the collecting solvent for three times. The hexane extract and ethyl ether extract were mixed, subsequently dried over anhydrous sodium sulfate, and the vapor condensed. Each essential oil extraction was running in duplicate.

1.4摇Gas chromatography coupled with mass spectrometry (GC-MS)

Analyses were performed using a Agilent Technologies 5973 mass selective detector coupled to a Agilent Technologies 6890N gas chromatograph. Sample volumes of 1 μL were injected in the splitless mode into gas chromatograph. Separation of analytes was achieved using a DB-1 MS (30 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas with velocity of 1 mL · min^{-1}. The oven temperature program was as follows. Initial temperature 40 ℃ for 1 min, 40 - 100 ℃ at 5 ℃ · min^{-1} the holding for 5 min, followed by 100 - 210 ℃ at 5 ℃ · min^{-1} holding for 10 min. The GC injector temperature was 200 ℃. The mass spectrometer parameters for EI mode were ion source temperature, 200 ℃; electron energy, 70 eV; filament current, 34.6 μA; electron multiplier voltage, 1 200 V.

Constituents were identified by matching experimental fragmentation patterns in mass spectra with those of NIST2002, as well as comparing their spectra with those reported in the literature. The relative percentage of the oil constituents was calculated from GC peak areas.

1.5摇DPPH assay

The free radical scavenging capacity of the oils was determined using the DPPH discoloration method (Silva *et al*., 2006). The oils was diluted in 95% ethanol giving a range of 1 - 6 mg · mL^{-1}. The dilutions 0.5 mL were placed in a test tube in duplicate. The reaction was initiated by addition of 2 mL DPPH solution (51.54 mg · L^{-1} in 95% ethanol). The absorbance was read at 517 nm over 50 min using a UV-Vis spectrophotometer until the reading reached a plateau.

IC_{50} value was determined from the plotted graph of scavenging activity versus the concentration of essential oils, which was defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH scavenged.

1.6摇Statistics

The volume of essential of oil producing 50% (IC_{50}) inhibition of oxidation or reduction in the DPPH assays were determined using the Table curve program. The standard errors at each concentration used lower than 2% and are therefore not shown in either the tables or figures. A significant difference was considered at the level of $P < 0.05$.

2摇Results and discussion

2.1摇Chemical compositions of the oils

The amount of essential oils obtained from the bamboo species was variable (Tab. 1). The greater yield was from *Bambusa vulgaris* (0.827%) and the least from *Phyllostachys pubescens* (0.391%). The chemical compositions in bamboo leaves were analyzed by GC-MS. The results showed that 168 chromatographic humps were gained, and 132 kinds of

composition were identified. The major volatile components detected and identified by GC-MS was also variable (Tab. 2). A major volatile was 3-methyl-2-butanol, detected in four bamboo species (maximum in *Dendrocalamus latiflorus* at 44.838%). Other major components detected were 2-methoxy-4-vinylphenol, 2-hexenal, 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, benzeneacetaldehyde, nonanal, phytol, 6, 10, 14-trimethyl-2-pentadecanone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2 (4H)benzofuranone and isophytol.

Tab.1摇Yield of essential oils from bamboo species after steam hydrodistillation

Bamboo species	Yield/%	Color
Bambusa vulgaris	0.872	Yellow
Bambusa multiplex	0.471	Yellow
Phyllostachys pubescens	0.391	Yellow
Dendrocalamus latiflorus	0.736	Yellow

Tab. 2摇Chemical composition of essential oil of *B. vulgaris* (Ⅰ), *B. multiplex* (Ⅱ), *P. pubescens* (Ⅲ), and *D. latiflorus* (Ⅳ)as determined by gas chromatography mass spectrometry①

Components	Retention time	Similarity/%	Ⅰ/%	Ⅱ/%	Ⅲ/%	Ⅳ/%
2-methyl-2- (1-methylethyl)- Oxirane	4.318	75	—	0.33	—	—
Unknown	4.335	*	0.14	—	—	—
2,4-dimethyl hexane	4.434	75	—	—	—	0.77
Hexanal	4.501	90	0.52	0.23	0.56	—
Unknown	4.601	*	0.08	0.14	0.08	—
Unknown	4.718	*	0.09	0.17	0.08	—
3-methyl-2-butanol	5.084	89	15.30	22.89	25.24	46.25
2-hexenal	5.533	95	1.78	0.76	3.97	2.31
(z)-3-hexen-1-ol	5.816	91	1.01	1.23	4.38	0.62
Ethylbenzene	5.982	90	0.13	0.19	—	—
2,3,5-trimethyl hexane	6.082	75	—	0.38	—	—
p-xylene	6.199	95	1.24	1.24	1.20	0.62
Unknown	6.532	*	0.12	—	—	—
o-xylene	6.748	93	—	0.46	—	—
Heptanal	6.781	94	0.39	—	0.42	—
Unknown	7.863	*	0.05	—	0.09	—
Benzaldehyde	8.063	96	0.16	0.18	0.27	—
Unknown	8.263	*	0.14	—	0.15	—
Unknown	8.363	*	0.28	0.42	0.33	0.34
Unknown	8.629	*	0.23	0.29	0.25	0.20
1-hepten-3-one	8.895	72	0.17	0.28	—	—
6-methyl-5-hepten-2-one	9.111	93	—	0.27	0.28	—
1-octen-3-ol	9.145	72	0.90	—	—	—
2-pentyl furan	9.577	94	1.04	0.52	—	0.14
(e,e)- 2,4-heptadienal	9.594	83	—	—	0.89	0.28
cis-2- (2-pentenyl)furan	9.761	97	0.25	0.23	0.33	—
Benzeneacetaldehyde	10.343	95	0.89	1.56	1.58	1.29
3,3,5-trimethyl-cyclohexanone	10.476	93	—	0.63	—	0.37
5,5-dimethyl-1,3-cyclohexanedione	10.493	76	0.40	—	0.38	—
Unknown	10.693	*	—	—	0.21	—
(e)-2-octenal	11.092	81	0.12	—	—	—
2-methyl benzaldehyde	11.175	64	0.34	0.37	0.34	0.21
3,5-octadien-2-one	11.425	87	0.20	—	—	—
Unknown	11.841	*	0.27	0.39	0.34	—
2,6-dimethyl- octadecane	11.974	83	0.09	0.15	0.11	0.14
3,5-octadien-2-one	12.074	80	0.18	—	—	—
Unknown	12.174	*	0.39	0.53	—	—
6-methyl-3,5-heptadiene-2-one	12.407	90	0.23	0.20	0.23	—
Nonanal	12.590	93	1.66	3.02	0.94	1.12
2,6,6-trimethyl-2-cyclohexene-1,4- dione	13.206	75	0.10	0.21	—	—
5-methyl- undecane	13.272	87	0.09	0.21	—	0.36
Unknown	13.455	*	—	0.13	—	—
2,3-dimethyl- heptane	13.755	72	—	0.31	—	0.31
4-ethyl benzaldehyde	14.055	74	0.26	0.14	0.33	—
(z)-2-nonenal	14.204	95	0.08	—	—	—

Continued

Components	Retention time	Similarity/%	Ⅰ/%	Ⅱ/%	Ⅲ/%	Ⅳ/%
Unknown	14.238	*	—	0.17	—	—
5,6-dimethyl-decane	14.354	90	—	0.20	—	0.17
Methyl salicylate	15.369	97	—	0.19	—	—
2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	15.586	97	0.44	0.47	0.41	0.28
Unknown	16.168	*	—	0.28	0.25	—
Unknown	16.185	*	0.24	—	—	—
2, 6,6-trimethyl-1-cyclohexene-1-carboxaldehyde	16.535	97	—	—	0.12	0.19
2,3-dihydro-benzofuran	16.634	70	2.05	1.27	—	—
Unknown	16.934	*	—	0.44	—	—
Unknown	17.317	*	0.16	—	—	—
Unknown	17.533	*	0.17	0.35	—	—
2,6,6-trimethyl-1-cyclohexene-1-acetaldehyde	18.515	85	0.20	0.18	—	—
Unknown	19.430	*	—	0.76	—	—
3,4-dimethoxy benzaldehyde	20.030	99	0.16	0.33	0.37	—
Ethyl 4-nitrobenzoate	20.529	95	0.13	0.17	0.23	—
2-methoxy-4-vinylphenol	20.862	94	6.93	5.19	2.87	1.02
(1,1-dimethylethyl)-phenol	21.345	91	0.28	0.67	—	0.26
Unknown	22.127	*	—	—	0.21	—
Unknown	22.360	*	0.10	—	0.20	—
1,6-trimethyl-1,2-dihydro-1-naphthalene	22.743	73	0.11	0.14	0.11	0.17
(e)-1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	23.791	98	1.07	1.67	1.37	0.36
1-ethyl-4-piperidinone	23.891	70	0.45	0.39	0.53	—
1,2-dihydro-1,4,6-trimethyl- naphthalene	24.141	93	—	0.28	—	—
1,2-dimethoxy-4-(2-propenyl)benzene	24.224	89	0.55	0.28	0.22	—
6,10-dimethyl-2-undecanone	24.823	80	0.27	0.34	0.22	—
2-ethyl-1,4-dimethyl benzene	25.023	70	0.30	0.39	0.57	—
(e)-4-(2, 6, 6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	25.289	96	1.42	1.36	0.60	1.72
Unknown	25.505	*	0.46	0.29	0.46	—
Unknown	25.655	*	—	0.13	0.48	—
(e)-2-methoxy-4-(1-propenyl) phenol	25.722	80	—	—	—	0.64
Unknown	25.755	*	0.23	—	0.31	—
(e)-6,10-dimethyl-5,9-undecadien-2-one	26.121	97	1.31	1.91	1.50	0.560
Unknown	26.404	*	0.14	—	—	—
α-farnesene	26.504	80	0.18	—	—	—
4-(2,2,6-trimethyl-7-oxabicyclo [4.1.0]hept-1-yl)-3-buten-2-one	26.937	96	1.79	1.57	1.36	2.61
4-(2, 6, 6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	27.053	96	1.82	—	—	—
tritetracontane	27.253	93	0.44	0.43	0.76	0.90
5, 6, 7, 7a-tetrahydro-4, 4, 7a-trimethyl-2(4H) benzofuranone	27.519	98	2.33	1.91	1.86	0.93
1-methyl-3-[(2-methylpropyl)thio]- benzene	27.802	59	0.95	0.95	1.06	0.35
(z)-11-pentadecenal	27.935	80	0.31	0.33	0.35	—
1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- naphthalene	28.168	98	—	0.25	0.20	—
pentadecane	28.268	95	0.75	0.56	0.49	0.56
(1S-cis)- 1, 2, 3, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl)-naphthalene	28.468	98	0.30	0.39	—	—
Unknown	28.768	*	0.42	—	0.37	0.25
Unknown	29.283	*	0.24	—	0.25	0.97
3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	29.467	92	3.31	2.45	0.42	—
Butyl pentanoate	29.550	78	—	0.94	—	—
4-(3, 4-dihydro-2H-quinolin-1-yl)-4-oxo-butyric acid hydrazide	29.566	75	0.87	—	0.59	—
6,10-dimethyl-3,5,9-undecatrien-2-one	29.700	80	0.54	0.48	0.65	—

Continued

Components	Retention time	Similarity/%	Ⅰ/%	Ⅱ/%	Ⅲ/%	Ⅳ/%
Caryophyllene oxide	29.799	95	0.88	0.72	—	—
2-methyl-pentadecane	29.966	96	—	0.39	0.26	—
6z-2, 5, 5, 10-tetramethyl-undeca-2, 6, 9-trien-8-one	29.983	75	0.44	—	—	—
di-tert-Dodecyl disulfide	30.149	60	—	0.28	—	—
Unknown	30.182	*	0.55	—	—	—
Megastigmatrienone	30.515	99	0.52	0.43	0.26	2.47
1-heneicosyl formate	30.632	75	0.19	—	—	—
Hexadecane	30.865	98	0.55	0.53	0.65	0.21
2-methyl-z-4-tetradecene	31.547	86	0.49	—	—	—
Unknown	31.813	*	0.28	—	—	—
2-(tetradecyloxy) ethanol	32.030	93	0.61	0.62	0.96	—
1-isopropenyl-3, 3-dimethyl-5-(3-methyl-1-oxo-2-butenyl)cyclopentane	32.213	87	1.60	1.01	0.58	0.52
2-methyl-hexadecane	32.379	96	—	0.37	—	—
2-(cyclohex-1-enyl) furan	32.479	70	0.21	0.22	0.71	—
Pentacosane	32.562	75	—	0.32	—	—
Unknown	32.612	*	0.43	—	—	—
6,10-dimethyl-2-undecanone	32.712	70	0.26	—	—	—
1-heneicosyl formate	33.062	93	—	—	1.02	—
2-dimethylamino-4-methyl-pent-4-enenitrile	33.078	84	3.84	1.91	1.06	0.38
2,6,10,14-tetramethyl pentadecane	33.444	93	0.49	0.83	0.87	0.30
Unknown	33.578	*	0.28	0.48	0.26	—
z-8-hexadecene	33.694	80	0.30	—	—	—
Phenanthrene	33.844	95	0.40	0.76	0.35	0.26
Unknown	34.210	*	0.18	0.14	0.27	—
n-(α-methyl-4-nitrobenzylidene)-o-(phenylcarbamoyl)hydroxylamine	34.326	75	0.53	0.40	0.65	—
Unknown	34.759	*	0.59	0.31	—	—
Hexadecyl- oxirane	35. 159	80	—	—	0.32	0.25
6-ethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-7-(1-methylethenyl)- naphthalene	35. 309	82	0.29	0.18	—	—
octadecane	35.408	98	0.24	0.25	0.82	0.21
2,6,10,14-tetramethyl- hexadecane	35.641	96	0.52	0.47	0.65	—
1,2-benzenedicarboxylic acid bis(2-methylpropyl) ester	35.874	93	0.88	1.09	0.86	0.92
6,10,14-trimethyl-2-pentadecanone	36.007	99	2.45	2.19	2.17	2.06
4,4-difluororetinol	36.157	93	0.28	0.14	0.47	0.18
Unknown	36.257	*	0.27	—	—	—
1,4-eicosadiene	36.640	70	0.28	—	0.36	—
Dibutyl phthalate	36.806	94	0.55	—	0.66	—
1,2-benzenedicarboxylic acid butyl 8-methylnonyl ester	36.790	78	—	0.30	—	0.34
Citronellyl isovalerate	37.006	80	0.38	0.10	0.51	—
6,10, 14-trimethyl-5,9,13-pentadecatrien-2-one	37.272	95	1.80	2.25	2.15	0.77
Heptadecyl oxirane	37.406	97	0.46	—	1.18	0.94
Methyl palmitate	37.605	97	—	0.53	0.53	0.18
Pentadecanoic acid 14-methyl-methyl ester	37.622	97	0.34	—	—	0.32
1,2-benzenedicarboxylic acid butyl 2-methylpropyl ester	37.722	94	1.43	1.41	1.45	1.27
isophytol	38.188	97	1.64	1.38	1.71	0.65
n-hexadecanoic acid	38.687	98	2.84	1.55	1.51	0.43
Octadecanoic acid	38.970	95	1.49	1.61	1.12	0.26
Eicosane	39.386	98	0.49	0.62	0.80	0.53
(z,e)- 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol	39.536	93	0.60	0.41	0.78	0.59
Unknown	39.786	*	0.47	0.37	0.68	—

Continued

Components	Retention time	Similarity/%	Ⅰ/%	Ⅱ/%	Ⅲ/%	Ⅳ/%
5-(1,3,5-trimethyl-4-pyrazolyl) amino-1,2,4-triazol-3-amine	40.102	75	0.64	0.37	0.76	0.56
1-(1,2-propadienyl)-cyclohexanol	40.218	83	0.47	1.05	0.92	0.72
(1S-cis)-1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	40.302	70	0.49	—	—	1.35
z-11-hexadecenoic acid	40.451	83	0.40	—	0.43	0.80
2-(2-furyl)-5-(1-pyrrolidyl)-1,3,4-oxadiazole	40.468	80	—	0.42	—	0.34
11-tricosene	40.651	85	0.26	—	—	—
8,11-octadecadienoic acid methyl ester	40.701	70	—	—	—	0.34
(z, z, z)-9, 12, 15-octadecatrienoic acid methyl ester	40.784	99	—	—	—	0.52
(2-dodecen-1-yl)succinic anhydride	40.917	70	—	0.26	—	—
1-nonadecene	41.067	80	0.27	—	—	—
Phytol	41.317	94	3.35	3.63	3.02	1.64
Unknown	41.666	*	0.14	0.29	—	—
(4-octyldodecyl)-cyclopentane	41.799	81	—	—	—	3.10
3,7,11-trimethyl-3-dodecanol	42.132	80	—	—	—	4.53
7,11-hexadecadienal	42.182	91	0.33	0.63	1.38	—
1-bromocyclobutanecarboxylic acid methyl ester	42.382	70	0.23	0.51	1.26	—
10-heneicosene	42.865	85	—	—	—	1.33
Octacosane	42.881	77	0.43	1.03	0.56	—
1-octadecene	43.597	98	0.42	0.66	0.50	0.34
17-pentatriacontene	43.630	83	—	—	—	1.33
2,3,4-trimethoxy-dibenz[b,d]cyclohepten	44.595	70	—	—	—	0.81
Hentriacontane	47.042	85	0.14	0.27	0.86	0.98
4,8,12,16-tetramethylheptadecan-4-olide	47.558	95	0.16	—	0.31	—
2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene	49.006	98	—	—	0.54	—
2,2′-methylenebis; 6-(1,1-dimethylethyl)-4-methyl-phenol	49.289	99	0.21	0.14	0.46	—

摇摇① —: Inexistence; *: < 70.

2.2摇Antioxidant capacity of the oils

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. DPPH is one of the compounds that possess a proton free radical and shows a maximum absorption at 517 nm for essential oil from bamboo leaf. There was a correlation between radical scavenging rate and the concentration of essential oil from bamboo leaves. The concentration-dependent scavenging of reactive oxygen species by the oil was depicted in Fig. 1.

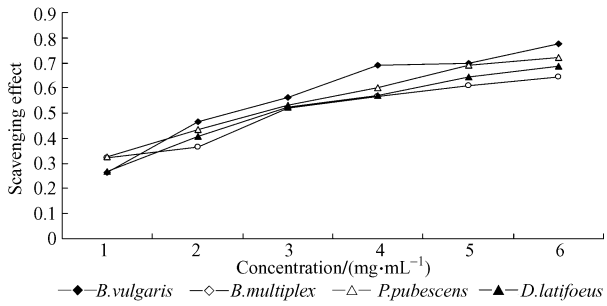

Fig. 1摇Scavenging effect of essential oil of bamboo leaves on DPPH radicals

Values are expressed as mean ± standard deviation ($n = 3$).

The antioxidant capacity of the oils correlated positively ($r = 0.91$, $P < 0.05$) with the concentration of essential oils. Radical scavenging rate was enhanced with increasing concentration of essential oils. *B. vulgaris* showed the highest scavenging effect, whereas *B. multiplex* exhibited the lowest activity among the bamboo species. However there was no significant difference between these bamboo species. The scavenging activity of essential oils on DPPH radicals rapidly increased from 1 to 6 mg·mL^{-1}. Results showed that scavenging activity was increased as the concentration of essential oils increased until a mild ascend state was reached after 4 mg·mL^{-1}. At a concentration of 3 mg·mL^{-1}, the essential oils showed higher scavenging activity than a concentration of 1 mg·mL^{-1}.

IC_{50} value was determined from the plotted graph of scavenging activity against the concentration of essential oils, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% (Tab. 3). The lowest IC_{50} indicates the strongest ability of the essential oils to act as DPPH scavengers. The IC_{50} value of *B. vulgaris* was 2.705, which was slightly lower than *B. multiplex*. However, no significant difference existed between these bamboo species. *B. vulgaris* exhibited a significant higher scavenging effect compared to *B. multiplex*. The scavenging activity of essential oils was in the order of *B. vulgaris* > *P. pubescens* > *D. latiflorus* > *B. multiplex*. Given that the production of secondary plant metabolites is mainly related to the preservation of the organism, it was of interest to determine whether the production of antioxidant volatile compounds showed any temporal variation in bamboo species.

Tab. 3摇Scavenging activity (IC_{50}) of essential oil of bamboo leaves on DPPH radicals

Sample	Regression equation	correlation coefficient (r)	IC_{50} value/ ($mg \cdot mL^{-1}$)
B. vulgaris	$Y = 0.095\ 9x + 0.240\ 6$	0.913 2	2.705
B. multiplex	$Y = 0.068\ 9x + 0.262\ 8$	0.937 6	3.442
P. pubescens	$Y = 0.080\ 3x + 0.270\ 2$	0.978 1	2.862
D. latiflorus	$Y = 0.081\ 6x + 0.230\ 9$	0.953 8	3.300
TBHQ	$Y = 0.013\ 9x + 0.488\ 4$	0.998 4	0.835

3摇Conclusion

A previous study had reported that antioxidant activity and the yield of phenolic content was influenced by different extracting solvents (Sun *et al*., 2005). For example, a water extract of *Terminalia chebuta* showed good antioxidant activity, compared to methanolic extracts of *Lycopersicon esculentum* (Cai *et al*., 2004). Moreover, from a toxicological point of view, ethanol and water are safer than acetone, methanol and other organic solvents (Oktay *et al*., 2003). The essential oils obtained by steam distillation from plant leaves may be safe for using. In the present study, the essential oils were obtained by steam distillation from four bamboo species of the *B. vulgaris*, *B. multiplex*, *P. pubescens*, and *D. latiflorus*. The yield of essential oils from bamboo species was variable, with *B. vulgaris* providing over two times more than *P. pubescens*. The steam distillation is an effective method for obtaining essential oils from bamboo leaves.

By adopting distillation of *P. pubescens* leaves and GC-MS analysis, the volatile composition of *P. pubescens* was extracted and identified in which 67 chromatographic humps were gained. 53 kinds of composition were identified. 3-hexen-1-ol and 2-hexenal were major components (Mao *et al*., 2001). However, the results of the present study showed that 168 chromatographic humps were gained, and 132 kinds of composition were identified. The major volatile components were 3-methyl-2-butanol, 2-methoxy-4-vinylphenol, 2-hexenal, nonanal, phytol, 6, 10, 14-trimethyl-2-pentadecanone, and isophytol. The difficulty of GC-MS analysis arises due to the complexity of the volatile compositions, this is particularly due to presence of natural essential oils and other ingredients consisting of complex chemical mixtures. The variety of extract solvents and different analysis method by GC-MS may be exactly the reasons for different results between two studies on essential oil from bamboo leaves.

DPPH is a free radical donor, which has been widely used to evaluate the free radical scavenging effect of natural antioxidants (Matsukawa *et al*., 1997; Jao *et al*., 2002). The IC_{50} values measured in the DPPH assay for essential oil of each bamboo species was extremely low, especially that of *B. vulgaris*, even in comparison with TBHQ, which was only over three times than TBHQ. Six major components detected were 2-methoxy-4-vinylphenol, 3, 7, 11-trimethyl-1, 6, 10-dodecatrien-3-ol, phytol, 6, 10, 14-trimethyl-2-pentadecanone, 5, 6, 7, 7a-tetrahydro-4, 4, 7a-trimethyl-2 (4H) benzofuranone and isophytol in four bamboo leaves, which may contribute to enhance antioxidant capacity of essential oils from bamboo leaves. The antioxidant capacity of the oils, however, is not clearly related to the proportion and profile of secondary plant compounds (Maria *et al*., 2006).

Other factors that also influence the antioxidants activity are antioxidants concentration, extraction medium, temperature, pH of medium (Gazzani *et al*., 1998)chemical structures and position in the molecule (Prior *et al*., 2005). A high antioxidant activity could

also be due to other compounds besides phenolics which are soluble in water. In the present study, a part of volatile components in essential oils were extract from water fraction using ethyl ether as extract solvent, which may be also positively correlated with antioxidant activity of essential oils from bamboo leaves.

Food such as fruits, vegetables and grains are reported to contain a wide variety of antioxidant components, including phenolic compounds. These compounds are found to be well correlated with antioxidant potential (Katalinic *et al*., 2004). Furthermore, an increase in the horticulure of bamboo leaves, such as *B. vulgaris*, would appear to be advantageous in terms of future application for incorporation into functional foods and pharmaceutical products. Also, benefits may accrue by the utilization of technologic processes or genetic manipulation to increase the yield of oil from this species. Furthermore, the oils are currently undergoing a battery of further in-vitro tests (Gerhauser *et al*.,2003) to clarify their preservation of pharmaceutical products and validity as food additives.

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