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Research Article

Chemical Constituents and Biological Activities of Essential Oils from Four Species of Bamboo Genus *Schizostachyum*

Charles Santhanaraju Vairappan[1*], Thilahgavani Nagappan[1,2], Liow Ting Hui[1], Julius Kulip[3]

1 Laboratory of Natural Products Chemistry, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
2 School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
3 Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
[*]Corresponding author: csv@ums.edu.my

Abstract

Volatile chemical constituents of four species of local bamboo (*Schizostachyum blumei* Nees., *Schizostachyum brachycladum* Nees., *Schizostachyum lima* (Blanco) Merrill and *Schizostachyum pilosum* S.Dransf.) were investigated. The oils were obtained from bamboo culm through steam distillation and profiled using Gas Chromatography-Mass Spectrometry (GCMS). A total of 59 volatile constituents were identified, and these contained oxygenated sesquiterpene, α-elemol (8.2~21.1 %), coumaran (6.7~32.3 %), guaiacol-4-vinyl (0.6~0.9 %), palmitic acid (1.5~25.6 %), pentacosane (0.1~0.2 %), phytol (1.0~12.6 %), phytol acetate (0.5~1.7 %) and trans-squalene (0.2~1.6 %) consistently in the specimens studied. Based on the observation, *S. pilosum* exhibited wider diameter of inhibition against *Escherchia coli* and *Staphylococcus aureus* compared to the other species of *Schizostachyum* essential oil.

Keywords: Wild bamboo, Volatile fingerprints, Antibacterial Activity.

Introduction

The medicinal applications of bamboo in the traditional medicine system were first mentioned around 500 AD. Bamboo sap and stem shavings were used in various therapeutic applications. The ancient Indian system of medicine, Ayurveda, recommends the use of bamboo and its products for treating various illnesses. Bamboo manna, also known as 'Banslochan' or 'Tabashir' in the Indo-Persian system of medicine, is a very important drug extracted from the substance accumulated at the hollow internodes of bamboo and is reported to have many medicinal properties (Singh & Das, 2011). In India, *Bambusa arundinacea* (Retez) Willd. is described as the major source of bamboo manna,

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though other species of *Bambusa* are also used. It is useful as a stimulant, a febrifuge, a cooling tonic, an antispasmodic agent as well as an aphrodisiac. It is also used to treat asthma, cough, paralytic complaints and other debilitating diseases. The stems and leaves are used to purify blood, in leucoderma and in inflammatory conditions. Infusion of the leaves can be used as eyewash or can be consumed for treating bronchitis, fever and gonorrhoea. The brunt roots are applied to ringworm, bleeding gums and painful joints. The black soot deposited on the culms can be used to heal cuts and wounds when mixed with lime. The bark is used to cure eruptions. Its tender shoots can be pickled or made into curries that are said to be able to promote appetite and facilitate digestion (Nath et al., 2009; Tanaka et al., 2011).

Apart from their difference in appearance, bamboos also vary in smell. The fragrance of the bamboos is produced by their culms. The culms of the moso-bamboo *Phyllostachys pubescens* (Pradelle) Mazel ex J.Houz contains 18 aroma-active compounds which were detected by aroma extract dilution analysis (AEDA) during sensory analysis. The most intense aroma-active compounds in *P. pubescens* were eugenol which is a sweet, clove-like green compound and (E)-2-nonenal which is also a green compound. These compounds were estimated to have a bamboo-like aroma and aldehyde compounds, such as a phenylacetaldehyde and C9-C10 unsaturated aldehydes, create the aroma of bamboo (Takahashi et al., 2010). Another interesting example is Incense Bamboo (*Phyllostachys atrovaginata* Nees.) that has a waxy coat on the culms that emits a pleasant fragrant similar to incense.

In Sabah, Malaysia, there are nine genera of bamboo comprising 34 recorded species. These are *Bambusa* (six species), *Dendrocalamus* (one species), *Gigantochloa* (two species), *Dinochloa* (nine species), *Schizostachyum* (seven species), *Racemobambos* (six species), *Yushania* (one species), *Sphaerobambos* (one species) and *Thyrsostachys* (one species) (Dransfield S, 1999). In this study, four species of native bamboos (*Schizostachyum blumei* Nees., *Schizostachyum brachycladum* Nees., *Schizostachyum lima* (Blanco) Merrill., and *Schizostachyum pilosum* S.Dransf.) were selected to investigate the presence of antibacterial properties and fragrance in these bamboos as it is frequently used by local indigenous people as medicine to treat various illnesses.

Methodology

Plant materials

The bamboo culm specimens of *Schizostachyum blumei* Nees., *Schizostachyum brachycladum* Nees., *Schizostachyum lima* (Blanco) Merrill and *Schizostachyum pilosum* S. Dransf were collected from Kg. Tikolod and Kg. Sinsuron, Tambunan, Sabah in January and September 2014. Identification of the plant material was based on its morphological features and confirmed by Mr Johnny Gisil, Botanist for BORNEENSIS. Voucher specimens (BORH 45699~45703) were deposited in BORNEENSIS, the herbarium at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

Extraction and isolation of the essential oil

Extraction of essential oil and analysis from the respective specimens was carried according to Nagappan et al., 2012 with slight modification. Four hundreds grams of sliced bamboo culm were steam distilled using a steam-distillation apparatus for 8 hours. Distilled oil was collected in GR-grade *n*-pentane (Merck, Germany), dried over sodium sulfate anhydrous (Sigma, USA), concentrated *in vacuo*, stored in air-tight glass vials, flushed with nitrogen (N_2) gas and kept at -81 ºC for further testing and chemical analysis.

GC-MS analysis of the essential oil

Analysis of the essential oils was performed using a Shimadzu QP-2010 chromatograph coupled with a Shimadzu GCMS QP-2010 plus detector (Shimadzu Corp., Japan) using a SGE BPX-5 (30.0 m X 0.25 µm i.d., film thickness 0.25 µm) fused silica capillary column. High purity helium was used as the carrier gas at a constant flow rate of 0.8 mL min^{-1}. A 1 µL sample was injected (split ratio 100:1) into the GCMS using an AOC5000 auto injector. The initial temperature was set at 50 °C, heated at a rate of 3°C min^{-1} to 280 °C and held isothermally for 5 minutes. The ion source temperature for these analyses was set at 200 °C, and the interface temperature at 280 °C. The mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV, and an acquisition mass range from 40 to 450 a.m.u. at 0.25 scan s^{-1}.

Identification of constituents was confirmed using two standard libraries, published EI-MS in the National Institute of Standard and Technology (NIST) 1998 and Shimadzu's Flavours and Fragrance of Natural and Synthetic Compounds (FFNSC) version 1.2 computerized mass spectral libraries. The retention indices were determined based on a homologous series of *n*-alkanes (C_8 – C_{40}) (Custom Retention Time Index Standard, Restek Corp, USA) external

standard analyzed under the same operating conditions and calibrated based on Automatic Adjustment of Compound Retention Time (AART) function of the GCMS. Relative concentrations of the essential oil components were calculated based on GC peak area with the AART correction factors.

Antibacterial activity

The antibacterial evaluation was based on diameter of the inhibition zone exhibited by the respective bamboo extracts using a method described by Ningappa et al., 2010. Microbes to assess the antibacterial activities of essential oils and their chemical constituents against two strains of food pathogenic bacteria: *Staphylococcus aureus* (ATCC 29213) and *Escherchia coli* (ATCC 7645) bacterial strains were obtained from the School of Food Science Culture Collection, Universiti Malaysia Sabah.

Results and Discussion

The essential oils obtained were whitish-clear, mild odour liquids. The yields were as follows; 3.2 %, 4.9 %, 5.1 % and 7.3 % for *S. blumei, S. brachycladum, S. lima* and *S. pilosum* respectively, calculated on a moisture free basis. A total of 59 volatile constituents were successfully identified by gas chromatography-mass spectrometry (GCMS). The details of the identified volatile constituents are presented in Table 1.

Overall, a majority of the volatile metabolites were oxygenated sesquiterpenes. A total of 50 volatile constituents were identified in the oil of *S. blumei*, 19 in *S. brachycladum*, 17 in *S. lima* and 19 in *S. pilosum*. Based on a detailed analysis, eight volatile markers were consistently present in the specimens studied; these were α-elemol (8.2~21.1 %), coumaran (6.7~32.3 %), guaiacol-4-vinyl (0.6~0.9 %), palmitic acid (1.5~25.6 %), pentacosane (0.1~0.2 %), phytol (1.0~12.6 %), phytol acetate (0.5~1.7 %) and trans-squalene (0.2~1.6 %). The essential oils also contained monoterpene hydrocarbons (0.3~22.1 %), oxygenated monoterpenes (0.8~39.4 %), sesquiterpene hydrocarbons (0.2~19.6 %) and oxygenated sesquiterpenes (41.2~70.4 %), diterpene hydrocarbon (0.2~2.8 %), oxygenated diterpenes (2.8~14.2 %) and triterpene hydrocarbons (0.5 %~1.9). The fluctuation and variations volatile present in the oil of each species could be highly influenced by ecological and plant growth factors. Lawrence and colleagues (Verma et al., 2013) also reported that composition of essential oil constituents rely on biochemical pathways, either the shikimic acid pathway which produces phenylpropanoids constituents or the mevalonic acid pathway which produce terpenes constituents.

Table 1. Volatile compositions (%) of essential oils of *Schizostachyum* spp.

Ret. Time (min)	Ret. Index	Compounds	Conc. (%)				Identification mode
			S.blumei	*S.brach.*	*S.lima*	*S.pilosum*	
18.08	1196	Isopulegol	0.5	-	-	-	NIST
18.17	1165	Citronellal	0.5	-	-	-	FFNSC
18.54	1169	Isoisopulegol	0.1	-	-	-	FFNSC
21.50	1036	Coumaran	6.7	22.1	32.3	25.8	NIST
21.63	1232	Citronellol	1.7	-	0.1	0.1	FFNSC
22.29	1238	Neral	0.2	-	-	5.1	FFNSC
22.69	1255	Geraniol	0.6	-	-	-	FFNSC
23.29	1285	Dodecane	0.3	-	-	-	NIST
23.66	1268	Geranial	0.4	-	-	7.3	FFNSC
25.41	1347	Citronellic acid	0.1	-	-	-	FFNSC
25.71	1309	Guaiacol-4-vinyl	0.9	0.7	0.7	0.6	FFNSC
27.04	1350	Citronellyl acetate	1.4	-	-	-	FFNSC
27.47	1392	Eugenol	0.1	-	-	-	NIST
28.20	1344	α-cubebene	0.1	-	-	-	NIST
28.35	1380	Geranyl acetate	2.9	-	-	-	FFNSC
28.79	1390	ß-elemene	0.7	-	-	-	FFNSC
29.04	1400	Tetradecane	0.2	-	-	-	FFNSC
29.68	1392	Vanillin	0.1	-	-	-	NIST
30.60	1432	α-trans-bergamotene	0.3	-	-	-	FFNSC
31.39	1452	Farnesene	0.1	-	-	-	FFNSC
31.72	1454	α-humulene	0.2	-	-	-	FFNSC
32.80	1512	ß-cadinene	3.5	-	-	-	FFNSC
33.48	1440	ß-muurolene	1.1	-	-	-	NIST
33.78	1500	ß-bisabolene	0.5	-	10.2	-	NIST
33.79	1555	Phenol	-	-	0.8	0.5	NIST
34.27	1518	α-cadinene	3.8	-	-	-	FFNSC
35.73	1546	α-elemol	12.8	21.1	10.1	8.2	FFNSC

(Continued on next page)

Table 4. (continued)

Ret. Time (min)	Ret. Index	Compounds	Conc. (%)				Identification mode
			S.blumei	*S.brach.*	*S.lima*	*S.pilosum*	
37.22	1600	Hexadecane	0.1	-	-	-	FFNSC
38.05	1710	Trans-farnesol	4.6	-	-	-	NIST
38.98	1632	ß-eudesmol	5.6	-	-	0.1	FFNSC
40.10	1593	Selina-6-en-4-ol	0.3	-	-	-	NIST
40.75	1661	2,3-dihydro-6-trans-farnesol	1.3	-	-	-	NIST
40.99	1700	Heptadecane	0.1	0.2	-	-	FFNSC
41.53	1696	Juniper camphor	0.1	-	-	-	FFNSC
41.73	2192	Geranyl geraniol	0.5	-	-	-	NIST
41.84	1710	Farnesol	1.7	-	-	-	NIST
42.73	1737	Farnesal	1.1	-	-	-	FFNSC
43.42	1769	Myristic acid	0.1	-	-	-	NIST
44.58	1800/	Octadecane	0.1	-	-	-	FFNSC
46.54	1869	Pentadecanoic acid	0.1	0.2	-	-	NIST
48.00	2109	Heneicosane	0.1	0.1	-	-	NIST
48.54	1624	Citronellyl valerate	0.4	-	-	-	FFNSC
49.66	1582	Neryl isovalerate	0.1	-	-	-	FFNSC
50.39	1977	Palmitic acid	1.5	15.4	25.6	11.9	FFNSC
54.15	1981	Heptadecanol	0.1	-	-	-	FFNSC
54.47	2085	Elaidic acid methyl ester	-	1.1	0.2	-	NIST
54.80	2045	Phytol	4.0	1.8	1.0	12.6	NIST
55.63	2183	Linoleic acid	-	10.8	0.6	10.3	NIST
55.81	2175	Oleic acid	-	11.5	55.8	10.6	NIST
56.49	2167	Stearic acid	-	10.1	-	-	NIST
56.70	2088	2-ethylhexyl methoxycinnamate	-	0.3	-	-	NIST

Table 4. (continued)

Ret. Time (min)	Ret. Index	Compounds	Conc. (%)				Identification mode
			S.blumei	*S.brach.*	*S.lima*	*S.pilosum*	
57.85	2212	Phytol acetate	0.9	0.5	1.7	0.8	FFNSC
60.23	2500	Pentacosane	0.1	0.1	0.2	0.2	FFNSC
62.84	2414	Adipic acid ester	-	0.5	1.2	0.7	NIST
62.97	2400	Tetracosane	-	0.1	0.2	1.1	NIST
65.62	3500	Pentatriacontane	-	-	-	0.2	NIST
65.63	3600	Hexatriacontane	0.4	0.2	0.3	-	NIST
66.68	2162	1,2-benzenedicarboxylic acid	0.7	-	-	0.1	NIST
73.30	2914	Trans-squalene	1.1	0.2	0.6	1.6	NIST
Composition of the volatiles (%)							
Monoterpene hydrocarbon			0.3	22.1	-	-	
Monoterpene oxygenated			30.8	0.8	33.8	39.4	
Sesquiterpene hydrocarbon			19.6	0.2	-	-	
Sesquiterpene oxygenated			37.9	70.4	57.9	41.2	
Diterpene hydrocarbon			0.7	0.3	0.2	2.8	
Diterpene oxygenated			5.0	2.8	2.8	14.2	
Triterpene hydrocarbon			1.5	0.5	1.1	1.9	
Percentage of detection			95.8	97.1	95.8	99.5	

*RRI, retention indices on BPX 5; Compounds are listed in order of elution based on BPX 5; Volatile compounds were identified based on their MS fragment pattern and retention index as compared with NIST and FFNSC data bases

All the essential oils were evaluated for their antibacterial activities against two strains of food pathogens. The detailed activities are given in Table 2. Based on the observation, *S. pilosum* exhibited wider diameter of inhibition against *E. coli* and *Staphylococcus aureus* compared to the other species of *Schizostachyum* essential oil. Upon testing, 1.0 mg/ml of *S. pilosum* essential oil exhibits diameter of inhibition as wide as 23.0 mm to 28.0 mm against *E. coli* and *Staphylococcus aureus*. Results also reflect the activity of essential oils against the food pathogen is dependent on the tested concentration.

Presence of common compounds like coumaran/benzofuran, palmitic acid, phytol, adipic acid ester and α-elemol are significant on the bioactive potential of these four bamboo species. According to Vijisaral et al., (2014), coumaran has the function of antihelminthic, and has anti-inflammatory and antidiarrhoeal properties. In addition, coumaran is reported to have insecticidal activity as it showed potent fumigant activity against stored grain insect pests (Rajashekara et al., 2013). Palmitic acid is known to have antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, anti-androgenic and 5α-reductase inhibitor (Afrin T., 2012). According to Mulyono et al., 2012, presence of fatty acids, esters, long chain alcohols and aldehydes could also enhance the antibacterial property. As such, the synergistic effects of this volatile metabolites could be the lead substances that inhibit *E. coli* and *Staphylococcus aureus*. In addition, α-elemol is a known plant derived fragrance that is widely used in decorative cosmetic products (Bhatia et al., 2008).

Table 2. Antibacterial activities of four species of bamboo against *Staphylococcus aureus* (ATCC 29213) *and Escherchia coli* (ATCC 7645)

EO	*S. aureus* Diameter of Inhibition Zone (mm)				*E. coli* Diameter of Inhibition Zone (mm)			
	0.25 mg/ml	**0.50 mg/ml**	**0.75 mg/ml**	**1.00 mg/ml**	**0.25 mg/ml**	**0.50 mg/ml**	**0.75 mg/ml**	**1.00 mg/ml**
S. blumei	13.0 ± 0.1	16.0 ± 0.3	17.0 ± 0.6	18.0 ± 0.5	11.0 ± 0.9	15.0 ± 0.1	15.0 ± 0.2	17.0 ± 0.5
S. brach.	12.0 ± 0.1	13.0 ± 0.1	19.0 ± 0.1	21.0 ± 0.7	12.0 ± 0.5	14.0 ± 0.1	17.0 ± 0.3	19.0 ± 0.5
S. lima	12.0 ± 0.1	13.0 ± 0.8	14.0 ± 1.0	16.0 ± 0.4	15.0 ± 0.5	16.0 ± 0.2	19.0 ± 0.5	20.0 ± 0.2
S. pilosum	24.0 ± 0.1	26.0 ± 0.9	27.0 ± 0.8	28.0 ± 0.2	20.0 ± 0.1	21.0 ± 0.1	22.0 ± 0.3	23.0 ± 0.8

**S. blumei: Schizostachyum blumei, S. brach.: Schizostachyum brachycladum, S. lima: Schizostachyum lima, S. pilosum: Schizostachyum pilosum.* Gentamicin was set as positive control and DMSO was set as negative for this assay.

Although *S. pilosum* exhibited the strongest antibacterial activity against the two microbes tested, all of the other species also showed evident inhibition activity against the bacteria. Different concentrations were used in antibacterial bioassay tests to determine the relationship between concentration of essential oils and the strength of their antibacterial activity. It was proven that higher concentrations incur stronger antibacterial activity. However, even the essential oil of least concentration (0.25 g/ml) showed distinct inhibition activity against the bacteria. Through this study, the pharmaceutical potential of these bamboo species are shown and they are worth to be further studied to fully utilize their potential as new source of medicines. Minimum Inhibitory Concentration (MIC) should be further investigated to achieve an ideal dosage for optimum antibacterial activity.

Antiphytofungal activity of the essential oils was assessed against a panel of eight phytopathogenic funguses using standardized bioautographic technique. Strong positive results were obtained against *Fusarium stilboides* and *Fusarium solani*, MIC evaluation via microdilution technique showed MIC value of 12.5 μg/mL and 25.0 μg /mL, respectively. In conclusion, it became apparent that essential oils from culms of bamboo genus *Schizostachyum* emits an important fragrance (α-elemol) as its major metabolite and contains a high concentration of coumaran that is a known bio-fumigant that acts as an antibacterial and antifungal agent.

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