

## DIVERSITY IN VOLATILE CHEMICALS AND ANTIBACTERIAL ACTIVITY AMONG SELECTED GENUS OF *Cinnamomum*, *Etilingera* AND *Schizostachyum* FROM SABAH

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**Abstract:** The volatile chemicals from species of wild *Cinnamomum* spp. (*C. racemosum*, *C. cuspidatum*, *C. politum*, *C. javanicum*), *Etilingera* spp. (*E. pyramidosphaera*, *E. megalochelios*, *E. coccinea*, *E. elatior*) and *Schizostachyum* spp. (*S. blumei*, *S. brachycladum*, *S. lima*, *S. pilosum*) found in Sabah were investigated. The oils were obtained from the bark, rhizome and culm of respective specimens by hydrodistillation and the profile of volatile chemicals was obtained using Gas Chromatography- Mass Spectrometry (GCMS). Dominance of eucalyptol, terpinen-4-ol and eugenol were consistent among the species from genus *Cinnamomum*. Aromadendrane oxide, lauryl aldehyde, elemicin, borneol and 1-dodecanol were predominant among the species from genus *Etilingera*.  $\alpha$ -elemol, coumaran, guiacol-4-vinyl, palmitic acid and phytol acetate predominate the species from genus *Schizostachyum*. Strong inhibition against *Staphylococcus aureus* (MIC:  $5.62 \pm 0.5 \mu\text{g mL}^{-1}$ ) were exhibited by essential oils of *C. cuspidatum* and *E. coccinea*, oil of *S. blumei* inhibited *Listeria monocytogenes* (MIC:  $4.60 \pm 0.5 \mu\text{g mL}^{-1}$ ), oil of *C. javanicum* inhibited *Salmonella typhimurium* (MIC:  $5.50 \pm 0.5 \mu\text{g mL}^{-1}$ ). Meanwhile the oil of *C. politum* suppressed *Salmonella enteritidis* (MIC:  $5.20 \pm 0.5 \mu\text{g mL}^{-1}$ ) was measured using microdilution method. These findings reveal the potential of selected plants used by indigenous communities of Borneo as antimicrobials in food, cosmetics and pharmaceutical industries

Keywords: Volatile chemicals, *Etilingera* spp., *Cinnamomum* spp., *Schizostachyum* spp., antibacterial activity.

### Introduction

The tropical rain forest of Malaysia is among the most diverse for medicinal plants. It has been estimated that 250,000 species of flowering plants exist in the world; 15,000 of these are found in Malaysian tropical rain forests (NRE, 2014). Peninsular Malaysia, in particular, has more than 2,000 species of medicinal plants with only about 10% of these being utilized by ethnic groups all around the country (Salleh & Latiff, 2002). In Borneo, members from the genus *Etilingera* are highly valued for their medicinal and culinary properties. *Etilingera coccinea*, locally known as "Tuhau", is consumed as pickles and utilized a traditional remedy for stomach aches, food poisoning and gastric problems. Poulsen (2006) mentioned in detail the traditional uses of the species as a food enhancer and as traditional

medicine by indigenous communities of Dusun, Iban and Kelabit. *Cinnamomum* spp. (family Lauraceae) is another genus known for its aromatic leaves and bark with more than 300 species widely distributed throughout Australasia and Southeast Asia. The commercial species sold as spices are *C. verum*, *C. cassia*, *C. burmannii*, *C. zeylanicum* and *C. loureiroi*. The barks of these species are highly valued in the food and beverage industries for food flavoring. Chang *et al.* (2001) documented that the oil of *C. zeylanicum* demonstrated an inhibitory effect on meat spoilage organisms and has fungitoxic properties against fungi involved in respiratory tract mycoses. Known as a source of cooling tonic, antispasmodic agent and remedy to treat asthma, cough, paralytic complaints and other debilitating diseases, members from the genus

*Schizostachyum* or bamboo have been used for alternative medicine since medieval times (Singh & Das, 2011). “Bamboo manna”, or 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 while the indigenous community in Borneo applies the brunt roots to treat ringworm, bleeding gums and painful joints (Dransfield, 1992).

To the best of our knowledge, research on the volatile chemical constituents of these three genus highly utilized by the indigenous community in Borneo is still lacking. Thus, Vairappan *et al.* (2012) initiated the investigation on a few species of *Etingera* from Borneo. The findings revealed that the essential oil composition of *E. pyramidosphaera*, *E. megalosphaera*, *E. coccinea*, *E. elatior* and *E. brevilabrum* is dominated by oxygenated monoterpene. *Etingera brevilabrum* and *E. pyramidosphaera* displayed the highest cytotoxicity against MCF-7 and HL-60 cancer cell lines and essential oils of *E. coccinea* and *E. megalosphaera* inhibit *Staphylococcus aureus* and *Streptococcus pyogenes* strains. The same group of researchers investigated a few species from the genus *Cinnamomum* (*C. crassinervium*, *C. racemosum*, *C. cuspidatum*, *C. politum*, *C. javanicum*, *Cinnamomum* sp) and documented high content of oxygenated monoterpene in the essential oils. The oils of *C. cuspidatum* and *C. crassinervium* were found to inhibit *Listeria monocytogenes*.

The scarcity of scientific information pertaining to the genus *Schizostachyum* from Borneo was confirmed after an exhaustive search. Scientific investigation on species of “bambusa” from Borneo is important, as this genus is commonly associated in culinary use and as folk medicine among indigenous community of Sabah, in particular. Due to the limited information on antimicrobial and diversity pertaining to volatile chemicals, we embarked on investigating a few ethnobotanically relevant species from the genus *Cinnamomum* (*C. racemosum* Kosterm, *C.*

*cuspidatum* Miq., *C. politum* Miq., *C. javanicum* Blume), *Etingera* (*E. pyramidosphaera* (K. Schum.), *E. megalosphaera* (Griff.), *E. coccinea* (Blume), *E. elatior* (Jack) and *Schizostachyum* (*S. blumei* Nees., *S. brachycladum* Nees., *S. lima* (Blanco) Merrill, *S. pilosum* S. Dransf).

## Materials and Methods

### Plant Materials

The bark specimens of *C. racemosum*, *C. cuspidatum*, *C. politum* and *C. javanicum* were collected from Klias Forest Reserve, Sabah, while rhizomes of *E. pyramidosphaera*, *E. megalosphaera*, *E. coccinea* and *E. elatior* were collected from Ranau, Sabah, whereas the culm of *S. blumei*, *S. brachycladum*, *S. lima* and *S. pilosum* were collected from Tambunan, Sabah. Specimens were collected from August 2014 to October 2014 throughout the mentioned sampling sites. Authentication of the plant materials was made based on morphological features. Voucher specimens (BORH 45660~45671) were deposited in BORNENSIS, Herbarium of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah (UMS).

### Essential Oil Isolation

Two hundred grams of powdered barks of *Cinnamomum* spp., 200 g of freshly-chopped rhizome of *Etingera* spp. and 200 g freshly chopped culms of *Schizostachyum* spp. were hydro-distilled using a Clevenger-type apparatus for 8 h. 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<sub>2</sub>) gas and kept at -80 °C for further bioassay and chemical analysis.

### GC-MS Analysis of the Essential Oils

Analysis of the essential oils were 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  $\mu\text{m}$ ) fused silica capillary column. High purity helium was used as the carrier gas. Accurately, 1  $\mu\text{L}$  sample was injected (split ratio 100:1) into the GCMS using an AOC5000 autoinjector. Identification of the constituents was confirmed using two standard libraries, published EI-MS in the National Institute for 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 – C<sub>40</sub>) (Custom Retention Time Index Standard,<sup>8</sup> Restek Corp, USA) external standard (Nagappan *et al.*, 2012).

### **Antibacterial Activity**

Minimum inhibitory concentrations (MIC) of the essential oils were determined using the microdilution method in 96-well plates as described by James *et al.* (2011) with slight modification against four strains of food pathogenic bacteria: *S. aureus* (ATCC 29213), *L. monocytogenes* (ATCC 7644), *S. typhimurium* (ATCC 25922) and *S. enteritidis* (ATCC 29213). The essential oils were diluted two-fold to obtain a series of different concentration range of 1.50 – 50  $\mu\text{g mL}^{-1}$ . Exactly, 10  $\mu\text{L}$  of each concentration of essential oils was added to 170  $\mu\text{L}$  nutrient broth growth medium in wells of a 96-well microtiter plate and 20  $\mu\text{L}$  of standardized (0.5 McFarland turbidity) suspension of the test organism was added to each well. The test and control microplates were incubated for 24 hours at 37 °C and MIC value of each essential oil was calculated by measuring the optical density using micro-plate reader at 700 nm. Gentamicin was set as reference antibacterial compound. The lowest concentration of essential oil at which no visible growth of organism was observed after the incubation period was defined as the MIC. These American Type Culture Collection (ATCC) bacterial strains were obtained from the School of Food Science Culture Collection, Universiti Malaysia Sabah.

### **Results and Discussion**

The pale-yellow, aromatic essential oils from the bark of four *Cinnamomum* species were extracted and the yields were as follows: 10.1%, 5.7%, 9.7% and 4.3% for *C. racemosum*, *C. cuspidatum*, *C. polatum* and *C. javanicum*, respectively. The clear, aromatic oils of *E. pyramidosphaera*, *E. megalocheilos*, *E. coccinea* and *E. elatior* yield were 5.3%, 6.0%, 9.0% and 5.7%, respectively, while the essential oils of *S. blumei*, *S. brachycladum*, *S. lima* and *S. pilosum* were calculated as 2.2%, 3.0%, 1.9% and 2.7% respectively. All the essential oils obtained were calculated on moisture-free basis. Volatile chemicals were identified based on their mass spectroscopy fragment pattern and retention index as compared with NIST and FFNSC databases. The summarized composition of major and minor volatile chemicals noted from the investigated species is shown in Table 1 while the details of identified volatile chemicals from genus *Schizostachyum* is presented in Table 2.

All the essential oils were evaluated for their antibacterial activities against four strains of food pathogenic bacteria; the details are presented in Table 3. Based on observations, *E. coccinea* and *S. blumei* displayed the best inhibition against *L. monocytogenes* with the lowest MIC values of 4.00  $\mu\text{g mL}^{-1}$  and 4.60  $\mu\text{g mL}^{-1}$  respectively, while *E. megalocheilos* displayed the best inhibition against *S. aureus* with MIC value of 4.40  $\mu\text{g mL}^{-1}$  followed by inhibition of *C. javanicum* against *S. typhimurium* with the MIC value of 5.50  $\mu\text{g mL}^{-1}$  and inhibition of *C. polatum* against *S. enteritidis* with the MIC value of 5.02  $\mu\text{g mL}^{-1}$ . It is also observed that essential oils of genus *Etilingera* displayed the best inhibition against all the strains of food pathogenic bacteria with MIC values below 8.20  $\mu\text{g mL}^{-1}$ .

Table 1: The composition of major and minor volatile chemicals from *Cinnamomum* spp., *Etilingera* spp. and *Schizostachyum* spp.

Constituents	Conc. (%)				RT (min)	RI	Id. Mode
	<i>C. rcm</i>	<i>C. csp</i>	<i>C. pol</i>	<i>C. jvc</i>			
<b>Major</b>							
Eucalyptol	15.5	8.1	12.3	7.5	12.58	1059	MS, NIST
Terpinen-4-ol	17.3	12.7	20.7	22.1	19.64	1137	MS, NIST
Eugenol	37.9	11.8	14.1	24.2	27.89	1392	MS, NIST
<b>Minor</b>							
$\alpha$ -Terpineol	1.0	4.1	3.9	2.7	20.39	1198	MS, FFNSC
Copaene	1.6	3.7	0.9	0.3	28.15	1221	MS, NIST
Eugenol methyl ether	0.6	2.0	1.8	0.3	29.58	1361	MS, NIST
$\alpha$ -trans Bergamotene	1.3	1.9	0.4	2.1	30.55	1432	MS, FFNSC
$\gamma$ - cadinene	0.4	1.9	0.2	0.6	32.43	1512	MS, FFNSC
$\delta$ - selinene	2.9	1.4	0.2	1.1	32.92	1481	MS, NIST
viridiflorol	0.4	1.7	1.9	2.3	37.43	1594	MS, FFNSC
$\alpha$ -cadinol	0.5	0.4	1.4	1.8	39.95	1581	MS, FFNSC
Constituents	<i>E. pyr</i>	<i>E. mgl</i>	<i>E. coc</i>	<i>E. ela</i>	RT (min)	RI	Id. Mode
<b>Major</b>							
Borneol	5.0	4.7	28.2	11.3	19.26	1148	MS, FFNSC
1-dodecanol	4.3	15.9	3.0	19.1	23.75	1278	MS, FFNSC
Lauryl aldehyde	30.0	7.9	5.9	5.7	29.77	1410	MS, NIST
Aromadendrene oxide	11.0	24.8	10.9	46.2	32.06	1462	MS, FFNSC
Elemicin	10.1	35.6	9.7	2.4	35.54	1551	MS, FFNSC
<b>Minor</b>							
Camphor	1.0	0.7	2.8	0.5	18.15	1149	MS, NIST
5-Decen-1-ol	-	-	1.3	2.1	22.92	1265	MS, FFNSC
Caryophyllene	2.3	0.5	-	0.7	30.11	1494	MS, FFNSC
$\beta$ -Caryophyllene oxide	3.7	1.2	-	1.6	30.82	1582	MS, NIST
$\delta$ - selinene	0.6	1.2	-	-	32.92	1481	MS, NIST
Constituents	<i>S. blu</i>	<i>S. brc</i>	<i>S. lima</i>	<i>S. pilo</i>	RT (min)	RI	Id. Mode
<b>Major</b>							
Coumaran	6.7	22.1	32.3	25.8	21.50	1036	MS, NIST
Guaiaicol-4-vinyl	2.9	4.4	10.7	6.9	25.71	1309	MS, FFNSC
$\alpha$ -elemol	12.8	21.1	10.1	8.2	35.73	1546	MS, FFNSC
Palmitic acid	1.5	15.4	25.6	11.9	50.39	1977	MS, FFNSC
Phytol acetate	2.9	1.5	4.9	7.2	57.85	2212	MS, FFNSC
<b>Minor</b>							
Citronellol	1.7	2.1	0.6	0.2	21.63	1232	MS, FFNSC
$\alpha$ -trans Bergamotene	0.3	-	1.9	0.7	30.55	1432	MS, FFNSC
Heptadecane	0.1	0.3	-	-	40.99	1700	MS, FFNSC
Elaidic acid methyl ester	2.2	4.2	1.1	0.7	54.47	2085	MS, NIST
Linoleic acid	1.0	0.8	0.6	6.3	55.63	2183	MS, NIST
Trans-squalene	1.1	0.2	0.6	1.6	73.30	2914	MS, NIST

\**C.rcm*: *C. racemosum*, *C. csp*: *C. cuspidatum*, *C. pol*: *C. politum*, *C. jvc*: *C. javanicum*; *E. pyr*: *E. pyramidosphaera*, *E. mgl*: *E. megalocheilos*, *E. coc*: *E. coccinea*, *E.ela*:*E. elatior*; *S.blu*: *S. blumei*, *S. brc*:*S. brachycladum*, *S. pilo*: *S. pilosum*. RT: retention time based on BPX-5 elution; RI: retention indices based on BPX5; Id mode: Identification mode. All sample were subjected to triplicate analysis to obtain the precision in detection.

A total of 65 volatile chemicals detected from investigated species of *Cinnamomum* consist mainly of oxygenated monoterpenes. The majority of the detected volatile chemicals were found to be similar to those reported by Vairappan *et al.* (2014) but differences in concentration of each volatile chemical were noted. Three

major volatile chemicals (eucalyptol, eugenol, terpinen-4-ol) were detected in highest concentrations ranging from 7.5% to 37.9% in the species of *Cinnamomum* studied. Meanwhile, 39 volatile chemicals were detected from species' of *Etilingera* consisting mixtures of oxygenated monoterpenes, sesquiterpenes,

Table 2: Volatile chemicals (%) of investigated *Schizostachyum* spp.

Ret. Time (min)	Ret. Index	Compounds	Conc. (%)				Identification mode
			<i>S.blu</i>	<i>S.brc.</i>	<i>S.lima</i>	<i>S.pilo</i>	
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	2.1	0.6	0.2	FFNSC
22.29	1238	Neral	0.2	-	-	5.1	FFNSC
22.69	1255	Geraniol	0.6	-	-	-	FFNSC
23.66	1268	Geranial	0.4	-	-	7.3	FFNSC
25.41	1347	Citronellic acid	0.1	-	-	-	FFNSC
25.71	1309	Guaiacol-4-vinyl	2.9	4.4	10.7	6.9	FFNSC
27.04	1350	Citronellyl acetate	1.4	-	-	-	FFNSC
27.47	1392	Eugenol	0.1	-	-	-	NIST
28.20	1344	$\alpha$ -cubebene	0.1	-	-	-	NIST
29.68	1392	Vanillin	0.1	-	-	-	NIST
30.60	1432	$\alpha$ -trans-bergamotene	0.3	-	1.9	0.7	FFNSC
31.39	1452	Farnesene	0.1	-	-	-	FFNSC
31.72	1454	$\alpha$ -humulene	0.2	-	-	-	FFNSC
32.80	1512	$\beta$ -cadinene	3.5	-	-	-	FFNSC
33.48	1440	$\beta$ -muurolene	1.1	-	-	-	NIST
33.78	1500	$\beta$ -bisabolene	0.5	-	10.2	-	NIST
33.79	1555	Phenol	-	-	0.8	0.5	NIST
34.27	1518	$\alpha$ -cadinene	3.8	-	-	-	FFNSC
35.73	1546	$\alpha$ -elemol	12.8	21.1	10.1	8.2	FFNSC
37.22	1600	Hexadecane	0.1	-	-	-	FFNSC
38.05	1710	Trans-farnesol	4.6	-	-	-	NIST
38.98	1632	$\beta$ -eudesmol	5.6	0.3	-	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	0.3	-	-	FFNSC
41.53	1696	Juniper camphor	0.1	-	-	-	FFNSC
41.73	2192	Geranylgeraniol	0.5	-	-	-	NIST
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
49.66	1582	Nerylisovalerate	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	2.2	4.2	1.1	0.7	NIST
54.80	2045	Phytol	4.0	1.8	1.0	12.6	NIST
55.63	2183	Linoleic acid	1.0	0.8	0.6	6.3	NIST
55.81	2175	Oleic acid	-	11.5	55.8	10.6	NIST
56.49	2167	Stearic acid	-	10.1	-	-	NIST
57.85	2212	Phytol acetate	2.9	1.5	4.9	7.2	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
<b>Composition of the volatiles (%)</b>							
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	

\**S.blu*: *S. blumei*, *S. brc*:*S. brachycladum*, *S. pilo*: *S. pilosum*.

Table 3: Minimum inhibition concentrations (MIC) ( $\mu\text{g mL}^{-1}$ ) of essential oils from investigated species against four strains of food pathogenic bacteria

Essential oil	<i>S. aureus</i> <sub>1</sub> MIC $\mu\text{g mL}^{-1}$	<i>L. monocytogenes</i> <sub>1</sub> MIC $\mu\text{g mL}^{-1}$	<i>S. typhimurium</i> <sub>1</sub> MIC $\mu\text{g mL}^{-1}$	<i>S. enteritidis</i> <sub>1</sub> MIC $\mu\text{g mL}^{-1}$
<i>C. racemosum</i>	9.00 $\pm$ 0.5	9.00 $\pm$ 0.5	-	10.00 $\pm$ 0.5
<i>C. cuspidatum</i>	5.62 $\pm$ 0.5	-	6.60 $\pm$ 1.5	5.20 $\pm$ 0.5
<i>C. politum</i>	7.70 $\pm$ 1.5	8.00 $\pm$ 0.5	6.90 $\pm$ 0.5	5.04 $\pm$ 1.5
<i>C. javanicum</i>	11.25 $\pm$ 0.5	7.00 $\pm$ 0.5	5.50 $\pm$ 0.5	6.30 $\pm$ 0.5
<i>E. pyramidosphaera</i>	8.20 $\pm$ 0.5	-	12.00 $\pm$ 0.5	-
<i>E. megalocheilos</i>	4.40 $\pm$ 0.5	8.00 $\pm$ 0.5	6.00 $\pm$ 0.5	9.00 $\pm$ 0.5
<i>E. coccinea</i>	5.00 $\pm$ 0.5	4.00 $\pm$ 0.5	8.00 $\pm$ 0.5	8.00 $\pm$ 0.5
<i>E. elatior</i>	8.00 $\pm$ 1.0	9.00 $\pm$ 1.5	7.00 $\pm$ 1.0	6.00 $\pm$ 1.5
<i>S. blumei</i>	10.35 $\pm$ 0.5	4.60 $\pm$ 1.0	8.00 $\pm$ 0.5	12.00 $\pm$ 0.5
<i>S. brachycladum</i>	7.90 $\pm$ 0.5	6.00 $\pm$ 1.5	11.50 $\pm$ 1.0	-
<i>S. lima</i>	8.30 $\pm$ 0.5	-	12.00 $\pm$ 0.5	10.00 $\pm$ 0.5
<i>S. pilosum</i>	6.00 $\pm$ 0.5	-	9.00 $\pm$ 1.0	10.70 $\pm$ 1.0

\*MIC - Minimum Inhibitory Concentration; *S. aureus*: *Staphylococcus aureus*; *L. monocytogenes*: *Listeria monocytogenes*; *S. typhimurium*: *Salmonella typhimurium* and *S. enteritidis*: *Salmonella enteritidis*. Gentamicin was set as positive control for this assay.

oxygenated diterpenes and diterpenes. Borneol, 1-dodecanol, lauryl aldehyde, aromadendrene oxide and elemicin were consistently detected, ranging from 2.4% to 46.2%.

Vairappan *et al.* (2014) reported similar pattern of volatile markers with slight changes in concentration. The differences in concentration of volatile chemicals observed between their report and our study could be attributed to differences in soil pH, altitude, physiological condition and maturity of the specimens studied (Kalua *et al.*, 2007). Verma *et al.* (2013) reported that the composition of volatile chemicals rely on biochemical pathways, either the shikimic acid pathway which produces phenylpropanoid constituents or the mevalonic acid pathway which produces terpene constituents.

The dominance of eucalyptol, terpinen-4-ol and eugenol in the oils of *Cinnamomum* spp. could be considered as the major influence for its positive antimicrobial activity. Terpinen-4-ol is reported to be responsible for diffusing into cell membrane structures, causing increase in fluidity, disordering the membrane structure and inhibiting membrane bound enzymes (Sikkema *et al.*, 1995). Eugenol was found to kill *L. monocytogenes*, *E. coli* and some antibiotic resistant bacterial strains (Gill & Holley, 2006). This could also be attributed by

high degrees of oxygenated monoterpene blend in oils of *Cinnamomum* spp. as classes of these volatile chemicals possess appreciable amount of bioactivity.

The antibacterial activities displayed by oils of *Etlingera* spp. were mainly due to oxygenated monoterpenes, sesquiterpenes and oxygenated diterpenes. It is also observed, that oils of *Etlingera* spp. had the best antibacterial activity among all the species investigated. The presence of borneol, camphor and  $\delta$ -selinene are among the principal chemicals that suppress the growth of gram-positive bacteria such as *S. aureus* (Elaissi *et al.*, 2011). The presence of  $\beta$ -caryophyllene oxide is known to suppress *S. aureus* and *V. parahaemolyticus* in food system (Kim *et al.*, 2008). These antibacterial data justify the usage of *Etlingera* spp., especially *E. coccinea* and *E. elatior*, by Kadazan-dusun community where the young shoots, inflorescences, fruits and flower buds are consumed as salad and medically used to treat stomach ache (Subramaniam *et al.*, 2010).

Overall, a total of 51 volatile constituents were identified in the oil of *Schizostachyum* spp., with the majority of the volatile chemicals are oxygenated sesquiterpenes. Based on detailed analysis, five major volatile markers

were consistently present in the specimens studied;  $\alpha$ -elemol (8.2~21.8%), coumaran (6.7~32.3%), guaiacol-4-vinyl (2.9~10.7%), palmitic acid (1.5~25.6%) and phytol acetate (1.5~2.9%). 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%). As oils of *Schizostachyum* spp. comprise mainly of oxygenated sesquiterpenes, the antimicrobial activity of this genus is found to be lower compared to genus *Etilingera* and *Cinnamomum*.

The presence of common compounds like coumaran, palmitic acid, phytol, adipic acid ester and  $\alpha$ -elemol are significant on the bioactive potential of the four bamboo species. According to Vijisara *et al.* (2014), coumaran functions as antihelminthic, anti-inflammatory and antidiarrhoeal agent and is reported to have insecticidal activity against stored grain insect pests (Rajashekara *et al.*, 2013). Palmitic acid is known to have antioxidant, hypocholesterolemic, nematocidal and pesticide, lubricant, anti-androgenic and 5 $\alpha$ -reductase inhibitor properties (Afrin, 2012). According to Mulyono *et al.* (2012), the presence of fatty acids, esters, long chain alcohols and aldehydes could also enhance the antibacterial property. As such, the synergistic effects of these volatile metabolites could be the lead substances that inhibit *S. aureus* and *Salmonella* strains.

## Conclusion

From the results of this study, the pharmaceutical potential of these three genera are revealed and should be further explored. The Minimum Inhibitory Concentration (MIC) of the essential oils for each species should be further investigated to achieve an ideal dosage for optimum antibacterial activity. These findings provide the scientific proof that supports the usage of a few wild species of *Cinnamomum*, *Etilingera* and *Schizostachyum* as part of

traditional medicine incorporated in dietary practices among the indigenous community of Borneo; and their nutraceutical potential should be further explored.

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