MJFAS MALAYSIAN JOURNAL OF FUNDAMENTAL AND APPLIED SCIENCES
PRINT ISSN: 2289-5981 | ONLINE ISSN: 2289-599X

RESEARCH ARTICLE

Chemical constituents, antioxidant, and cytotoxicity of essential oils of *Piper arborescens* and *Piper caninum*

Nicholas Daniel [a, b,*], Fasihuddin Badruddin Ahmad [a], Zaini Assim [a], Chua Hun Pin [b]

[a] *Faculty of Resource Science and Technology, University Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia*
[b] *Food Science & Technology Research Centre, Malaysian Agricultural Research and Development Institute, MARDI Kuching, Lot 411 Block 14, Sultan Tengah Road, Petra Jaya, 93055 Kuching, Sarawak, Malaysia*

* *Corresponding author: nicholas@mardi.gov.my*

Article history
Received 27 July 2018
Revised 10 October 2018
Accepted 15 April 2019
Published Online 3 December 2019

Abstract

Essential oils of the stem bark of Sarawak's wild pepper species namely the *Piper arborescens* and *Piper caninum* were extracted by using Clevenger's water distillation method, and analysis using gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectroscopy (GC-MS) have identified a total of 54 and 57 chemical components in the essential oils, respectively. Three major compounds have been identified in the essential oil of *Piper arborescens* namely the pentadecanal (18.88%), guaiol (11.19%), and *β*-guaiene (11.12%). In the essential oil of *Piper caninum*, four main compounds identified were isocaryophyllene (20.60%), (*E*)-*α*-bergamotene (13.74%), (*E*)-isoeugenol (13.46%), and (*E,Z*)-3,6-nonadien-1-ol (9.35%). Evaluation of antioxidant properties showed the EC_{50} values of essential oils of *Piper arborescens* and *Piper caninum* were 249.30 and 238.70 µg/mL, respectively, indicating low scavenging activity against DPPH as compared to ascorbic acid as standard with EC_{50} value of 2.72 µg/mL. Cytotoxicity assay showed that average death of *Artemia salina* brine shrimp in the essential oil of *Piper arborescens* was higher, with LC_{50} 57.95 µg/mL, as compared to 249.74 µg/mL of essential oil of *Piper caninum.* The cytotoxic level does not always indicate its outright toxicity but may also indicate the presence of potential natural cytotoxic components, especially in essential oil of *Piper arborescens* as suggested by Elumba *et al.* (2013).

Keywords: *Piper arborescens*, *Piper caninum*, essential oil, antioxidant, cytotoxicity

INTRODUCTION

Piper species, widely distributed over the tropical and subtropical regions of the world are used medicinally in various manners. There are many species of the genus of *Piper* can be found in Borneo, mostly in the wild. Most of the Sarawak's indigenous *Piper* plants possess promising potential as medicinal herbs but are underutilized due to the lack of scientific information. Besides the well-studied black pepper (*Piper nigrum*) and *Piper aduncum*, further studies on other wild *Piper* species, particularly on their phytochemical properties must be explored.

This paper focuses on two wild *Piper* species that can be found in the forest mostly throughout Sarawak, namely *Piper arborescens* (locally known as *lada hutan*) and *Piper caninum* (locally known as *sireh hutan*). The essential oils from these two *Piper* species were extracted by using water distillation, followed by analyses by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectroscopy (GC-MS) to identify their chemical constituents.

Besides, the antioxidant and cytotoxic activities of essential oils of these two *Piper* species were also studied to support its future applications as natural products or customary medicines. Antioxidant activity was tested by using free radical, 2,2-diphenyl-1-pycrylhydrazyl (DPPH) as it is a rapid, simple, and inexpensive method to measure the antioxidant capacity of the compounds to act as free radical scavengers, as described by Tailor & Goyal (2014). Brine shrimp (*Artemia salina* Leach) was used for cytotoxicity assay as suggested by Ramachandran *et al.* (2011) to determine the toxicity of plants by estimating the medium lethality concentration LC_{50}.

EXPERIMENTAL

Plant material

The stem bark of *Piper arborescens* was collected from Betong division in Sarawak, while the stem bark of *Piper caninum* was collected from Serian division. The samples were then air-dried, cut into pieces, and grind prior analysis. It was then deposited into polymer laboratory (specimen numbers: *Piper arborescens*/Betong/2015/ND, and *Piper caninum*/Serian/2015/ND) at Faculty of Resource Science and Technology, UNIMAS.

Extraction of essential oils

The plant samples were subjected to water distillation for 8 hours using Clevenger apparatus to extract the oils quantitatively, following a method described by Samsiah *et al.* (2015) and Fasihuddin & Ibrahim (2003). Approximately 100 g of fresh cut sample was weighed, transferred to 2 L round bottle flask, and mixed with 1.35 L of distilled water. The flask was assembled to the Clevenger trap, connected to the condenser and heated. The sample was heated for 8 hours for hydrodistillation process. After 8 hours, the oil trapped in the Clevenger was then cooled to room temperature. The water layer at the bottom of the oil was first drained out while the oily layer was treated with anhydrous sodium sulphate to absorb any trace of water remained inside. The experiment was performed in triplicates for each sample. The essential oils obtained were kept in vial bottles and stored at 4 °C prior to analysis. The percentage of the oil collected was calculated based on the dried weight of the sample, according to the method mentioned by Costa *et al.* (2014).

GC-FID and GC-MS analysis of essential oils

The essential oils were characterized by chromatography methods. The essential oils were first analyzed with a gas chromatograph equipped with a flame ionization detector (GC-FID) (Fasihuddin & Ibrahim, 2003). The GC-FID was performed on a Perkin Elmer gas chromatography model Clarus 680, equipped with HP-5 fused capillary column (5 % phenylmethylpolysiloxane stationary phase) with 30 m length, 0.25 µm of film thickness, and 0.25 mm internal diameter. The temperature for injector and detector were programmed at 260 ºC and 280 ºC, respectively. The GC oven temperature was programmed from 60 ºC for 2 minutes, then increase 5 ºC/min to 300 ºC, and hold at the final temperature for 5 minutes. Prior to injection, 1.0 µL of essential oil was diluted with 199 µL of dichloromethane. Exactly 1 µL of the prepared essential oil sample was then injected using microsyringe into the GC column. Hydrogen was used as carrier gas with a flow rate of 1 mL/min.

The essential oils were further analyzed by gas chromatography-mass spectrometry (GC-MS). The analysis was performed on a Shimadzu GC-MS model QP 2010 Plus, equipped with BPX-5 column (5 % phenyl polysilphenylene-siloxane) of 30 m length, 0.25 µm of film thickness, and 0.25 mm internal diameter. The injection mode and temperatures programmed used were similar to GC-FID analysis. Helium was used as carrier gas with a flow rate of 1mL/min. The chemical constituents of the oils were identified based on their Kovat's indices (KI) calculation from GC-FID data, and confirmation of the compound name was made by comparing their mass spectra with GC-MS data, with the guidance of name suggested by NIST library. The standard used was n-alkanes (C_9 to C_{32}) (Fouziah *et al*., 2012). The Kovat's indices were calculated using n-alkanes homologous series. A standard hydrocarbon was run beforehand in GC-FID and GC-MS and its retention times were compared with those of the sample. The semi-quantitative data of the oils were obtained using peak area of each component in the gas chromatogram without applying correction factors.

DPPH (2,2-diphenyl-1-pycryl-hydrazyl) free radical scavenging assay

The free radical scavenging assay was used to evaluate the antioxidant properties of essential oil of *Piper arborescens* and *Piper caninum*. The measurement was based on the method as described by Wang *et al.* (2008) with some modifications. The sample was prepared by diluting 10 µL of essential oil into 10 mL of methanol, making a concentration of 1000 µg/mL. Three other concentrations were prepared in 10, 50, and 100 µg/mL, diluted from the 1000 µg/mL master stock solution. A sample of concentration 5000 µg/mL was prepared separately by diluting 25 µL of essential oil into 5 mL of methanol.

Approximately 3 mL from 0.1 mM methanol solution of 2,2-diphenyl-1-pycrylhydrazyl (DPPH) was each added into five series of prepared concentrations (10, 50, 100, 1000, and 5000 µg/mL) of sample solutions (1 mL). The analysis was done in triplicates. The solution was mixed vigorously and left to stand at room temperature for 30 min in the darkness after which its absorbance was measured spectrophotometrically at a wavelength of 517 nm, performed using Jasco ultraviolet spectrophotometer model V-630. Methanol was used as both blank (only methanol) and negative control (1 mL methanol mixed with 3 mL DPPH), while ascorbic acid (vitamin C) as the standard. The concentration of the sample required to inhibit 50 % of the DPPH free radical was calculated as EC_{50}, and the value was determined using Log dose inhibition curve, which performed by using PRISM software, based on the calculated values of the DPPH scavenging activity (%) of the sample (Tailor & Goyal, 2014).

Brine shrimp (*Artemia salina*) lethality test

Cytotoxicity against brine shrimp (*Artemia salina*) developed by McLaughlin (1991) was used in this study. Leached brine shrimp eggs were hatched in seawater and incubated for 48 hours of at 25 ºC. Exactly 3 mg of sample was dissolved in 3 mL of methanol. From this solution, 500 µL, 250 µL, 50 µL, and 5 µL samples were transferred into NUNC multidish in triplicates. The solvent was allowed to evaporate under a running fume hood overnight and followed by the addition of 4.8 mL seawater and 0.2 mL dimethylsulphoxide (DMSO) to give a final concentration of 100, 50, 10, and 1 µg/mL, respectively. Ten nauplii were transferred into each treatment in NUNC multidish and were observed every 6 hours for 24 hours. Thymol was used as positive control, whereas a mixture consists of 0.2 mL DMSO and 4.8 mL seawater was used as negative control. A number of dead nauplii were calculated. In this study, LC_{50} refers to the concentration of the samples that kill 50 % of brine shrimp at 24 hours. LC_{50} was calculated and determined by performing Probit analysis in IBM SPSS statistic software.

RESULTS AND DISCUSSION

Chemical constituent of essential oils

Water distillation method was carried out for 8 hours, producing 0.40 % yield of pale yellowish essential oil from the stem bark of *Piper arborescens*. Based on the GC-FID and GC-MS analyses, this essential oil consisted of a total of 54 chemical compounds (Table 1). The result showed that there were three major compounds in this *Piper* species, namely pentadecanal, guaiol, and *β*-guaiene with the percentage of 18.88 %, 11.19 % and 11.12 %, respectively. Other compounds were identified as *δ*-dodecalactone (6.71 %), erucin (6.01 %), (*E*)-*α*-bergamotene (5.89 %), tetradecanol (4.38 %), bicyclogermacrene (3.38 %), (*Z*)-oak-lactone (2.75 %), (*E*)-2-dodecen-1-ol (2.30 %), *β*-selinene (2.14 %), *α*-cadinol (2.02 %), dill apiol (1.85 %), *α*-curcumene (1.81 %), *α*-ionone (1.71 %), tridecanal (1.63 %), butyl decanoate (1.46 %), methyl eugenol (1.06 %), *β*-ionone (1.04 %), and some other compounds which were each presence less than 1 %. Approximately 0.74% of chemical constituents in *Piper arborescens* remained unidentified by GC-FID and GC-MS.

Previous study by Hakimi *et al*. (2016) reported that important constituents identified in the stem oil of *Piper arborescens* were *β*-phellandrene (2.04 %), methyl eugenol (11.0 %) and *β*-caryophyllene (9.0 %). Their finding was slightly different as compared to the important constituents identified in *Piper arborescens* from this study. The difference in the important constituents between the two studies probably due to locality differences of the samples as suggested by Pattamapan *et al*. (2015). *Piper arborescens* in this study was of Betong origin, while *Piper arborescens* used by Hakimi *et al*. (2016) was collected from Forest Research Centre of Kuching, Sarawak. The two studies however reported the same chemical composition of bicyclogermacrene (3.38 % identified in this study; while 4.50 % reported by Hakimi *et al.* (2016)), *β*-selinene (2.14 %; 3.00 %), *α*-cadinol (2.02 %; 1.40 %), *α*-bisabolol (0.79 %; 0.40 %), *α*-cubebene (0.29 %; 0.70 %), and *α*-muurolene (0.44 %; 0.20 %) in the essential oil of *Piper arborescens*.

Hydrodistillation process produced a light yellowish essential oil from the stem bark of *Piper caninum*, with a yield of 0.48 %. GC-FID and GC-MS have identified a total of 57 chemical components in *Piper caninum* essential oil (Table 1). Chromatographic result showed that there were four major compounds identified in this sample, namely isocaryophyllene (20.60 %), (*E*)-*α*-bergamotene (13.74 %), (*E*)-isoeugenol (13.46 %), and (*E,Z*)-3,6-nonadien-1-ol (9.35 %).

In addition, the analysis also identified the presence of isopropyl palmitate (6.81 %), (*E,E*)-farnesylacetone (4.26 %), *β*-selinene (3.57 %), ethyl salicylate (3.05 %), *β*-caryophyllene (3.01 %), palmitaldehyde (2.91 %), ethyl dihydrocinnamate (2.34 %), bornyl isovalerate (1.61 %), 6-methoxyeugenol (1.23 %), (*E*)-isoelemicin (1.13 %), and other constituents that each consist of less than 1 % in the stem bark oil of *Piper caninum*. It was observed in this study that the identified chemical constituents in the stem bark oil of *Piper caninum* were mostly similar to those reported by Hakimi *et al*. (2011) with the presence of similar components such as *β*-caryophyllene (3.01 % identified in this study; while 9.80 % reported by Hakimi *et al*., 2011), *δ*-elemene (0.08 %; 4.10 %), eugenol (0.04 %; 2.40 %), *β*-bourbonene (0.03 %; 1.10 %), aromadendrene (0.09 %; 0.80 %), *α*-zingiberene (0.74 %; 0.60 %), bicyclogermacrene (0.74 %; 2.30 %), *α*-bisabolol (0.25 %; 0.40 %) and *α*-cadinol (0.17 %; 1.00 %). Besides, (*Z*)-nerolidol (0.19 %) was identified in this study, while Hakimi *et al*. (2011) reported the presence of (*E*)-nerolidol (1.60 %)

in the stem oil of *Piper caninum*. In this study, approximately 1.63 % of chemical constituents were remained unidentified in *Piper caninum* due to the poor similarity of the calculated Kovat's indices with the reference available in www.flavornet.org. Besides, the similarity index of these unidentified components was low compared to NIST library.

Table 1 Chemical composition identified in the essential oils of *Piper arborescens* and *Piper caninum.*

	Chemical composition	KI *a*	KI *b*	Percentage of concentration (%)	
				Piper arborescens	***P. caninum***
	Monoterpene hydrocarbones				
1	Isocaryophyllene	1439	1438	0.48	20.60
	Sub-total			**0.48**	**20.60**
	Oxygenated monoterpane				
2	1,3-p-menthadien-7-al	1294	1293		0.90
3	Ethyl dihydocinnamate	1351	1351		2.34
4	Citronellyl acetate	1356	1357	0.78	
5	Methyl eugenol	1400	1407	1.06	0.04
6	*α*-ionone	1423	1422	1.71	
7	Wine lactone	1456	1456	0.31	0.19
8	Butyl decanoate	1466	1467	1.46	
9	Asaricin	1479	1479		0.23
10	Epoxy-2-undecenal	1484	1484	0.27	
11	Bornyl butyrate	1488	1490	0.84	
12	*β*-ionone	1493	1493	1.04	
13	Tridecanal	1505	1503	1.63	
14	Methyl laurate	1511	1509	0.99	0.88
15	Myristicin	1531	1532	0.16	0.79
16	12-methyltridecanal	1574	1576		0.08
17	Isopropyl benzoate	1567	1567	0.27	
18	(*E*)-isoelemicin	1595	1596		1.13
19	Dill apiole	1601	1602	1.85	
20	*δ*-undecalactone	1607	1606		0.04
21	Epoxy-*β*-ionone	1611	1610	0.11	
22	(Z)-6-dodecen-*γ*-lactone	1656	1656		0.65
23	*γ*-dodecalactone	1686	1685	0.21	0.09
24	(*E*)-2-dodecen-1-ol	1692	1692	2.30	0.05
25	Methyl cinnamate	1700	1700		0.03
26	*δ*-dodecalactone	1717	1721	6.71	
27	Benzyl benzoate	1723	1723	0.09	
28	*γ*-undecalactone	1921	1922		0.04
29	(*E*)-isoeugenol	2025	2024		13.46
30	Tetradecanol	2128	2116	4.38	
31	6-methoxyeugenol	2225	2222		1.23
	Sub-total			**26.17**	**22.17**
	Sesquiterpene hydrocarbons				
32	*δ*-elemene	1340	1340		0.08
33	*α*-cubebene	1344	1345	0.29	
34	*β*-elemene	1394	1393	0.07	
35	*β*-bourbonene	1418	1417	0.05	0.03
36	(*E*)-*α*-bergamotene	1433	1431	5.89	13.74
37	*β*-selinene	1434	1436	2.14	3.57
38	*β*-farnesene	1442	1445		0.99
39	*α*-guaiene	1453	1453		0.08
40	*β*-caryophyllene	1464	1467		3.01
41	Aromadendrene	1473	1475		0.09
42	*β*-guaiene	1483	1483	11.12	0.34
43	*α*-zingiberene	1494	1494		0.74
44	Alloaromadendrene	1496	1496	0.41	
45	*α*-farnesene	1499	1500	0.42	0.18
46	Bicyclogermacrene	1520	1517	3.38	0.74
47	*α*-muurolene	1523	1523	0.44	0.23
48	Cadinadiene	1527	1527	0.56	
49	*γ*-cadinene	1542	1543	0.20	0.29
50	*α*-curcumene	1552	1553	1.81	
51	*β*-sesquiphellandrene	1559	1560	0.18	0.09

	Sub-total			**26.97**	**24.20**
	Oxygenated sesquiterpenes				
52	Bornyl isovalerate	1530	1529		1.61
53	Elemol	1546	1547	0.40	
54	Caryophyllene alcohol	1556	1556	0.21	
55	(*Z*)-nerolidol	1565	1565		0.19
56	Guaiol	1580	1589	11.19	0.04
57	Citronellyl valerate	1627	1625		0.04
58	*β*-caryophyllene alcohol	1642	1642	0.06	0.35
59	(-)-cubenol	1645	1645	0.38	
60	Geranyl valerate	1650	1649	0.18	
61	Bulnesol	1652	1651		0.04
62	*α*-bisabolol	1660	1662	0.79	0.25
63	oxo-*β*-ionone	1664	1665	0.49	
64	*δ*-cadinol	1675	1674	0.63	0.04
65	*α*-cadinol	1679	1676	2.02	0.17
66	Pentadecanal	1712	1711	18.88	
67	Perhydrofarnesylacetone	1769	1770	0.30	
68	Hexadecanone	1798	1798		0.04
69	Palmitaldehyde	1813	1813		2.91
70	(*E*,*E*)-farnesyl acetate	1934	1935	0.04	
71	Isopropyl palmitate	2010	2010	0.05	6.81
72	(*E*,*E*)-farnesylacetone	2015	2015		4.26
73	Octadecanaldehyde	2052	2052		0.06
74	Hydroxycalamenene	2098	2085	0.16	0.74
	Sub-total			**35.78**	**17.55**
	Miscellaneous compound				
75	Methylethylpyrazine	1034	1035		0.22
76	*γ*-nonalactone	1369	1366		0.05
77	(*E*,*Z*)-3,6-nonadien-1-ol	1383	1383	0.83	9.35
78	Ethyl salicylate	1435	1436		3.05
79	Erucin	1447	1447	6.01	
80	(*Z*)-oak-lactone	1535	1538	2.75	
81	(*E*)-whiskey lactone	1628	1629	0.14	
82	(*Z*)-whiskey lactone	1637	1637		0.03
83	Diethyl-2-hydroxyglutarate	1822	1823		0.04
84	4-Carbethoxybutyrolactone	1890	1893		0.07
85	4-vinylphenol	2076	2079		0.73
86	Acetovanillone	2286	2292	0.14	0.31
	Sub-total			**9.87**	**13.85**
	Total identified compounds			99.26	98.37
	Unidentified compounds			0.74	1.63
	Total			100	100

KI *a* = Calculated Kovat's Indices;
KI *b* = Reference Kovat's Indices (www.flavornet.org)

The result showed that both essential oils of *Piper arborescens* and *Piper caninum* contained a total of 25 same compounds namely the isocaryophyllene, methyl eugenol, wine lactone, methyl laurate, myristicin, *γ*-dodecalactone, (*E*)-2-dodecen-1-ol, *β*-bourbonene, (*E*)-*α*-bergamotene, *β*-selinene, *β*-guaiene, *α*-farnesene, bicyclogermacrene, *α*-muurolene, *γ*-cadinene, *β*-sesquiphellandrene, guaiol, *β*-caryophyllene alcohol, *α*-bisabolol, *δ*-cadinol, *α*-cadinol, isopropyl palmitate, hydroxycalamenene, (*E*,*Z*)-3,6-nonadien-1-ol, and acetovanillone. In *Piper arborescens* and *Piper caninum*, some compounds were present in almost very similar quantity such as *γ*-cadinene (0.20 % in *Piper arborescens*; while 0.29% in *Piper caninum*), methyl laurate (0.99 %; 0.88 %) and *β*-bourbonene (0.05 %; 0.03 %). The presence of isocaryophyllene (20.60 %) as a major component in *Piper caninum*, but only 0.48% in *Piper arborescens*, and (*E*)-*α*-bergamotene (13.74 %) that presence as major component in *Piper caninum*, while only 5.89 % in *Piper arborescens*. Besides, an important constituent of (*E*)-isoeugenol (13.46 %) in *Piper caninum* was absent in the essential oil of *Piper arborescens*. These three constituents can be used to differentiate between essential oils of *Piper caninum* and *Piper arborescens*.

Antioxidant activity

In this study, the absorbance measured at a wavelength of 517 nm by UV spectrophotometer at different concentrations of essential oils of *Piper arborescens* and *Piper caninum* is shown in Table 2. The results obtained show that both plants species possess low scavenging activity against DPPH with the EC_{50} values of 249.30 and 238.70 µg/mL, respectively, as shown in Table 2. As comparison, the EC_{50} value of ascorbic acid is 2.72 µg/mL.

The result suggested that in the form of essential oil, *Piper arborescens* and *Piper caninum* might captured less of the free radicals formed by DPPH, resulting in a low absorbance and high EC_{50} value, a similar result pattern discussed by Tailor & Goyal (2014). A study on the essential oil of *Piper caninum* by Hakimi *et al.* (2011) reported that the essential oil showed weak activity towards DPPH free-radical scavenging, with EC_{50} value for the leaf oil was 187.60 mg/mL, whereas EC_{50} for the stem oil was 452.40 mg/mL. The low antioxidant activity of the stem bark oils of *Piper caninum* and *Piper arborescens* in this study corresponded well to the low antioxidant activity of the essential oils reported by Hakimi *et al.* (2011). Besides, low antioxidant activity was also reported for the

essential oil of *P. nigrum* from China with EC_{50} value of 1335 mg/mL (Zhang & Xu, 2015). Pattamapan *et al.* (2015) has reported on the weak radical scavenging activity of Thai black pepper (*P. nigrum*) and also mentioned that the black pepper essential oil of the same species collected from a different country might give a different result. The differences in essential oil's antioxidant activity might be due to the variation in their chemical composition (Pattamapan *et al.*, 2015).

Table 2 Absorbance of different sample concentration and free radical scavenging activities against DPPH radical of the essential oils of *Piper arborescens and Piper caninum.*

Essential oils	Absorbance at different sample concentration					EC_{50} (µg/mL)
	10 µg/mL	50 µg/mL	100 µg/mL	1000 µg/mL	5000 µg/mL	
Piper arborescens	0.5804 ± 0.01	0.5576 ± 0.00	0.5926 ± 0.00	0.5488 ± 0.00	0.5027 ± 0.01	249.30
Piper caninum	0.3985 ± 0.00	0.4056 ± 0.00	0.4047 ± 0.00	0.3871 ± 0.00	0.3718 ± 0.00	238.70
Ascorbic acid	0.5906 ± 0.01	0.0866 ± 0.00	0.0873 ± 0.00	0.0896 ± 0.00	0.0950 ± 0.00	2.72

Table 3 Average death of *Artemia salina* brine shrimp at different concentrations of the essential oils of *Piper arborescens* and *Piper caninum.*

Essential Oils Samples	Average death of *Artemia salina*				LC_{50} (µg/mL)
	Concentration (µg/mL)				
	1	10	50	100	
Piper arborescens	0	1 ± 0.57	3 ± 0.57	8 ± 0.57	57.95
Piper caninum	0	0	2 ± 0.57	3 ± 0.00	249.74
(-ve Control)	0	0	0	0	-
(+ve Control) Thymol	5 ± 0.57	7 ± 0.57	10 ± 0.00	10 ± 0.00	1.15

Cytoxicity

The average number of death of *Artemia salina* brine shrimp in different concentration of essential oils of *Piper arborescens* and *Piper caninum* after 24 hours is shown in Table 3, while the average death of *Artemia salina* brine shrimp (%) as a function of the concentration of essential oils of *Piper arborescens* and *Piper caninum* is shown in Fig. 1. In this study, thymol, the positive standard has the LC_{50} value against the brine shrimp of 1.15 µg/mL.

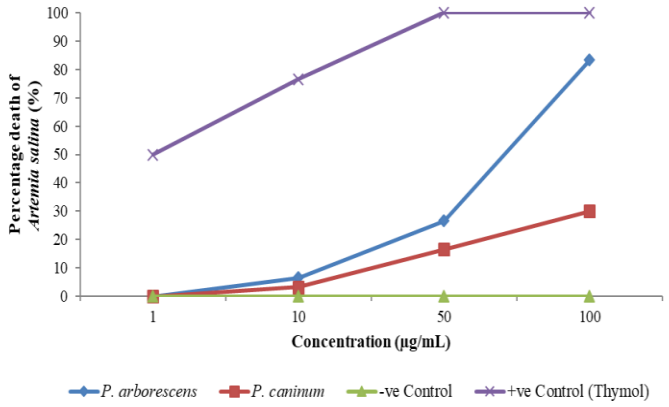

Fig. 1 Average death of *Artemia salina* brine shrimp (%) as a function of a concentration of the essential oils of *Piper arborescens* and *Piper caninum.*

Moshi *et al*. (2010) reported that if the test sample showed LC_{50} between 30–100 µg/mL, it is categorized as mildly toxic, whereas those with LC_{50} more than 100 µg/mL are considered as being practically low or non-toxic. Referring to this guideline, essential oil of *Piper arborescens* can be categorized as mildly toxic (LC_{50} value of 57.95 µg/mL), whereas essential oil of *Piper caninum* was non-toxic (LC_{50} value of 249.74 µg/mL) (Table 3). Greater cytotoxicity of essential oil of *Piper arborescens* towards brine shrimp indicated the presence of potent cytotoxic components in this *Piper* spp. The presence of three major compounds such as pentadecanal, guaiol, and *β*-guaiene in essential oil of *Piper arborescens* may have influenced its cytotoxic properties.

Previous study by Magdalene *et al.* (2014) suggested that some of the plant extracts and essential oils with LC_{50} below 100 µg/mL which are categorized as toxic, does not always indicated its toxicity toward human, but may also suggest a potential antitumor or anticancer activities. Exposure or consuming this type of plant is unlikely to have any detrimental effect on human (Moshi *et al.*, 2010). As a reference, previous cytotoxicity studies done by Tsai *et al.* (2005) on the isolated compound from *Piper arborescens* have identified certain compounds that showed significant cytotoxicity against various cancer and human cells, which also indicate that this *Piper* species is a source of potential natural product for anticancer.

ACKNOWLEDGEMENT

The FRGS research grants GL(F07)/MARDI/2014(12) and FRGS/1/2014/SG03/MOA/02/1 from Ministry of Education of Malaysia are gratefully acknowledged. The supports received through the collaborative work of Universiti Malaysia Sarawak and Malaysian Agricultural Research and Development Institute (MARDI) in this research project are appreciated.

REFERENCES

Costa, O. B., Menezzi, C. H. S. D., Resck, I. S., Vieira, R. F., and Bizzo, H. R. (2014). Essential oil constituents and yields from leaves of *Blepharocalyx salicifolius* (Kunt) *Berg* and *Myracrodruon urundeuva* (Allemao) collected during daytime. *International Journal of Forestry Research*, *2014*, 1-6.

Elumba, Z. S., Teves, F. G., and Madamba, M. R. S. B. (2013). DNA-binding and cytotoxic activities of supercritical-CO2 extracts of *Ganoderma lucidum* (Curt.:Fr.) P. Karst. collected from the wild of Bukidnon province, Philippines. *International Research Journal of Biological Sciences*, *2*(3), 62-68.

Fasihuddin, B. A., and Ibrahim, J. (2003). Chemical constituents of the essential oils of *Goniothalamus uvariodes King*. *Flavour and Fragrance Journal*, *18*(2), 128-130.

Fouziah, A., Assim, Z. B., Ismail, J., and Fasihuddin, B. A. (2012). Chemical constituents of essential oils from resin and bark of *Agathis bornensis*. *Borneo Journal of Resources Science and Technology*, *2*(1), 28-32.

Salleh, W. M. N. H. W., Ahmad, F., and Khong, H. Y. (2016). Essential oil compositions and antimicrobial activity of *Piper arborescens*. *Roxb. Marmara Pharmaceutical Journal*, *20*(2), 111-115.

Salleh, W. M. N. W., Ahmad, F., Khong, H. Y., and Hasnah, M. S. (2011). Chemical compositions, antioxidant and antimicrobial activities of essential oils of *Piper caninum* Blume. *International Journal of Molecular Sciences*, *12*(11), 7720-7731.

Magdalene, M., Del, S., Clifford, P. B., and Charity, M. L. D. (2014). Cytotoxic effects of Betel vine, *Piper betle* Linn. leaf extracts using *Artemia salina* leach (brine shrimp lethality assay). *Journal of Multidisciplinary Studies*, *3*(1), 100-111.

McLaughlin, J. L. (1991). Crown gall tumours on potato discs and brine shrimp lethality: Two simple bioassay for higher plants screening and fractionation. In K. Hostettmann (Ed.), *Methods for Plant Bioactivity: Assay for Bioactivity* (pp. 2-32). Academic Press, San Diego.

Moshi, M. J., Innocent, E., Magadula, J. J., Otieno, D. F., Weisheit, A., Mbabazi, P. K., and Nondo, R. S. O. (2010). Brine shrimp toxicity of

some plants used as traditional medicines in Kagera region, North Western Tanzania. *Tanzania Journal of Health Research*, *12*(1), 1-6.

Pattamapan, L., Kittisak, S., Phanida, P., Worawan, K., Krit, T., and Nuntavan, B. (2015). *In vitro* biological activities of black pepper essential oil and its major components relevant to the prevention of Alzheimer's disease. *The Thai Journal of Pharmaceutical Sciences*, *39*(3), 94-101.

Ramachandran, S., Vamsikrishna, M., Gowthami, K. V., Heera, B., and Dhanaraju, M. D. (2011). Assessment of cytotoxic activity of *Agave catula* using brine shrimp (*Artemia salina*) lethality bioassay. *Asian Journal of Scientific Research*, *4*(1), 90-94.

Samsiah, J., Fasihuddin, B. A., Laily, D., and Zuriati, Z. (2015). Essential oils from different parts of *Goniothalamus ridleyi* plant. *Sains Malaysiana*, *44*(11), 1579-1585.

Tailor, C. S., and Goyal, A. (2014). Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides Linn*. leaves. *American Journal of Ethnomedicine*, *1*(4), 244-249.

Tsai, I. L., Lee, F. P., Wu, C. C., Duh, C. Y., Ishikawa, T., Chen, J. J., Chen, Y. C., Seki, H., and Chen, I. S. (2005). New cytotoxic cyclobutanoid amides, a new furanoid lignan and anti-platelet aggregation constituents from *Piper arborescens*. *Planta Medica*, *71*(6), 535-542.

Wang, H., Zhao, M., Yang, B., Jiang, Y., and Rao, G. (2008). Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. *Food Chemistry*, *107*(4), 1399-1406.

Zhang, L. L., and Xu, J. G. (2015). Comparative study on antioxidant activity of essential oil from white and black pepper. *European Journal of Food Science and Technology*, *3*(3), 10-16.