

Brine Shrimp Toxicity of Essential Oils from *Musa* spp.

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ABSTRACT

Essential oils of seven *Musa* spp.'s fruits namely, *Musa acuminata* colla 'gros michel' (PE), *Musa acuminata* colla 'lakatan' (PB), *Musa acuminata* colla 'sucrier' (PM), *Musa acuminata* × *balbisiana* 'horn plantain' (PT), *Musa acuminata* × *balbisiana* colla 'saba' (PN), *Musa acuminata* colla 'inarnibal' (PO) and *Musa acuminata* colla 'red' (PJ) were extracted by hydrodistillation method using Clevenger apparatus. The essential oils were analysed using GC-FID and identified using Kovat Indices compared with published information. PE gave the highest yields of oils compared to the other species with yield of 0.43% and 0.28% for its peels and flesh, respectively. The most abundant groups present in most of the essential oils are ester and alcohol groups. The brine shrimp toxicity of PT, PM, PN and PO essential oils were tested and showed toxicity against brine shrimp in dose dependent manner. It can be concluded that the essential oils showed some biological activities which may be a potent medicine in curing tumor. This study implies that the presence of ester and alcohol groups are a good marker for the biological activities of plants. Further studies should focus on the potential of the essential oils of *Musa* spp. as an antitumor medicine.

Keywords: *Artemia salina*, banana, essential oil, hydrodistillation

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INTRODUCTION

Essential oils are very valuable natural product that are used as raw materials in many fields such as food preservation, pharmaceuticals, alternative medicine, perfumes, cosmetics, aromatherapy, spiritual uses, spices and nutrition (Buchbauer, 2000).

The presence of chemical constituent in the essential oil are very valuable due to their biological activities such as antibacterial (Dutra *et al.*, 2019), antifungal (Sienkiewicz *et al.*, 2015), anti-inflammatory (Murbach *et al.*, 2014; Koh *et al.*, 2002), anti-lice (Di *et al.*, 2012), anti-tumour (Calcabrini *et al.*, 2004), antioxidant (Aazza *et al.*, 2014) and act as insect repellent (Gkinis *et al.*, 2003).

Musa spp. commonly known as banana, is a tropical plant that has been consumed since many years ago by mankind and animals for its nutrients and delicious taste. In Malaysia, *Musa* spp. is known as *pisang*. It is one of local favorite fruits, which is used for cooking and

making desserts.

Musa spp. comes from Musaceae family and approximately 1200 species of varieties are reported (Guylène *et al.*, 2008). Analytical research on the volatile compound of *Musa* spp. had been carried out for more than 55 years with various extraction methods (Shiota, 1993; Brat *et al.*, 2004; Aurore *et al.*, 2011). Generally, volatile compound of *Musa* spp. consists of three major components, which are ester, alcohol, and carbonyl groups (ketones and aldehydes) (Jordán *et al.*, 2001; Pérez *et al.*, 1997). This chemical constituent that is present in *Musa* spp. is valuable, especially in traditional and alternative medicine in countries such as India, Pakistan, United States and Asian countries such as Malaysia, Thailand, Philippines and Indonesia because of the biological activities of the fruit (Pari & Umamaheswari, 2000; O'Hara *et al.*, 1998; Orhan, 2001). Thus, the objective of this study was to evaluate the brine shrimp toxicity and antibacterial properties of this plants' essential oils which were extracted using hydro-distillation technique.

MATERIALS AND METHODS

Sample Preparation

Seven *Musa* spp. fruits namely *M. acuminata* colla 'gros michel' (PE), *M. acuminata* colla 'lakatan' (PB), *M. acuminata* colla 'sucrier' (PM), *M. acuminata* × *balbisia* 'horn plantain' (PT), *M. acuminata* × *balbisia* colla 'saba' (PN), *M. acuminata* colla 'inarnibal' (PO) and *M. acuminata* colla 'red' (PJ) were purchased from local markets in Kuching and Kota Samarahan. The fresh ripe samples were separated into peels and flesh. Each part of the samples was sliced to small pieces. About 100 g to 500 g of the sample was weighed using a single pan electronic weighing balance and placed into a 3 L flat-round bottom flask.

Extraction of Essential Oils

The essential oil of seven *Musa* spp.'s fruits were extracted using hydrodistillation method in a Clevenger-type apparatus based on method suggested by Sim *et al.* (2013). Fresh samples, which were placed into a 3 L flat-round bottom flask were added together with 1.5 L of distilled water. The sample was hydro-distilled continuously for 6 hours. The essential oils were diluted in *n*-hexane upon the collection and left to cool at room temperature. The oils were separated from water by passing them over anhydrous sodium sulphate. The essential oils were kept in 2-mL amber glass vial and kept in a refrigerator at 4 °C before further analysis. The percentage yield of essential oils was calculated based on the weight of the essential oils extracted over dry weight of the sample after the extraction.

Gas Chromatography – Flame Ionisation Detector (GC-FID) Analysis

The constituents of the essential oils were analysed using Gas Chromatography-Flame Ionisation Detector (GC-FID). Exactly, 1 µL of sample was diluted with 199 µL *n*-hexane prior to the analysis. The samples were analysed in gas chromatography (GC) model of Agilent Hewlett-Packard (HP) 6890 equipped with flame ionisation detector (FID) using capillary HP-5 column (30 m × 250 µm × 0.25 µm). The carrier gas used was purified helium. The initial temperature for the oven was set at 50 °C for 5 minutes and increased to 280 °C with the rate of 6.5 °C min⁻¹ and hold for 7 minutes.

Exactly, 1 µL of diluted sample was injected in the GC-FID by splitless injection mode. Standard of *n*-alkanes with internal standard of C₂₀H₄₂ was also analysed as a standard for the calculation of Kovat's Indexes. The individual components present in the essential oils were determined based on their calculated Kovat's Index by comparison with previous published data.

Kovat's Index

Kovat's Index was used to identify the chemical constituents that are present in the essential oils. The Kovat's Index was calculated using Eq. 1.

$$I_x = 100 \left[\frac{t_x - t_n}{t_{(n+1)} - t_n} \right] + 100n \quad \text{Eq. 1}$$

Where,

I_x = Kovat's Index

t_x = Retention time for compound x

t_n = Retention time of alkane eluted before the compound

$t_{(n+1)}$ = Retention time of alkane eluted after the compound

n = Number of carbon atom

Semi-Qualitative Analysis

The percentage of individual chemical components in the essential oil were calculated using a normalised calculation as in Eq. 2.

$$\%X = \frac{A_x}{\Sigma A_1} \times 100\% \quad \text{Eq. 2}$$

Where,

A_x = Peak area of X

ΣA_1 = Sum of all area (that identified in the compound of essential oil only)

Percentage Yield

The percentage yield of essential oils was calculated based on the dry weight of sample used for each extraction. The percentage yields were calculated using Eq. 3.

$$\text{Percentage yield (\%)} = \frac{\text{Weight of oil (g)}}{\text{Weight of the dried sample (g)}} \times 100\% \quad \text{Eq. 3}$$

Cluster Analysis

Cluster analysis was performed using SPSS version 22. Cluster analysis was used to identify whether the species are related to each other. From this information, the data can be used for the chemotaxonomy classification.

Brine Shrimp Toxicity Test

Cytotoxicity test of essential oils of *Musa* spp. was tested against brine shrimp, *Artemia salina* using method described by McLaughlin and Rogers (1998). The brine shrimp eggs were hatched in a beaker containing artificial sea water which was prepared using 38 g of artificial sea salt in 1 L of distilled water and left for 2 days at room temperature. Stock solutions of samples with a concentration of 1000 ppm were prepared. Exactly 2 μL , 20 μL and 200 μL of stock solution transferred were into a separate hole of 24 well NUNC multiwell and left to dry the solvent. Methanol was used as negative control as it was added in the multiwell and were left to dry. Exactly 5 μL of dimethylsulphoxide (DMSO) and 2 mL of artificial seawater was transferred into each hole including the control holes. Exactly 10 *Artemia salina* larvae were added into each hole of the multiwell plates. The multiwell plates were left for one day under room temperature with exposure to light. The number of dead shrimp was counted and percentage of mortality was determined.

Antibacterial Assay

The antibacterial activity of essential oils of *Musa* spp. was determined by disc diffusion based on method by Sim *et al.* (2013). The bacteria used were *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The pure cultures of all bacteria were inoculated into Mueller-Hinton broth and incubated at 37 °C for 24 hours. The optical density (OD) of each bacterium was standardised to 0.168 OD at 610 nm wavelength using UV-Vis spectrophotometer. Commercial penicillin (10 μg disc⁻¹) and streptomycin (10 μg disc⁻¹) were used as positive controls. Sterile discs loaded with methanol were used as the negative control. The agar plates were prepared using Mueller-Hinton agar. The agar plates were swabbed with bacterial culture uniformly using sterile cotton uniformly. Stock solutions of samples were prepared at a

concentration of 1000 ppm. Then 100 μL of stock solution was loaded on 6 mm sterile discs. The discs were placed on the surface of medium and the agar plates were incubated at 37 °C for 24 hours. The inhibition zones were measured in millimeter. The assay was conducted in triplicates.

RESULTS AND DISCUSSION

Essential Oil Yields

The percentage yield of essential oils obtained from seven *Musa* spp. are varied. The percentage yield, the physical appearance of the extracted essential oils are shown in Table 1.

The percentage yield of essential oils extracted from the flesh part of the fruits are in a range of 0.06% (w/w) to 0.28% (w/w). The highest percentage yield among the flesh samples was shown by *M. acuminata colla 'gros michel'* with a yield of 0.28% (w/w), while the lowest yield was obtained from *M. acuminata colla 'red'* with a yield of 0.06% (w/w). The essential oils extracted from the peels part gave yields in a range of 0.05% (w/w) to 0.43% (w/w). *M. acuminata colla 'gros michel'* peels have the highest yield with a yield of 0.43% (w/w), while the lowest yield was given by *M. acuminata* \times *balbisiana colla 'saba'* with a percentage of 0.05% (w/w).

Chemical Composition of Essential Oils

All essential oils of *Musa* spp. were extracted by hydrodistillation method showed different chemical composition between the studied species. The chemical compositions of essential oils from seven *Musa* spp. is shown in Table 2.

The chemical constituents in essential oil of *Musa* spp. consist of a complex mixture mainly esters, alcohols, carboxylic acids, aldehydes, ketones, and terpenes with a total of 247 components were identified. The essential oils of *Musa* spp. studied showed most of the components are from the alcohol, ester and carbonyl group. This is similar to previous studies by Selli *et al.* (2012), Nogueira *et al.* (2003) and Jordán *et al.* (2001), where these three groups were the most abundant groups in *Musa* spp. essential oils. Each class of chemical components in *Musa* spp.'s fruit are important in determining the properties and aromatic quality of the *Musa* spp. (Aurore *et al.*, 2011).

Table 1. The percentage yield and the physical appearance of the extracted essential oils

Species	Part	Percentage yield of oil (%)	Colour	Major Component	Percentage Composition (%)
PM	Peels	0.32	Light yellow	α -Terpinyl acetate	7.72
	Flesh	0.23	Light yellow	β -Phellandrene	9.56
PN	Peels	0.05	Colourless	Eugenol acetate	19.75
	Flesh	0.08	Light yellow	α -Cadinene	49.39
PT	Peels	0.09	Light yellow	Ethyl decanoate	36.63
	Flesh	0.12	Light yellow	13-Epi-manool	18.77
PE	Peels	0.43	Colourless	Piperitenone	8.52
	Flesh	0.28	Colourless	Ethyl pentanoate	33.80
PO	Peels	0.32	Light yellow	Epi-1-cubenol	22.77
	Flesh	0.13	Colourless	Isospathulenol	5.26
PJ	Peels	0.09	Colourless	Dehydro-1,8-cineole	34.47
	Flesh	0.06	Colourless	Palustrol	20.20
PB	Peels	0.22	Pale yellow	Ethyl tetradecanoate	16.26
	Flesh	0.12	Pale yellow	Benzyl benzoate	12.48

Notes : PM - *M. acuminata* colla 'sucrier', PN - *M. acuminata* \times *balbisiana* colla 'saba', PT - *M. acuminata* \times *balbisiana* 'horn plantain', PE - *M. acuminata* colla 'gros michel', PO - *M. acuminata* colla 'inarnibal', PJ - *M. acuminata* colla 'red', PB - *M. acuminata* colla 'lakatan'

Table 2. Chemical constituents of seven *Musa* spp.'s fruit essential oils

Compound	PM		PN		PO		PT		PJ		PE		PB	
	F	S	F	S	F	S	F	S	F	S	F	S	F	S
Alcohol														
(2E,6E)-Farnesol	0.15	0.44	0.40	0.30	-	0.18	-	-	2.36	0.27	0.39	0.78	1.01	5.28
(2Z,6Z)-Farnesol	0.30	0.24	-	-	-	-	-	-	-	-	-	-	-	-
(E)-2-Hexen-1-ol, acetate	-	-	-	-	0.27	-	-	-	-	-	-	-	-	-
(E)-2-Octen-1-ol	-	-	-	-	-	-	-	-	0.07	-	-	-	-	-
(E)-Nerolidol	-	-	-	-	-	-	-	-	0.19	-	-	-	-	-
(Z)-Nerolidol	-	-	0.54	0.48	0.07	-	-	0.10	0.94	-	-	-	-	0.53
1,10-di-epi-Cubenol	-	0.63	-	-	-	-	-	-	-	-	-	-	-	-
10-epi- γ -Eudesmol	0.88	0.26	-	-	-	-	-	0.12	0.04	-	0.13	0.26	0.57	-
13-epi-Manool	0.05	-	-	-	-	-	18.77	-	0.07	0.31	-	0.39	-	-
1-Heptanol	-	-	-	-	-	-	-	-	0.06	0.34	0.17	-	-	-
1-Hexadecanol	2.64	-	-	-	0.11	-	0.25	-	-	0.31	0.09	0.31	0.11	0.20
1-Octadecanol	0.59	-	-	-	0.27	-	-	-	-	-	-	-	0.21	-
1-Octanol	0.10	-	-	-	-	-	-	-	0.07	-	-	-	-	-
1-Tetradecanol	0.90	0.12	2.00	5.22	-	2.54	-	-	-	-	0.34	0.80	0.44	-
2-methoxy- <i>p</i> -Cresol	-	-	-	-	-	-	-	-	-	-	-	0.12	-	-
3-Octanol	0.13	-	-	8.24	-	-	-	-	-	-	-	-	-	-
6-methyl-5-Hepten-2-ol	-	-	-	-	-	6.46	-	-	-	-	-	27.33	-	-
Carotol	-	-	-	-	-	-	-	0.28	-	-	-	-	-	0.36
Carvacrol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Caryophylla-4(12),8(13)-dien-5 α -ol	0.16	-	0.39	-	5.88	-	-	-	-	-	-	0.17	-	-
<i>cis</i> -1(7),8- <i>p</i> -Menthadien-2-ol	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-
<i>cis</i> -Carveol	-	-	-	-	0.07	-	-	-	0.43	-	-	-	0.45	-
<i>cis</i> -Piperitol	-	-	-	-	0.09	-	-	-	0.07	-	-	-	-	-
Citronellol	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-
Cubebol	-	-	-	13.13	3.49	-	-	0.26	2.92	-	0.51	-	-	-
Elemol	-	0.42	0.29	1.98	-	-	-	-	-	-	-	0.06	1.55	-
<i>epi</i> -1-Cubenol	-	-	0.24	0.48	5.51	22.77	-	-	0.04	-	-	0.02	0.22	0.07
<i>epi</i> -Globulol	-	0.35	-	-	-	-	-	-	-	-	-	-	-	-
<i>epi</i> - α -Bisabolol	0.17	0.40	0.71	0.46	-	-	0.30	-	0.13	0.14	-	-	-	-
<i>epi</i> - α -Cadinol	0.04	0.15	-	-	-	-	-	1.74	-	-	-	-	-	-
Eudesma-7(11)-en-4-ol	-	-	0.42	0.91	-	0.42	0.19	-	1.41	-	-	0.09	-	-
Eudesma-4(15),7-dien-1 β -ol	0.30	-	-	-	-	-	-	0.11	-	-	-	-	0.17	0.09
Eugenol	8.83	-	-	-	-	-	-	-	-	-	-	0.03	-	-
Germacrene- δ -4-ol	-	3.26	-	0.58	-	-	-	-	-	-	-	-	1.57	-
Guaiol	0.95	6.10	-	-	-	-	-	-	-	-	-	-	-	-
Himachalol	-	-	-	-	1.11	0.09	-	0.20	-	-	0.17	0.31	1.18	0.34
Intermedeol	-	-	0.94	1.12	0.65	11.64	-	0.20	2.50	-	0.13	-	0.45	-
Isoborneol	-	-	-	-	-	-	-	-	-	-	-	4.54	-	-
iso-Dihydrocarveol	-	-	-	-	0.23	-	-	-	-	-	-	0.02	-	-
Isophytol	-	-	-	-	-	-	-	-	0.12	-	-	-	-	-
Isophytol	-	0.56	-	-	-	-	0.99	1.11	-	0.37	-	0.38	0.66	0.07
Isospathulenol	0.93	0.53	-	-	8.86	-	-	0.17	-	-	-	0.33	0.08	0.45
Linalool	-	-	-	-	-	-	-	-	-	-	-	0.40	-	-
Manool	-	-	-	-	-	-	-	0.12	-	-	-	0.33	-	-
Methyl ether thymol	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-

Continue from Table 2

Methyl eugenol	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-
Palustrol	3.15	-	-	-	-	-	-	20.20	-	-	-	-	-	-
Patchouli alcohol	0.43	0.32	-	-	2.54	-	0.26	-	1.06	0.14	0.39	1.36	0.90	0.22
<i>p</i> -Cresol	-	0.21	-	-	-	-	2.67	1.20	-	-	-	-	-	0.23
Phenol	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-
Phytol	0.34	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>p</i> -vinyl-Guaiacol	3.02	-	-	-	-	-	-	-	-	-	-	-	-	-
Selin-11-en-4 α -ol	0.16	-	-	-	1.16	-	0.13	-	-	-	0.09	0.20	-	-
<i>trans</i> -Carveol	0.19	0.03	-	-	-	-	-	-	-	-	-	0.14	0.07	-
<i>trans</i> - <i>p</i> -Menth-2-en-1-ol	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-
α -Acorenol	0.16	-	-	-	-	0.06	0.81	-	-	-	-	-	0.19	-
α -Bisabolol	0.21	0.93	-	-	-	0.73	0.28	0.69	-	0.14	0.51	0.39	0.91	0.38
α -Eudesmol	0.21	1.05	-	-	0.96	-	-	-	-	-	-	-	0.39	0.06
α -Muurolool	-	0.09	-	0.62	-	-	1.83	-	1.72	-	0.13	0.24	-	-
β -Copaen-4 α -ol	-	1.25	-	0.96	-	-	-	-	0.29	-	-	-	-	-
γ -Eudesmol	-	7.07	-	-	-	-	-	-	0.15	-	-	-	-	-
Sub Total	25.0	24.4	5.92	34.4	31.3	44.9	26.5	6.29	34.8	2.08	30.4	11.8	11.5	7.90
Aldehyde and Ketone														
(2 <i>E</i> ,4 <i>E</i>)-2,4-Decadienal	-	0.07	0.42	0.25	0.06	-	-	-	-	-	-	0.05	-	-
(<i>E</i>)-2-Decenal	0.85	0.04	-	-	0.06	0.18	-	-	-	-	-	0.32	0.14	0.07
(<i>E</i>)-2-Dodecenal	-	0.72	-	-	0.58	-	-	-	-	0.20	0.09	0.24	-	-
(<i>E</i>)-2-Heptenal	-	-	-	-	0.14	-	8.91	6.02	-	0.14	-	-	-	-
(<i>E</i>)-2-Undecenal	-	-	-	-	0.09	-	-	-	-	-	-	-	-	0.24
(<i>E</i>)-4,5-Epoxy-2-decenal	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-
(<i>E</i>)- α -Ionone	0.28	1.43	-	-	-	-	-	-	-	-	-	0.05	0.05	-
(<i>E</i>)- β -Ionone	0.03	0.54	-	-	0.04	-	-	-	-	-	-	-	-	-
1-Octen-3-one	-	-	-	-	-	-	-	-	-	-	-	-	0.37	-
2-Heptadecanone	-	0.82	-	-	-	0.26	-	0.46	1.34	-	0.30	5.31	1.62	1.79
2-Pentadecanone	0.14	0.09	-	-	-	0.22	-	-	1.95	0.24	-	1.62	-	-
2-Undecanone	-	-	-	-	-	-	-	-	0.01	-	-	0.02	0.80	-
6-Methyl-5-hepten-2-one	-	-	5.40	-	-	-	-	-	-	-	-	3.11	-	-
Acetophenone	-	-	-	-	-	-	-	-	-	-	-	-	-	0.65
Artemisia ketone	-	-	-	-	0.79	-	-	-	-	-	-	-	-	-
Carvone	-	-	0.34	-	-	-	-	-	-	-	-	-	0.14	-
<i>cis</i> -Cinnamaldehyde	-	-	-	-	0.44	-	-	-	-	-	-	-	-	-
<i>cis</i> -Thujone	0.26	0.12	-	-	-	-	-	-	-	-	-	-	-	-
Cumin aldehyde	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-
Farnesyl acetone, (5 <i>E</i> ,9 <i>E</i>)	3.09	0.88	-	-	-	-	-	-	0.23	-	-	0.28	0.49	0.06
Fenchone	0.25	-	-	-	-	-	-	-	-	-	-	-	-	-
Geranial	-	0.09	-	-	0.17	-	0.58	0.27	-	-	-	-	-	-
Hexadecanal	1.06	-	-	-	-	-	-	-	0.69	-	-	0.17	1.72	-
Hexahydrofarnesylacetone	0.07	0.95	-	-	-	-	-	0.15	-	-	-	1.55	-	-
Methional	-	-	-	-	1.48	-	-	-	-	-	-	-	-	-
Nonanal	-	-	-	-	1.84	-	-	-	-	-	-	-	-	-
Nootkatone	0.24	1.03	-	-	-	0.34	-	-	-	-	-	-	0.13	0.15
Pentadecanal	-	1.25	-	-	-	0.21	0.25	1.52	-	-	0.09	0.26	-	2.29
Pinocarvone	-	0.52	-	-	2.69	-	-	0.38	-	-	-	-	-	0.96
Piperitenone	-	-	-	-	2.58	-	-	1.09	-	-	0.43	8.52	-	0.13
Tetradecanal	0.94	0.40	0.11	-	-	-	0.25	0.80	0.35	-	0.13	1.01	0.98	1.23
<i>trans</i> -Cinnamaldehyde	0.13	-	-	-	-	-	-	-	-	-	-	0.03	0.14	-
<i>trans</i> -Dihydrocarvone	0.02	-	-	-	-	-	-	-	-	-	-	-	0.04	0.05
Tridecanal	-	0.25	-	2.20	-	-	-	-	1.27	-	0.94	0.93	-	0.27
Undecanal	-	-	1.00	0.79	-	-	-	-	0.14	-	-	0.91	-	-
Valeranone	-	0.35	-	-	1.52	-	1.74	-	0.48	-	-	-	-	-
Verbenone	-	-	-	0.16	-	-	-	-	-	-	-	-	-	-
α -Sinensal	-	-	-	-	-	-	-	-	-	-	-	0.19	-	-
α -Sinensal	-	0.85	0.33	0.60	-	0.22	-	-	-	-	-	0.05	0.11	-
β -Oplophenone	0.49	0.52	-	-	-	-	-	-	-	-	-	-	0.68	0.20
γ -Decalactone	0.04	-	-	-	-	0.07	-	0.27	-	0.24	0.09	-	2.00	-
δ -Decalactone	0.07	-	-	-	-	-	-	-	-	1.29	-	2.03	-	-
Sub Total	7.98	10.8	7.19	3.75	12.4	1.51	11.7	11.0	6.48	2.11	2.06	26.6	9.42	8.09
Carboxylic acid														
2-methyl-Butanoic acid	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-
(<i>Z</i>)-Hexadec-9-enoic acid	0.42	-	-	-	-	2.28	0.13	3.27	0.06	0.24	-	-	0.82	1.00
Decanoic acid	0.70	-	-	-	-	-	-	-	-	-	0.13	0.44	-	-
Heptanoic acid	0.16	-	-	-	0.72	-	-	-	-	-	-	-	-	-
Hexadecanoic acid	0.56	-	0.36	0.60	-	-	1.09	-	0.92	0.14	-	0.61	0.21	0.44
Hexanoic acid	-	0.41	-	-	-	-	-	-	-	-	-	3.30	1.84	13.34
Linoleic acid	-	-	-	-	-	-	-	-	-	-	-	0.11	-	-
Nonanoic acid	-	-	0.27	-	-	-	-	-	0.10	-	-	-	-	-
Octadecanoic acid	0.27	0.53	-	-	-	-	-	-	0.06	0.07	-	-	-	0.10
Octanoic acid	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-
Oleic acid	0.35	-	-	-	-	-	-	-	-	-	-	-	0.47	-
Pentadecanoic acid	0.10	-	-	-	-	-	-	0.43	0.21	-	0.47	0.39	0.15	0.68
Tetradecanoic acid	-	0.14	0.49	0.74	-	-	-	-	-	0.68	-	0.09	-	0.05
Tridecanoic acid	0.28	0.70	0.50	-	-	1.81	-	-	-	-	-	0.06	1.57	0.31
Undecanoic acid	-	0.28	-	-	1.01	-	-	0.40	-	-	-	-	-	-
Sub Total	2.88	2.05	1.62	1.34	1.83	4.09	1.22	4.11	1.35	1.12	0.60	5.01	5.07	15.9

Continue from Table 2

Esters														
(2E,6E)-Farnesyl acetate	-	0.33	-	-	-	-	-	0.65	0.06	-	1.80	-	2.57	0.66
(Z)-3-Hexenyl benzoate	-	-	-	-	1.24	-	-	0.40	0.13	-	-	0.34	1.87	0.55
2-Methylpropyl 3-methylbutanoate	2.42	-	-	-	0.11	-	-	-	0.02	0.24	0.13	-	-	-
2-Phenylethyl acetate	-	-	-	-	0.04	-	-	-	-	-	-	0.22	-	-
Benzyl benzoate	-	-	-	-	0.55	-	-	-	-	-	-	-	12.48	0.44
Benzyl salicylate	-	-	-	-	-	1.15	-	-	-	-	-	-	-	-
Bornyl acetate	0.07	-	-	-	0.47	-	-	-	-	-	-	-	-	-
Bornyl formate	-	-	0.21	-	-	-	-	-	0.02	-	-	-	-	-
β -Phenylethyl tiglate	-	-	-	0.32	-	0.09	-	1.90	0.06	-	-	0.39	0.23	0.05
Carvacrol acetate	-	0.24	1.09	1.24	0.66	-	-	-	-	-	0.09	-	-	0.05
cis-Carvyl acetate	2.70	-	-	-	0.34	-	-	-	-	-	-	0.11	-	-
cis-Chrysanthenyl acetate	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-
cis-Sabinene hydrate	0.16	-	-	-	-	-	-	-	-	-	-	-	-	-
cis-Sesquibinene hydrate	0.48	0.03	0.22	2.15	-	-	-	-	0.08	-	-	0.29	0.47	0.11
Citronellyl butanoate	-	-	-	-	-	-	-	-	0.34	-	-	0.42	-	0.15
Decyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	0.12	-
endo-Fenchyl acetate	0.15	-	-	-	0.20	-	-	-	0.01	-	-	-	-	-
Ethyl decanoate	-	0.12	0.38	0.50	-	-	-	36.63	-	-	-	1.39	-	-
Ethyl dodecanoate	5.14	0.02	-	-	-	-	0.21	-	0.43	-	-	-	0.15	0.73
Ethyl hexadecanoate	-	-	-	-	-	-	-	-	2.28	0.24	0.21	0.65	-	-
Ethyl hexanoate	-	-	4.37	-	-	0.15	-	-	-	-	-	-	-	-
Ethyl linoleate	-	-	0.27	-	-	-	-	-	-	-	-	-	0.41	-
Ethyl pentanoate	0.64	0.38	-	11.17	1.47	4.45	8.78	7.39	3.76	31.71	33.80	-	1.82	15.85
Ethyl tetradecanoate	0.30	1.74	-	-	1.36	0.08	0.89	0.96	-	-	-	-	0.46	16.26
Eugenol acetate	0.13	1.82	-	19.75	-	-	-	-	-	0.18	-	0.43	0.72	0.12
Geranyl acetate	-	0.42	0.31	0.84	0.34	0.35	-	-	-	-	-	-	-	0.15
Geranyl butanoate	0.22	-	0.21	0.19	-	-	1.61	1.66	-	-	-	-	-	0.71
Geranyl isovalerate	-	-	-	-	-	-	-	0.10	3.93	-	1.20	-	0.42	0.20
Geranyl propanoate	0.14	-	0.52	-	-	-	-	-	-	-	-	-	0.04	-
Geranyl tiglate	0.01	-	-	-	-	0.81	-	-	-	-	-	0.60	-	-
Hexyl 2-methyl butanoate	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-
Hexyl benzoate	-	0.19	-	-	-	-	-	0.29	0.01	-	1.03	0.86	1.12	0.57
Isopentyl isovalerate	-	-	-	-	-	-	-	-	-	-	-	-	-	0.48
Lavandulyl acetate	0.66	-	-	-	-	-	-	-	-	-	-	-	-	-
Linalool acetate	-	-	-	-	-	-	-	-	-	-	-	0.28	-	-
Linalool propanoate	0.16	0.08	-	-	-	-	-	-	-	-	-	-	-	-
Menthyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	0.07	-
Methyl 3-phenylpropionate	-	-	-	0.25	0.08	-	-	-	-	-	-	0.02	0.64	-
Methyl decanoate	0.10	-	-	-	-	-	-	-	0.04	-	-	-	-	-
Methyl geranate	-	-	-	-	0.03	-	-	-	-	-	-	0.57	-	0.33
Methyl hexadecanoate	0.25	-	-	-	-	1.09	-	0.66	-	-	-	0.05	0.47	-
Methyl hexanoate	-	-	0.60	0.22	-	-	-	-	0.51	-	0.47	-	-	-
Methyl linoleate	-	0.86	0.29	0.26	0.88	1.08	-	-	0.45	0.48	-	-	0.60	-
Methyl octadecanoate	-	-	-	-	-	-	-	0.60	-	-	-	-	-	0.09
Methyl oleate	-	0.52	-	-	-	0.63	-	-	-	-	0.17	-	-	-
Methyl tetradecanoate	-	-	0.93	2.23	-	-	-	-	-	-	0.34	0.10	0.21	0.10
Myrtenyl acetate	0.10	0.17	0.12	-	0.04	-	-	-	-	-	-	0.37	-	-
Neryl propanoate	0.01	0.07	-	-	-	-	-	-	-	-	0.13	0.08	-	-
Nonyl acetate	-	0.20	-	0.59	1.03	-	-	0.27	-	-	-	0.11	-	1.16
Octanol acetate	2.62	-	-	-	-	-	-	-	-	-	-	-	0.69	-
Phenylethyl 3-methylbutanoate	-	-	0.19	-	0.05	0.39	-	-	-	-	-	0.06	-	-
Terpinen-4-ol acetate	0.08	-	-	-	-	-	-	-	-	-	0.13	0.06	-	-
trans-Pinene hydrate	-	-	-	-	0.18	-	-	-	-	-	-	-	-	-
α -Terpinyl acetate	-	7.72	-	0.52	-	-	-	0.85	-	-	-	-	-	-
Sub Total	16.6	14.6	9.70	40.2	7.83	10.3	11.5	51.3	15.9	32.7	38.1	7.35	20.4	37.6
Miscellaneous Compound														
1,8-Cineole	-	-	-	-	-	-	-	-	-	-	-	0.26	-	-
cis-Limonene oxide	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-
cis-Linalool oxide, (furanoid)	-	-	-	-	0.32	0.85	-	-	-	-	-	0.13	-	-
Dehydro-1,8-cineole	-	-	-	-	-	-	-	-	-	34.47	-	-	-	-
Dendrolasin	0.43	-	-	-	-	-	-	-	-	-	-	1.13	-	-
Dill apiole	0.26	0.06	-	-	-	-	-	2.43	-	-	-	-	0.23	0.41
Dimethyl trisulfide	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-
Humulene epoxide II	3.19	0.28	-	-	-	-	-	-	-	-	1.03	1.79	-	-
Indole	-	-	-	0.53	-	-	-	-	-	-	-	-	-	-
Manoyl oxide	0.05	-	0.13	-	-	-	1.33	-	-	-	-	-	0.75	0.09
Myristicin	-	-	5.11	2.92	-	-	-	-	-	-	-	-	1.46	-
Piperitenone oxide	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-
trans-Limonene oxide	-	-	-	-	0.12	-	-	-	-	-	-	0.05	-	-
trans-Linalool oxide, (furanoid)	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-
α -Humulene oxide	-	-	-	-	-	-	-	-	0.44	-	0.43	-	-	-
Sub Total	3.99	0.35	5.24	3.45	0.12	0.00	1.33	2.43	0.44	0.00	1.46	2.98	2.44	0.51
Terpenes														
7-epi- α -Selinene	-	-	-	-	-	-	-	-	-	-	-	0.08	-	-
(E)-Caryophyllene	1.01	-	-	-	-	-	-	0.31	-	-	-	-	1.52	-
(E)- β -Farnesene	-	-	-	0.23	-	-	-	-	-	-	-	-	-	-
(E,E)- α -Farnesene	-	-	0.86	1.24	-	-	-	0.40	-	-	-	-	-	-

Continue from Table 2

(Z)-Caryophyllene	7.69	-	-	-	-	-	-	-	-	-	-	-	-	-
(Z)- γ -Bisabolene	-	-	-	-	-	0.24	-	-	-	-	-	-	-	-
2,5-Dimethoxy- <i>p</i> -cymene	-	-	-	-	-	-	0.12	-	-	-	-	-	-	-
Abietatriene	0.09	0.49	-	-	-	-	-	0.29	0.14	-	-	-	-	-
Alloaromadendrene	0.19	0.45	-	-	-	-	1.15	-	-	-	-	-	0.05	-
α -Pinene	-	-	0.61	-	0.45	-	-	-	0.04	-	-	0.84	0.83	6.76
<i>Continue..</i>														
Aromadendrene	0.11	-	-	-	-	-	-	-	-	-	0.14	-	-	-
β -Copaene	-	-	-	-	-	-	0.35	-	-	-	-	-	0.09	-
β -Humulene	-	-	0.22	-	0.56	-	-	0.12	-	-	-	-	-	-
Bicyclogermacrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cadalene	0.21	-	-	-	1.47	-	-	0.15	-	-	-	-	-	-
Chamazulene	0.33	0.21	-	-	0.57	0.29	-	-	0.17	-	1.86	-	-	-
<i>cis</i> -Cadina-1(2),4-diene	-	1.58	-	-	0.65	-	-	0.11	0.10	-	-	-	-	-
<i>cis</i> -Muurolo-4 (14),5-diene	-	-	-	-	2.59	-	-	-	-	-	-	-	-	-
<i>cis</i> - α -Bergamotene	-	-	-	-	-	-	-	-	0.75	0.14	-	0.54	-	-
<i>cis</i> - β -Farnesene	-	-	-	-	0.33	0.04	-	0.09	-	0.10	0.09	0.05	0.43	-
Cuparene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
δ -Elemene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Elemicin	0.52	-	-	-	-	-	-	-	-	-	-	-	-	-
Germacrene B	-	-	-	-	-	0.93	-	-	-	-	-	-	-	-
Isoitalicene	-	1.01	-	-	0.13	-	-	-	-	-	-	-	-	-
<i>m</i> -Cymene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.22
Myrcene	-	-	-	2.79	-	-	-	-	-	-	3.35	-	-	-
<i>o</i> -Cymene	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-
Santolina triene	0.83	-	-	-	-	-	-	-	-	-	-	-	-	-
Thuja-2,4(10)-diene	0.19	0.66	-	-	0.94	-	8.40	-	0.02	0.10	-	-	1.12	-
<i>trans</i> - α -Bergamotene	-	0.08	-	-	1.47	-	-	-	-	-	-	-	-	-
Tricyclene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Verbenene	-	-	-	-	-	-	-	3.61	-	-	-	-	-	-
α -Amorphene	0.09	0.03	-	-	-	-	-	-	-	-	-	-	-	-
α -Cadinene	-	0.68	49.39	4.35	-	-	-	-	-	-	-	-	0.93	0.12
α -Cedrene	5.10	0.02	5.20	-	-	-	-	-	-	0.30	-	-	0.09	0.06
α -Cubebene	-	3.01	-	-	-	-	-	-	-	-	-	0.02	-	-
α -Fenchene	0.13	-	-	-	-	3.41	-	-	-	0.37	0.17	-	-	-
α -Guaiene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α -Gurjunene	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-
α -Himachalene	0.15	0.17	-	-	-	-	0.24	0.11	-	-	-	-	-	-
α -Humulene	0.24	0.18	-	-	-	-	-	-	-	-	-	0.33	-	-
α -Thujene	0.51	-	1.00	3.25	-	1.83	3.91	3.16	1.17	16.74	14.55	0.35	-	-
α -Ylangene	0.03	-	-	-	-	-	-	-	0.22	-	-	0.18	-	-
α -Zingiberene	-	0.18	-	-	-	-	-	0.20	-	-	0.56	-	0.08	-
β -Acoradiene	0.02	0.09	-	-	1.03	-	-	-	-	-	-	-	0.07	-
β -Bisabolene	-	-	-	-	-	-	-	-	0.25	-	-	-	2.30	0.19
β -Calacorene	-	0.19	-	-	-	-	-	-	0.06	0.07	1.16	3.43	0.15	-
β -Cedrene	0.08	-	8.18	-	2.56	-	-	-	-	-	-	-	-	-
β -Chamigrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β -Cubebene	2.30	-	-	-	0.09	-	-	-	0.07	-	0.09	-	-	-
β -Elemene	-	1.16	-	-	0.11	-	-	0.31	0.09	-	0.19	2.94	-	0.22
β -Gurjunene	0.04	0.33	-	-	0.29	-	-	-	-	-	-	0.07	-	-
β -Himachalene	0.99	0.10	-	-	-	-	-	-	-	-	-	0.60	-	-
β -Phellandrene	9.56	-	-	-	-	-	-	-	-	-	-	-	-	-
β -Santalene	0.05	-	-	-	-	-	-	-	-	-	-	0.11	-	-
γ -Curcumene	0.08	0.04	-	-	-	-	-	-	-	-	-	0.06	-	-
γ -Elemene	0.61	-	-	-	-	-	-	-	-	-	-	-	-	-
Sub Total	31.3	10.7	65.5	11.9	13.2	6.50	14.3	8.68	3.07	18.1	20.5	11.6	7.66	7.57
Total	87.7	62.9	95.1	95.1	66.9	67.2	66.5	83.8	62.0	56.0	93.1	65.3	56.5	77.5

Notes : PM - *M. acuminata* colla 'sucrier', PN - *M. acuminata* \times *balbisiana* colla 'saba', PT - *M. acuminata* \times *balbisiana* 'horn plantain', PE - *M. acuminata* colla 'gros michel', PO - *M. acuminata* colla 'inarnibal', PJ - *M. acuminata* colla 'red', PB - *M. acuminata* colla 'lakatan', S-Peels, F-Flesh

Three compounds in the alcohol group namely, 1-tetradecanol, (2*E*,6*E*)-farnesol and epi-1-cubanol are the major components that were found in essential oil almost all seven species of *Musa*'s essential oils. 1-Tetradecanol is the major component found in *M. acuminata* \times *balbisiana* colla 'saba' peels (5.22%) and *M. acuminata* colla 'inarnibal' peels (2.54%). Alcohol group contributes to the green-woody smell in the *Musa* spp. (Aurore *et al.*, 2011).

Phenylpropanoids compounds such as eugenol, methyl eugenol and carveol were also found in the

essential oil for almost all seven *Musa* spp. However, carveol is identified in the *Musa* spp. in Malaysia but was not detected in *M. acuminata* colla 'gros michel' in Madeira Island (Nogueira *et al.*, 2003). The highest percentage of eugenol (8.83%) was found in *M. acuminata* colla 'sucrier' flesh oil.

Ester group is an important volatile component in *Musa* spp. as this group plays a major role in the aroma of fully ripe banana fruits and responsible for the essence that carry by *Musa* spp. (Selli *et al.*, 2012). A total of 54 components were identified in the

essential oils are from the ester group, where acetate esters were found in a higher concentration compared to the other esters. Carvacrol acetate, methyl hexanoate, methyl-3-phenylpropionate, eugenol acetate and ethyl pentanoate were the components from ester group that were identified in all of the studied species. However, the percentage abundance of the components differs between each species.

Ethyl pentanoate present in all *Musa* spp.'s fruit with the highest percentage of the component is identified in *M. acuminata colla 'gros michel'* flesh (33.8%). Besides that, eugenol acetate is also a common ester that present in the essential oil of *Musa* spp.'s fruit. This component was detected in *Musa* spp. essential oils with the highest abundance in *M. acuminata × balbisiana colla 'saba'* (19.75%). However, the component was not detected in the essential oil of *M. acuminata × balbisiana 'horn plantain'* and *M. acuminata colla 'inarnibal'*.

Besides alcohol, carbonyl components are also contributed to the aroma characteristic in *Musa* spp. In this study, a total of 42 components of carbonyl group were identified in *Musa* spp.'s fruits essential oil. Hexadecanal and (*E*)-2-hexanal are minor constituents detected in *M. acuminata colla 'inarnibal'* flesh (0.14%) and *M. acuminata colla 'gros michel'* peels (0.17%). In contrast, previous studies found that the carbonyl group was the major constituent in their research (Shiota, 1993).

Tridecanoic acid and hexadecanoic acid are carboxylic acids that were found in all *Musa* spp.'s fruit essential oils. Hexadecanoic acid were also found in the study done by Nogueira *et al.*, (2003), while tridecanoic acid is not reported in any previous studies. These carboxylic acids are associated with the aroma of the banana and the ripening of banana (Pino & Febles, 2013).

The chemical constituents in the essential oils of *Musa* spp. are different due to their different maturity stage of the fruits (Brat *et al.*, 2004; Macku and Jennings 1987). Pentan-2-one was reported as the major constituent in *Musa* spp. from Philippines (Shiota, 1993). However, in this study the compounds were not detected in all seven *Musa* spp. that were studied. As shown in Table 1, the major components percentage abundance of seven *Musa* spp.'s fruit is in a range of 5.26% to 49.39%. Most of the major components present in the essential oils studied are from the ester and alcohol groups.

Besides that, the differences between chemical constituents and the percentage of the constituent in all seven *Musa* spp. studied are different from the literature. This may be due to the different extraction method used in both studies. In this study, hydrodistillation method was employed for the extraction of the essential oils from *Musa* spp. This method requires the oil collector to be heated and this may have resulted in the degradation of volatile and thermolabile compounds by the thermal reaction which include transformation reaction and oxidation reactions (Chen *et al.*, 2014). This explains why in previous studies researchers prefer headspace-solid phase microextraction method in isolating volatile oil of *Musa* spp.

Cluster Analyses

The statistical analysis of the essential oils was carried out to show the relationship between the studied species. The analysis was carried out by SPSS version 22 on the flesh and peels the essential oils. The dendrogram for the flesh and peels are shown in Figure 1 and Figure 2, respectively. Figure 1 shows that there is a strong relationship between the flesh oil of *M. acuminata colla 'inarnibal'*, *M. acuminata × balbisiana 'horn plantain'*, *M. acuminata colla 'lakatan'*, *M. acuminata colla 'red'* and *M. acuminata colla 'sucrier'*.

M. acuminata colla 'gros michel' and *Musa acuminata colla 'red'* also showed significant relationship between the two species. In contrast, *M. acuminata × balbisiana colla 'saba'* have no correlation with the other six species. The species that were studied had a uniform qualitative essential oil composition but differed in quantitative content. This difference in morphology is also one of the factors that causes the difference in the chemical variability among the species (Joshi *et al.*, 2009).

Based on Figure 2, it shows that there is a strong relationship between *M. acuminata colla 'sucrier'*, *M. acuminata colla 'gros michel'*, *M. acuminata colla 'inarnibal'* and *M. acuminata colla 'lakatan'*. *M. acuminata × balbisiana colla 'saba'* and *M. acuminata × balbisiana 'horn plantain'* also showed a significant relationship between the cultivar. However, for *M. acuminata colla 'red'* it has no colleration with the other six species. A strong relationship between *M. acuminata colla 'sucrier'*, *M. acuminata colla 'gros michel'*, *M. acuminata colla 'inarnibal'* and *M. acuminata colla 'lakatan'* was due to the same AA genome group, while for *M. acuminata × balbisiana colla 'saba'* and *M.*

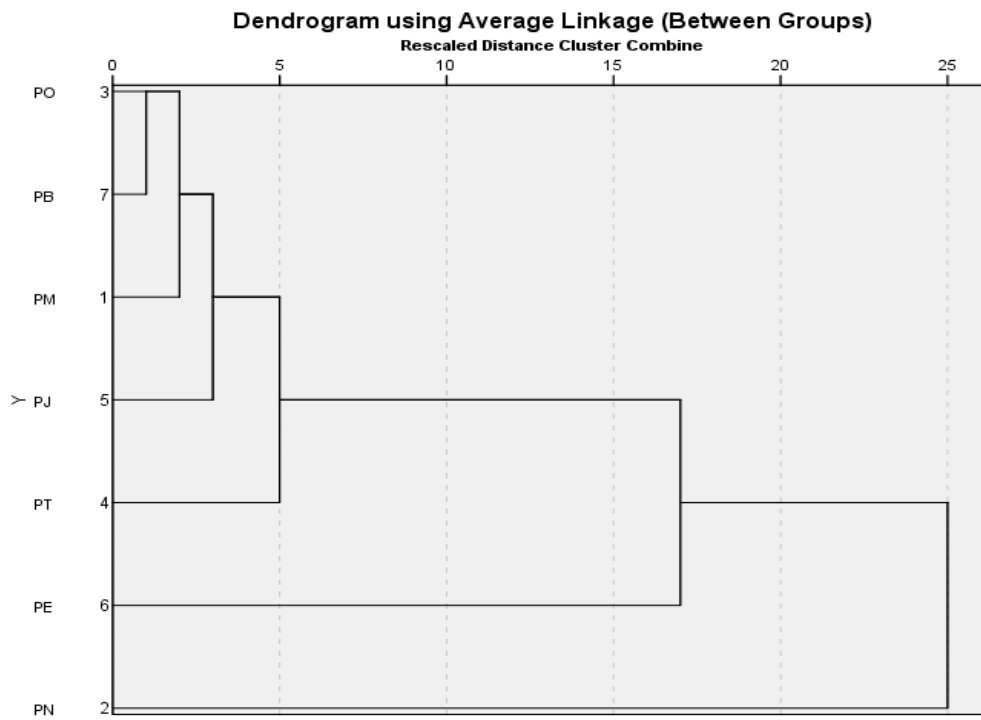


Figure 1. Dendrogram of cluster analysis for the flesh oils of seven *Musa* spp.'s fruits

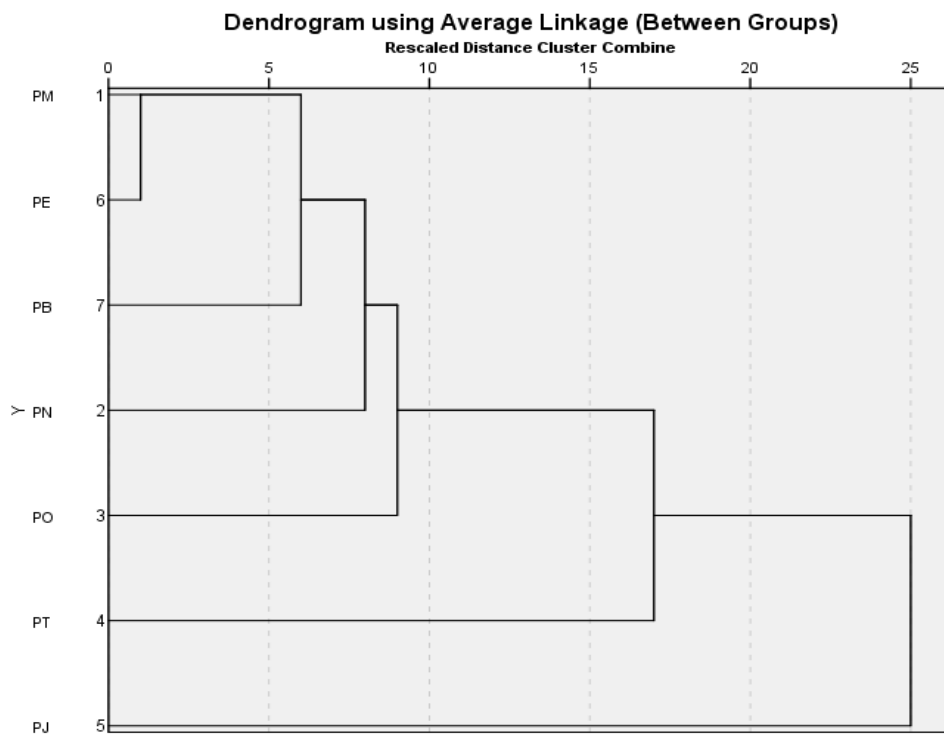


Figure 2. Dendrogram of cluster analysis for the peels oils of seven *Musa* spp.'s fruits

acuminata × *balbisiana* 'horn plantain' are from the combination of AA and BB genome group. BB genome group has different unique characteristic and varies with each other (Ploetz *et al.*, 2007).

Brine Shrimp Toxicity

Toxicity against *Artemia salina* had been carried out on the essential oils of *M. acuminata* colla 'sucrier' (flesh and peels), *M. acuminata* × *balbisiana* colla 'saba' (flesh and peels), *M. acuminata* × *balbisiana* 'horn plantain' (flesh and peels) and *M. acuminata* colla 'inarnibal' (peels). After 24 hours, the number of death *Artemia salina* was observed and recorded. The percentage of death for *Artemia salina* against the essential oil of *Musa* spp. at different concentrations are shown in

Figure 3.

According to Kalala *et al.* (2015), LC₅₀ value greater than 100 ppm is considered non-toxic, while LC₅₀ below or equal to 99 ppm is considered toxic. All seven sample tested were interpreted as non-toxic (LC₅₀>100 ppm). Based on the results, the samples showed a dose of dependent toxicity activity indicating the potential of the sample to be studied for their antitumor properties. McLaughlin (1991) stated the toxicity of the sample on brine shrimp test was correlated with the antitumor activity. Besides, another study by Bagya *et al.* (2011) also showed a good correlation between toxicity and brine shrimp with antitumor properties of *Kaempferia galangal* ethanolic extract.

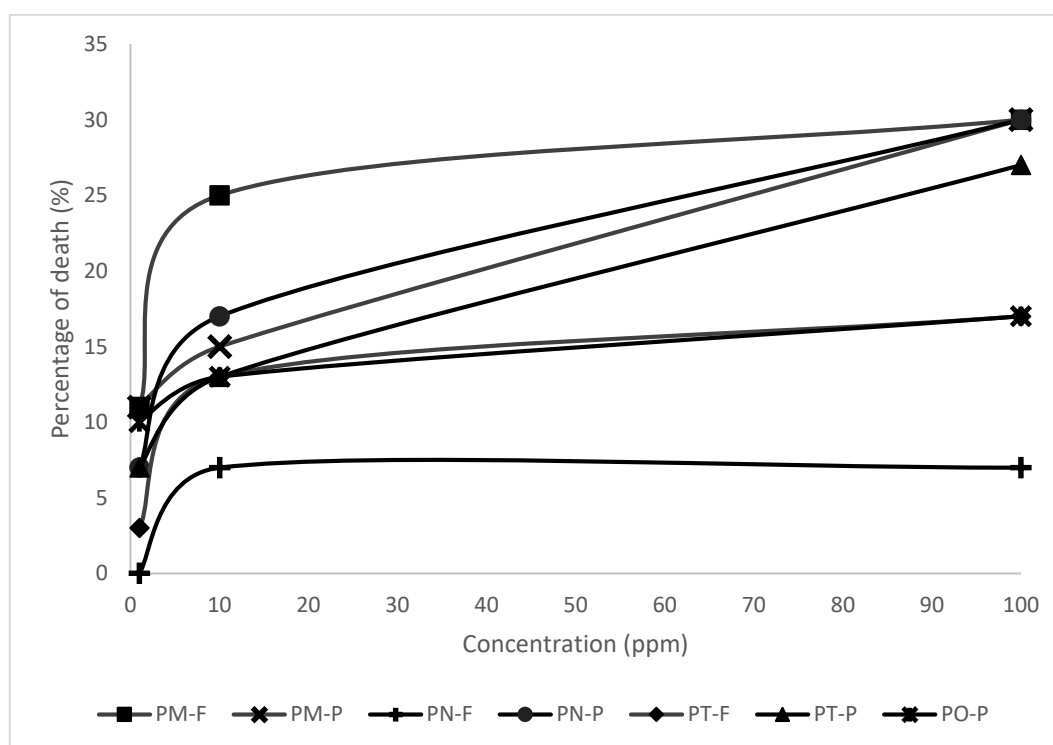


Figure 3. Percentage of death brine shrimp against concentration graph

Notes : PM-F - *Musa acuminata* colla 'sucrier' flesh oil, PM-P - *Musa acuminata* colla 'sucrier' peels oil, PN-F - *Musa acuminata* × *balbisiana* colla 'saba' flesh oil, PN-P - *Musa acuminata* × *balbisiana* colla 'saba' peels oil, PT-F - *Musa acuminata* × *balbisiana* 'horn plantain' flesh oil, PT-S - *Musa acuminata* × *balbisiana* 'horn plantain' peels oil, PO-P - *Musa acuminata* colla 'inarnibal' peels oil

Antibacterial Assay

An antibacterial test had been carried out on essential oils of *M. acuminata* colla 'sucrier'

(peels and flesh), *M. acuminata* × *balbisiana* 'horn plantain' (peels and flesh), *M. acuminata* colla 'gros michel' (peels and flesh) and *M.*

acuminata × *balbisiana* colla 'saba' (peels and flesh). Three types of bacteria used were *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. Sample with a concentration of 100 µg disc⁻¹ was used for the antibacterial activity. However, there was no inhibition zone observed for all the essential oils tested. This may be due to the concentration of the tested essential oils (100 µg disc⁻¹) that was insufficient for antibacterial activity.

Research done by Fagbemi *et al.* (2009) on unripe ethanol extract of *M. sapientum* showed minimum inhibitory concentration of 32000 µg/ml on the bacteria *Escherichia coli* and *S. aureus*. The difference between the method of extracting essential oils and hydrodistillation and solvent extraction can influence the antimicrobial properties. This was shown by the essential oils extracted by hexane that has greater antimicrobial activity compared to steam hydrodistillation of the corresponding essential oil (Packiyasothy & Kyle, 2002).

CONCLUSION

In conclusion, the study revealed that ester and alcohol groups are the most dominant components in the flesh and peel oils of *Musa* spp. This has contributed to the biological activities of the essential oils. Weak brine shrimp toxicity of the tested samples may hint the potential of the essential oil as an antitumor medicine. Further studies on essential oil of *Musa* spp. should focus on their antitumor properties.

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