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Comparison of Chemical Composition, Antimicrobial and Antioxidant Activities of Essential Oils Extracted from Different Parts of Bambangan (*Mangifera pajang*) Fruit

LAM NYEE FAN[1], MOHD FADZELLY ABU BAKAR[2,3,*], NOOR ATIEKAH MD NOR[1], AZLEN CHE RAHIM[2,3], FAZLEEN IZZANY ABU BAKAR[2,3] and MOHD ASPOLLAH MD SUKARI[4]

[1]Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, Malaysia
[2]Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Hab Pendidikan Tinggi Pagoh, KM1, Jalan Panchor, 84600 Muar, Malaysia
[3]Centre of Research for Sustainable Uses of Natural Resources (CoR-SUNR), Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia
[4]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

*Corresponding author: Fax: +60 6 9742191; Tel: +60 6 9742021; E-mail: fadzelly@uthm.edu.my

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Mangifera pajang produces a fruit that is similar to mango, but characterized by brownish exterior colour that contained yellowish flesh and strong mangoish-turpentine aroma. This study identified the chemical composition, antimicrobial and antioxidant activities of the essential oil extracted from flesh, peel and kernel of *M. pajang*. Different parts of *M. Pajang* were extracted using hydrodistillation and the essential oils obtained were analyzed using gas chromatography-mass spectrometry (GC-MS). The antioxidant activity was determined spectrophotometrically while the antimicrobial activity was evaluated using disc diffusion method. Total of 56 (flesh), 9 (peel) and 26 (kernel) volatile compounds were identified from the essential oil with the major component obtained were 3-methyl-4-cyclohexene (14.91 %), α-pinene (34.73 %) and linoleic acid (16.58 %), respectively. The essential oils extracted from different parts of the fruit displayed weak inhibition in antimicrobial activity. Antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging showed that essential oil from *M. pajang* peel displayed the highest antioxidant capacity. It is clear that the essential oils extracted from different parts of *M. pajang* possess beneficial phytochemicals that can contribute to human's health.

Keywords: *Mangifera pajang*, Flesh, Peel, Kernel, Essential oils, Antimicrobial, Antioxidant activity.

INTRODUCTION

There are approximately 70 species in the genus of *Mangifera*. 25 species of *Mangifera* can be found in Peninsular Malaysia, meanwhile 17 species of *Mangifera* can be found in Sabah, Malaysian Borneo. Bambangan or *Mangifera pajang* Kosterm. is the biggest fruit in the genus of *Mangifera*; up to 2 kg per fruit [1]. This species is endemic to Borneo Island and categorized as vulnerable by IUCN Red List of Threatened Species. It is found mainly in Borneo Island (Brunei Darussalam, Kalimantan (Indonesia), Sabah (Malaysia) and Sarawak (Malaysia) [2]. The flesh is yellowish, fibrous, thick with sweet and sour taste. The flesh of *M. pajang* is eaten fresh while the peel and kernel are used in cooking and made into pickle [1].

Several studies have been conducted on nutritional compositions of *M. pajang* [1,3,4]. Seed kernel of *M. pajang* was found to contain carbohydrate (38.68 %), protein (3.08 %), crude fibre (4.79 %), fat (9.85 %), ash (2.23 %) and water (41.38 %). Another study showed that the edible portion of fruit contained carbohydrate (21.02 %), protein (1.13 %), crude fibre (5.26 %), fat (1.98 %) and ash (0.43 %) while fibre-rich peel contained carbohydrate (7.3 %), protein (4.6 %), total dietary fibre (72.3 %), fat (2.9 %), ash (2.7 %) and moisture (3.9 %) [4].

Kernel extract of *M. pajang* showed a high potential as a potent cytotoxic agent against breast cancer cell lines as it induced cytotoxicity in MCF-7 (hormone-dependent breast cancer) cells and MDA-MD-231 (non-hormone dependent breast cancer) cells with IC_{50} values of 23 and 30.5 μg/mL respectively [5]. Besides, kernel and peel of *M. Pajang* considered as the waste of the fruit showed better anticancer potential as compared to flesh [2].

For the antioxidant effects,the methanolic extract of *M. pajang* kernel displayed the highest free radical scavenging and ferric reducing activities. Kernel possessed the highest 2,2-diphenyl-1-pycryl-hydrazyl (DPPH) free radical scavenging (23.23 mg AEAC/g), followed by peel (20.32 mg AEAC/g) and flesh (9.94 mg AEAC/g) [6]. Meanwhile, DPPH assay on

carotenoids extract of peel exhibited higher radical scavenging activity than its pulp [7]. Crude polysaccharide of this fruit and its fractions also showed strong antioxidant activities; the acidic polysaccharides had the highest antioxidant activity with 81.4 % at 100 µg/mL extract [8]. A study found that the optimum extraction conditions for highest recovery of antioxidant capacities were 68 %, 56 ºC and 31.8 mL/g, respectively where the predicted values agreed well with the experimental values [9]. In addition, Abu Bakar *et al*. [10] further confirmed the antioxidant activity at the cellular level of which *M. Pajang* kernel extract displayed cytoprotective activity against *tert*-butyl hydroperoxide induced oxidative damage [10].

There were many studies done on the volatile compounds from the *Mangifera* species [11,12]. However, there is still lack on the study of volatile constituents specifically from *M. pajang*. This study, therefore, aimed to identify the chemical composition, antimicrobial and antioxidant activities of essential oil extracted from flesh, peel and kernel of *M. pajang* (bambangan) using hydrodistillation.

EXPERIMENTAL

Sample preparation: The fruits of *M. pajang* were collected from Pasar Tamu Gaya, Kota Kinabalu, Sabah, Malaysia. The fruits were cleaned and separated into peel, flesh and kernel. Authentication of the fruits was done by Mr. Johnny Gisil from Institute for Tropical Biology and Conservation of Universiti Malaysia, Sabah, Malaysia. The peel, flesh and kernel were separated, cut into small pieces and stored in freezer (-20 ºC).

Extraction: The flesh (520 g), peel (500 g) and kernel (500 g) of *M. pajang* were extracted separately by hydrodistillation method using Clavenger apparatus for 8 h.

Gas chromatographic-mass spectral analysis (GC-MS): The *M. pajang* essential oils were then analyzed using Shimadzu GS-MS - QP5050 spectrophotometer equipped with Shimadzu GC-17A, HP5MS (5 % phenyl methylsilane) capillary column (30 × 250 µm × 0.25 µm) and helium as gas carrier. The GC oven temperature was programmed from 50 ºC to 250 ºC at rate of 5 ºC/min with an initial hold 1 min and final hold 10 min. The constituents of oils were identified using GC-MS technique by comparing their mass spectral data with those from the Wiley mass spectral database and Kovat retention indices with the literature values.

2,2-Diphenyl-1-pycryl-hydrazyl (DPPH) free radical scavenging assay: The free radical scavenging ability of extract was determined according to the method described elsewhere [13]. DPPH solution (1 mL, 0.3 mM) was added to a 2.5 mL of sample extracts or standards. The mixture was then allowed to stand for 30 min in room temperature. The absorbance value was measured spectrophotometrically at 518 nm and the antioxidant activity (AA) was calculated as:

AA% = 100 - [(Absorbance sample - Absorbance empty sample)/ (Absorbance control)] × 100

The result obtained was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) using the following equations:

AEAC = (IC_{50} (AA)/IC50 (sample)) × 105, where AA is ascorbic acid.

Antimicrobial assay: The disc diffusion method was used for screening the antimicrobial activity of isolated volatile oils using 6 mm sterile of filter discs [13]. Two Gram-positive bacteria (*Stapyhlococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*) were used. Microorganisms were cultured at 37 ºC for 24 h and prepared to turbidity equivalent to 0.5 McFarland Standard. The suspension was added to the top of agar. Sterile discs were impregnated with four different concentration of essential oil (1000, 2000, 4000 and 5000 ppm) and places on the test plate (Muller Hinton agar). Distilled water was used as the negative control while kanamycin (50 µg/mL) was used as positive reference standards to determine the sensitivity of bacteria in each plates. Plates were subsequently incubated at the appropriate temperature for 24 h and zone of inhibition were calculated by using the diameter in mm.

Statistical analysis: Data were expressed as mean ± standard deviation of three triplicates. Statistical analysis was analyzed using SPSS (Statistical Package for the Social Sciences) through one-way ANOVA test followed by comparison of Tukey's post hoc. Difference on statistical analysis were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Gas chromatographic-mass spectral analysis: The yields of essential oil from *M. Pajang* were 0.46 % (flesh), 0.36 % (peel) and 0.16 % (kernel). In total, 56 volatile compounds from the essential oil of flesh, 9 volatile compounds from essential oil of peel and 26 volatile compounds from essential oil of kernel were identified by gas chromatography-mass spectrometry (GC-MS). These compounds are listed in Table-1 with their retention time and percentage of concentration. Many volatile compounds were found in flesh, followed by kernel and peel. For the flesh part, the dominant compound was 3-methyl-4-cyclohexene (14.91 %) while α-pinene (34.73 %) and linoleic acid (16.58 %) were abundant in peel and kernel, respectively. Essential oil extracted from the flesh of *M. pajang* consisted of butanoic acid (1.0 %), 2-furancarboxalde (1.14 %), 1-hexanol (0.14 %), cyclopentane carboxylic acid (0.14 %), 2,7-nonadien-5-one (0.25 %), butanoic acid (0.25 %), 2-furancarboxaldehyde (0.26 %), 8-chlorocapric acid (0.16 %), α-phellandrene (0.52 %), bicyclo-2-hept-2-ene (0.40 %), benzene (0.63 %), 2-furanmethanol (10.33 %), 3-methyl-4-cyclohexene (14.91%), α-methyl (2.07 %), undecane (1.95 %), benzene (1.24 %), 1,6-octadien-3-ol (0.37 %), phenylethyl alcohol (0.30 %), cyclohexen-1-ol (0.33 %), 2-cyclohexen-1-ol (0.91 %), cyclohexanol (1.92 %), *p*-flouroethylbenzene (0.06 %), 4,4-dimethyl-2-cyclopenten-1-one (0.11 %), cyclopropane (1.14 %), 3-cyclohexen-1-ol (0.68 %), benzene methanol (5.96 %), 3-cyclohexene-1-methanol (13.32 %), bicyclohexan-3-ol (7.51 %), bicyclohept-3-en-2-one (0.56 %), 2-oxabicycloctanol-6-ol (0.54 %), 2-acetylcyclopentanone (0.43 %), ethanol (0.32 %), 2-cyclohexen-1-one (3.60 %), 2-cycloyexen-1-one (6.32 %), acetic acid (2.40 %), 3-hexyne-2,5-diol (2.13%), cyclohexene (0.36%), 3-hexyne-2,5-diol (0.34%), phenol (0.53 %), cyclohexene (0.33 %), 10-methyl-8-tetradecen-1-ol acetate (1.79 %), phenol (0.48 %), 7-octen-3-ol (0.30 %), pentasiloxane (9.68 %), 2-acetoxydodecane (0.54 %), 5,7-octadien-3-ol (0.86 %) and 2-bicycloheptanol (0.49 %). On the other hand, α-pinene (34.73%), *cis*-pinen-3-ol (0.09 %), β-pinene (0.89 %), β-mycrene (1.62 %), α-phellandrene (28.57

TABLE-1
CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF *M. pajang*

RT (min)	Compound	Concentration (%)		
		Flesh	Peel	Kernel
5.305	Butanoic acid	0.57	–	–
6.187	2-Furancarboxalde	1.14	–	–
6.694	Butanoic acid	0.11	–	–
7.023	1-Hexanol	0.14	–	–
7.588	α-Pinene	–	34.73	0.21
8.082	*cis*-Pinen-3-ol	–	0.09	–
8.593	β-Pinene	–	0.89	–
8.766	β-Mycrene	–	1.62	0.27
8.575	Cyclopentanecarboxylic acid	0.14	–	–
8.704	2,7-Nonadien-5-one	0.25	–	–
9.327	Butanoic acid	0.25	–	–
10.207	2-Furancarboxaldehyde	0.26	–	–
10.684	8-Chlorocapric acid	0.16	–	–
11.145	α-Phellandrene	0.52	28.57	6.03
	1,3-Cyclohexadiene	–	0.15	0.03
11.477	Bicycle-2-hept-2-ene	0.40	–	–
11.850	Benzene	0.63	7.65	1.15
	D-Limonene	–	12.55	2.16
	β-Phellandrene	–	7.18	1.83
13.442	2-Furanmethanol	10.33	–	–
13.477	3-Methyl-4-cyclohexene	14.91	–	–
13.938	α-Methyl	2.07	–	–
14.017	Undecane	1.95	–	–
14.100	Benzene	1.24	–	–
14.340	1,6-Octadien-3-ol	0.37	–	–
15.096	Phenylethyl alcohol	0.30	–	–
15.212	Cyclohexen-1-ol	0.33	–	–
15.432	Butanoic acid	0.32	–	–
15.806	2-cyclohexen-1-ol	0.38	–	0.54
16.043	Cyclohexanol	0.60	–	–
16.383	*p*-Flouroethylbenzene	0.06	–	–
16.613	Cyclohexanol	0.22	–	–
16.684	4,4-Dimethyl-2-cyclopenten-1-one	0.11	–	–
16.796	Cyclopropane	1.14	–	–
16.971	3-Cyclohexen-1-ol	0.68	–	–
17.292	Benzenemethanol	5.96	–	–
17.464	3-Cyclohexene-1-methanol	12.41	–	–
17.564	3-Cyclohexene-1-methanol	0.91	–	–
17.725	Bicyclohexan-3-ol	4.97	–	–
17.878	Octadecanoic acid	–	–	0.59
17.917	Bicyclohept-3-en-2-one	0.56	–	–
18.054	2-Oxabicycloctanol-6-ol	0.54	–	–
18.108	Cyclohexanol	0.31	–	–
18.182	2-Cyclohexen-1-ol	0.53	–	–
18.344	2-Acetylcyclopentanone	0.43	–	–
18.600	Ethanol	0.32	–	–
18.761	Benzophenone	–	–	1.11
18.863	Bicyclohexan-3-ol	2.54	–	–
19.011	Cyclohexanol	0.79	–	–
19.044	Heptadecane	–	–	0.78
19.108	2-Cyclohexen-1-one	3.60	–	–
19.259	2-Cycloyexen-1-one	0.60	–	–
19.433	Acetic acid	2.40	–	–
19.910	3-Hexyne-2,5-diol	2.13	–	–
20.140	Octadecanoic acid	–	–	2.28
20.357	Cyclohexene	0.36	–	–
20.442	3-Hexyne-2,5-diol	0.34	–	–
20.537	Phenol	0.53	–	–
20.913	Cyclohexene	0.33	–	–
21.027	1,2-Benzenedicarboxylic acid	–	–	1.43
21.191	10-Methyl-8-tetradecen-1-ol acetate	1.79	–	–
21.324	2-Heptadecanone	–	–	1.04
21.528	Hexadecanoic acid	–	–	1.05
21.967	Phenol	0.48	–	–
22.021	Dibutyl phthalate	–	–	0.98
22.192	Hexadecanoic acid	–	–	5.86
23.207	9,12-Octadecadienoic acid	–	–	1.26
23.304	2-Nonadecanone	–	–	2.27
23.449	7-Octen-3-ol	0.30	–	–
23.819	Linoleic acid	–	–	16.58
23.750	Pentasiloxane	9.68	–	–
23.860	9-Octadecenoic acid	–	–	12.35
23.960	2-Cyclohexen-1-one	5.72	–	–
24.065	Octadecanoic acid	–	–	2.39
24.288	2-Acetoxydodecane	0.54	–	–
24.492	2-Octanol	–	–	0.98
24.820	Cyclopentane	–	–	0.71
26.006	5,7-Octadien-3-ol	0.86	–	–
26.575	2-Bicycloheptanol	0.49	–	–
27.003	1,2-Benzenedicarboxylic acid	–	–	1.43
28.971	Squalene	–	–	2.99

RT = Retention time

%), 1,3-cyclohexadiene (0.15 %), benzene (7.65 %), D-limonene (12.55 %) and α-phellandrene (7.18%) were found in *M. pajang* peel. For *M. pajang* kernel, the volatile compounds found were α-pinene (0.21 %), β-mycrene (0.27 %), α-phellandrene (6.03 %), 1,3-cyclohexadiene (0.03 %), benzene (1.15 %), D-limonene (2.16 %), β-phellandrene (1.83%), 2-cyclohexen-1-ol (0.54 %), benzophenone (1.11 %), heptadecane (0.78 %), octadecanoic acid (5.26 %), 1,2-benzene dicarboxylic acid (1.43 %), 2-heptadecanone (1.04 %), hexadecanoic acid (6.91 %), dibutyl phthalate (0.98 %), 9,12-octadecadienoic acid (1.26 %), 2-nonadecanone (2.27 %), linoleic acid (16.58 %), 9-octadecenoic acid (12.35 %), 2-octanol (0.98 %), cyclopentane (0.71 %), 1,2-benzene dicarboxylic acid (1.43 %) and squalene (2.99 %). Other study isolated 50 volatile compounds from the pulp of *M. pajang* with 15 monoterpene hydrocarbons were found to be dominant chemical class. For instance, α-pinene (67.2 %) and α-phellandrene (11.0 %) [14]. Apart from this, the second most abundant chemical class in *M. pajang* pulp was esters (n = 19) and of these, ethyl butanoate (3.85 %) and butyl butanoate (3.38 %) were the most abundant [14].

2,2-Diphenyl-1-pycryl-hydrazyl (DPPH) free radical scavenging assay: DPPH free radical scavenging assay is commonly used to determine the antioxidant activity. In this study, *M. pajang* peel displayed the highest radical scavenging activity with 64.4 %, followed by flesh and kernel with 61.6 and 52.2%, respectively (Table-2). Other study reported that the DPPH radical scavenging activity of *M. pajang* pulp and juice powder were 43.25 and 52.61 %, respectively [4], showing that the results were lower than that of *M. pajang* peel and flesh in this study. Crude polysaccharide isolated and purified from *M. pajang* fibrous pulp displayed low radical scavenging activity (38.3 %) [9] compared to this study. The ethyl acetate and methanolic extracts of *M. pajang* kernel also showed high radical scavenging activity with IC_{50} values of 7.28 and 8.84 µg/mL, respectively [15].

Antimicrobial assay: To the best of our knowledge, this is the first report on antimicrobial of essential oils extracted

TABLE-2
FREE RADICAL SCAVENGING ACTIVITY
OF ESSENTIAL OIL OF *M. Pajang*

Samples	DPPH*	Radical scavenging activity (%)
Flesh	68.8 ± 0.19	61.6
Peel	80.7 ± 0.35	64.4
Kernel	48.7 ± 0.25	52.2

*DPPH free radical scavenging was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC).

from *M. pajang* against several pathogenic microorganisms by disc diffusion method (Table-3). Essential oils from flesh, peel and kernel of *M. pajang* were tested against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Salmonella enteritidis* and *Escherichia coli*). The results revealed that the essential oil extracted from different parts of the fruit were more sensitive against Gram-positive bacteria. However, *M. pajang* peel was considered to show weak inhibition against *B. subtilis* (5.3 ± 0.4 mm) followed by flesh against *B. subtilis* (5.1 ± 0.3 mm) and kernel against *S. aureus* (4.0 ± 1.3 mm). The antimicrobial activity against Gram-negative bacteria was lower due to the existence of outer membrane layer which prevent absorption of hydrophobic component through the lipopolysaccharide layer [16]. Previous study also indicated that most of the crude extracts of this fruit did not show significant inhibition activity against targeted microbes of which most of them displayed either weak or moderate activities with inhibition zones between 6 and 13 mm [15].

TABLE-3
ANTIMICROBIAL ACTIVITIES (DIAMETER OF
INHIBITION ZONE) ESSENTIAL OIL OF *M. Pajang*

Samples	Inhibition zone (mm)			
	S. aureus	*B. subtilis*	*E. coli*	*S. enteritidis*
Flesh	2.2 ± 0.5	5.1 ± 03	2.1 ± 1.1	1.1 ± 0.1
Peel	1.7 ± 0.8	5.3 ± 0.4	2.0 ± 0.3	1.3 ± 0.5
Kernel	4.0 ± 1.3	2.8 ± 0.5	2.6 ± 1.4	1.1 ± 0.1

Conclusion

The volatile compounds of *Mangifera pajang* peel showed the highest concentration with α-pinene (34.73 %), followed by kernel and flesh with linoleic acid (16.58 %) and 3-methyl-4-cyclohexane (14.91 %), respectively. Meanwhile, the essential oil of peel displayed the highest free radical scavenging for antioxidant activity *in vitro*. For antimicrobial activity, the essential oil of all parts of *M. pajang* showed weak inhibition.

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