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CHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITIY OF *PHALERIA MACROCARPA* (SCHEFF.) BOERL.

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ABSTRACT: Phytochemical studies on the fruits of *Phaleria macrocarpa* yielded 2,6,4'-trihydroxy-4-methoxy-benzophenone **(1)** and 6,4'-dihydroxy-4-methoxybenzophenone-2-*O*-*β*-D-glucopyranoside **(2)** together with two triterpenoids, 24-methylenecycloartan-3-one (**3**) and 24-methyl-9,19-cyclo- lanost-25-en-3-ol (**4**). Investigation on the leaves part led to the isolation of β-sitosterol (**5**) and stigmasterol (**6**). All extracts and isolated compounds were tested for their antibacterial activity using disc diffusion method followed by minimal inhibitory concentration (MIC) against two Gram-positive bacterial strains, *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 29737) and two Gram-negative bacterial strains, *Escherichia coli* (ATCC 10536) and *Pseudomonas putida* (ATCC 49128). In general, the result has demonstrated that all extracts and isolated compounds were exhibited weak activity against all tested bacteria with the inhibition zone having diameter between 6.1 – 7.3 mm. This is the first report on the isolation of triterpenoids (**3** and **4**) from this plant and the antibacterial activity for compounds (**1**-**4**).

INTRODUCTION: *Phaleria macrocarpa* (Scheff.) Boerl (Thymelaeaceae), known as 'mahkota dewa', originates from Irian Jaya (Papua province of Indonesia). This plant is used as a therapeutic healing alternative in health system of the Indonesians and lower course of Malaysia [1].

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Particularly, all parts of this plant including fruits, seeds, stems and leaves have well known therapeutic properties and have been extensively used in traditional medicine [2]. The fruits are used to treat flu, rheumatism, heart diseases and cancer; the leaves are used to treat dysentery, allergy, tumor and impotency, while the stems are beneficial in the treatment of bone cancer. The eggshells of seeds are used to counter breast cancer, cervix cancer, lung disease, liver and heart diseases. This plant, especially the seed part, cannot be consumed directly due to its high level of toxicity, which may cause inflammation, numbness and unconsciousness.

However, the seeds can be used as an external medicine for the treatment of skin conditions and as an ornamental plant, which acts as a traditional biopesticide [3, 4].

From the chemical point of view, *P. macrocarpa* contains various types of secondary metabolites belonging to the classes of benzophenones, lignans, sesquiterpenes, triterpenoids and xanthones and related compounds, often present as glycosides [5, 6, 7, 8]. Qualitative analysis revealed the presence of kaempferol, myricetin, naringin, and rutin as the major flavonoids in the pericarp, while naringin and quercetin were found in the mesocarp and the seeds *of P. macrocarpa* [10]. These compounds are thought to be responsible for the valuable medicinal properties ascribed in this plant including anticancer, antidiabetic, antihyperlipidemic, anti-inflammatory, antibacterial, antifungal, antioxidant and vasorelaxant effects [11, 12].

To date, various studies have been carried out to evaluate the antibacterial activities of *P. macrocarpa* on the crude extracts but there is no study has been conducted on the phytochemical composition [13]. We now report the antibacterial activity of *P. macrocarpa* on the basis of the crude extracts and the isolated constituents. To the best of our knowledge, this is the first report on the isolation of triterpenoids (**3** and **4**) from this plant, as well as the antibacterial activity of compounds (**1**-**4**).

MATERIALS AND METHODS:

Instruments and materials: Melting points were recorded on a Leica Gallen III and mass spectral data were provided by Kent Mass Spectrometry Service, UK. Fourier Transform-Infra Red (FTIR) spectrophotometer (Shimadzu 8300 series) was used to record the IR spectra. Nuclear Magnetic Resonance, ^{1}H NMR and ^{13}C NMR (Bruker Avance) spectra were recorded at 400 and 100 MHz, respectively, and Tetramethyl silane (TMS) was used as an internal standard. Column chromatography (CC) was performed using Merck silica gel 60 (230-400 mesh and 70-230 mesh) and thin layer chromatography (TLC) was carried out using pre-coated silica gel aluminium plate (Merck Kieselgel 60 F_{254}) with thickness of 0.20 mm.

Plant materials: Fruits and leaves of *P. macrocarpa* were collected from Pontian, Johor, Malaysia, in May 2010, and were identified by Dr. Shamsul Kamis, Plant Taxonomist, Biodiversity Unit, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. A voucher specimen (SK 2248/13) representing this collection has been deposited in the Biodiversity Unit's Herbarium, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia.

Extraction and isolation: The dried and ground fruits of *P. macrocarpa* (500 g) were extracted twice with ethanol (EtOH) (2 L) at room temperature for 48 h. The resulting extract was filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness using vacuum distillation and rotary evaporator at 50°C. The EtOH extract was partitioned with water-chloroform-ethyl acetate to give chloroform ($CHCl_3$), ethyl acetate (EtOAc) and aqueous extracts, respectively (Oshimi et al., 2008). Evaporation of the $CHCl_3$ and EtOAc extracts afforded 7.55 g and 2.40 g of respective extracts.

A portion of the EtOAc extract (1.00 g) was subjected to CC on silica gel using a gradient elution of petroleum ether (PE)-EtOAc to give 13 fractions (Fr 1-13). Benzophenone (**1)** (50 mg) was obtained from Fr. 10 using an eluent system PE-EtOAc (85:15), while benzophenone glucoside (**2)** (70 mg) was obtained from purification of Fr. 11 using a gradient elution $CHCl_3$-MeOH (90:10). The $CHCl_3$ extract (7.55 g) was submitted to vacuum liquid chromatography (VLC), eluted with *n*-hexane-EtOAc-MeOH mixture of increasing polarity to give twelve fractions. VLC-fraction 4 (1.48 g) was purified using CC eluted with *n*-hexane-diethyl ether to give triterpenoids **3** (52 mg) and **4** (35 mg) as white solids.

The dried and ground leaves (500 g) were extracted sequentially using (2 L) each of *n*-hexane, dichloromethane (DCM), EtOAc and MeOH at room temperature for 48 h each. Evaporation of all extracts afforded *n*-hexane (10.63 g), DCM (9.47 g), EtOAc (8.06 g) and MeOH extracts (15.66 g). VLC-fractionation of the *n*-hexane extract (10.63 g) using a solvent system comprising *n*-hexane-EtOAc-MeOH of increasing polarity afforded seven fractions.

Fraction 4 (1.51 g) was subjected to CC eluted with *n*-hexane-EtOAc to yield β-sitosterol **(5)** (31 mg) and stigmasterol **(6)** (64 mg) as white needles.

Disc diffusion assay: Inoculum of 400 µL suspension containing 10^8 CFU/mL of bacterial was spreaded on the nutrient agar (NA) and potato dextrose agar (PDA) medium. The discs (6 mm diameter) impregnated with 10 µL of the sample and DMSO (negative control) was placed on the inoculated agar, and was incubated for 24 h at 37°C. Streptomycin sulfate (10 µg/mL) was used as the positive control. Clear inhibition zones around the discs indicated the positive antimicrobial activity. The experiment was replicated two times and zones of inhibition reported as mean ± SD.

Minimum Inhibitory Concentration (MIC): Inoculates of the microbial strains were prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Each sample (3.6 mg) was dissolved in DMSO (2 mL) to get 1800 µg/mL stock solution. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 µL) was added to well from row B to H. The stock solutions of samples (100 µL) were added to wells at row A and B. Then, the mixture of samples and sterile broth (100 µL) at row B were transferred to each well in order to obtain a twofold serial dilution of the stock samples (concentration of 1800, 900, 450, 225, 112.5, 56.25, 28.13 and 14.06 µg/mL). The inoculated bacteria (100 µL) were added to each well. The final volume in each well was 200 µL. Plates were incubated at 37°C for 24 h. Microbial growth was indicated by the turbidity and the presence of pellet at the bottom of the well.

RESULTS AND DISCUSSION:

Phytochemistry: As part of our on-going phytochemical study on the medicinal plants from Malaysia [13, 14], we now report on the isolation, identification, and antibacterial activity of the phytochemicals and crude extracts from *Phaleria macrocarpa*. Purification on the EtOAc extract of the fruits led to the isolation of benzophenones **(1** and **2)**. From the qualitative TLC analyses of the extracts it was evident that aglycone benzophenone (**1**) was only present in the EtOAc extract of the fruits, while benzophenone glycoside (**2**) was detected in the EtOAc extracts of the fruits and the leaves. Benzophenones (**1** and **2**) were isolated previously from the leaves and stems of this plant [6, 15].

Two triterpenoids, 24-methylenecycloartan-3-one **(3)** and 24-methyl-9,19-cyclolanost-25-en-3-ol (**4**) were isolated from the $CHCl_3$ extract of the fruits. Comparison on the TLC analyses showed that both triterpenoids (**3** and **4**) were absent in all extracts of the leaves. Purification of the *n*-hexane extract of the leaves afforded β-sitosterol (**5**) and stigmasterol (**6**), which was also detected in the $CHCl_3$ extract of the fruits. To the best of our knowledge, this is the first report on the isolation of triterpenoids (**3** and **4**) from this plant. All structures (**Figure 1**) were elucidated by spectroscopic means and by comparison with the previously published data [16, 17, 18, 19].

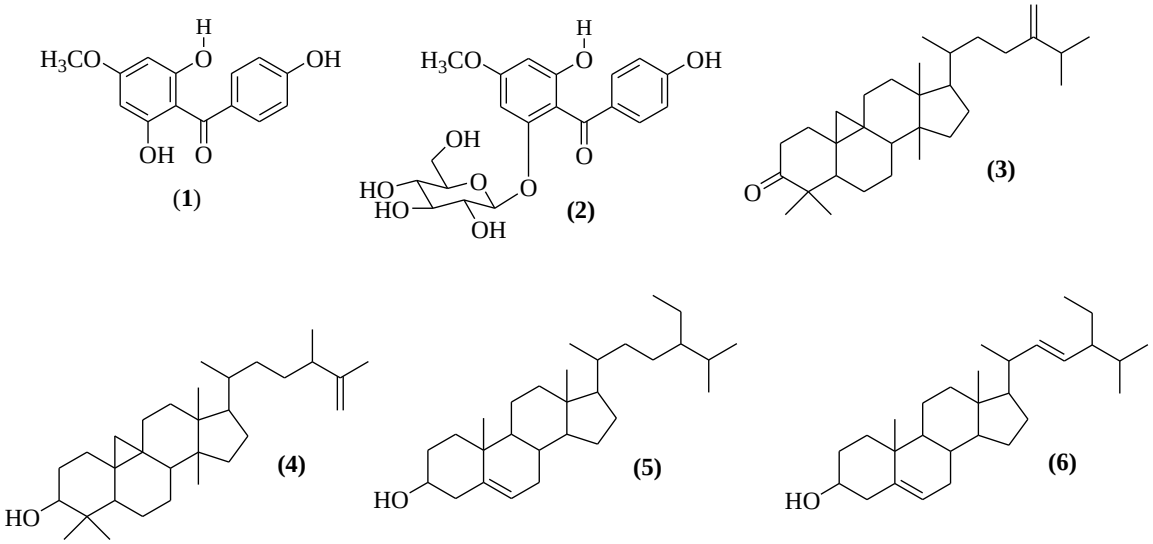

FIGURE 1: STRUCTURES OF COMPOUNDS (1-6) ISOLATED FROM *P. MACROCARPA*

In the present study, the finding from the phytochemical study on *P. macrocarpa* was slightly different from the previous report. Chemical investigation of *P. macrocarpa* by other researchers reported the isolation of xanthone derivative, sesquiterpene glycoside, lignan and 29-norcucurbitacin derivatives [5, 6, 7, 8]. The variance in compounds content may be due to the influence of environmental variables on plant growth and micronutrients of the soil from diverse geographical regions. The existences of triterpenoids (**3-6**) from our results may enhance information on the distribution of chemical composition of *P. macrocarpa*.

Antibacterial activity: The use of plant extracts and phytochemicals, both with known antibacterial properties, can be of great significance in therapeutic treatments. There are several antibacterial activities have been conducted on the extracts of *P. macrocarpa* [9, 10] and to the best our knowledge, there is no study has been reported regarding the antibacterial activity of isolated compounds from this plant to date.

In general, the extracts and pure compounds from *P. macrocarpa* exhibited weak antibacterial activity with the inhibition zone ranging from 6.1 – 7.3 mm, suggested the resistance of tested bacterial strains towards all samples (**Table 1**). Previous studies on the antibacterial activity of the *n*-hexane and $CHCl_3$ extracts of the leaves was in agreement with our results as the extracts were found to have low antibacterial activity against *B. cereus, E. coli, K. pneumonia and P. aeruginosa* with the inhibition zone of < 10mm each [10, 20]. Meanwhile research conducted on various parts of the fruits reported good antibacterial activity of the extracts against gram positive bacterial strains (*B. cereus, B. subtilis, M. luteus, S. aureus*) with inhibition zone ranging between 13.3 – 23.3 mm [9]. The presences of flavonoids identified as kaempferol, mycricetin, naringin, quercertin and rutin in the extracts contribute to antibacterial activity with some mechanism of action against pathogenic microorganism [12]. Thus, it was proposed that the weak antibacterial activity of extracts and phytochemicals in the present study might due to the absence of the flavonoids.

TABLE 1: ANTIBACTERIAL ACTIVITY OF EXTRACTS AND ISOLATED COMPOUNDS FROM *P. MACROCARPA* FRUITS AND LEAVES

Samples	Inhibition zone (mm)				MIC (µg/mL)			
	Gram-positive bacteria		Gram-negative bacteria		Gram-positive bacteria		Gram-negative bacteria	
	B. s	***S. a***	***E. c***	***P. p***	***B. s***	***S. a***	***E. c***	***P. p***
Extracts								
Fruits part								
Ethanol	7.2 ± 0.14	6.2 ± 0.21	6.3 ± 0.14	6.2 ± 0.07	900	900	900	900
Ethyl acetate	6.3 ± 0.42	6.0 ± 0.00	6.1 ± 0.07	6.4 ± 0.21	900	900	900	900
Chloroform	6.5 ± 0.71	6.4 ± 0.07	6.5 ± 0.14	6.1 ± 0.07	1800	1800	1800	1800
Leaves part								
Methanol	6.3 ± 0.42	6.1 ± 0.14	6.4 ± 0.14	6.1 ± 0.07	900	900	900	900
Ethyl acetate	6.3 ± 0.35	6.1 ± 0.07	6.2 ± 0.28	6.0 ± 0.00	900	900	900	900
Dichloromethane	7.1 ± 0.07	7.3 ± 0.42	6.1 ± 0.07	6.3 ± 0.14	900	900	900	900
n-Hexane	7.3 ± 0.21	6.9 ± 0.14	6.0 ± 0.00	6.2 ± 0.21	1800	1800	1800	1800
Isolated compounds								
Benzophenone **(1)**	6.2 ± 0.21	6.8 ± 0.64	6.0 ± 0.00	6.1 ± 0.07	1800	1800	900	900
Benzophenone **(2)**	6.0 ± 0.00	6.1 ± 0.07	6.2 ± 0.21	6.2 ± 0.07	1800	1800	900	900
Triterpenoid **(3)**	9.0 ± 0.00	8.8 ± 0.35	8.2 ± 0.21	8.9 ± 0.14	1800	1800	1800	900
Triterpenoid **(4)**	9.1 ± 0.07	8.1 ± 0.14	8.5 ± 0.14	8.0 ± 0.00	1800	1800	1800	1800
β-sitosterol **(5)**	8.1 ± 0.28	7.5 ± 0.71	8.0 ± 0.00	8.6 ± 0.57	450	900	900	900
Stigmasterol **(6)**	8.5 ± 0.64	8.0 ± 0.00	8.1 ± 0.21	8.3 ± 0.07	450	900	900	450
Positive control								
Streptomycin sulphate	19.5 ± 0.71	17.0 ± 1.41	16.5 ± 0.71	18.0 ± 01.41	14.1	14.1	14.1	14.1

B.a=Bacillus subtilis (ATCC 6633); *S.a=Staphylococcus aureus* (ATCC 29737); *E.a=Escherichia coli* (ATCC 10536); *P.a=Pseudomonas putida* (ATCC 49128). For inhibition zone of disc diffusion method, data were expressed as mean ± STDEV of two independent experiments performed in triplicates; Zone of inhibition including diameter of the Whatman disc (6 mm).

The antibacterial activity of benzophenones **(1** and **2)** and triterpenoids **(3** and **4)** were reported for the first time in this study. All isolated compounds were displayed larger diameter inhibition zone (6.1 – 9.1 mm) compared to the extracts and indicated that the compounds were slightly more active than the extracts. However, the antibacterial activity of the phytochemicals was considered very low and these compounds may associate to the weak antibacterial properties of the extracts.

Previous study was discovered the good antibacterial activity of benzophenones against variety of pathogenic bacteria was contributed by the presence of prenyl and geranyl substituents in the structure [21]. The absence of these substituents in benzophenones **(1** and **2)** suggested the weak antibacterial activity of these compounds. Meanwhile, β-sitosterol (**5**) and stigmasterol (**6)** exhibited as moderate inhibitor with MIC value of 450 µg/mL against *B. subtilis*.

The finding in the present study is consistent with the weak antibacterial activity as only benzophenones and triterpenoids were detected presence in all extracts of *P. macrocarpa* fruits and leaves. The inhibition zones of all samples were comparable to the standard, streptomycin sulphate (16.5 – 19.5 mm) which acts as a positive control.

CONCLUSION: Two benzophenones (**1-2**) and four triterpenoids (**3-6**) have been isolated from *P. macrocarpa*. Triterpenoids (**3** and **4**) have been reported here for the first time from this plant. The extracts and isolated compounds from *P.macrocarpa* did not show significant antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas putida*. Geographical growing region of *P. macrocarpa* contribute to the difference of phytochemical presence and variable levels of antibacterial properties.

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REFERENCES:

1. Ali RB, Atangwho IJ, Kuar N, Mohamed EAH, Mohamed AJ, Asmawi MZ, Mahmud R: Hypoglycemic and anti-hyperglycemic study of Phaleria macrocarpa fruits pericarp. J. Med. Plants Research 2012; 6:1982-1990.
2. Harmanto N 2003. Conquering Disease in Unison with mahkota dewa, Phaleria macrocarpa, Mahkota dewa. PT Indonesia: North Jakarta, p 14.
3. Aditama TY: Kanker. Medisinal Jurnal Kedokteran, Vol 2, 2001: 1-5.
4. De Padua LS, Bunyapraphatsara N, Lemmens RHMS: Plant resources of South East Asia, Medical and poisonous plants 1 (PROSEA). Backhuys Publishers: Leiden 1999.
5. Kurnia D, Akiyama K, Hayashi H: 29-Norcucurbitacin derivatives isolated from the indonesian medicinal plant, Phaleria macrocarpa (Scheff.) Boerl. Biosci. Biotechnol. Biochem 2008; 72:618-620.
6. Oshimi S, Zaima K, Matsuno Y, Hirasawa Y, Iizuka T, Studiawan H: Studies on the constituents from the fruits of Phaleria macrocarpa. J Nat Med 2008; 62:207-210.
7. Susilawati S, Matsjeh S, Pranowo HD, Anwar C: Macronone, a novel diepoxylignan from bark of mahkota dewa (Phaleria Macrocarpa (Scheff.) Boerl.) and its antioxidant activity. Indo J Chem 2012; 12:62-69.
8. Zhang SY, Zhang QH, Zhao W, Zhang X, Zhang Q, Bi YF: Isolation, characterization and cytotoxic activity of benzophenone glucopyranosides from mahkota dewa (Phaleria macrocarpa (Scheff.) Boerl). Bioorg Med Chem Lett 2012; 22:6862-6866.
9. Hendra R, Ahmad S, Sukari A, Shukor MY, Oskoueian E: Flavonoid analyses and antimicrobial activity of various parts of Phaleria macrocarpa (Scheff.) Boerl fruit. Int J Mol Sci 2011; 12: 3422-3431.
10. Yosie A, Effendy M, Sifzizul T, Habsah M: Antibacterial, radical-scavenging activities and cytotoxicity properties of Phaleria macrocarpa (Scheff.) Boerl. leaves in HEPG2 cell lines. Int J Pharmac Sci Res 2011; 2:1700-1706.
11. Ali RB, Atangwho IJ, Kaur N, Abraika OS, Ahmad M, Mahmud R: Bioassay-guided antidiabetic study of Phaleria macrocarpa fruit extract. Molecules 2012; 17:4986-5002.
12. Hendra R, Ahmad S, Oskoueian E, Sukari A, Shukor MY: Antioxidant, anti-inflammatory and cytotoxicity of Phaleria macrocarpa (Boerl.) Scheff fruit. BMC Complement Altern Med 2011; 11:1-10.
13. Sirat HM, Basar N, Jani NA: Chemical compositions of the rhizome oils of two Alpinia species of Malaysia. Nat Prod Res 2011; 25:982-986.
14. Jantan I, Yasin YHM, Jamil S, Sirat H and Basar N: Effect of prenylated flavonoids and chalcones isolated from Artocarpus species on platelet aggregation in human whole blood. J Nat Med 2010; 64:365-369.
15. Susilawati, Matsjeh S, Pranowo HD, Anwar C: Antioxidant activity of 2,6,4'-trihydroxy-4-methoxy benzophenone from ethyl acetate extract of leaves of mahkota dewa (Phaleria macrocarpa (Scheff.) Boerl.). Indo J Chem 2011; 11:180-185.
16. Anjaneyulu V, Ravi K, Prasad KH, Connolly JD: Triterpenoids from Mangifera indica. Phytochemistry 1989; 28:1471-1477.
17. Chen YH, Chang FR, Lu MC, Hsieh PW, Wu MJ, Du,YC: New benzoyl glucosides and cytotoxic pterosin sesquiterpenes from Pteris ensiformis Burm. Molecules 2008; 13:255-266.
18. Patra A, Jha, S, Murthy P, Sharone A: Isolation and characterization of stigmast-5-en-3-β-ol (β-sitosterol) from

the leaves of Hygrophila spinosa T. Anders. Int J Pharma Sci Res 2010; 1:95-100.
19. Chaturvedula VSP, Prakash I: Isolation of stigmasterol and β-sitosterol from the dichloromethane extract of Rubus suavissimus. Int Curr Pharm J 2012; 1:239-242.
20. Muhammad AS: Antimicrobial activity of Mahkota Dewa [Phaleria macrocarpa (Scheff). Boerl.] leaf extract against Pseudomonas aeruginosa by agar dilution and scanning electron microscopy. Folia Medica Indonesiana 2010; 46:172-178.
21. Naldoni FJ, Claudino AL, Cruz JW Jr, Chavasco JK, Faria e Silva PM, Veloso MP, Dos Santos MH: Antimicrobial activity of benzophenones and extracts from the fruits of Garcinia brasiliensis. J Med Food. 2009; 12:403-407.

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