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Isolation and identification of compounds from *Phaleria macrocarpa* (Scheff.) Boerl fruit extract

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ABSTRACT

Objective: To identify and isolate the chemical compounds of *Phaleria macrocarpa* (*P. macrocarpa*) fruit ethanolic extract.
Methods: Dried fruit of *P. macrocarpa* was extracted with 90% ethanol and partitioned between *n*-hexane/H_2O and ethyl acetate/H_2O. The organic layer was fractionated by various stationary phase and identified by using combined data of 1D [(proton nuclear magnetic resonance (NMR), carbon-13 NMR)], 2D-NMR (heteronuclear multiple-quantum correlation and heteronuclear multiple-bond correlation), and mass spectrum.
Results: Purification of *n*-hexane and ethyl acetate fractions from ethanolic extract of *P. macrocarpa* fruit resulted in isolation of nine compounds.
Conclusions: A new compound was isolated and identified as glyceryl pentacosanoate. Also, two xanthones, which are 1,7-dihydroxy-3,6-dimethoxyxanthone and 1,6,7-trihydroxy-3-methoxyxanthone, are firstly reported to be isolated from *P. macrocarpa*.

1. Introduction

Phaleria macrocarpa (Scheff.) Boerl (*P. macrocarpa*), traditionally known as "mahkota dewa", is a plant from Thymelaeaceae family. It grows in tropical countries such as Indonesia and originates from Papua [1]. This plant has been known as a medicinal plant since antiquity. Traditionally, people use its fruit for treating numerous types of diseases such as hypertension, diabetes, cancer, dysentery, rheumatism, and kidney disorder. Furthermore, scientific studies have shown different bioactivities of *P. macrocarpa*. Hendra *et al.* reported that different parts of *P. macrocarpa* fruit can generate several activities such as antioxidative, anti-inflammatory, and cytotoxic effects [2]. Other studies indicate other bioactivities of *P. macrocarpa* fruit such as anti-cancer, anti-oxidant, anti-hyperglycemic, anti-hyperlipidemia, anti-bacterial, and vaso-relaxant activity [3–7].

These previous findings have led us to focus on the bioactivity of *P. macrocarpa* fruit. Recently, we have conducted a study on aqueous ethanolic extract of *P. macrocarpa* to evaluate its effect on women with premenstrual syndrome (PMS). Our study showed the potential of this *P. macrocarpa* fruit extract as a novel treatment for PMS [8]. Moreover, the extract was able to alleviate primary dysmenorrhea as well as abdominal pain and other symptoms related to PMS [9]. The extract has also been evaluated on its potential as treatment for endometriosis and breast cancer on a cellular level. It shows how *P. macrocarpa* fruit produces anti-inflammatory, pro-apoptotic, and anti-angiogenic activity by reducing nuclear factor-κB and inhibiting the eicosanoid pathway [10–12].

As a potential source of novel bioactive compounds, isolation and identification of the chemical constituents of *P. macrocarpa* are important. Few compounds have been successfully isolated and identified from every part of *P. macrocarpa* such as 2,6,4′-trihydroxy-4-methoxy benzophenone from the leaves [13], 29-norcucurbitacin derivatives from the seed [14], and Mahkosides A and B from the nut shell part [15]. Mahkoside A also has been isolated from *P. macrocarpa* bark alongside with phalerin (5) [16,17]. Lastly, DLBS1425-F1 (5) and DLBS1425-

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E2.2 (6) compounds have been isolated from the flesh of *P. macrocarpa* fruit [18]. In this present study, compounds from *P. macrocarpa* fruit extract were further evaluated to improve the compound library of *P. macrocarpa* fruit.

2. Materials and methods

2.1. General experiment

Flash chromatography was performed in a Sepacore flash chromatography (BUCHI, Switzerland) using silica gel 60, 0.063–0.200 mm mesh (Merck, Germany). Preparative high performance liquid chromatography (HPLC) was done on a preparative HPLC instrument with a UV absorbance detector (Waters, United Kingdom). Thin layer chromatography (TLC) was executed by Automatic TLC Sampler 4 (CAMAG, Germany) in silica gel 60 F254 TLC plates (Merck, Germany) and the results were documented by Reprostar 3 (CAMAG, Germany). High resolution mass spectra were obtained from LCT Premier XE time-of-flight (TOF) using electrospray ionization (ESI) instrument (Waters, United Kingdom) by direct injection. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-ECA 500 (JEOL, USA) NMR spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively). Results were recorded as follows: chemical shift values were expressed as units acquired in CD3OD, CD3Cl, or dimethyl sulfoxide (DMSO)-d_6 with tetramethylsilane as reference, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet), coupling constants (*J*) in Hertz and integration.

2.2. Chemical reagents

HPLC-grade methanol was purchased from Merck (Germany). Analysis-grade solvents, which are chloroform, methanol and acetonitrile, were obtained from Merck (Germany). Distilled water was purified using an arium® 611 UV ultrapure water system (Sartorius Stedim Biotech, Germany). Technical grade solvents were provided by different suppliers: ethanol was purchased from PT Molindo Raya Industrial (Indonesia), *n*-hexane was purchased from ExxonMobil (USA), ethyl acetate was purchased from PT Showa Esterindo (Indonesia), and methylene chloride was purchased from the Dow Chemical Company (USA).

Open column chromatography was performed with several stationary phases: silica gel from Merck (Germany), Diaion-HP20 from Sigma–Aldrich (USA) and Sephadex LH-20 from GE Healthcare (USA).

2.3. Plant materials

Dried *P. macrocarpa* fruits were collected from Central Java, Indonesia. Determination of plant species had been conducted by Herbarium Bogoriense, Research Center of Biology, Indonesian Institute of Science (Bogor, Indonesia) with certificate number 1261/IPH.1.02/lf.8/XII/2009.

2.4. Extraction

As much as 4 kg of dried and sliced *P. macrocarpa* fruits were extracted with 90% ethanol. The solvent was evaporated and the viscous extract was partitioned by *n*-hexane to produce *n*-hexane phase. The aqueous phase was then further partitioned by ethyl acetate to give ethyl acetate phase. The organic phase was dried with a rotary evaporator to attain *n*-hexane and ethyl acetate fractions. These fractions were proceeded into fractionation and isolation process.

2.5. Further fractionation of n-hexane fraction

n-Hexane fraction was fractionated using Sepacore flash chromatography with silica gel as stationary phase and eluted using gradient systems of *n*-hexane: ethyl acetate. The obtained fractions were observed with TLC and fractions that produced yellow spot at 366 nm were combined. The combined fractions were further fractionated on silica gel column chromatography using isocratic method with methylene chloride: methanol (20:1) as solvent system. This fractionation resulted in two fractions and fraction A was further fractionated on silica gel column chromatography using isocratic method with *n*-hexane: ethyl acetate (70:30) as solvent system. This process gave result to three fractions and fraction 2 was concentrated using rotary evaporator. To remove fatty acids in this fraction, acetonitrile was added. The insoluble compound was named compound 1. The soluble part resulted in compound 2 as yellow crystals after crystallization using acetone.

2.6. Further fractionation of ethyl acetate fraction

Ethyl acetate fraction was dissolved in methanol and mixed with silica gel. The mixture was loaded into vacuum column chromatography. Elution was done using methylene chloride, ethyl acetate, and methanol respectively to give rise to three fractions (A, B, and C).

Fraction A (6 g) was subjected to flash chromatography and eluted by gradient *n*-hexane: ethyl acetate. Fraction A5, which produced brown colored spot on TLC after being sprayed by 10% sulfuric acid in water, was subjected to silica gel (0.063–0.200 mm) column chromatography. Further fractionation was eluted by *n*-hexane: chloroform (1:1) system and resulted in crude crystal. Recrystallization process gave colorless needle-shaped crystal, compound 3.

Some of fraction B was dissolved in methanol and mixed with silica gel. The mixture was loaded into flash chromatography and eluted with chloroform: methanol (85:15). Four fractions (B1, B2, B3, and B4) were obtained from this separation process. Fractions B1 and B2 were combined and further fractionated in silica gel (0.063–0.200 mm) column chromatography. Separation was performed in chloroform: methanol (8:2) mobile system. Fractions, which produced black spot at R_f 0.58 when eluted with chloroform: methanol (85:15), were collected and further separated in Diaion-HP20 column chromatography (eluted by gradient water-methanol) followed by separation in Sephadex LH-20 (eluted by aqueous methanol). The process gave white solid form, named compound 4. Recrystallization in aqueous ethanol was performed to obtain a more pure compound.

Fraction B3 was further separated using silica gel column chromatography and eluted with dichloromethane: methanol (9:1). Fractions which produced black spot at R_f 0.31 when eluted by chloroform: methanol (8:2) were collected and separated in Diaion HP-20. Fractionation on Diaion HP-20 was

performed in water: methanol gradient system. Targeted fractions were further fractionated in silica gel column chromatography and eluted by methylene chloride: methanol (10:1). Desired fractions were combined and slowly evaporated to give rise to a white solid (compound 5), known as DLBS1425-F1 or phalerin [16,18]. Recrystallization in water was performed to obtain purer crystal.

Compound 5 was hydrolyzed using 2 mol/L chloric acid (6 h, 60 °C) and the reaction mixture was left to stand overnight at room temperature until orange solid was formed. The solid was then separated in silica gel (0.063–0.200 mm) and eluted with methylene chloride: methanol (25:1) to produce yellow crystal (compound 6), known as DLBS1425-E2.2 [18]. Recrystallization in aqueous methanol was performed to obtain crystal in a more pure state.

The remaining fraction B was subjected to vacuum column chromatography and eluted by gradient methylene chloride–acetone. Fractions which produced yellow spot on TLC and produced R_f 0.45 when eluted by methylene chloride: methanol (10:1) were combined and resulted in crude crystal. The solid was washed by acetone and gave a yellowish-brown crystal, as compound 7.

2.7. Preparative HPLC for isolation of compounds 8 and 9

Fraction A8 was dissolved in methylene chloride and subjected into preparative HPLC, equipped with XterraPrep® MS C18 OBD™ (5 μm; 30 mm × 100 mm; Waters) column. The isocratic elution was performed in methanol-MiliQ water (1:1) for 7 min at a flow rate of 42.53 mL/min. Detection was recorded with a Waters 2489 UV–vis detector at 271 nm. Two compounds (8 and 9) were isolated from the result of this fractionation.

2.8. ESI-TOF/mass spectrometry (MS) analysis

The mass of each isolated compound was analyzed using mass spectrometry LCT Premier XE (TOF) instrument (Waters, United Kingdom). Samples were dissolved in methanol and directly injected into the instrument. The analysis was performed in negative ESI mode with source temperature at 120 °C and desolvation temperature at 250 °C. The desolvation gas flow was set at 500 L/h.

3. Results

3.1. Analysis results

3.1.1. Glyceryl pentacosanoate

White powder, $C_{28}H_{56}O_4$, molecular weight (MW) 456. TLC: ($CHCl_3$—MeOH, 10:1) R_f 0.37; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, J = 6.85 Hz, H-25), 1.25 (42H, m), 1.64 (2H, m, J = 6.85 Hz, H-3), 2.36 (2H, t, J = 6.25 Hz, H-2), 3.60 (1H, dd, J = 11.45 Hz, H-3 propyl), 3.70 (1H, dd, J = 11.45 Hz, H-3 propyl), 3.93 (1H, m, J = 4.5 Hz, H-2 propyl), 4.22 (1H, dd, J = 9.6; 12.25 Hz, H-1 propyl), 4.15 (1H, dd, J = 8.9; 12.25 Hz, H-1 propyl); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.32 (C-25), 22.88 (C-24), 25.10 (C-3), 29.31–29.88 (C-4–C-22), 32.11 (C-23); 34.33 (C-2), 63.49 (C-3 propyl), 65.35 (C-1 propyl); 70.45 (C-2 propyl); 174.56 (C═O).

3.1.2. 1,7-Dihydroxy-3,6-dimethoxyxanthone (2)

Light-yellow crystal, $C_{15}H_{12}O_6$, MW 288.06. ESI-MS m/z (negative) 287.01 $[M-H]^-$. TLC: (n-hexane–ethyl acetate, 7:3) R_f 0.35; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.86 (3H, s, $-OCH_3$); 3.89 (3H, s, $-OCH_3$); 6.35 (1H, d, J = 2.6 Hz, H-4); 6.60 (1H, d, J = 2.6, Hz, H-2), 6.93 (1H, s, H-5); 7.45 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6): δ 55.92 ($-OCH_3$ ring B), 56.07 ($-OCH_3$ ring A), 92.49 (C-2), 96.87 (C-4), 102.48 (C-5), 102.73 (C-1a), 104.68 (C-8), 111.52 (C-9a), 146.22 (C-10a), 152.03 (C-7), 154.89 (C-6), 157.15 (C-1), 162.32 (C-4a), 165.71 (C-3), 179.02 (C═O).

3.1.3. β-Sitosterol (3)

Colorless needle-shaped crystal, $C_{29}H_{50}O$, MW 414.71. TLC: ($CHCl_3$ 100%) R_f 0.32; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.69 (3H, s, H-18), 0.82 (3H, d, J = 7.1 Hz, H-26), 0.83 (3H, d, J = 7.15 Hz, H-27), 0.84 (3H, t, J = 7.8 Hz, H-29), 0.92 (3H, d, J = 6.5 Hz, H-21), 1.15 (3H, s, H-19), 3.52 (1H, m, H-3), 5.35 (1H, br s, H-6); ^{13}C NMR (125 MHz, $CDCl_3$): δ12.04 (C-18), 12.17 (C-29), 18.96 (C-21), 19.21 (C-27), 19.59 (C-19), 20.02 (C-26), 21.26 (C-11), 23.23 (C-28), 24.49 (C-15), 26.21 (C-23), 28.44 (C-16), 29.31 (C-25), 31.83 (C-2), 32.07 (C-7), 32.09 (C-8), 34.10 (C-22), 36.33 (C-20), 36.68 (C-10), 37.42 (C-1), 39.94 (C-12), 42.47 (C-4), 42.50 (C-13), 45.99 (C-24), 50.29 (C-9), 56.21 (C-17), 56.94 (C-14), 71.99 (C-3), 121.91 (C-6), 140.92 (C-5).

3.1.4. 2,4′,6-Trihydroxy-4-methoxy-6″-acetyl-benzophenone-2-O-β-D-glucoside (4)

Colorless powder, $C_{22}H_{24}O_{11}$, MW 464.42, TLC: ($CHCl_3$ —MeOH, 85:15), R_f 0.29; ^{1}H NMR (500 MHz, CD_3OD): δ 2.04 (3H, s, CH_3 acetyl), 3.09 (1H, t, J = 7.8 Hz, H-2″), 3.23 (1H, t, J = 9.1 Hz, H-4″), 3.37 (1H, t, J = 9.1 Hz, H-3″), 3.58 (1H, m, H5″), 3.81 (3H, s, OCH_3), 4.20 (1-H, dd, J = 6.5; 11.7 Hz, H-6″), 4.35 (1H, dd, J = 1.95; 11.7 Hz, H-6″), 4.85 (2H, d, J = 7.75 Hz, H-1″), 6.20 (1H, d, J = 2.5 Hz, H-5), 6.32 (1H, d, J = 1.95 Hz, H-3), 6.79 (2H, d, J = 9.0 Hz, H-5′), 7.67 (2H, d, J = 9.0 Hz, H-6′); ^{13}C NMR (125 MHz, CD_3OD): δ 20.79 (CH_3), 56.06 (OCH_3), 64.84 (C-6″), 71.53 (C-4″), 74.74 (C-2″), 75.51 (C-5″), 77.80 (C-3″), 95.49 (C-3), 96.48 (C-5), 102.16 (C-1″), 111.75 (C-1), 115.95 (C-5′ and C-3′), 132.07 (C-1′), 133.66 (C6′, and C-2′), 158.30 (C-2), 159.48 (C-6), 163.80 (C-4′), 164.37 (C-4), 172.82 (C═O acetyl), 197.13 (C═O).

3.1.5. 1,6,7-Trihydroxy-3-methoxyxanthone (7)

Yellow crystal, $C_{14}H_{10}O_6$, MW 274.05. ESI-MS m/z (negative) 272.97 $[M-H]^-$. TLC: ($CHCl_3$—MeOH–formic acid, 90:1:1) R_f 0.56; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.85 (3H, s, $-OCH_3$), 6.31 (1H, d, J = 2.6 Hz, H-4), 6.54 (1H, d, J = 2.6 Hz, H-2), 6.86 (1H, s, H-5), 7.37 (1H, s, H-8); ^{13}C NMR (125 MHz, DMSO-d_6): δ 55.97 ($-OCH_3$), 92.29 (C-2), 96.65 (C-4), 102.43 (C-1a), 102.59 (C-8), 107.93 (C-5), 111.78 (C-8a), 143.94 (C-5a), 151.08 (C-6), 154.36 (C-7), 157.17 (C-1), 162.32 (C-4a), 165.55 (C-3), 179.08 (C═O).

3.1.6. Coumarin (8)

Colorless crystal, $C_9H_6O_2$, MW 146.04. RP-TLC: (MeOH—H_2O, 8:2), R_f 0.57; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.42 (1H, d, J = 9.8 Hz, H-3), 7.28 (1H, dt, J = 1.25 Hz; 8.3 Hz, H-6), 7.34 (1H, d, J = 8.3 Hz, H-8), 7.48 (1H, dd, J = 1.25 Hz; 8.3 Hz, H-5), 7.53 (1H, dt, J = 1.25; 8.3 Hz, H-7), 7.71 (1H, d, J = 9.8 Hz,

H-4); ^{13}C NMR (125 MHz, $CDCl_3$): δ 116.89 (C-3), 117.10 (C-8), 119.01 (C-10), 124.62 (C-6), 128.03 (C-5), 132.02 (C-7), 143.64 (C-4), 154.22 (C-9), 161.00 (C═O).

3.1.7. 2,3-Dihydroxybenzoic acid (9)

White crystal, $C_7H_6O_4$, MW 154.12, RP-TLC: (MeOH−H_2O, 8:2), R_f 0.73; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.32 (^{1}H, d, J = 7.1 Hz, H-4), 7.35 (^{1}H, t, J = 7.1 Hz, H-5), 7.55 (^{1}H, d, J = 7.1 Hz, H-6); ^{13}C NMR (125 MHz, DMSO-d_6): δ 113.1 (C-1), 119.6 (C-4), 121.3 (C-5), 121.4 (C-6), 146.6 (C-3), 151.6 (C-2), 172.9 (C-7).

3.2. Structure elucidation

The molecular formula of compound 1 was indicated as $C_{27}H_{54}O_4$, which was shown by the ^{1}H NMR and ^{13}C NMR spectra. Also, both NMR data gave the indication that 1 was an ester of saturated fatty acid. It was known that ^{1}H NMR presented a signal of methyl group (−CH_3), which was established by the value of the chemical shift at 0.88 (3H, t, J = 6.85 Hz), and methylene group 20 × CH_2, which was indicated by δ 1.25 (m, 40H). The presence of glycerol group could be easily identified because the proton region of low field chemical shifts at 3.70 (dd, J = 4.55; 11.45 Hz), 3.60 (dd, J = 11.45 Hz), 3.93 (m), 4.22 (dd) and 4.15 (dd) were correlated with the ^{13}C NMR chemical shifts at 63.49 (C-3′), 70.45 (C-2′), and 63.35 (C-1′). Carbon of methyl group was detected at δ 14.1 (C-24) and methylene groups were detected at 29.23–29.64 (C-4–C-22). Prediction of this structure was also supported by a distortionless enhancement by polarisation transfer (DEPT), heteronuclear multiple-quantum correlation (HMQC) and the data correlation heteronuclear multiple-bond correlation (HMBC) (Figure 1). Based on these data, compound 1 was probably a monoester of glycerol at C-1 with a chain length of 24 (C24), and given the name glyceryl pentacosanoate (1).

The molecular formula of compound 2 was assumed to be $C_{15}H_{12}O_6$, which was shown by ^{1}H NMR and ^{13}C NMR spectra and MS data. The *m/z* value of the isolated compound, according to the MS data in ESI negative ion mode was 287.01. Also, based on ^{1}H and ^{13}C NMR, this compound consisted of two aromatic rings and two methoxy groups, which were shown at δ 3.86 (3H, s) and 3.89 (3H, s). Both were attached to each aromatic ring at C-3 (165.71) and C-6 (154.89) respectively. Chemical shifts at δ 6.60 (d, ^{1}H, 2.6 Hz) and 6.35 (d, ^{1}H, 2.6 Hz) showed meta position of protons at aromatic ring B, while chemical shifts δ at 7.45 (s, ^{1}H) and 6.93 (s, ^{1}H) showed two singlet protons at aromatic ring A. There was also the presence of a carbonyl group, which was shown by ^{13}C NMR chemical shift at δ 179.02 ppm, and it indicated that this compound was a xanthone derivate. In addition, ^{1}H NMR chemical shift at δ 13.1 (1H, s) indicated the presence of hydrogen bonding between hydroxyl and carbonyl group. According to these data interpretations, compound 2 was possibly a xanthone derivative which was 1,7-dihydroxy-3,6-dimethoxyxanthone (2). This was supported by the DEPT, HMQC and HMBC experiment.

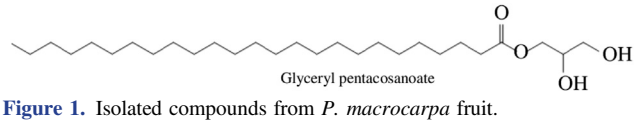

Figure 1. Isolated compounds from *P. macrocarpa* fruit.

According to ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, and correlation spectroscopy, it was hypothesized that compound 7 was another xanthone derivative with molecular formula of $C_{14}H_{10}O_6$. There were two aromatic rings which were shown by specific ^{13}C NMR signals. Chemical shifts at δ 92.29 and 96.65 exposed an aromatic ring (ring B) with two doublet protons at meta position, which were recognized from ^{1}H NMR chemical shifts at δ 6.54 and 6.31 with J coupling 2.6 Hz. Another aromatic ring (ring A) was known from chemical shifts at δ 102.59 and 107.93. Two singlet protons, which were shown by chemical shifts at δ 7.37 (s, ^{1}H) and 6.86 (s, ^{1}H), attached to it. In addition, there was a carbonyl group, which was shown by a signal at δ 179.08.

The difference between compound 7 and compound 2 was in the presence of methoxy group. In compound 2, there were two methoxy groups, while in compound 7 there was only one methoxy group, which was shown by signal at δ 3.85 (3H, s) and attached to aromatic carbon at a chemical shift of δ 165.55, based on long range coupling in HMBC experiment. It was confirmed by the *m/z* value of the compound 7 which was 272.97. According to these data interpretations, compound 7 was known as 1,6,7-trihydroxy-3-methoxyxanthone or 3-methoxymangiferitin.

According to the ^{13}C NMR, compound 3 consisted of 29 carbon atoms. Also, ^{1}H NMR spectrum exhibited a broad triplet at 5.35 and a multiple at 3.52 corresponding to H-6 olefinic proton and H-3α proton, respectively. Rest of protons appeared in the high field region between 0.7 and 2.0 ppm. These spectrum were similar to beta-sitosterol which was previously isolated. Therefore, compound 3 was beta-sitosterol.

Based on ^{1}H NMR, ^{13}C NMR and DEPT data, it was indicated that molecular formula of compound 4 was $C_{22}H_{24}O_{11}$. Both NMR, combined with HMQC, HMBC, and correlation spectroscopy, also indicated that compound 4 consisted of two aromatic rings. Chemical shifts at δ 6.20 (d, 1H, 2.55 Hz) and 6.32 (d, ^{1}H, 1.95 Hz) showed meta position of proton at ring A, while chemical shifts at δ 6.79 (d, 2H, 9.05 Hz) and 7.67 (d, 2H, 9.05 Hz) showed ortho position of proton at ring B. There was also a ^{13}C NMR chemical shift at δ 197.13 ppm which showed the presence of a carbonyl group in this compound. Another proton chemical shift at δ 3.8 ppm (s, 3H) showed the occurrence of a methoxy group (−OCH_3). There was a glucose group in this compound which was shown by chemical shifts of proton at δ 3.09 (dd, 1H, 7.8 Hz), 3.23 (t, 1H), 3.37 (t, 1H), 3.57 (m, 1H), 4.21 (dd, 1H, 6.5 Hz), 4.33 (dd, 1H, 1.95 Hz, 2.6 Hz), and 4.85 (d, 2H, 7.75 Hz) combined with ^{13}C NMR chemical shifts at δ 74.74 (C-2″), 71.53 (C-3″), 77.80 (C-4″), 75.51 (C-5″), 64.84 (C-6″), and 102.16 (C-1″). A signal singlet (3H) at 2.04 ppm indicated the presence of acetyl group (CH_3CO). From HMQC and HMBC data, it was indicated that the glucosyl group was attached to ring A at C-2. According to these data, compound 4 was elucidated as benzophenone with an acetylated glycoside group attached to C-6″, 2,4′,6-trihydroxy-4-methoxy-6″-acetyl-benzophenone-2-*O*-β-D-glucoside (4).

According to ^{13}C NMR, compound 8 consisted of nine carbons. Also, from DEPT analysis, it could be seen that 3 carbon atoms at δ 161.00, 154.22, and 119.01 were quaternary carbon atoms. Combining DEPT analysis data and ^{1}H NMR, it was shown that six other carbons were tertiary carbon atoms. There was a presence of carbonyl group which was shown by ^{13}C NMR chemical shift at 161.00. Also, the existence of an aromatic ring was shown by ^{13}C NMR signal at 117.10 (C-8),

119.01 (C-10), 124.62 (C-6), 128.03 (C-5), 132.02 (C-7), and 154.22 (C-9) combined with ^{1}H NMR at δ 7.28 (1H, dt, *J* = 1.25; 8.3 Hz, H-6), 7.34 (1H, d, *J* = 8.3 Hz, H-8), 7.48 (1H, dd, *J* = 1.25; 8.3 Hz, H-5), 7.53 (1H, dt, *J* = 1.25; 8.3 Hz, H-7). Its *m/z* value was 147.008. According to the result, compound 8 was elucidated as coumarin, a common compound which had been isolated from numerous different plants but was newly found in this species.

It was recognized from ^{1}H NMR and ^{13}C NMR data that compound 9 probably had molecular formula of $C_7H_6O_4$. It had an aromatic group with ABC system, shown at δ 7.32 (1H, d, *J* = 7.1 Hz), 7.35 (1H, t, *J* = 7.1 Hz), 7.55 (1H, d, *J* = 7.1 Hz). On the other hand, ^{13}C NMR data indicated that the compound had six carbon atoms and one carboxyl group at 172.9 ppm. Based on this result, compound 9 was suggested to be 2,3-dihydroxybenzoic acid, or commonly known as pyrocatechuic acid.

4. Discussion

P. macrocarpa has been confirmed for its activity towards many diseases, and recently ethyl acetate fraction of its fruit has been proved to have some activities towards endometriosis and breast cancer [10–12]. Further study is needed to identify the chemical compounds content from *P. macrocarpa* fruit so that the bioactive compound could be recognized.

As the identification of chemical compounds from *P. macrocarpa* has become necessary, the present study was focused on enhancing the chemical compound database. Nine compounds were isolated from the ethanolic extract of *P. macrocarpa* fruit. Five of them are known compounds which are β-sitosterol (3), 2,4′,6-trihydroxy-4-methoxy-6″-acetyl-benzophenone-2-*O*-β-D-glucoside (4), 2,4′,6-trihydroxy-4-methoxybenzophenone-2-*O*-β-D-glucoside (5), 2,4′,6-trihydroxy-4-methoxybenzophenone (6), coumarin (8), and 2,3-dihydroxy-benzoic acid (9). Three other compounds are firstly isolated from *P. macrocarpa* fruit. Compound 1, which is glyceryl pentacosanoate, is a new compound. Other compounds, which are 1,7-dihydroxy-3,6-dimethoxyxanthone (2) and 1,6,7-trihydroxy-3-methoxyxanthone (7), have been isolated before from *Calophyllum inophyllum* and *Athyrium mesosorum* leaves respectively [19]. However, they are firstly reported to be isolated from *P. macrocarpa* fruit.

Xanthones and benzophenones groups are commonly found in plants. They have been evaluated for the anticancer activity. Benzophenone derivatives, structurally related to combretastatin, demonstrated excellent cytotoxic activities against a panel of human cancer cell lines including multi-drug resistant cell lines [20]. On the other hand, α-mangostin is the most widespread studied and exhibits the highest activity against breast cancer, human leukemia, lung cancer, pheochromocytoma, and colorectal carcinoma [21]. In light of these findings, isolated compounds from *P. macrocarpa* fruit are suggested to be further tested so that the activity would be recognized.

Nine compounds have been isolated and identified from *P. macrocarpa* fruit extract. One of them was identified as glyceryl pentacosanoate, which is a newly isolated compound. Also, two xanthones, which are 1,7-dihydroxy-3,6-dimethoxyxanthone and 1,6,7-trihydroxy-3-methoxyxanthone, are firstly reported to be isolated from *P. macrocarpa*.

Conflict of interest statement

We declare that we have no conflict of interest.

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