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Antimalarial and Antioxidant Activities of Isoprenylated Coumarins from the Stem Bark of *Mesua borneensis* L.

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Abstract: The aim of this study was to assess the antimalarial and antioxidant effects of the *n*-hexane and ethyl acetate extracts together with three isolated isoprenylated coumarins, mammea A/BA(**1**),mammea A/AA cyclo D **(2)** and mesuol **(3)** from the stem bark of *M. borneensis* L. The *n*-hexane and ethyl acetate extracts, as well as compounds **1-3** were evaluated for their antimalarial activity against *Plasmodium falciparum* strain 3D7 (chloroquine-sensitive) and their antioxidant activity against DPPH radical scavenging. Compound **2** exhibited slightly more active than chloroquine. Compounds **1** and **3** showed very high activity against DPPH radical.

Key words: Isoprenylated coumarin, *Mesua borneensis* L., Antimalarial, Antioxidant.

Introduction

Malaria is a major cause of death in the word caused by a protozoan of the genus *Plasmodium* and transmitted by *Anopheles* mosquito vectors, especially in tropical developing countries. This disease has been found endemic at all of region in Indonesia. Recently, chloroquine and artemisinin have been used as antimalarial drugs and showed resistance against *Plasmodium* parasites in Indonesia. *Mesua* belongs to the family Calophyllaceae. This plant has produced a number of secondary metabolites such as coumarins, flavonoids, xanthones, and terpenoids that showed biological activities as anticancer, antioxidant, antimicrobial and antimalarial [1,2,3,4]. Based on ethno-botanical survey, the aqueous decoction from the stem bark or leaves of *M. borneensis* L. has been used in the Dayak people as malaria traditional medicine. Literature survey revealed that the extracts and the isolated compounds of isoprenylated coumarins from *Mesua borneensis* L. have not yet reported for their antimalarial and antioxidant activities. In continuation of our phytochemical work of Indonesian tropical plants aiming to find new antimalarial and antioxidant activities from *M. borneensis* L., this study focused on the structure-activity relationship of the antimalarial toward *Plasmodium palcifarum* strain 3D7 (chloroquine-sensitive) and antioxidant effects toward DPPH radical from the *n*-hexane and ethyl acetate extracts, mammea A/BA (**1**), mammea A/AA cyclo D **(2)** and mesuol **(3)** from the stem bark of *M. borneensis* L.

Materials and methods

Plants material

The stem bark of *M. borneensis* L. were collected in August 2014 from the conserved forest of Bukit Bangkirai, Semboja, East Kalimantan, Indonesia. The plant was identified at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia.

Extraction and isolation

The dried powder of stem barks of *M. borneensis* L. (3.0 kg) were macerated in metha-

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nol at room temperature for 24 h two times. The methanol extract was evaporated under reduced pressure to give methanol extract as dark brown residue (300 g). The methanol extract was partitioned with *n*-hexane and then evaporated by rotavapor to give *n*-hexane extract (152 g). Furthermore, the methanol extract was suspended in water (9:1) and partitioned futher with ethyl acetate and then evaporated by rotavapor to give ethyl acetate extract (32 g). The ethyl acetate extract (30 g) was separated by vacuum liquid chromatography on silica gel. Elution with *n*-hexane-ethyl acetate with increasing amount of ethyl acetate (9:1, 8:2; 7:3; 1:10 and 3:7) gave three fraction A-C. On TLC analysis, fraction A (1.2 g) showed one major spot with other several minor spots. On separation of this fraction using flash chromatography eluted with *n*-hexane-ethyl acetate mixture (19:1, 9:1; and 8:2) yielded three subfractions, A_1-A_3. Purification of subfraction A_1 by planar radial chromatography using eluent *n* hexane-acetone (from 19:1 to 9:1), yielded compound **1** (30 mg). The separation of fraction B (2.6 g) by flash chromatography eluted with *n*-hexane-diisopropylether mixture (9:1; and 8:2) yielded two subfractions, B_1-B_2. The subfraction B_1 (250 mg), purified using planar radial chromatography eluted with *n*-hexane-diisopropyl ether mixture (9:1; and 8:2) to yielded compound **2** (16 mg). Using the same method, separation of fraction C (3.8 g) with flash chromatography eluted with chloroform and chloroform-methanol 9:1 gave one major spot and then purified further by planar radial chromatography using the same eluent afforded compound **3** (12 mg).

Mammea A/BA (**1**), yellow solid, m.p. 126-127°C. UV/Vis (MeOH) λ_{maks} (nm) (log ε): 234 (3.94), 297 (3.90), and 334 (3.95). ^{1}H-NMR ($CDCl_3$) δ_H ppm: 14.61 (1H, s, 7-OH), 7.55 (3H, m, H-3'/4'/5'), 7.42 (2H, m, H-2'/6'), 5.99 (1H, *s*, H-3), 5.98 (1H, *s*, 5-OH), 5.09 (1H, t, *J* = 6.8 Hz, H-2''), 3.29 (2H, d, *J* = 6.9 Hz, H-1''), 3.19 (2H, d, *J* = 6.7 Hz, H-2'''), 2.32 (1H, m, H-3'''), 1.70 (3H, s, H-4''), 1.65 (3H, s, H-5''), 1.06 (6H, d, *J* = 6.6 Hz, H-4'''/5'''). ^{13}C-NMR ($CDCl_3$) δ_C ppm: 206.1 (C-1'''), 166.8 (C-7), 158.6 (C-2), 157.1 (C-5), 155.9 (C-8a), 154.0 (C-4), 136.8 (C-1'), 134.1 (C-3''), 130.2 (C-4'), 129.5 (C-3'/5'), 127.5 (C-2'/6'), 120.8 (C-2''), 112.5 (C-6), 112.2 (C-3), 104.5 (C-8), 100.4 (C-4a), 53.6 (C-2'''), 25.7 (C-4''), 25.6 (C-3'''), 22.7 (C-4'''/5'''), 21.6 (C-1''), 17.9 (C-5''). HR-ESI-MS: *m/z* $[M+H]^+$ calcd. for $C_{25}H_{27}O_5$ 407.1858, found 407.1865. The structure of **1** was elucidated from their physical properties and by spectroscopic methods as well as comparison with previous literature data [5].

Mammea A/AA cyclo D (**2**), yellow solid, m.p. 144-146°C. UV/Vis (MeOH) λ_{maks} (nm) (log ε): 230 (4.24), 285 (4.38), and 335 (3.94). ^{1}H-NMR ($CDCl_3$) δ_H ppm: 14.80 (1H, s, 7-OH), 7.40 (3H, m, H-3'/4'/5'), 7.31 (2H, m, H-2'/6'), 6.89 (1H, d, *J* = 10.2 Hz, 1'''), 5.99 (1H, s, H-3), 5.62 (1H, d, *J* = 10.2 Hz, 2'''), 2.96 (2H, d, *J* = 6.8 Hz, H-2''), 2.23 (1H, m, H-3''), 1.57 (6H, s, H-4'''/5'''), 0.96 (6H, d, *J* = 6.7 Hz, H-4''/5''). ^{13}C-NMR ($CDCl_3$) δ_C ppm: 206.1 (C-1''), 164.4 (C-5), 159.6 (C-2), 157.4 (C-8a), 156.4 (C-4), 154.8 (C-7),

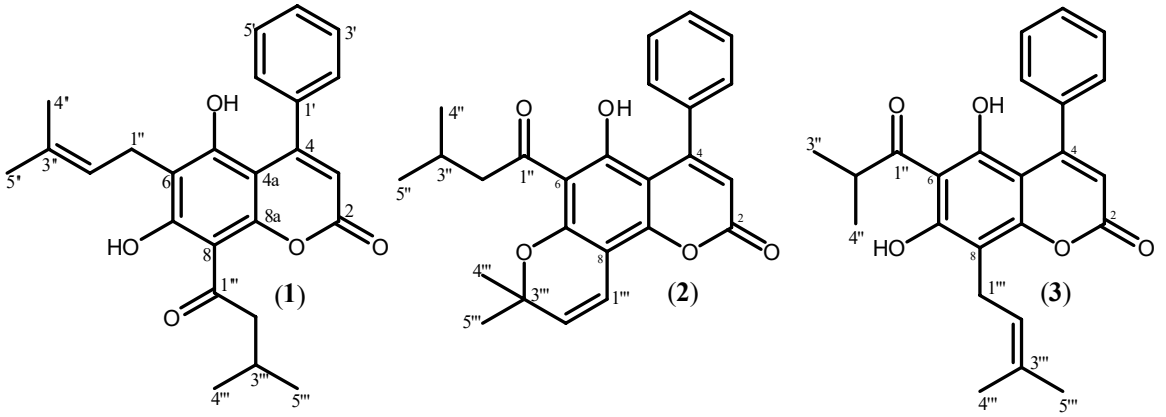

Figure 1. Structures of isoprenylated coumarin *Mesua*

139.2 (C-1'), 128.2 (C-4'), 127.6 (C-3'/5'), 127.2 (C-2'/6'), 126.3 (C-2'''), 115.5 (C-1'''), 112.7 (C-3), 107.2 (C-6), 102.4 (C-4a), 101.5 (C-8), 79.9 (C-3'''), 53.6 (C-2''), 28.3 (C-4'''/5'''), 25.1 (C-3''), 22.7 (C-4''/5''). HR-ESI-MS: *m/z* $[M+H]^+$ calcd. for $C_{25}H_{25}O_5$ 405.1736, found 405.11748. The physical properties and spectra data of **2** was compare with literature data [5].

Mesuol (**3**), yellow solid, m.p. 154-156°C. UV/Vis (MeOH) λ_{maks} (nm) (log ε): 206 (4.68), and 303 (4.16). ^{1}H-NMR ($CDCl_3$) δ_H ppm: 14.48 (1H, s, 7-OH), 7.40 (3H, m, H-3'/4'/5'), 7.30 (2H, m, H-2'/6'), 5.93 (1H, s, H-3), 4.91 (1H, t, *J* = 6.8 Hz, 2'''), 3.31 (2H, d, *J* = 7.2 Hz, 1'''), 2.90 (1H, m, H-2''), 1.43 (3H, s, H-4'''), 1.31 (3H, s, H-5'''), 0.97 (3H, d, *J* = 6.7, H-3''), 0.96 (3H, d, *J* = 6.7, H-4''). ^{13}C-NMR ($CDCl_3$) δ_C ppm: 205.1 (C-1''), 164.4 (C-5), 164.1 (C-7), 159.8 (C-2), 156.6 (C-4), 155.6 (C-8a), 139.0 (C-1'), 134.8 (C-3'''), 128.3 (C-4'), 127.6 (C-3'/5'), 127.2 (C-2'/6'), 112.8 (C-2'''), 112.1 (C-3), 105.1 (C-8), 103.3 (C-6), 102.4 (C-4a), 52.0 (C-2''), 25.9 (C-4'''), 26.7 (C-1'''), 24.4 (C-5'''), 22.6 (C-3''/4''). HR-ESI-MS: *m/z* $[M+H]^+$ calcd. for $C_{24}H_{25}O_5$ 393.1630, found 393.1635. The structure of **3** was elucidated from their physical properties and by spectroscopic methods as well as comparison with previous literature data [1].

Parasite cultivation

Plasmodium palcifarum strain 3D7 (chloroquine-sensitive) was obtained from the culture collection of the Institute of Tropical Diseases, Airlangga University, Surabaya, Indonesia. *P. palcifarum* are cultivated in human O Rh^+ red blood cells using RPMI 1640 medium with O Rh^+ serum (10 %), 5 % sodium bicarbonate and 40 µg/mL of gentamysin sulphate. Hematocrits were adjust at 5 % and parasite cultures were used when they exhibited 2 % parasitaemia [6,7].

***In vitro* antimalarial activity**

Antimalarial assay of the *n*-hexane extract, ethyl acetate extract, and compounds **1-3** in comparison with that of the control drug chloroquine against *Plasmodium palcifarum* strain 3D7 (chloroquine-sensitive) were carried out according to a modified method of Trager and Jensen. Fresh red blood cells were used as an negative control. The *n*-hexane extract, ethyl acetate extract, compounds **1**, **2** and **3** at various concentration (200, 100, 50, 10, 1 and 0,1 µg/mL) were incorporated in 96 well tissue culture plates containing 200 µL of *P. palcifarum* culture with fresh blood cells diluted to 2 % hematocrit [6,7]. Parasitaemia was evaluated after 48 by Giemsa stain and the average percentage suppression of parasitaemia was calculated by following equation:

% average suppression of parasitaemia = 100 x

(% average parasitaemia in control - % average parasitaemia in active compound/ % average parasitaemia in control).

Antiplasmodial activity calculation

The antiplasmodial activity of the *n*-hexane extract, ethyl acetate extract, and compounds **1-3** were expressed by the the 50 % inhibitory concentrations (IC_{50}), representing the concentration of chloroquine that induced a 50 % parasitaemia decrease compared to the positive control culture referred as 100 % parasitaemia.

DPPH scavenging activity assay

Determination of the antioxidant activity of the *n*-hexane extract, ethyl acetate extract, as well as compounds **1-3** performed using reagent DPPH (2,2-diphenyl-1-pikrihidrazil) using methods of reduction of free radicals as measured by UV spectrometer at λ 517 nm [8,9]. Determination of antioxidant activity was done by dissolving a compounds assay with methanol, then added solution of 0.1 M buffer acetate (pH 5.5) and added DPPH radical solution of 5.10^{-4} M. Determination of the inhibition of isolated compounds against DPPH radical was observed using a spectrometer at λ 517 nm after incubation for 30 min at 20°C. Samples were dissolved in ethanol at various concentrations (4000, 2000, 1000, 500, 100, 10 and 1 µg/mL). The inhibition percentage (%) of radical scavenging activity was calculated using the following equation:

$$\text{Inhibition (\%)} = (A_o - A_s/A_{o)} \times 100$$

Where A_o is the absorbance of the control reaction (containing all reagents except the test com-

pound), and A_s is the absorbance of the test compound. The inhibitory concentration (IC_{50}) of the samples were calculated using a regression linear from the graph plotting scavenging activity against concentration. Assays were carried out in triplicate.

Determination of melting point

Determination of melting point using Fisher-Johns melting point apparatus. The melting point of the solid compound was observed through the lens and record the temperatures at which the compound begins melting until all of disappear [10].

Results and discussion

In continuation of our phytochemical work of Indonesian tropical plants aiming to find new antiplasmodial and antioxidant compounds from the stem bark of *M. borneensis* L. In this paper, we report antiplasmodial and antioxidant activities of the *n*-hexane and ethyl acetate extracts, as well as three isolated compounds, mammea A/BA (**1**), mammea A/AA cyclo D (**2**) and mesuol (**3**).

The *n*-hexane and ethyl acetate extracts from the stem bark of *M. borneensis* L.were tested for *in vitro* inhibitory effects against *Plasmodium falciparum* strain 3D7 (chloroquine-sensitive) by Tragger and Jensen methods showing their IC_{50} were 23.56 and > 100 µg/mL, respectively. The results of ethyl acetate extract showed moderate activity and *n*-hexane extract was inactive.

The antimalarial evaluation of compounds **1-3** was carried out against *Plasmodium falciparum* strain 3D7 (chloroquine-sensitive) showing their IC_{50} were 3.72; 1.02 and 8.81 µg/mL, respectively of parasitaemia inhibition. Chloroquine was used as positive controlin the entire assay, IC_{50} 1.02 µg/mL). Based on the result of the extracts and the pure compounds on antimalarial evaluation, it indicated that the antimalarial activity of the pure compounds is higher compared to the exctracts. These antimalarial data suggested that the compound **2** showed very high activity, compounds **1** and **3** against *Plasmodium falciparum* have moderate activity [11]. The results indicate that compound **2** slightly more active than chloroquine. The structure-activity relationship of the antimalarial of compounds **1-3** showed typical of 4-phenylcoumarin derivatives with variation substitutent groups on their skeleton such as 3-methyl-2-butenyl, 3-methylbutanoil, 2-methylpropanoil, and 2,2-dimethylpyrano at C-7 and C-8. The presence of 3-methylbutanoil at C-6 and the fusion 2,2-dimethylpyrano ring fused at C-7 and C-8 of compound **2** increased antimalarial activity. The presence of pyrano ring seems play a role on antimalarial activity, since the presence of pyrano ring makes the structure becomes more planar. However, the presence of 3-methyl-2-butenyl at C-6 and 3-methylbutanoil at C-8 of compound **1** showed slightly higher activity than 2-methylpropanoil at C-6 and 3-methyl-2-butenyl at C-8 of compound **3**. This showed that the posistion of substituent with carbonyl group on C-8 increased the antimalarial activity compared to the position on C-6. The result of *n*-hexane extract, ethyl acetate extract and compounds **1-3** shown in Table 1.

DPPH radical is a paramagnetic and unstable radical that able to accept an electron or hydrogen radical to become a stable diamagnetic mol-

Table 1. Antimalarial and antioxidant activities of extract and compounds 1-3

Compound	Antimalarial (µg/mL)	DPPH (µg/mL)
n-Hexane extract	> 100	3575.25
Ethyl acetate extract	23.56	1058.35
Mammea A/AB (**1**)	3.72	60.26
Mammea A/AA cyclo D (**2**)	1.02	124.31
Mesuol (**3**)	8.81	71.63
Chloroquine	1.03	-
Ascorbic acid	-	57.87

ecule. Classification of a highly active compound if in small concentrations can change the color DPPH from purple to yellow. The scavenging capacity of the *n*-hexane extract, ethyl acetate extract, and compounds **1-3** were evaluated for their antioxidant properties against DPPH radical by using a spectrophotometric assay based on the ability of extract or pure compound to decrease DPPH oxidation. The ethyl acetate extract and *n*-hexane extract on evaluation for antioxidant activity against DPPH radical scavenging showing their IC_{50} were 1058.35 and 3575.25, respectively. The results of ethyl acetate extract showed more active than *n*-hexane extract and suggested that the ethyl acetate extract contained high phenolic compound. The result of compounds **1-3** with ascorbic acid as a positive control (Table 1) exhibited DPPH radical scavenging its IC_{50} value of 60.26; 124.31; 71.63 and 57.87 µg/mL, respectively. The results of radical DPPH scavenging assay from compounds **1** and **3** showed very high activities and compound **2** moderate activity [9]. The results indicate that ascobic acid as positive control slightly more active than compounds **1** and **3**. Compounds **1** and **3** have two hydroxyl groups at C-5 and C-7, while mammea A/AA cyclo D have an hydroxyl group at C-5. Based on number of hydroxyl group, the ability of hydrogen donor from compounds **1** and **3** to DPPH radical cause the delocalization and stabilization resonance effect of the phenolic compound. In contrast with the antimalarial activity, the influence of 3-methyl-2-butenyl, 3-methylbutanoil, 2-methylpropanoil, and 2,2-dimethylpyrano showed no significant toward antioxidant properties.

Conclusion

Three isoprenylated coumarins, mammea A/BA (**1**), mammea A/AA cyclo D (**2**), mesuol (**3**), the *n*-hexane extract and ethyl acetate extract were evaluated antimalarial and antioxidant activities from the stem bark of *Mesua borneensis* L. The antimalarial assay of mammea A/AA cyclo D (**2**) against *P. falciparum* showed high activity and slightly more active than chloroquine. Therefore, potential of mammea A/AA cyclo D (**2**) will be continued to *in vivo* antimalarial and cytotoxicity assay for development of antimalarial drug. The potential of three coumarins by others antioxidant test will be evaluated further to know the influence of hydroxyl, 3-metil-2-butenil , 3-methylbutanoil , 2-methylpropanoil , and the 2,2-dimethylpyrano toward structure-activity relationship, so that it could be used as new antioxidant in health or food.

Conflict of interest

The authors declare no conflict of interest.

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