

# The anticancer, antimalarial, and antibacterial activities of moracalkon A isolated from *Artocarpus kemando* Miq

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## ABSTRACT

*Artocarpus kemando* Miq. (pudau) is a species of plant from the genus *Artocarpus*, family Moraceae. Some compounds have previously been isolated from the genus *Artocarpus* and they showed some bioactivities as antimicrobial, anticancer, antifungal, antiplatelet, anti-inflammatory, antioxidant, antidiabetic and cytotoxic properties. This study aims to isolate and characterize the flavonoid compounds in the root wood of the pudau plant (*A. kemando* Miq.) which was obtained from Karang Anyar, Klaten, Penengahan, South Lampung, Indonesia. A chalcone compound namely moracalkon A (1) is successfully isolated from the root wood of the pudau plant, *A. kemando* Miq.. Compound 1 was obtained as a yellow solid with a melting point range of 122-125°C. It was well characterized with spectroscopy techniques of UV-Vis, IR, and NMR, and its anticancer activity was tested using murine leukemia cells P-388 and IC<sub>50</sub> value was 1.54 g/mL. The antimalarial activity test against *Plasmodium falciparum* gave IC<sub>50</sub> value of 0.08 g/mL and it is categorized as a very active. Furthermore, the antibacterial activity test towards *Bacillus subtilis* and *Escherichia coli* showed that compound 1 was strong.

**Keywords:** *A. kemando*, Antikanker, Antimalaria, Moracalkon A

## Introduction

The isolation and activity tests of flavonoid compounds from *Artocarpus* plants are interesting to study objects. This study showed that some flavonoids isolated from this plant have the potential for many biological tests, such as anticancer [1-5], antioxidants [6], anthelmintic drug [7], antimalarial [8-11], antidiabetic [12] and antibacterial [3]. *Artocarpus* plants could be divided into those that produce edible fruit and those that do not. These two plant types mostly produce flavone derivatives, flavonoids.

Chalcone compounds are flavonoids isolated from *Artocarpus* and are fewer than flavones. They include canzonol C and

artoindonesianin J from *A. bracteata* [13], AC-3-2, AC-5-1, and AC-3-1 from *A. communis* [14], and moracalkon A from *A. champeden* [10]. Of these chalcone compounds, the only tested antimalarial was moracalkon A [10, 15].

*A. kemando* is a rare endemic plant in Indonesia that produces inedible fruit. For the first time, moracalkone A has been isolated from the root wood of *A. kemando*. Antimalarial, anticancer, and antibacterial activity tests show that moracalkone A is highly active. The antimalarial test of compound 1 strengthens the results obtained by Hafid *et al.* [10] and Nindatu *et al.* [15].

The bark of the *A. kemando* Miq plant has been isolated and identified by previous studies. Therefore, this study examines the root wood of *A. kemando* Miq. to determine its content of flavonoid compounds and test its bioactivity. The sample used was the root wood of the pudau plant (*A. kemando* Miq.) which grows in Karang Anyar, Klaten Village, Penengahan District, South Lampung, Lampung Province.

## Materials and Methods

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## Instrumentation

The instruments used in this study include glassware, rotary vacuum evaporator, gravity column chromatography (KKG), vacuum liquid chromatography (VLC), thin-layer chromatography (TLC), UV lamp, capillary pipette, and point meter. Other instruments used are MP-10 Stuart melt, analytical balance, autoclave, Laminar Air Flow (LAF), loop needle, petri dish, incubator, Bunsen, and micropipette. Also, this study used Shimadzu Prestige 21 FT-IR spectroscopy and Agilent Technologies Cary Series UV-Vis brand of ultraviolet-visible spectroscopy spectrophotometer Cary 100 UV-Vis. NMR

## Materials

Pudau root wood (*A. kemando* Miq.) was obtained from Karang Anyar, Klaten Village, Penengahan District, South Lampung. The sample plant species were determined at the Herbarium Bogoriense for Botany at the Indonesian Institute of Sciences (LIPI) Biology Research Center, Cibinong, West Java.

## Isolation and purification of the compound

Maceration was performed three times on 2.4 kg fine powder of root wood with 11 L methanol solvent for 1x24 hours. Using a rotary evaporator, the maceration results were evaporated and 90.7 grams of the extract was obtained. The extract was fractionated by the Vacuum Liquid Chromatography (VLC) method, with ethyl acetate, eluent n-hexane, and adsorbent silica gel with variations in an increase of polarity. The fractionation obtained five main fractions from A to E with 0.8415 grams of A, 1.1750 grams of B, 1.5500 grams of C, 0.8700 grams of D, and 0.4800 grams of E. Column chromatography was performed on B Fraction 1.1750 grams repeatedly using silica gel adsorbents and variations in ethyl acetate and n-hexane, DCM ethylacetate acetone, and acetone and n-hexane. The test obtained 15 mg of compound 1, a yellow amorphous solid, with a melting point of

122 - 125°C. Also, one stain is obtained in TLC compound 1 with three eluent systems, including n-hexane/acetone 70%, ethyl acetate/n-hexane 50%, and ethyl acetate/dichloromethane 40%.

## Results and Discussion

### Spectrometry analysis

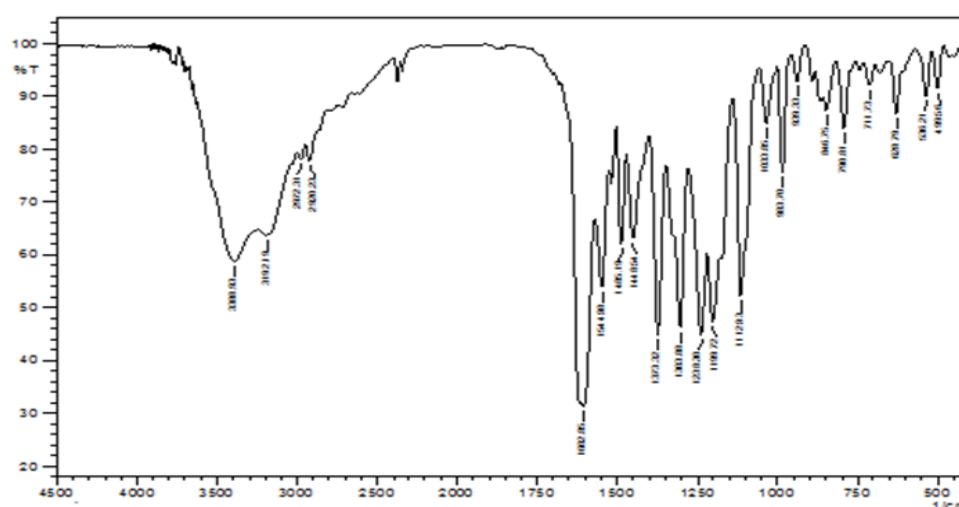
#### Analysis of spectrometry UV-Vis

The UV spectrum of Compound 1 showed maximum absorptions at  $\lambda_{\max}$  386 nm (band I) and 212 nm (band II), characteristic of chalcone-type flavonoid compounds [16].

There was a bathochromic shift in the band I from  $\lambda_{\max}$  386 nm to 449 nm on adding NaOH reagent or 63 nm, indicating the presence of an OH group at C-4. However, there was no bathochromic shift on adding other reagents, such as NaOAc (sodium acetate), NaOAc + H<sub>3</sub>BO<sub>3</sub>, AlCl<sub>3</sub>, and AlCl<sub>3</sub> + HCl. This indicates the absence of free OH at C 4', OH group, or the presence of a free OH group adjacent to the prenyl group.

#### Infrared spectrometry analysis

The infrared spectrum analysis of compound 1 in **Figure 1** showed a wide band in the wavenumber region of 3388 cm<sup>-1</sup>. This is the stretching vibration of the hydroxyl group strengthened by vibrations at wavenumbers of 1238, 1199, and 1112 cm<sup>-1</sup>, indicating the presence of the CO group. Similarly, aromatic C-H vibrations are indicated by the absorption at a wavenumber of 3192 cm<sup>-1</sup>. The absorption peaks in the 2972 and 2920 cm<sup>-1</sup> regions indicate the aliphatic C-H group derived from the isoprenyl group. Furthermore, the wavenumber of 1602 cm<sup>-1</sup> indicates the presence of a carbonyl group (C=O) conjugated with C=C. The absorption peaks at 1544, 1485, and 1448 cm<sup>-1</sup> indicated the presence of aromatic C=C vibrations.



**Figure 1.** IR Spectrum of the compound 1

## Nuclear magnetic resonance spectrometry (NMR) analysis

The <sup>1</sup>H-NMR spectrum of compound **1** shows the presence of a prenyl group as  $\gamma,\gamma$ -dimethylallyl, 1.63 s; 1.77 s, 5.26 m, and 3.39 ppm (d, J = 7.25 Hz). The presence of two adjacent aromatic protons occurs at 7.88 (d, J = 8.6 Hz) and 6.52 ppm (d, J = 9 Hz). Additionally, there are three aromatic protons with the ABC system at 7.66 (d, J = 8.6 Hz); 6.45 (dd, J = 2.5 and 8.5 Hz); and 6.50 ppm (d, J = 2.3 Hz).

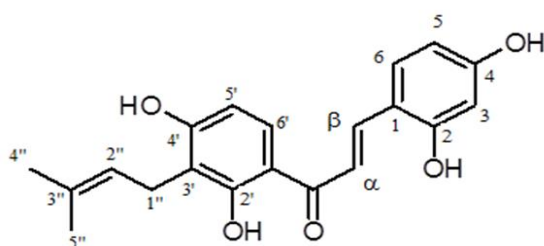
The presence of trans protons in alkenes is indicated by 7.78 (d, J = 15.4 Hz) and 8.20 ppm (dd, J = 15.4 Hz). This <sup>1</sup>H-NMR spectrum analysis supports UV spectrum data, which shows that compound (**1**) is chalcone. The chalcone compound estimation is

supported by the <sup>13</sup>C-NMR spectrum analysis. It shows 20 carbon atoms, comprising one carbonyl side by side, with two alkene carbons at 192.50, 116.70, and 140.00 ppm. Also, there is one tri-substituted aromatic ring at 113.6, 129, 106.9, 164.2, 115, and 161.5 ppm. One of the substituents in this aromatic is an alkyl of isoprenyl at 21.4, 122.4, 130.5, 17, and 24.9 ppm. Moreover, one aromatic ring is substituted at 114, 159, 102.6, 163.8, 108, and 131 ppm. The value of the chemical shear and the bond between the C and H atoms in compound **1** was confirmed by HMQC and HMBC spectral analyses.

The one and two-dimensional analysis of UV, IR, and NMR spectra showed that compound **1** and morachalcone A had similar structures, as shown in **Figure 2**. **Table 1** shows the comparison of data for <sup>1</sup>H-NMR and <sup>13</sup>C-NMR compounds **1** with the literature.

**Table 1. Comparison of NMR data for compound 1 and Moracalkon A [10]**

Carbon number	Chemical Shift of Moracalkon A (ppm) in CD <sub>3</sub> OD		The chemical shift of compound 1, ppm (CD <sub>3</sub> ) <sub>2</sub> CO	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
$\alpha$	7.73 dd. (15.25 Hz)	117.93	7.78 (1H. dd. J = 2.3; 15.4 Hz)	116.72
$\beta$	8.1 dd (15.9 Hz)	142.15	8.20 (1H. dd. J = 2.3; 15.4 Hz)	139.91
C=O	-	194.25	-	192.56
1'	-	114.68	-	113.60
2'	-	163.50	-	161.36
3'	-	116.62	-	115.13
4'	-	165.32	-	164.20
5'	6.43 q (8.55. 17.75 Hz)	108.26	6.52 (1H. dd. J = 1.8; 9 Hz)	106.93
6'	7.76 d (8.55 Hz)	130.47	7.88 (1H. d. J = 8.6 Hz)	129.01
1	-	115.74	-	114.32
2	-	160.85	-	159.03
3	6.36 m	103.69	6.50 (1H. d. J = 2.3 Hz)	102.65
4	-	162.77	-	163.75
5	6.36 m	109.23	6.45 (1H. dd. J = 2.5; 8.5 Hz)	108.18
6	7.52 dd (8.55. 3.7 Hz)	132.56	7.66 (1H. d. J = 8.6 Hz)	130.83
CH <sub>2</sub> , 1''	3.34	22.60	3.34 (2H. d. J = 7.25 Hz)	21.42
CH=, 2''	5.22	123.70	5.26 (1H. m)	122.50
C=	-	131.59	-	130.49
E-Me, 4''	1.65 s	26.09	1.63 (3H. s)	24.98
Z-Me, 5''	1.77 s	18.04	1.77 (3H. s)	17.02



**Figure 2.** Molecular structure of compound (1), Moracalkon A

## bioactivity test

Compound **1** was tested for cytotoxicity against murine leukemia cells P-388, for its bioactivity against *P. falciparum*, *B. subtilis*, and

*E. coli*. The results showed that compound **1** was highly active in cytotoxicity, with IC<sub>50</sub> values of 0.08 g/mL. Also, the antimalarial test results indicated that compound **1** gave IC<sub>50</sub> 1.54 g/mL, categorized as very active, while the antibacterial activities were very strong. Compound **1** is highly active due to four phenolic groups [17] and one free prenyl group in the molecule. Flavonoid compounds with free phenolic and prenyl groups are highly active, potentially being used as anticancer, antimalarial, and antibacterials.

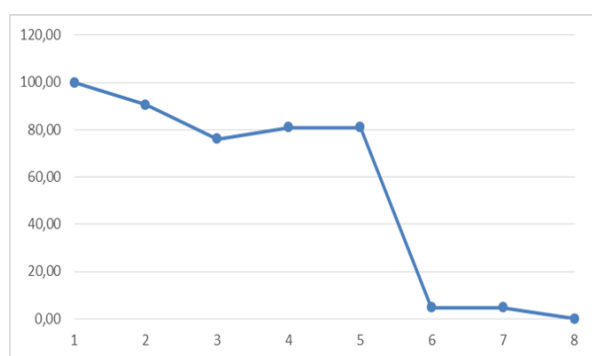
## Antimalarial activity

The in vitro antimalarial assays were performed in the Laboratory of Malaria, Eijkman Institute for Molecular Biology, Jakarta. The malaria parasite *P. falciparum* 3D7 clone was propagated based on

the previously published procedure [18]. Furthermore, the in vitro antimalarial assay was performed following the previous procedures used in the literature [19-21], as shown in **Table 2** and **Figure 3**. The calculation of IC<sub>50</sub> of compound **1** was 0.08 µg/mL.

**Table 2. In vitro antimalarial assay of compound 1**

Compound 1		Plate 1	Plate 2	Average	Growth Rate (%)
1	Untreated	11	10	10.5	100.00
2	10 <sup>-7</sup>	10	9	9.5	90.48
3	10 <sup>-6</sup>	7	9	8	76.19
4	10 <sup>-5</sup>	9	8	8.5	80.95
5	10 <sup>-4</sup>	9	8	8.5	80.95
6	10 <sup>-3</sup>	1	0	0.5	4.76
7	10 <sup>-2</sup>	1	0	0.5	4.76
8	10 <sup>-1</sup>	0	0	0	0.00



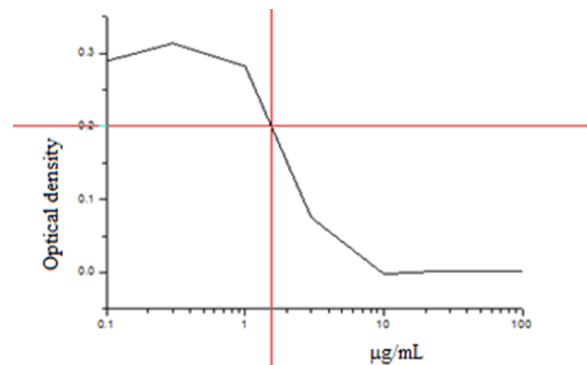
**Figure 3. In vitro antimalarial assay of compound 1**

### Anticancer activity test

A cytotoxicity test of compound **1** was performed using the method adopted from Alley *et al.* [22]. It was conducted at the Laboratory of Natural Product Chemistry, Department of Chemistry, Bandung Institute of Technology, Indonesia. Using murine leukemia cancer cells P-388, the cytotoxicity test revealed that compound **1** was active, with an IC<sub>50</sub> 1.54 µg/mL. Also, IC<sub>50</sub> was determined from the data in **Table 3** using Origin 8.5 software, as shown in **Figure 4**.

**Table 3. The data of murine leukemia cancer cells P-388 test of compound 1**

ppm	The optical density of compound 1			The average optical density of compound 1 Average
	1	2	3	
100	0.003	0.001	0	0.001333
30	0.003	0.004	0.001	0.002667
10	0	-0.002	-0.004	-0.002
3	0.14	0.025	0.06	0.075
1	0.393	0.296	0.158	0.282333
0.3	0.383	0.293	0.265	0.313667
0.1	0.302	0.337	0.229	0.289333



**Figure 4. The graph of cytotoxicity test of compound 1 using leukemia cells P-388**

### Antibacterial activity

Using the paper disc method, the antibacterial activity test was performed. The results showed that compound **1** has an antibacterial activity towards the growth of *B. subtilis* by forming an inhibition zone at concentrations of 0.3, 0.4, and 0.5 mg/disc, with diameters of 14, 18, and 15 mm, respectively. Furthermore, amoxicillin used as control positive formed an inhibition zone at concentrations of 0.05, 0.10, and 0.15 mg/disc, with a diameter of 25, 26, and 25 mm, respectively. Similarly, *E. coli* formed an inhibition zone at concentrations of 0.3, 0.4, and 0.5 mg/disc, with diameters of 11, 11, and 10 mm, respectively. Additionally, the chloramphenicol used as control positive formed an inhibition zone at concentrations of 0.05, 0.10, and 0.15 mg/disc, with diameters of 26, 27, and 28 mm, respectively. According to Davis and Stout [23], when the inhibition zone diameter is 5 mm or less, it is categorized as weak antibacterial activity. Similarly, 5-10 mm is categorized as a medium, 10-19 is strong, while 20 mm or more is categorized as a very strong activity. These results show that compound **1** is categorized as strong in the antibacterial activity test and its activity is comparable to some organotin (IV) compounds [24-26] or even stronger.

### Conclusion

From the root wood of *A. kemando* Miq. (a pudau plant), 15 mg of moracalkon A was isolated as a yellow amorphous solid with a melting point of 122-125°C. Moracalkon A compound was isolated in *A. kemando* for the first time. It is highly active in antimalarial tests using *P. falciparum* with IC<sub>50</sub> of 0.08 g/mL, anticancer using P-388 leukemia cells with IC<sub>50</sub> 1.54 g/mL. Also, its antibacterial activity against *B. subtilis* and *E. coli* is categorized as strong.

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**Conflict of interest:** None

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**Ethics statement:** None

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