Identification and antibacterial activity of flavonoid compounds from wood branches of the pudau plant (Artocarpus kemando Miq.)

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Identification and antibacterial activity of flavonoid compounds from wood branches of the pudau plant (*Artocarpus kemando* Miq.)

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Abstract. In this research, isolation, identification, and antibacterial assay of flavonoid compounds have been carried out. These compounds were obtained from the wood branches of the pudau endemic plant from Karang Anyar village, Penengahan, South Lampung Regency, Lampung Province. Flavonoid compound extraction was carried out by maceration method using methanol, while fractionation and purification were conducted using chromatography technique. The purity of the compounds was determined by the melting point test and thin layer chromatography, the identification of compounds was assigned by UV-Vis and IR spectroscopy. The results of the isolation obtained two compounds, artocarpin (1) and cycloartocarpin (2). Compound (1), 92 mg, in the form of a yellow needle crystal with a melting point of 184.9 -187.5 °C; compound (2), 20 mg, in the form of yellowish white needle crystals with melting point 280 - 283 °C. In the antibacterial assay against *Bacillus subtilis* and *Escherichia coli*, compounds (1) showed very strong antibacterial activity at a concentration of 0.4 mg/disk and 0.5 mg/disk, respectively, whereas compound (2) had strong antibacterial activity against *B. subtilis* and *E. coli* at the same concentration of 0.5 mg/disk.

Key words: *Artocarpus kemando* Miq, flavonoids, artocarpin, cycloartocarpin, *B. subtilis*, *E. coli*.

1. Introduction

According to the Indonesian health ministry, diarrheal disease is still a public health problem in developing countries such as Indonesia, also can be a major cause of death in humans, especially in children and infants [1]. Sources of this disease can be derived parasites, viruses and bacteria. The bacteria that give the second largest diarrheal disease are mainly *Escherichia coli* [2], besides that, also *Bacillus subtilis*. These bacteria can damage canned food that can cause gastroenteritis in humans who consume them [3].

Many people used natural ingredients as traditional medicine and modern medicine to treat diarrhea. Utilization of natural materials such as traditional medicinal plants, conventionally has been widely carried out by the community, in 1985 it was predicted that more than 80% of the world's population use plants as medicinal ingredients to maintain their primary health [4] and to treat various diseases caused by microorganisms such as bacteria and viruses [5]. Plants that are commonly used, are plants that contain many flavonoid compounds.

Flavonoids are secondary metabolites found in green plants, and algae [6]. In general, flavonoids are found in all parts of the plant including leaves, roots, wood, bark, flowers, fruit, and seeds [7]. Among the plants that are widely used, which is derived from the genus Artocarpus which has been

proven to contain a lot of flavonoid compounds that have the potential as medicinal compounds [8-11].

Artocarpus kemando Miq. reportedly contains many flavonoid compounds that are effective as antibacterial drugs such as diarrheal disease. Previous studies have successfully isolated the flavonoid compounds Artonin E, Artonin O, artobiloxanton and cycloartobiloxantone from bark *A. kemando* Miq. which has cytotoxic activity against HL-60 cancer cells and IMR-32 cells [12], further research is needed regarding secondary metabolite compounds, specifically flavonoids from plants *A. kemando* Miq. on the branch wood, and the bioactivity test of the isolated compound was carried out. This plant was taken from Karang Anyar Hamlet, Klaten Village, Penengah District, South Lampung, Lampung, Indonesia.

The method of isolating flavonoid compounds was done by maceration using methanol as a solvent. The obtained methanol extract was partitioned by liquid-liquid extraction using *n*-hexane solvent and further separation was carried out by chromatography. The purity of the isolated compound was determined using Thin Layer Chromatography (TLC) and melting point tests; identification of isolated compounds, determined using UV-Vis and infrared spectrophotometry, and antibacterial bioactivity tests was carried out against *B. subtilis* and *E. coli* bacteria.

2. Experimental

2.1 General

Melting point determination using the MP-10 Stuart melting point tool, IR spectrum using the Prestige 21 Shimadzu FT-IR spectrophotometer, and UV spectrum using the UV-Vis Cary-100 UV-Vis Agilent spectrophotometer. Vacuum liquid chromatography (KCV) was carried out using Merck 60 Si-gel, and thin layer chromatography (TLC) analysis was carried out on Si-gel plates (Merck Kieselgel 60 F254, 0.25 mm).

2.2 Sample preparation

The sample used in this study was the branch of *A. kemando* Miq. obtained from Karang Anyar, Klaten village, Panengahan, South Lampung, Lampung and was identified at the Bogoriense Herbarium, Research Center for Biology, Indonesia, Bogor Institute of Sciences, Indonesia.

2.3. Extraction and isolation

Branch sawdust *A. kemando* Miq. 2.5 kg was macerated with 12 L methanol solvent for 3x24 hours, then the solvent was evaporated with a rotary evaporator and a crude extract of methanol was obtained. This extract was further partitioned by liquid-liquid extraction with *n*-hexane solvent to obtain the methanol fraction (13 g) and the hexane fraction (10 g).

The methanol fraction (13 g) was then chromatographed using a liquid liquid silica gel adsorbent and eluted with EtOAc/*n*-hexane which increased polarity from 0-100%, producing five main fractions (A-E). Fraction C (0.6 g), then was fractionated by chromatography column with silica gel adsorbent and acetone / *n*-hexane eluent with a ratio of 25:75 to obtain a yellow needle crystal (92 mg), is called KC compound (1). Whereas fraction B (0.17 g) was fractionated by column chromatography with silica gel adsorbent and acetone / *n*-hexane eluent at a ratio of 20:80% so that a yellow needle crystal (20 mg) was obtained, referred to as KB compound (2). The purity of the compound obtained was determined by measuring the melting point and a TLC chromatogram was made together with standard artocarpin using three eluent systems. Compounds (1) and (2) were characterized by UV-Vis, and IR spectroscopy.

2.4 Antibacterial bioactivity test

In the antibacterial activity test the paper disc diffusion method is used [13-15]. Antibacterial test using Nutrient Agar (NA) media. 4.2 grams of NA were dissolved in 150 mL distilled water and then heated until homogeneous. Samples (1) and (2) compounds, prepared for antibacterial testing, were made with variations in three concentrations: 0.5 mg/disk; 0.4 mg/disk and 0.3 mg/disk. Each compounds (1.5 mg) were dissolved in 150 µL methanol, then taken 50 µL; 40 µL; and 30 µL to be impregnated onto a paper disk.

In the antibacterial test against *B. subtilis* and *E. coli*, amoxycillin and chloramphenicol were used as positive control, respectively. After the media solidified, added agar media that has been mixed with distilled water contain 1 ose bacteria. Then the disc paper containing the sample, positive control and negative control is placed on the surface of the media that has been made. Petri dishes are covered with paper and plastic wrap, then put in an incubator for 1x24 hours.

3. Results and Discussion

3.1 Isolation of compounds from A. kemando Miq.

In determining the purity of compound (1), the melting point of 184.9-187.45 °C and TLC together with standard artocarpin using three eluent systems obtained the same Rf results (Figure 1). From the results of TLC it was estimated that the compound (1) was an artocarpin compound.

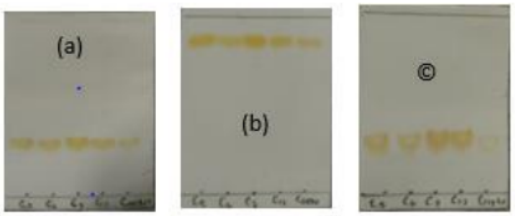

Figure 1. TLC chromatograms of compound (1) and artocarpin (standard, far right) use three eluent systems (a) acetone: *n*-hexane 20% Rf = 0.31, (b) DCM: ethyl acetate 40% Rf = 0.85, and (c) ethyl acetate: *n*-hexane 30% Rf = 0.28.

In determining the purity of compound (2), the melting point of 280-283 °C and TLC together with standard artocarpin using three eluent systems results in unequal Rf results (Figure 2). From the TLC results, it is estimated that compound (2) was not artocarpin compound.

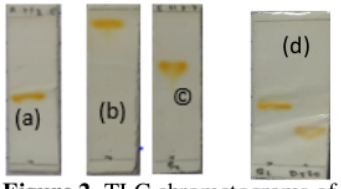

Figure 2. TLC chromatograms of compound (2) and artocarpin (standard) using three eluent systems (a) acetone: n-hexane 20% Rf = 0.45, (b) DCM: ethyl acetate 40% Rf = 0.91, (c) ethyl acetate: n-hexane 30% Rf = 0.65, and (d) compound (2) and standard artocarpin (right)

3.2 Spectroscopic analysis

The UV spectrum of the compounds (1) in the MeOH solvent, at λ_{max}, nm (log ε): 321 (2.97) and 278 (3.45), and (2), at λ_{max}, nm (log ε): 368 (3.25) and 292 (3.33), respectively, resulted in the absorption

of band I typical of flavones which came from cinnamoyl chromophore, while band II which came from benzoyl chromophore (Figure 3).

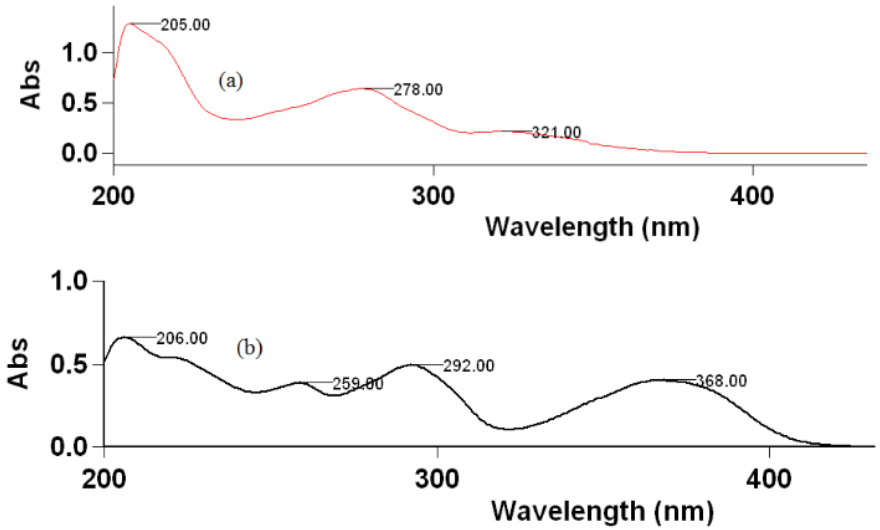

Figure 3. UV spectrum (a) compound (1) (b) compound (2) in the MeOH solvent.

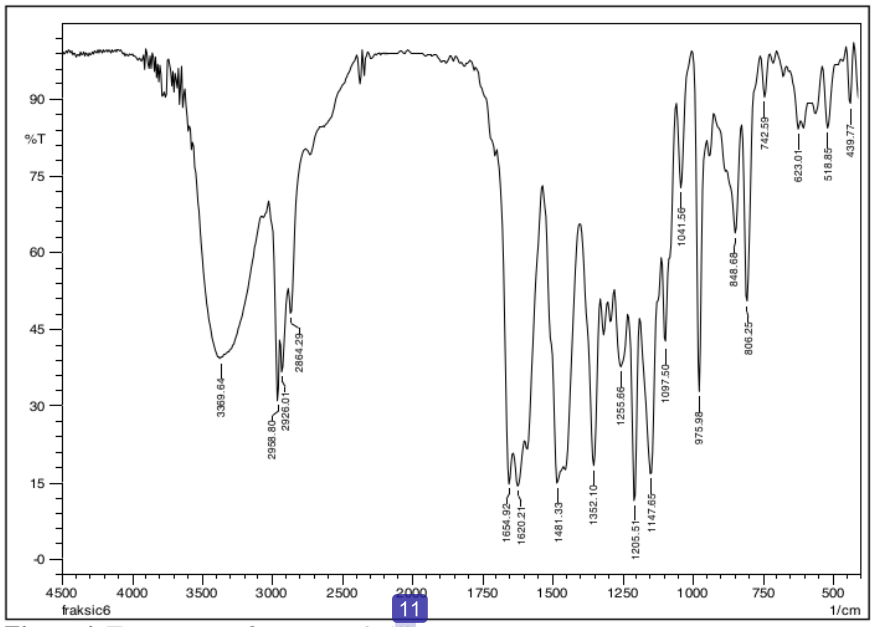

Figure 4. IR spectrum of compounds (1)

The IR spectrum of compound (1) (Figure 4) showed a wide band in the area of the wave number 3369 cm^{-1} indicating the presence of stretching vibrations of free hydroxyl groups that can form

hydrogen bonds, vibrations of aliphatic CH groups, indicated by absorption peaks of 2958, 2926, and 2864 cm^{-1} which probably originated from the prenyl group; vibrations of the carbon-carbon benzene ring at the absorption peak of 1620 -1352 and 1481 cm^{-1}, and the associated carbonyl at the absorption peak of 1654 cm^{-1} and the presence of C-O alcohol which was shown at the absorption peak in the region of 1205 cm^{-1}. The absorption peak in the area of 1481 cm^{-1} provides important information regarding the presence of aromatic rings, this is reinforced by the presence of aromatic C-H absorption at wave numbers 900-600 cm^{-1}. The IR spectrum of compound (1) is similar in comparison to the artocarpin in the literature [16-18].

The IR spectrum of compound (2) (Figure 5), an absorption band at wave number 3396 cm^{-1} indicated the presence of stretching vibration of the hydroxyl group. This assumption is supported by the appearance of the absorption peaks at wave numbers 1296 and 1209 cm^{-1} indicating the C-O stretching vibration of alcohol. The absorption peaks in the 2943 and 2882 cm^{-1} regions indicated the presence of aliphatic C-H groups; the absorption peak at 1649 cm^{-1} originates from the correlated carbonyl group. The sharp absorption peaks at wave numbers 1620, 1583, 1552, 1479 cm^{-1} indicated C-C vibrations of the benzene ring, this is reinforced by the presence of aromatic C-H absorption at wave numbers 800-600 cm^{-1}. The appearance of absorption peaks at wave numbers 1134, 1083, 1041 and 981 cm^{-1} indicated that compound (2) has an alkene group. The IR spectrum of compound (2) is similar in comparison to the cycloartocarpine in the literature [17].

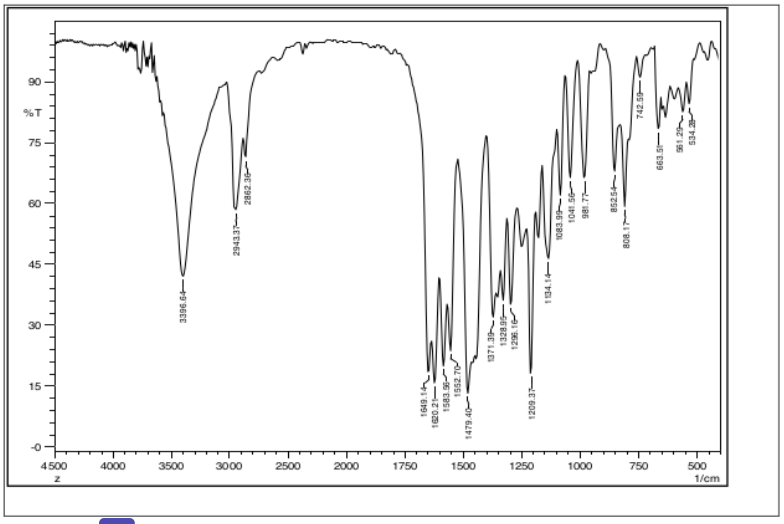

Figure 5. IR spectrum of compound (2)

Based on the comparison of UV-Vis and IR spectra data, as well as the TLC chromatogram, compound (1) and (2) were identified as artocarpin and cycloartocarpin, respectively. The structures of (1) and (2) can be seen in Figure 6.

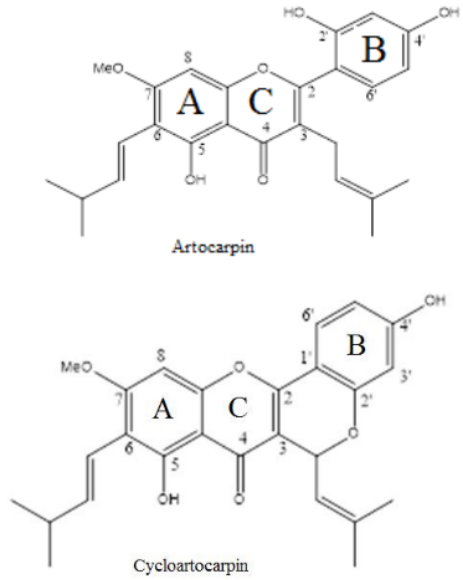

Figure 6. Structure of artocarpin and cycloartocarpin compounds

3.3 Antibacterial bioactivity test

Compounds (1) and (2) were tested for antibacterial bioactivity against *B. subtilis* and *E. coli* using the diffusion method based on the diameter of the inhibition zone. Antibacterial activity based on the inhibition zone diameter is divided into categories: very strong (inhibition zone more than 20 mm), strong (inhibition zone 10-20 mm), moderate (inhibition zone 5-10 mm), and weak (inhibition zone less than 5 mm) [18].

The sample concentration in this study was made into three variations of concentration, that is 0.5; 0.3; 0.1 mg / disk. Likewise for positive control and negative control made in the same three variations with the sample. The positive controls used were chloramphenicol in *B. subtilis*, and amoxicillin used in *E. coli*. The use of *B. subtilis* and *E. coli* bacteria in this experiment aims to represent gram-positive and gram-negative. Meanwhile, the negative control used methanol p.a.

Based on the results of the antibacterial assay for compounds (1) and (2), it was shown that compound (1) had a very strong and strong category of antibacterial activity against *B. subtilis* at a concentration of 0.5; 0.4; 0.3 mg/disk is indicated by measuring the formed inhibition zone, namely 25, 24, and 17 mm; compound (2) at a concentration of 0.5 mg / disk was in the strong category, while at 0.4 and 0.3 mg/disk in the moderate category, with an inhibition zone formed, respectively, 9 and 8 mm. Meanwhile, for *E. coli* compounds (1) and (2) have strong antibacterial activity at a concentration of 0.5 mg/disk with an inhibition zone of 11 and 10 mm, respectively; moderate at a concentration of 0.4 and 0.3 mg/disk the antibacterial activity of the two compounds, moderate category, was indicated by the inhibition zone formed less than 10 mm each. The results of the antibacterial bioctivity test against *B. subtilis* and *E. coli* compounds (1) and (2) can be seen in Table 1.

Based on the structure, compound (1) has two hydroxy groups on ring B, and two free prenyl groups; whereas compound (2) has only one hydroxy group on ring B, and one free prenyl group. The additional of phenol and prenyl groups on compound (1) [19, 20] may increase the hydrophilic

properties of flavonoid compounds and could enhance the antibacterial properties. Therefore, compound (1) has a higher antibacterial activity than compound (2).

Table 1. The results of the antibacterial test for compounds (1) and (2) against bacteria *B. subtilis* dan *E. coli*

Compound	Inhibition zone (mm)					
	Concentration 0.5 mg/*disk*		Concentration 0.4 mg/*disk*		Concentration 0.3 mg/*disk*	
	B. subtilis	*E. coli*	*B. subtilis*	*E. coli*	*B. subtilis*	*E. coli*
(1)	25	11	24	7	17	6
(2)	10	10	9	8	8	7
Control (+)	38	33	30	32	30	31
Control (-)	0	0	0	0	0	0

4. Conclusions

Artocarpin (1) and cycloartocarpin (2) compounds have been isolated from *A. kemando* Miq branch wood. Compounds (1) had very strong antibacterial activity against *B. subtilis* bacteria at a concentration of 0.4 mg/disk and against *E. coli* in the strong category at a concentration of 0.5 mg/disk; whereas compound (2) had antibacterial activity against bacteria *B. subtilis* and *E. coli* in the strong category at a concentration of 0.5 mg/disk.

5. Acknowledgments

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