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ISOLATION, CHARACTERIZATION, MODIFICATION, AND BIOACTIVITY TEST OF ARTONIN E FROM ROOT BARKS OF Kenangkan (*Artocarpus rigida*)

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Abstract. Diarrheal disease is an endemic in Indonesia and it is a potential illness of Extraordinary Occurrence which is often followed by death. Several organic compounds have been isolated from natural products which are common used as drugs. *Artocarpus rigida* or Kenangkan is one of natural products that contains flavonoid as derivate compounds. Flavonoid is known as a good antibacterial agent. The research phases were conducted following; preparation, extraction, isolation, purification, identification, modification, and antibacterial test. The isolated flavonoid was obtained from semipolar fraction from root barks of Kenangkan and visualized by TLC. Purification process was used VLC method. The molecular structure of the compound was determined by physical and spectroscopic data (UV-Vis and IR). The obtained compound was 1.6 g Artonin E has a yellow solid with melting point 250-252^{o}C, mean while a modified artonin E with acetic anhydride has a white solid with melting point 192-194^{o}C. The result of bioactivity test, an isolated artonin E has better antibacterial activity than a modified artonin E towards *Bacillus subtilis*, but the activities are not included in a high category.

INTRODUCTION

Diarrheal disease is an endemic in Indonesia and it is a potential illness of Extraordinary Occurrence which is often followed by death. In 2015 there are 18 outbreaks of diarrhea spread in 11 provinces, 18 districts / cities, with the number of 1,213 people and 30 people dead. The Case Fatality Rate (CFR) when the outbreak is expected to be <1%. In Lampung province, especially Pesisir Barat's fatality rate achieves 100%, whereas in Pesawaran's fatality rate is 3.33% (Kemenkes, 2016).

There are several causes of diarrheal diseases, including bacterial infections and food poisoning (Widoyono, 2008). The damage by microorga-nism presence in can is decreasing quality product and endangered health even death (Pratiwi, 2004). One of bacterias that can cause damage in canned food and also cause gastroenteritis in humans, is Bacillus subtilis (Nursal, 1998).

Bacillus subtilis can contaminate food and cause food poisoning (Constantin *et al*., 2009). These bacterias produce extracellular subtilisin toxins which can cause hypersensitivity reactions if exposed continously (Sundaram *et al*., 2011). These bacterias contaminate and release acid and CO_2 gas (Ray, 2004).

Antibiotics are the most widely used drugs for bacterial infections. The intensity of using antibiotics is relatively too high caused various problems and is a global threat to health, especially bacterial resistance to antibiotics. Initially resistance occurred at the hospital level, but gradually also developed in the community (Menkes RI, 2011). Likewise Bacillus subtilis also shows multi-drug resistance at hospital environment in Duhok City, Iraq (Yassin and Ahmad, 2012).

Many organic compounds are successfully isolated from natural products that have been used to treat various diseases. However, the necessity for drugs for various types of

diseases grow continuesly. Therefore, the research of novel compounds from natural products conducted continuously to outgrow various diseases (Herbert, 1996).

Artocarpus rigida, known as a fruit, has been studied previously, and obtained several flavonoid derivative compounds such as artonin E (Hernawan, 2008). Artonin E has high cytotoxic properties for attacking murine leukemia P-388 cells (Suhartati *et al*., 2008), then artonin E also has antimalarial activity. Artonin E used is the result of isolation from Artocarpus rigida (Suhartati *et al*., 2010).

Once has been isolated previously secondary metabolite compounds from the root bark of *A. rigida*, but it has not been determined its structure (Faizar, 2010). Meanwhile, Artocarpol A has been successfully isolated from the root bark (Chung *et al*., 2000). Artocarpol F has also been isolated (Ko *et al*., 2001). In addition, it has also been successfully isolated Artocarpol I and J from *A. rigida* (Lu *et al*., 2003).

In the range 1981 - 2010, 4.4% of the 1355 medicines in circulation came from purification of natural ingredients, 0.4% of the extract, and 43% were natural Modification (Newman *et al*., 2012). Purification of molecules from natural materials is not a final job and can be used as medicine immediately, but still another step is needed. So only about 5% of the compound produced by direct extraction can be used for medicine, most find the model compounds to be synthesized or further modified (Saifuddin, 2014).

In this paper, we reported the results of isolate the flavonoid compounds from semipolar fraction from the root bark of Kenangkan (*Artocarpus rigida*), determine the structure of flavonoid were isolated, modify flavonoid and antibacterial activity test of isolated and modified flavonoids.

EXPERIMENTAL

Plant Materials

Root bark samples of Kenangkan (*Artocarpus rigida*) were collected from Kaputren Village, Sukoharjo, Pringsewu, Lampung, Indonesia. They were identified by the staff at the Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Scineces, Bogor, Indonesia and a voucher speciment has been deposited at the herbarium.

Isolation

The dried bark of *A. rigida* (3 Kg) was extracted with n-hexane at room temperature for 3 days and such was repeated two more times. The residue was extracted successively with methanol: ethyl acetate (1: 1). Evaporation of methanol: ethyl acetate solutions to dryness yield 150 g of the residue. This extract (150 g) was chromatographed by Vacuum Liquid Chromato-graphy (VLC) on silica gel with *n*-hexane containing increasing amount of ethyl acetate as an eluent by 13 VLC stages, each fraction being monitored by Thin Layer Chromatography (TLC). We were focused on two of six main fractions (A and B fraction). Into A fraction, there were yellow granules (K1 – K9 = 0,6 g) from several VLC stages. A part of the fraction eluted with n-hexane containing 40 – 50% ethyl acetate was evaporate to give residue (38,7 g), which was rechromatographed with the same eluent like the first VLC to give three main fractions, each fraction being monitored by TLC. From all of this fraction, we were got some yellow granules (K10 – K15 and K17 – K19 = 0,4 g). B fraction of the fraction eluted with n-hexane containing 60 – 70% ethyl acetate was evapotare to give a residue (23,87 g), which was rechromatographed with the same eluent like the first VLC to give two main fractions, each fraction being monitored by TLC. From the first fraction, we were got some yellow granules (K16 and K20 = 0,08 g). Three fractions of A and two fractions of B then analyzed the similarity of Rf by TLC so that give 3 fractions were obtained, including A1, A2, and A3 fractions. In the fraction of A3 obtained yellow granules, which was purified by recrystallization from n-hexane-ethyl acetate to give yellow granules (K21 and K22 = 0,52 g).

Modification

The modification of the isolated compound was carried out using a method performed by Hano (1990). A mixture of isolated compounds (9.5 mg), acetic anhydride (0.3 ml), and pyridine (0,1 ml) was kept at room temperature for 3 days and treated as usual. The modified compounds was crystallized from *n*-hexane-ether to give colorless granules.

RESULT AND DISCUSSION

Purity Analysis

Based on the results of purification analysis of the isolated compounds can be taken hypothesis that a pure compound had been well isolated because of the Rf value of isolated compound is as same as artonin E standard . The yield of yellow granule (yellow solid) was 1.6 g which have a melting point of 250 - 252^{0}C. Then the modified compound has a colorless granules (white solid) with a melting point of 190 - 192 ^{0}C.

The Rf value of the modified compound has a lower degree of polarity compared to the isolated compound. It caused by esterification or acetylation which the hydroxyl groups had been replaced into the acetyl group.

That hydroxyl group is more polar than the acetyl group. General reactions of esterification or acetylation using acetic anhydride with pyridine at Figure 1.

Characterization

The modified compound was analyzed using a ultraviolet-visible spectrophotometer (UV-Vis), which the spectrum are shown in Figur 2.

The isolated compound from root barks of *A. rigida* has maximum absorption (λ_{max}) at 211 nm, 268 nm, and 347 nm in methanol with a concentration of 5 ppm (1 mg / 20 mL). UV-Vis spectrum data shows characteristics of flavone compounds. The typical spectrum of flavones in band I at λ_{max} 347 nm shows a change in the B and C rings of flavonoid structure. The typical spectrum of flavones in band II (250 - 280 nm) at λ_{max} 267 nm shows a characteristic for benzoyl groups in ring A.

The modified compounds has maximum absorption at 211 nm, 236 nm (shoulder), 261 nm, and 319 nm in methanol with a concentration of 5 ppm (1 mg / 20 mL). UV spectrum data shows characteristics of flavone compounds. The typical spectrum of flavones in band I at λ_{max} 319 nm shows a change in the B and C rings of the flavonoid structure. There was a decrease in absorption value of the isolated compound towards the modified compound. The decrease of absorbance value of 347 nm into 319 nm is 28 nm. Hydroxylation may cause a band shift to a higher wavelength (batochromic), and otherwise (Markham, 1988). From this fact, it can be predicted that a decrease of absorbance was caused by dehydroxy (removal of hydroxy effect) of approximately 3 or 4 hydroxy groups in band 1 (B and C rings).

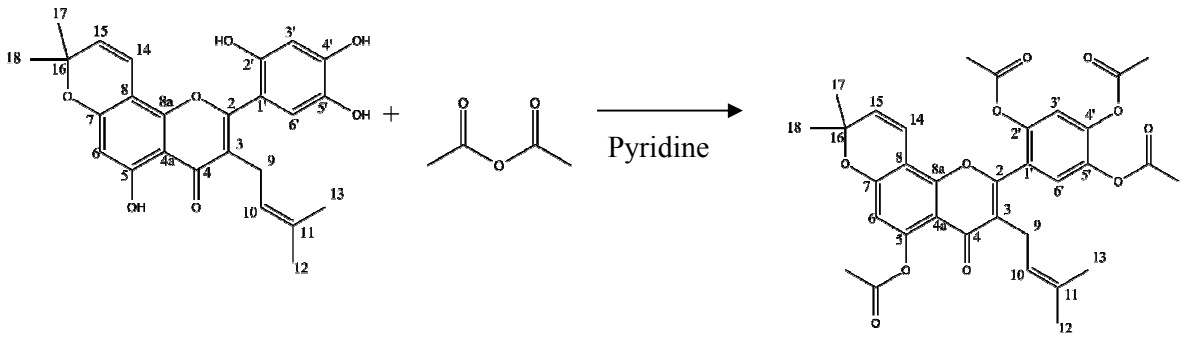

Figure 1. The reaction of artonin E with acetic anhydride, pyridine catalyst.

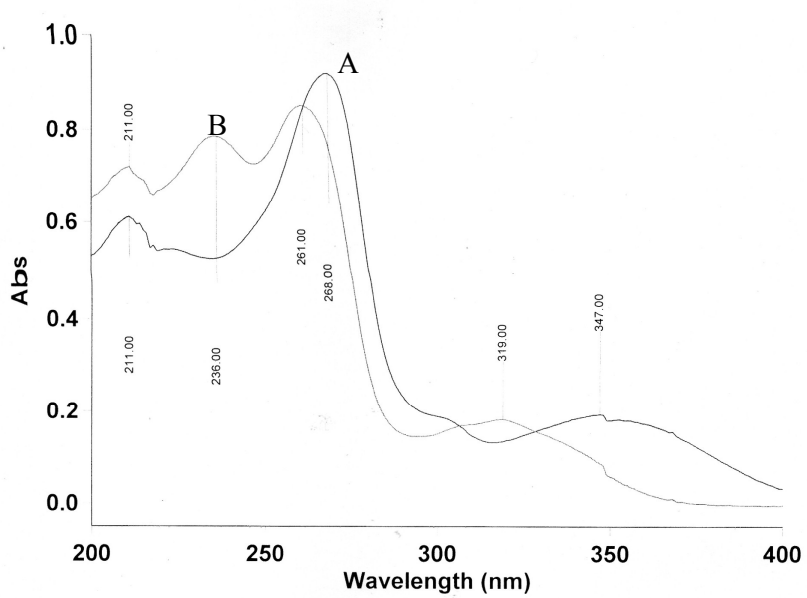

Figure 2. UV-Vis spectrum; (A) the isolated compound and (B) the modified compound in methanol

The typical spectrum of flavones in band II (250 - 280 nm) at λ_{max} 261 nm shows a characteristic of benzoyl groups in A ring. The decrease in absorption value from 268 nm into 261 nm was caused by dehydroxy on one hydroxy group in band II (A ring). From the results, this research had been welldone to convert the hydroxy group into an acetyl group to form an acetylated flavonoid.

Infrared spectrophotometry (IR), spectrum data can be used to analyze the presence of functional groups of an organic compound. The IR spectrum of modified compound can be seen in Figure 3.

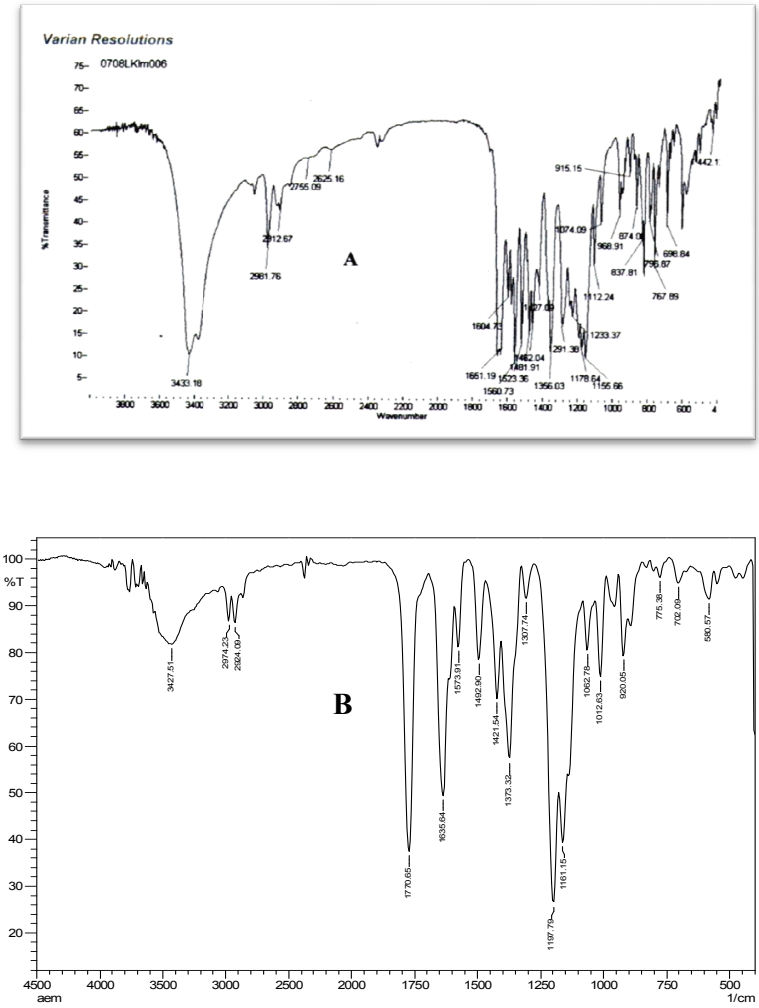

Figure 3. IR spectrum; (A) artonin E (Hernawan, 2008); (B) the modified compound.

Based on Figure 5, there is a widening band on the wavelength region of 3427 cm^{-1} which is the vibration of the hydroxy group which can form hydrogen bonds, but the percent of transmitance was higher than artonin E. The absorption peak at 2924 cm^{-1} and 2974 cm^{-1} areas were indicated of aliphatic C-H groups.

The absorption peaks at regions 1770 cm^{-1} and 1635 cm^{-1} indicate the presence of carbonyl groups (C = O) conjugated with (C = C). Absorption peaks in regions 1573, 1492, 1421, and 1373 cm^{-1} show the presence of C = C aromatics.

Antibacterial Test

In this study, the antibacterial test was used an agar diffusion method which the final result was an inhibitory zone diameter. Negative control was used methanol as pure solvent, while the positive control was used amoxycilin. The concentrations of negative control, positive control, an isolated compound, and a modified compound were 0.5 mg / disc and 1 mg / disc.

The result of antibacterial bioactivity test towards *Bacillus subtilis* of isolated and modified compounds are showed in Table 1.

The result of antibacterial bioactivity test of an isolated and modified artonin E showed that both compounds had intermediate category antibacterial activity against *Bacillus subtilis* were shown in resistor zone size. Inhibitory zone of an artonin E (AE) has wider size than a modified (AEM) inhibitory zone. Acetylation on the isolated artonin E decreases antibacterial activity towards *Bacillus subtilis*. Inhibitory zone of the compound about 5 – 10 mm means the compounds were include in medium as antibacterial activity (Davis and Stout, 1971).

Table 1. Inhibitory zone of the isolated and modified compounds toward *Bacillus subtilis*

The concentration of compound	**0.5 mg/*disk***	**1 mg/*disk***
Control (+)	27 mm	32 mm
Control (-)	-	-
Isolated compound (AE)	8 mm	12 mm
Modified compound (AEM)	6.5 mm	7 mm

CONCLUSIONS

The conclusions of this research are; It has been isolated and esterified artonin E from root bark of *A. rigida* from Kaputren Village, Sukoharjo, Pringsewu, Lampung, Indonesia. The antibacterial activity test against *Bacillus subtilis* from both compounds show intermediate activity.

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