ISOLATION, CHARACTERIZATION, MODIFICATION OF ARTOCARPIN COMPOUND FROM PUDAU PLANT (*Artocarpus kemando* Miq.) AND BIOACTIVITY ANTIBACTERIAL ASSAY OF ARTOCARPIN COMPOUND AND THEIR MODIFICATION RESULT

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Abstract : *Artocarpus kemando* Miq. is a species from Moraceae family in Indonesia know as Pudau plant. The purposes of this research are to isolate, characterize, modify and test the antibacterial activity to the artocarpin compounds isolated from pudau plants. The research stages includeed sample preparation, extraction, isolation, and purification of compounds using chromatographic techniques (TLC, VLC, and CC), characterization of compounds using spectroscopy. Isolated compound was obtained as a yellow crystal with a melting point of 185-187 °C. Based on the results of spectroscopic analysis indicated that it has been successfully isolated a prenylated flavonoid, artocarpin in the amount of 35.2 mg. The modification of artocarpin with acetic anhydride produced acetyled artocarpin as yellow crystal. Artocarpin and the modified compounds showed an antibacterial activity against *Bacillus subtilis* and *Escherichia coli*.

Keywords : antibacterial, *A. kemando* Miq., Artocarpin, *B. subtilis*, *E. coli*

INTRODUCTION

Indonesia is known as a country that have a large biodiversity, some of plants are consumed as food and the others have a potential to be developed as medicines. Based on this, the scientists continue to develop the potential of these plants as raw material for medicines to overcome various diseases. One of the family of medicinal plants is Moraceae which consists of 60 genus including 1400 species [1]. The main genus of the Moraceae family is *Artocarpus*.

Artocarpus consists about 50 species, this genus is widely distributed in tropical and subtropical regions, including Indonesia [2]. The bark, leaves, seeds, fruits and roots of some species are reported have medicinal properties and used for the treatment of diseases such as diarrhea, fever, cirrhosis, hypertension, inflammation, malaria, ulcers, sores and for tapeworm infections [3,4,5] One of the species of *Artocarpus* which is an endemic plant in Indonesia is *Artocarpus kemando* Miq. can produce many prenylated flavonoid compound by means of isolation and some compounds obtained show interesting biological activity [6,7,8]. One of the compound contained in *A. kemando* Miq. is artocarpin [9].

Artocarpin is a prenylated flavon compound which has three hydroxyl groups. Two of them are in an metha position, so that the group is easily to oxidized. In order to avoid oxidation reactions, it is necessary to modify with esterification [10]. In this study, artocarpin was isolated from *A. kemando* Miq. This plant was picked from Karang Anyar, Klaten Village, Penengahan, South Lampung, Lampung, Indonesia. After that artocarpin esterified with acetic anhydride using pyridine catalyst. The ester compound were characterized by spectroscopy methods and then tested for antibacterial bioactivity against the bacteria *B. subtilis* and *E. coli*.

METHOD

General

The melting point was determined by the MP-10 Stuart melting point. The UV-vis and IR spectra were measured with Agilent Cary 100 and 21 Shimadzu Prestige FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained with an Agilent spectrometer with DD2 console system at 500 MHz and 125 MHz, respectively. Vacuum liquid chromatography (VLC) was performed using a Merck Si-gel 60, and thin layer chromatography (TLC) analysis was Si-gel plates (Merck Kieselgel 60 F254, 0.25 mm).

Sample Preparation

Root wood of *A. kemando* Miq. obtained from Karang Anyar, Klaten village, Penengahan, South Lampung, Lampung, in March 2019 and identified at Bogoriense Herbarium, Research Biology Center, Indonesia, Institute of Sciences Bogor, Indonesia.

Extraction and Isolation

A.kemando Miq. root wood powder (3 kg) macerated with methanol as a solvent for 3x24 hours. Then to remove the solvent, evaporated with a rotary evaporator to obtained a crude extract (41 g). The extract was fractionated with hexane solvent to obtained a methanol fraction (29.8 g). the methanol fraction in vacuum liquid chromatography was eluted with EtOAc / n-hexane in a ratio of 0-100%, to produce five main fractions (A - E). Fraction C 1.2455 g was then chromatographed with silica gel adsorbent and acetone / hexane eluent at a ratio of 20:80%. Chromatography results obtained yellow crystals from the C8 fraction (140 mg), then purified further by column chromatography using silica gel adsorbents and hexane / acetone eluents in a ratio of 20:80%, producing yellow crystals from the C8f fraction as much as 35.2 mg, expressed as compound (1). The purity of the crystals obtained was determined by measuring the melting point and TLC chromatograms were made with three eluent systems. The structure of the compound (1) was determined by UV-Vis, IR, and NMR spectroscopy.

Modification of Artocarpin Compound

Modification of artocarpin compounds by using acetic anhydride and pyridine catalyst. 10 mg of artocarpin compound was mixed with 0.1 mL of pyridine catalyst, then added 0.24 mL of acetic anhydride reagent and put into a closed reaction flask. The mixture is allowed to stand for up to 3 x 24 hours and spotted with TLC every 24 hours. After that, the mixture with aquades, filtered and vacuum so that yellow precipitate is obtained. The modified results were recrystallized with ether/hexane solvent to produce 6,6 mg yellow crystal. Expressed as compound (2) and analyzed by determining the melting point and TLC with three eluent systems. The success of the esterification was determined using UV-Vis and IR spectroscopy.

Antibacterial Bioactivity Test

In the antibacterial bioactivity test, the paper disc diffusion method was used [11]. Antibacterial test using Nutrient Agar (NA) media. 4.2 grams of NA were dissolved in 150 mL aquades and then heated until homogeneous. artocarpin compound and modified compound were made variations in three concentrations: 0.5 mg/disk; 0.4 mg/disk and 0.3 mg/disk. 1.5 mg compound (1) are dissolved in 150 µL, then taken 50 µL; 40 µL; and 30 µL to be bottled into a paper disk.

In the antibacterial test against *B. subtilis* a amoxicillin was used as a positive control, while the antibacterial test on *E. coli* used chloramphenicol as a positive control. After the media solidifies, inserted NA added with aquades containing 1 ose of bacteria. Then the paper disk containing the sample, positive control and negative control is inserted into the NA that has been made. Petri dishes are covered with paper and plastic wrap and then put in an incubator for 1x24 hours.

RESULT AND DISCUSSION

Isolation of compounds from *A. Kemando* Miq.

To determine the purity of compound (1),the sample was spotted in TLC together with standard artocarpin using three different eluent systems and obtained the same Rf results (**Figure 1**). From the TLC results it is estimated that the compound (1) is an artocarpin compound. Melting point of compound (1) 185-187 °C.

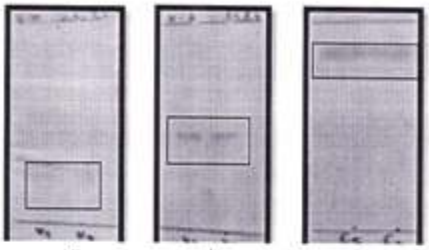


Figure 1. Chromatograms compound (1) and standard artocarpin use three eluent systems (a) ethyl acetate: n-hexane 75% Rf = 0.20, (b) acetone: n-hexane 65% Rf = 0.34, (c) ethyl acetate: dichloromethane 60% Rf = 0.85.

Modification of Artocarpin Compound

The purity (1) was determined, so that a melting point of 150-152 °C was obtained and diKLT with compound (2) used three eluent systems and a higher Rf was obtained than compound (1). This shows that compound (2) is more nonpolar compared to the compound isolated because the hydroxyl group is estimated to have been esterified (**Figure 2**).

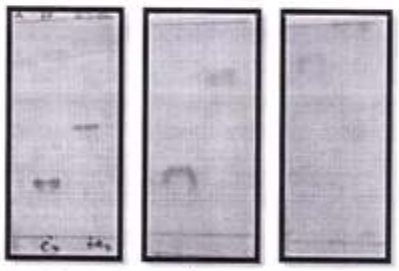


Figure 2. Chromatogram of the compound(1) (left) and compound (2) (right) using three eluent systems (a) acetone: n-hexane 75% Rf = 0.20;0.42, (b) ethyl acetate: n-hexane 75% Rf = 0.26;0.71, (c) ethyl acetate: dichloromethane 85% Rf = 0.82;1.00.

Spectroscopic analyses

The UV-Vis spectrum of compound (1) in the MeOH solvent produces absorption at λmax 321 and 221 nm which is a typical spectrum of flavones. Band I which shows the characteristics of the B and C rings of the flavon structure as sinamoil chromophore. Maximum peak at λmax 221 nm is a typical absorption of flavones in band II that shows the characteristics of benzoyl chromophore.

UV-Vis spectrum of compound (2) in the MeOH solvent obtained λmax: 222 and 300 nm The maximum absorption in the ultraviolet region at λmax 300 nm represents the spectrum in band I and the maximum absorption at λmax 222 nm is the spectrum in band II. Based on this data it can be seen that band 1 for compound (2) has decreased wavelength when compared to compound (1) which is equal to 21 nm. This shows that there has been a hydroxy substitution in compound (1) into an ester group in compound (2) (**Figure 3**).

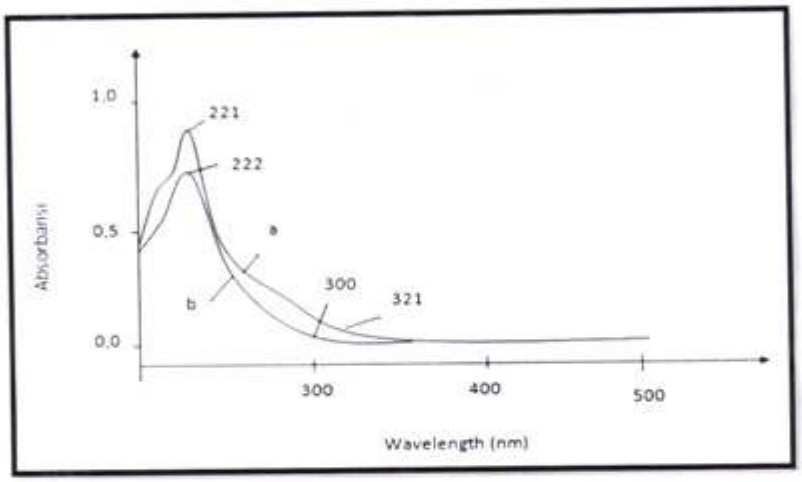


Figure 3. UV-vis spectrum of (a) compound (1) and (b) compound (2) (in methanol).

The results of IR analysis isolated compound showed artocarpin (KBr) Vmax. : 3390, 2958, 2866, 1680, 1481, 1471,1352, 1259, 1205, 1149,1097 and 979 cm^{-1} (**Figure 4a**). The absorption peak at 3390 cm^{-1} is a stretching vibration from hidroxy group, 2958 cm^{-1} dan 2866 cm^{-1} is C-H alifatik, 1620 cm^{-1} is carbonyl group (C=O) conjugated with C=C, 1205 cm^{-1} is bond C-O alcohol, 1481 cm^{-1} provide information about the presence of aromatic ring which is strengthened by the absorption C-H at 900-600 cm^{-1}.

In the compound (2) was obtained data IR (KBr) Vmax. : 3510,3093, 2956, 2866, 1772, 1647, 1587, 1485, 1450, 1354, 1301, 1201, 1145, 1099, 1041, 1012, 974 cm^{-1} (**Figure 4b**). Absorption at wave numbers 3200-3500 cm^{-1} is absorption from O-H stretching vibration[12]. The compound (2) there is still visible absorption of 3510 cm^{-1} but the intensity is smaller than the compound (1). In addition, there was also a change in the absorption of the wave number of the carbonyl group (C = O) seen at 1772 cm^{-1} with a greater intensity compared to the compound (1). This shows that the compound (2) experienced a change in the hydroxyl group into an ester group due to the esterification reaction.

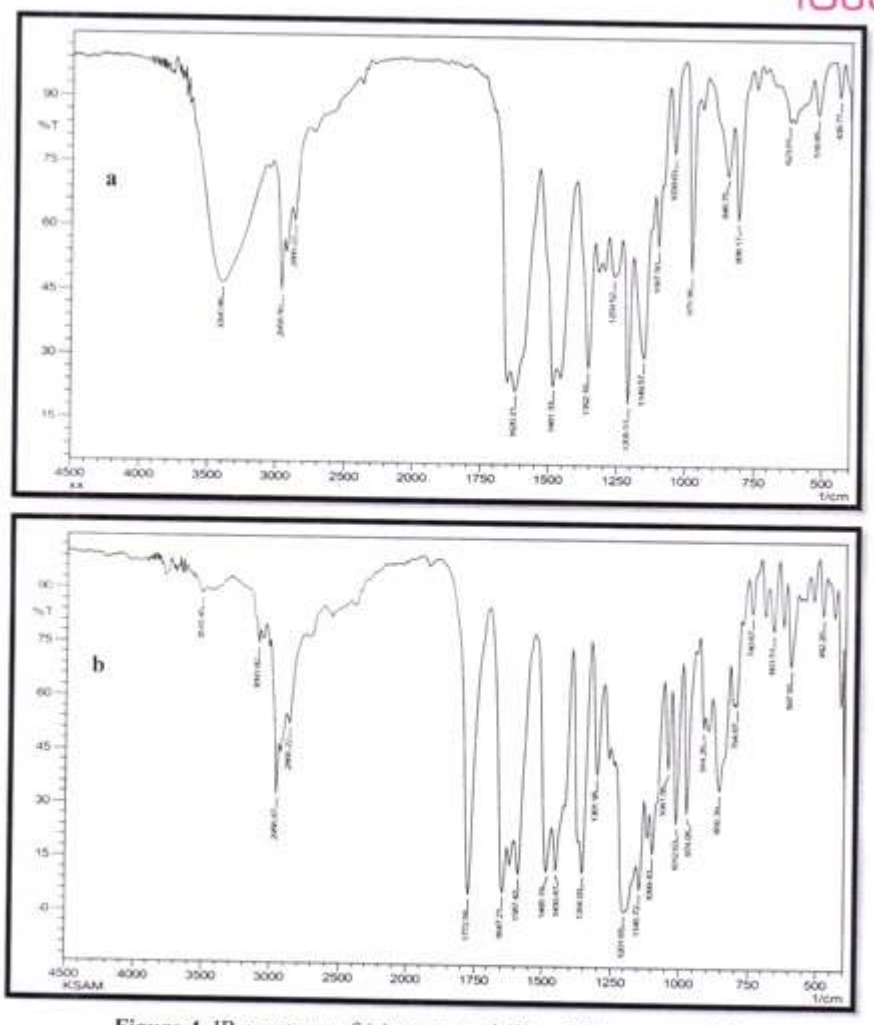


Figure 4. IR spectrum of (a) compound (1) and (b) compound (2).

The ^{1}H-NMR spectrum data shows that the presence of proton C sp^3 at δ 1.56 ppm (3H, s), and at δ 1.43 ppm (3H, s), which shows the peak of the singlet. The peak appears at δ 5.12 ppm (1H, d, J = 7.0 Hz) due to the coupling of protons at δ 3.12 ppm. Proton C sp^3 at a chemical shift of δ 1.09 ppm (3H, d, J = 6.7 Hz) and at δ 1.09 ppm (3H, d, J = 6.7 Hz) indicates a double peak. At δ 2.43 ppm (1H, m) which shows the peak of the multiplet due to

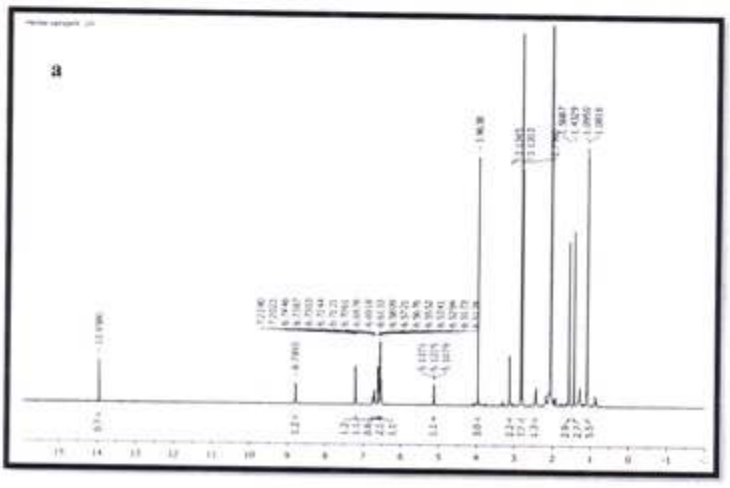
coupled protons at δ 6.73 ppm, δ 1, 08 ppm, and δ 1.09 ppm. The proton at δ 6.57 ppm (1H, d, J = 3Hz) is a proton of C sp^2 alkene that is coupled to a proton at δ 6.73 ppm.

Proton of C sp^2 aromatic rings detected at δ 6.61 ppm (1H, s) is aromatic in ring A. Proton at δ 6.51 ppm (1H, d, J = 2.2 Hz) is possible from ring B coupled by a meta-spaced proton at a chemical shift of δ 6.52 ppm. Proton at δ 6.52 ppm (1H, dd, J = 8.4 Hz and 2.3 Hz) are coupled by protons at δ 7.20 ppm. Proton at δ 7.20 ppm (1H, d, J = 8.6 Hz) is coupled to one proton at δ 6.52 ppm. So that the spectrum appears 2 peaks (doblet, d). In addition, there is a proton C sp^3 at δ 3.96 ppm (3H, s) which appears as the peak of the singlet and is likely to originate from the proton methoxy group (**Figure 5a**).

Spectrum data of ^{1}H-NMR is estimated that the shift leads to the position of the proton: δ 1, 08 ppm and δ 1.09 ppm (H-17 and H-18), δ 2.43 ppm (H-16), δ 6.73 ppm (H-15), δ 6.57 ppm (H-14), δ 1.43 ppm (H-13), δ 1.56 ppm (H-12), δ 5.12 ppm (H-10) , δ 3.12 ppm (H-9), δ 6.61 ppm (H-8), δ 3.96 ppm (H-OCH3), δ 6.51 ppm (H-3'), δ 6.52 ppm (H-5'), and δ 7.20 ppm (H-6').

The results of the ^{13}C-NMR spectrum which showed the presence of 26 carbons, including oxygenated aromatic carbon atoms at δ 157.4; δ 159.8; δ 163.8; δ 142.3; δ 142.3; and δ 132,3 ppm. The chemical shift at δ 183.3 ppm indicates a carbonyl group. Carbon atom of the methoxy group at δ 56.6 ppm. There is C sp^3 at δ 34.3; δ 25.8; δ 24.3; δ 23.1 and δ 17.6. There is an aromatic C sp^2 at δ 105.6; δ 108.1; δ 109.8; δ 117 and δ 121.9 ppm (**Figure 5b**).

Based on the ^{1}H-NMR and ^{13}C-NMR spectrum data compare with the TLC cromatogram show that compound (1) is an artocarpin compound, this result also matched with the standard artocarpin compound literature data [13] (**Table 1**).



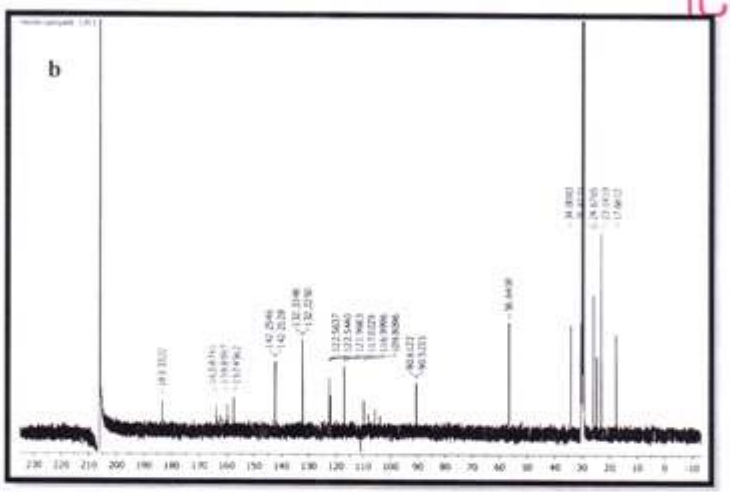


Figure 5. Results of data (a) ^{1}H-NMR and (b) ^{13}C-NMR compound (1).

Table 1. Comparison of ^{13}C-NMR data between literature artocarpin compound [13] and compound (1).

	^{13}C-NMR, (ppm)	
No C	standard artocarpin [13]	(1)
2	163,6	159,9
3	122,2	122,0
4	183,9	183,3
4a	106,0	105,6
5	159,7	142,3
6	110,4	109,8
7	164,3	163,9
8	90,7	90,5
8a	158,0	157,5
1'	113,2	116,2
2'	157,8	142,21
3'	103,7	90,6
4'	161,9	132,3
5'	108,0	108,1
6'	132,8	132,3
9	24,9	24,6
10	122,8	122,5
11	132,4	121,9
12	25,9	25,8
13	17,7	17,6
14	117,2	117,0
15	142,8	122,6
16	34,5	34,0
17	23,2	23,1
18	23,2	23,1
OCH_3	56,6	56,6

Antibacterial Bioactivity Test

In the antibacterial bioactivity test with variations in three concentrations, that is 0.3 mg/disk, 0.4 mg/disk, and 0.5 mg/disk. In the antibacterial bioactivity test, the isolated artocarpin compound (1) against *E. coli* bacteria was obtained by inhibiting zones of 9 mm, 10 mm, and 12 mm, respectively (0.3; 0.4; 0.5 mg / disk). These results indicate that compound (1) can inhibit bacteria in the medium category. In the antibacterial bioactivity test the modified artocarpin compound (2) produces inhibition zones of 5 mm, 6 mm, and 7 mm (0.3; 0.4; 0.5 mg/disk), so these results indicate that the antibacterial bioactivity of the compound (2) also included in the medium category (**Table 2**).

compound (1) and compound (2) were also tested for antibacterial bioactivity against *B. subtilis* bacteria. Compound (1) produces inhibition zones of 11 mm, 15 mm, and 17 mm (0.3; 0.4; 0.5 mg/disk). Whereas the compound (2) shows inhibition zones of 10 mm, 11 mm, and 14 mm (0.3; 0.4; 0.5 mg/disk). Based on the results, it can be seen that both compounds have antibacterial bioactivity in the strong category [14] (**Table 3**).

From the results of the antibacterial bioativity test, it is known that the size of the inhibition zone of compound (1) is greater than that of compound (2), both against *E. coli* bacteria and *B. Subtilis* bacteria. This happen because the compound (1) has three hydroxyl groups which cause denaturation of proteins through an adsorption process involving hydrogen bonds so that the antibacterial bioactivity of compound (1) is better than compound (2).

Table 2. Size of inhibition zones from compound (1) and compound (2) against *E. coli* bacteria.

Concentration	Compound (1)	Compound (2)	Control +	Control -
0.5 mg/*disk*	11 mm	7 mm	30 mm	-
0.4 mg/*disk*	9 mm	6 mm	30 mm	-
0.3 mg/*disk*	8 mm	5 mm	28 mm	-

Table 3. Size of inhibition zones from compound (1) and compound (2) against *B. Subtilis* bacteria.

Concentration	Compound (1)	Compound (2)	Control +	Control -
0.5 mg/*disk*	17 mm	14 mm	29 mm	-
0.4 mg/*disk*	15 mm	11 mm	28 mm	-
0.3 mg/*disk*	11 mm	10 mm	28 mm	-

CONCLUSION

Artocarpin has been successfully isolated from root wood *A. kemando* Miq. and has also been modified into ester; artocarpin acetate. The compound has been indicate to have antibacterial activity against *E. coli* in the medium category and against *B. subtillis* in the strong category.

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REFERENCES

[1] Hakim, E.H., Achmad, S.A., Juliawaty, L.D., Makmur, L., Syah, Y.M., Aimi, N., Kitajima,M., *and* Ghisalberti, E.L. Prenylated flavonoids and related compounds of the Indonesian *Artocarpus* (Moraceae). *J. Nat. Med.* **6**, pp. 161–184, 2006.

[2] Amarasinghe, N.R., Jayasinghe, L., Hara, N., *and* Fujimoto, Y. Chemical constituents of the fruits of *Artocarpus altilis*. *Biochem. Syst. Ecol.* **36** (4), Pp. 323-325, 2008.

[3] Khan, M.R., Omoloso, A.D., and Kinara, M. Antibacterial activity of *Artocarpus heterophyllus*. *Fitoterapia*. **74**, pp. 501-505, 2003.

[4] Patil, A.D., Freyer, A.J., Killmer, L., Offen, P., Taylor, P.B., Votta, B.J., and Johnson, R.K. A new dimeric dihydrochalcone and a new prenylated flavone from the budcovers of *Artocarpus altilis*: Potent inhibitors of cathepsin. *J. Nat. Prod.* **65**, pp. 624-62, 2002..

[5] Su, B.N., Cuendet, M., Hawthorne, M.E., Kardono, L.B.S., Mehta, R.G., Pezzuto, J.M., and Kinghorn, A.D. Constituents of the bark and twigs of *Artocarpus dadah* with cyclooxgenase inhibitory activity. *J. Nat. Prod.* **65**, pp. 163-169, 2002.

[6] Ee, G.C.L., Teo, S. H., Rahmani, M., Lim, C.K., Lim, Y.M., *and* Go, R. Artomandin, a new xanthone from *Artocarpus kemando* (Moraceae). *J. Nat. Prod. Res.* **25** (10), pp. 995-1003, 2011.

[7] Hashim, N. M., Rahmani, M., Shamaun, S. S., Ee, G. C. L., Sukari, M. A., Ali, A. M., *and* Go, R. Dipeptide and xanthones from *Artocarpus kemando* Miq. *J. Med. Plant Res.* **5** (17), pp. 4224-4230, 2011.

[8] Seo, E.K., D. Lee, Y.G. Shin, H.B. Chai, H.A. Navarro, L.B. Kardono, I. Rahman, G. A. Cordell, N. R. Farnsworth, J. M. Pezzuto, A. D. Kinghorn, M. C. Wani, *and* M.E. Wall. Bioactive prenylated flavonoids from the stem bark of *Artocarpus kemando*. *Archives Pharmacal Research*. **26** (2), pp. 124-127, 2003.

[9] Suhartati, T., Hernawan, Suwandi, J.F., Yandri, and Hadi,S. Isolation Of Artonin E From The Root Bark Of *Artocarpus Rigida*, Synthesis Of Artonin E Acetate And Evaluation Of Anticancer Activity. *Macedonian Journal of Chemistry and Chemical Engineering*. **37** (1), pp. 35–42, 2018.

[10] Suhartati, T. Phenol Compounds of Several Indonesian Cempedak Plant Species. *Dissertation*. ITB. Bandung, 2001.

[11] Banjara,R.A., Jadhav, S.K., Bhoite,S.A. Antibacterial activity of di-2ethylaniline phosphate screened by paper disc diffusion method. *Journal of Applied Pharmaceutical Science*. Vol.2 (7), 2012.

[12] Indarto. Isolation and Identification of Phenolic Compoundsfrom root bark of the plant *Artocarpus* dadah. *J. Physics Education*. **04**(2), pp. 145-153. 2015

[13] Hakim, E. H., Marlina, E.E., Mujahidin, D., Achmad, S.A., Ghisalberti, E.L., and Makmur, L. Artocarpin and heteroflavanon-A, two bioactive flavonoid compounds from Artocarpus champeden. *Proc.* ITB. **30** (1), 1998.

[14] Davis W.W. and T.R. Stout. Disc Plate Method of Microbiological Antibiotic Assay. *Appl Microbiol*. 22 (4), pp. 659 – 665, 1971.