Original Article

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

**Tati Suhartati[1]*, Nurul Fatimah[1], Yandri Yandri[1], Rahmat Kurniawan[2], Syaiful Bahri[1], Sutopo Hadi[1]**

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Bandar Lampung 35145, Indonesia. [2]Chemistry, Science Departement, Institut Teknologi Sumatera, Lampung Selatan 35365, Indonesia.

**Correspondence:** Tati Suhartati, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Bandar Lampung 35145, Indonesia. tati.suhartati@fmipa.unila.ac.id

## 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 succesfullyisolated 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 $IC_{50}$ value was 1.54 g/mL. The antimalarial activity test against *Plasmodium falciparum* gave $IC_{50}$ 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.

| Access this article online | |
|---|---|
| **Website:** www.japer.in | **E-ISSN:** 2249-3379 |

**How to cite this article:** Suhartati T, Fatimah N, Yandri Y, Kurniawan R, Bahri S, Hadi S. The anticancer, antimalarial, and antibacterial activities of moracalkon a isolated from Artocarpus kemando Miq. J Adv Pharm Educ Res. 2021;11(4):105-10. https://doi.org/10.51847/9NHxpCqzUD

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 moracalcon A [10, 15].

*A. kemando* is a rare endemic plant in Indonesia that produces inedible fruit. For the first time, moracalcone A has been isolated from the root wood of *A. kemando*. Antimalarial, anticancer, and antibacterial activity tests show that moracalcone 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

### *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 + $H_3BO_3$, $AlCl_3$, and $AlCl_3$ + 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 $cm^{-1}$. This is the stretching vibration of the hydroxyl group strengthened by vibrations at wavenumbers of 1238, 1199, and 1112 $cm^{-1}$, indicating the presence of the CO group. Similarly, aromatic C-H vibrations are indicated by the absorption at a wavenumber of 3192 $cm^{-1}$. The absorption peaks in the 2972 and 2920 $cm^{-1}$ regions indicate the aliphatic C-H group derived from the isoprenyl group. Furthermore, the wavenumber of 1602 $cm^{-1}$ indicates the presence of a carbonyl group (C=O) conjugated with C=C. The absorption peaks at 1544, 1485, and 1448 $cm^{-1}$ indicated the presence of aromatic C=C vibrations.

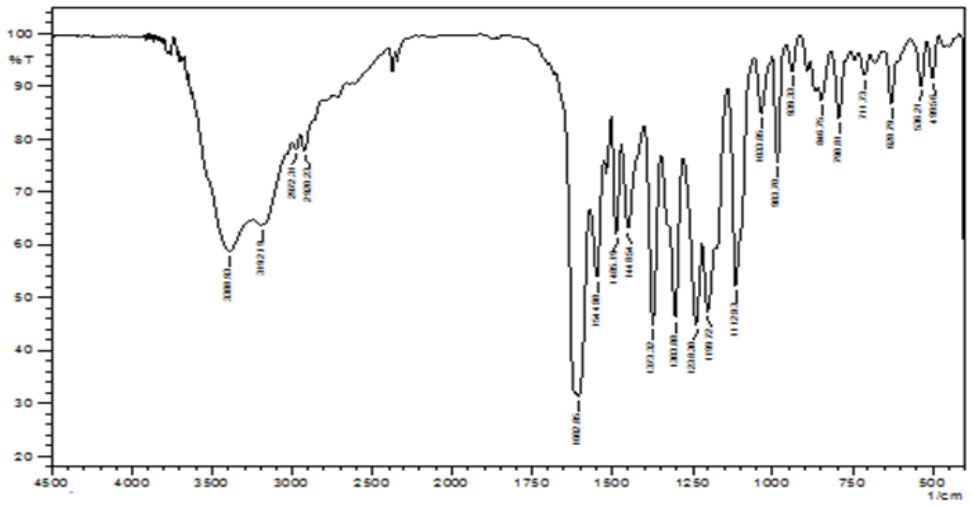


**Figure 1.** IR Spectrum of the compound 1

## *Nuclear magnetic resonance spectrometry (NMR) analysis*

The $^1$H-NMR spectrum of compound **1** shows the presence of a prenyl group as γ,γ-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 $^1$H-NMR spectrum analysis supports UV spectrum data, which shows that compound (1) is chalcone. The chalcone compound estimation is supported by the $^{13}$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 $^1$H-NMR and $^{13}$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 $CD_3OD$ | | The chemical shift of compound **1**, ppm $(CD_3)_2CO$ | |
|---|---|---|---|---|
| | $^1$H | $^{13}$C | $^1$H | $^{13}$C |
| α | 7.73 dd. (15.25 Hz) | 117.93 | 7.78 (1H. dd. J = 2.3; 15.4 Hz) | 116.72 |
| β | 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 |
| $CH_2$ , 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 |

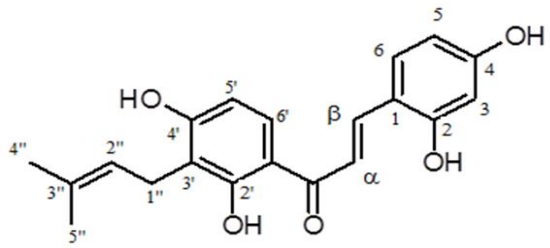


**Figure 2.** Molecular structure of compound (1), Moracalkon A

## *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 $IC_{50}$ values of 0.08 g/mL. Also, the antimalarial test results indicated that compound **1** gave $IC_{50}$ 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 $IC_{50}$ 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 | $10^{-7}$ | 10 | 9 | 9.5 | 90.48 |
| 3 | $10^{-6}$ | 7 | 9 | 8 | 76.19 |
| 4 | $10^{-5}$ | 9 | 8 | 8.5 | 80.95 |
| 5 | $10^{-4}$ | 9 | 8 | 8.5 | 80.95 |
| 6 | $10^{-3}$ | 1 | 0 | 0.5 | 4.76 |
| 7 | $10^{-2}$ | 1 | 0 | 0.5 | 4.76 |
| 8 | $10^{-1}$ | 0 | 0 | 0 | 0.00 |

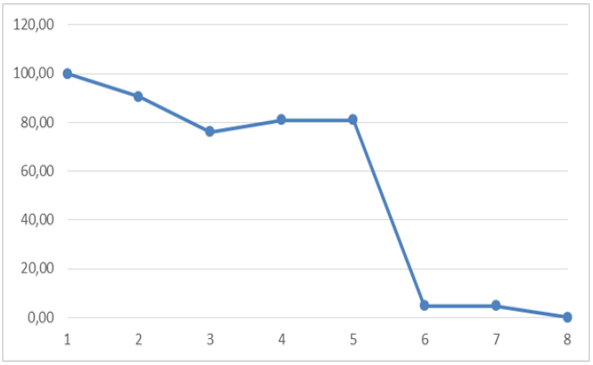


**Figure 3.** In vitro antimalarial assay of compound **1**

## *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 $IC_{50}$ 1.54 µg/mL. Also, $IC_{50}$ 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**

| | The optical density of compound 1 | | | The average optical density of compound 1 Average |
|---|---|---|---|---|
| **ppm** | **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 |

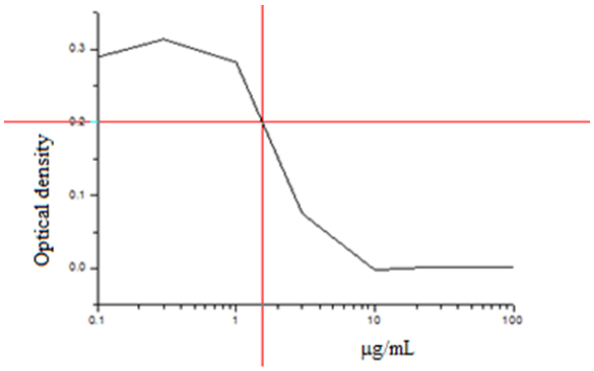


**Figure 4.** The graph of cytotoxicity test of compound **1** using leukemia cells P-388

## *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 $IC_{50}$ of 0.08 g/mL, anticancer using P-388 leukemia cells with $IC_{50}$ 1.54 g/mL. Also, its antibacterial activity against B. subtilis and E. coli is categorized as strong.

**Acknowledgments:** The authors would like to thank Dr. Elvira Hermawati, M.Si. and the late Ms. Suzany Dwi Ellita, S.Si. from Laboratory of Natural Product Chemistry, Bandung Institute of Technology for their help in the anticancer activity test and Ms. Puji Budi Setia Asih of Eijkman Institute for Molecular Biology, Jakarta, Indonesia for her help in the antimalarial activity test.

**Conflict of interest:** None

**Financial support:** This research was funded by the Ministry of Education, Culture, Research, and Technology, for financial support in the form of Basic Research 2021 with contract numbers of 120/E4.1/AK.04.PT/2021, 12 July 2021 and 3972/UN26.21/PN/2021, 14 July 2021.

**Ethics statement:** None

## References

1. Suhartati T, Achmad SA, Aimi N, Hakim EH, Kitajima M, Takayama H, et al. Artoindonesianin L, a new prenylated flavone with cytotoxic activity from Artocarpus rotunda. Fitoterapia. 2001;72(8):912-8. doi:10.1016/S0367-326X(01)00343-4
2. Suhartati T, Yandri, Hadi S. The Bioactivity Test of Artonin E from the Bark of Artocarpus rigida Blume. Eur J Sci Res. 2008;23(2):330-7.
3. Suhartati T, Hadi S. Artonin O, a Xanthone Compound from Root Wood of Artocarpus rigida. Orient J Chem. 2016;32(5):2777-84. doi:10.13005/ojc/320552
4. Suhartati T, Hernawan, Suwandi JF, Yandri, Hadi S. Isolation of Artonin E from The Root Bark of Artocarpus rigida, Synthesis of Artonin E Acetate and Evaluation of Anticancer Activity. Maced J Chem Chem Eng. 2018;37(1):35-42. doi:10.20450/mjcce.2018.1406
5. Musthapa I, Hakim EH, Syah YM, Juliawaty LD. Cytotoxic Activities of Prenylated Flavonoids from Artocarpus heterophyllus. ARPN J Eng Appl Sci. 2016;11(16):9754-8.
6. Gupta AK, Rather MA, Kumar Jha A, Shashank A, Singhal S, Sharma M, et al., Artocarpus lakoocha Roxb. and Artocarpus heterophyllus Lam. Flowers: New Sources of Bioactive Compounds. Plants. 2020;9(10):1329. doi:10.3390/plants9101329
7. Aneklaphakij C, Bunsupa S, Sirichamorn Y, Bongcheewin B, Satitpatipan V. Taxonomic Notes on the 'Mahat' (Artocarpus lacucha and A. thailandicus, Moraceae) Species Complex in Thailand. Plants. 2020;9(3):391. doi:10.3390/plants9030391
8. Suhartati T, Yandri, Suwandi JF, Hadi S. In vitro and in vivo antiplasmodial activity of oxyresveratrol and artonine isolated from two Artocarpus plants in Indonesia. Orient J Chem. 2010;26(3):825-30.
9. Pan WH, Xu XY, Shi N, Tsang SW, Zhang HJ. Antimalarial Activity of Plant Metabolites. Int J Mol Sci. 2018;19(5):1382. doi:10.3390/ijms19051382
10. Hafid AF, Ariantari NP, Tumewu L, Hidayati AR, Widyawaruyanti A. The Active Marker Compound Identification of Artocarpus champeden spreng Stembark Extract, Morachalcone A as Antimalarial. J Pharm Sci. 2012;4(Supp. 5):246-9.
11. Suhartati T, Epriyanti E, Borisha I, Yandri, Suwandi JF, Yuwono SD, et al. In Vivo Antimalarial Test of Artocarpin and in vitro Antimalarial Test of Artonin M Isolated from Artocarpus. Rev Chim. 2020;71(5):400-8.
12. Wongon M, Limpeanchob N. Inhibitory effect of Artocarpus lakoocha Roxb and oxyresveratrol on alpha-glucosidase and sugar digestion in Caco-2 cells. Heliyon. 2020;6(3):e03458. doi:10.1016/j.heliyon.2020.e03458
13. Ersam T, Achmad SA, Ghisalberti EL, Hakim EH, Makmur L, Syah YM. A New Isoprenylated Chalcone, Artoindonesianin J, from the Root and Tree Bark of Artocarpus bracteata. J Chem Res. 2002;2002(4):186-7. doi:10.3184%2F030823402103171618
14. Nomura T, Hano Y, Aida M. Isoprenoid-Substituted Flavonoids From Artocarpus Plants (Moraceae). Heterocycles. 1998;47(2):1184-99.
15. Nindatu M, Widyawaruyanti A, Asih PBS, Syafruddin D, Dahlan YP, Zaini NC, et al. Effects of Morachalcon a Compound Isolated from Artocarpus champeden Spreng Stembark on the Morphology of the Malaria Parasites. Res J Pharm Biol Chem Sci. 2015;6(2):1622-7.
16. Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids. Springer Verlag. Berlin, 1970.
17. Aldulaimi O, Uche FI, Hameed H, Mbye H, Ullah I, Drijfhout F, et al. A characterization of the antimalarial activity of the bark of Cylicodiscus gabunensis Harms. J Ethnopharm. 2017;198:221-5. doi:10.1016/j.jep.2017.01.014
18. Promchai T, Jaidee A, Cheenpracha, S, Trisuwan K, Rattanajak R, Kamchonwongpaisan, S, et al. Antimalarial Oxoprotoberberine Alkaloids from the Leaves of Miliusa cuneata. J Nat Prod. 2016;79(4):978-83. doi:10.1021/acs.jnatprod.5b01054
19. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria. XXII. The value of drug-resistant strains of P. berghei in screening for blood schizonticidal activity. Ann Trop Med Parasit. 1975;69(2):155-71. doi:10.1080/00034983.1975.11686997
20. Hadi S, Noviany N, Qudus HI, Wattana-Amorn P. The Potency Study of Organotin (IV) 3-Nitrobenzoate Compounds as Antimalarial Agents. J Phys Conf Ser. 2019;1338(1):012012. doi:10.1088/1742-6596/1338/1/012012
21. Hadi S, Fenska MD, Noviany N, Satria H, Simanjuntak W, Naseer MM. Synthesis and antimalarial activity of some triphenyltin (IV) aminobenzoate compounds against Plasmodium falciparum. Main Metal Group Chem. 2021;44(1):256-60. doi:10.1515/mgmc-2021-0028
22. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, et al. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 1988;48(3):589-601.
23. Davis WW, Stout TR. Disc Plate Method of Microbiological Antibiotic Assay. Appl Microbiol. 1971;22(4):659-65.
24. Samsuar S, Simanjuntak W, Qudus HI, Yandri Y, Herasari H, Hadi S. In Vitro Antimicrobial Activity Study of Some Organotin (IV) Chlorobenzoates against Staphylococcus

aureus and Escherichia coli. J Adv Pharm Edu Res. 2021;11(2):17-22.

25. Hadi S, Lestari S, Suhartati T, Qudus HI, Rilyanti M, Herasari D, et al. Synthesis and comparative study on the antibacterial activity organotin (IV) 3-hydroxybenzoate compounds. Pure Appl Chem. 2021;93(5):623-8. doi:10.1515/pac-2020-1103
26. Hadi S, Samsuar S, Simanjuntak W, Qudus HI. In Vitro Antibacterial Activity of Some of Dibutyltin(IV) Chlorobenzoate Derivatives against Staphylococcus aureus and Escherichia coli. ARPN J Eng Appl Sci. 2021;16(15):1623-8. doi:10.1515/pac-2020-1103.