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Anticancer Activity Study of Modified Artocarpin Compound from Pudau Plant (*Artocarpus kemando* Miq.)

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Abstract

This research is a continuation of the successful isolation of artocarpin from the root of Artocarpus kemando Miq reported in our previous study. In the previous study, the artocarpin was characterized with UV-Vis and FTIR techniques. In this follow-up investigation, the artocarpin was subjected to a transesterification reaction using acetic anhydride and pyridine as catalysts, and the product of the reaction was specified as compound 1. The compound 1 was further characterized with different techniques to gain more complete data and then tested for anticancer activity test against P-388 murine leukemia cells. Characterizations of the compound 1 using ^{1}H-NMR and ^{13}C-NMR techniques suggest that the modification reaction resulted in the conversion of the -OH groups at C2' and 4' at the artocarpin molecule to -OOCH$_3$, and based on the MS analysis, the compound was proposed to have the molecular formula of $C_{30}H_{32}O_8$. Another important feature of compound 1 that should be noted is the significant improvement in stability compared to the unmodified artocarpin. Anticancer activity tests against P-388 murine leukemia cells revealed that compound 1 has an IC_{50} of 2.35 µg/mL, confirming that the compound is categorized as an active anticancer agent and suggesting that the compound has promising potential that deserves further investigations.

Keywords:
Acetic Anhydride;
Artocarpin;
Leukemia P-388 Cells;
Modification.

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1- Introduction

In the search for bioactive compounds, natural products isolated from plants are of particular importance for several reasons. The diversity, availability, and accessibility of plants offer attractive opportunities to discover many useful compounds, even novel compounds, with various potentials, such as natural medicines to treat different diseases. Natural medicines produced from plants are also very useful in medical research and drug development [1]. Many secondary metabolites contained in plants, including active chemicals and bioactive components, have been recognized to have physiological and pharmacological effects, and therefore they have been utilized as antioxidants, antimicrobials, anticancer, anti-hemolytic, antibiotics, antiplatelet, anticoagulants, antidiabetics, and antifungals [2–6].

Of various plants, Artocarpus (which belongs to the Moraceae family, which encompasses around 50 species) is a promising source of bioactive compounds since different parts of this plant, including leaves, bark, stems, and fruits, contain a variety of bioactive compounds [7]. The results of extensive phytochemical studies on Artocarpus plant species have revealed that this plant species is rich in a variety of secondary metabolites, including triterpenoids, prenylated stilbene, arylbenzofurans, and flavonoids [8–11]. *A. kemando* Miq. is a genus of Artocarpus that has been reported as a plant rich in secondary metabolites, such as moracalcone A, artoindonesianin C, artomandin, artonol B, artosimmin, artonin E, artonin O, artobilosanthone, and cycloartobilosanthone [12–14].

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Cancer is one of the diseases that remains a serious health concern and is reported as the cause of the second-highest death rate in the world. As an example, in the United States, it is predicted that new cases will reach a total of 1,918,030 in 2022, with a mortality rate of 609,360 cases [15]. According to the International Agency for Research on Cancer, 396,914 new cases of cancer were reported in Indonesia in 2020, with a mortality rate of 234,511 [16]. The most common cause of cancer is changes (mutations) in the genes of cells, leading to an uncontrolled proliferation of abnormal cells that spreads throughout the body. Up to the present time, cancer treatment is mostly based on chemotherapy, although this treatment also causes detrimental effects on health such as drug resistance, nausea, and vomiting [17]. Furthermore, chemotherapy is associated with long-term harmful influences on health.

Harmful side effects of chemotherapy are the driving force behind extensive investigations devoted to finding alternative and safer anticancer drugs. In this respect, one of the most promising groups of plant-derived compounds are flavonoids. This group of compounds is reported to have significant properties in relation to cancer, including anti-inflammation, protein kinase enzyme inhibitors, inhibitors of metastatic processes, stimulation of autophagy processes, targeting cancer stem cells, anti-angiogenicity, and inducing cancer cell differentiation [18]. For example, in a previous study by Musthapa et al. [19], flavonoids were reported to exhibit anticancer activity toward leukemia P-388 cells. In other works [20, 21], the anticancer activity of artonin E, artocarpin, and artoindonesianin L compounds has been reported. Furthermore, the inhibitory effects of cathepsin K have also been described by other workers [22, 23]. One of the plants from which flavonoid compounds have been isolated by many workers is *A. kemando* Miq [13, 14, 24-27], and it has been reported that in many cases, the flavonoids from this plant were found to exhibit positive bioactivity. Another plant reported to contain flavonoids with useful biological activities is Artocarpus. A flavonoid compound isolated from this plant and the derivatives of the compound have been reported to display biological activities worthy of further study, including antibacterial, antimalarial, anticancer, antivirus, and antifungal [28–32].

It has been acknowledged that the activities of flavonoid compounds are associated with the prenyl group on C3 in their molecular structure and the existence of phenolic groups [33, 34], especially on the A ring, where two or three hydroxyl groups are attached. The presence of several hydroxyl groups on the A ring makes the compound more prone to oxidation into quinone. The change in the function group as a result of the oxidation may reduce the activity of the compound, and therefore it is necessary to alleviate this drawback without causing the compound to lose its activity.

One of the effective methods to avoid this problematic oxidation is modification of the molecular structure of the flavonoid by converting the hydroxyl group into an ester [35]. In addition to increased stability, modification of the structure was also reported to boost the biological activities of flavonoid compounds. In a previous study [36], it was found that the addition of acetate groups at C-5 and C-7 of the flavone framework played a crucial role in the antidiabetic action of glucose absorption processes [36]. The results of the work by Sakao & Hou [37] indicated that, when compared to the parent compound, the modified flavonoid by acylation displayed higher antioxidant activity, anti-inflammatory activity, cytoprotective impact, and the potential to trigger apoptosis in cancer cells [37]. In our previous investigation [25], successful modification of artocarpin using acetic anhydride was achieved. The modified artocarpin was confirmed by physical properties, UV-Vis measurement, and FTIR characterization. The compound was also found to exhibit antibacterial activity against *Bacillus subtilis* and *Escherichia coli* [25]. As a continuation of the previous study, in this work the modified artocarpin compound was further characterized using NMR and MS techniques, followed by the determination of the anticancer cytotoxicity of the compound against leukemia P-388 cancer cells.

2- Methods

2-1- General

The main apparatus and instruments used in this study were a Stuart MP-10 melting point apparatus, an FT-IR spectrophotometer (*Prestige 21 Shimadzu),* and an NMR Spectrophotometer Agilent 500MHz ^{1}H-NMR, 125 MHz on ^{13}C-NMR, UV-Vis spectrophotometer *Cary*-100 UV-*Vis Agilent*. Vacuum Liquid Chromatography (VLC) using Si-gel Merck 60 and thin layer chromatography (TLC) with Si-gel plates (Merck Kieselgel 60 F254, 0,25 mm).

2-2- Sample Preparation

A. kemando Miq. roots were collected from Karang Anyar, Klaten Village, Penengahan, South Lampung, Lampung Province, on March 2019. The plant was identified and confirmed by Herbarium Bogoriense, Biological Research Center, Indonesia, and the Indonesian Institute of Science, Bogor, Indonesia. The procedures for extraction, isolation, and esterification of artocarpin to produce modified artocarpin (specified as compound **1**) are described in Sa'Diah et al. [25].

2-3- Bioactivity Test

The bioactivity test of the compound **1** was conducted based on the method of Alley et al. [38]. The flowchart of the research is presented in Figure 1.

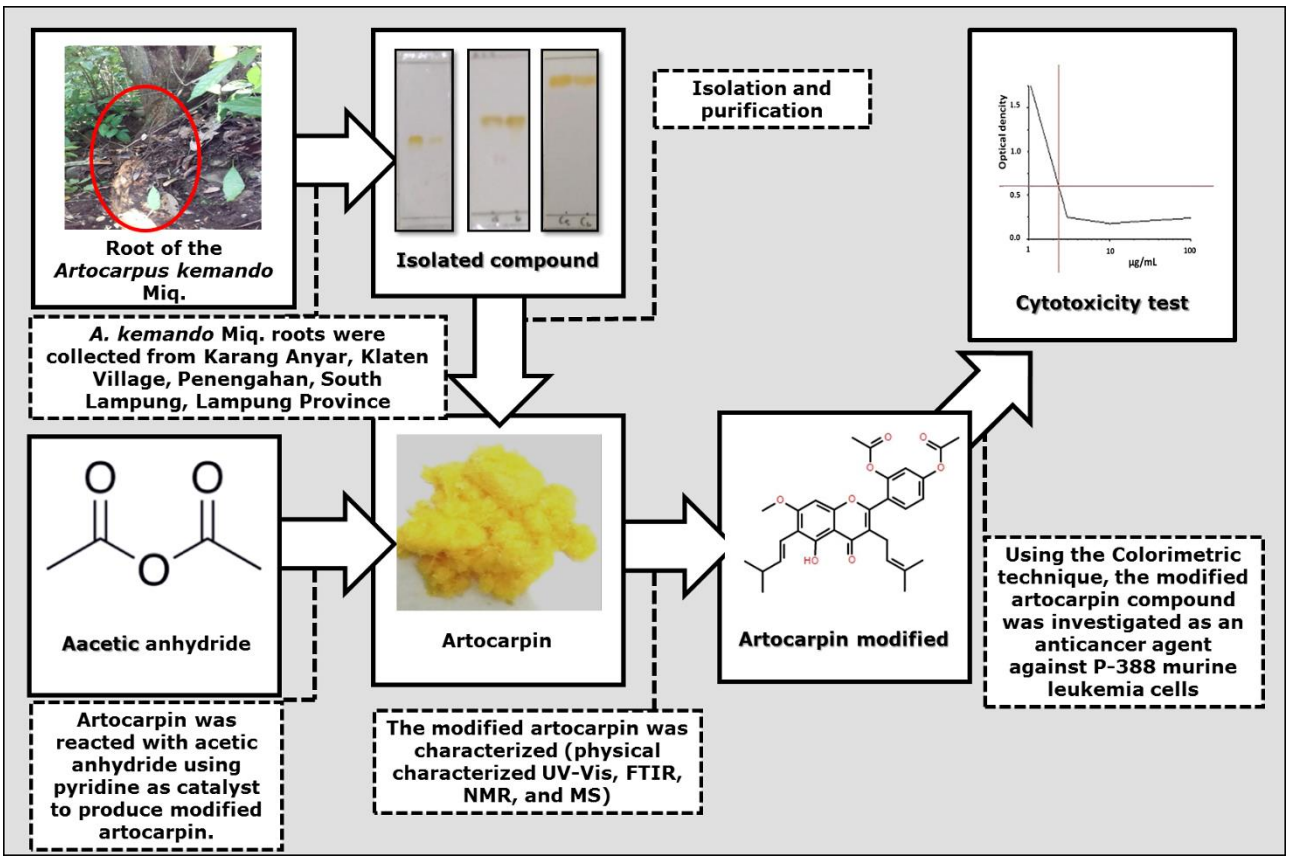

Figure 1. The flowchart of the research

3- Results and Discussions

As previously mentioned, characterizations of the compound **1** using UV-Vis and FTIR technique have been described in Sa'idah et al. [25].

3-1- NMR Characterization

Characterization of the compound **1** using NMR technique produced the ^{1}H-NMR spectrum presented in Figure 2 and the ^{13}C-NMR spectrum presented in Figure 3.

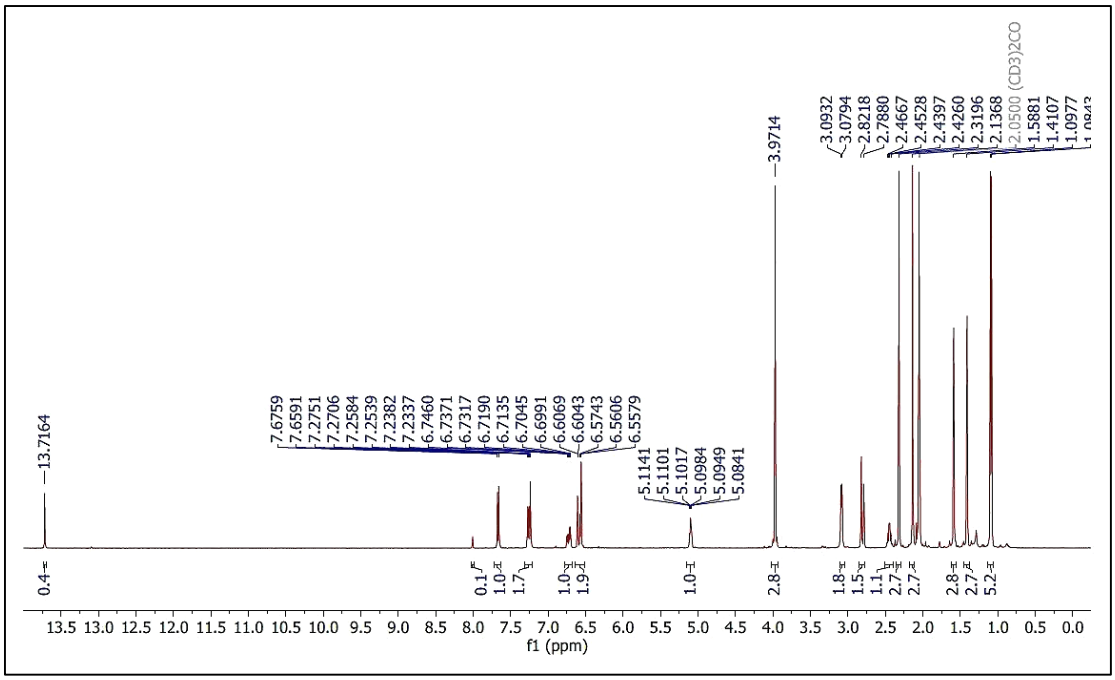

Figure 2. ^{1}H-NMR spectrum of compound 1

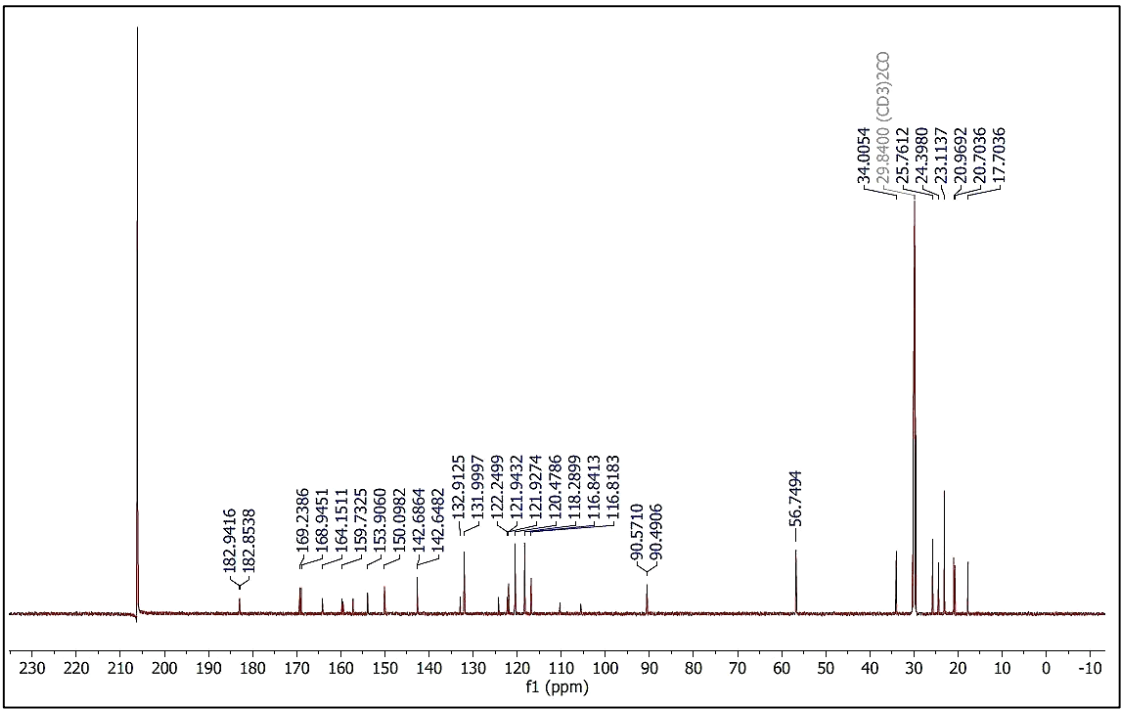

Figure 3. ^{13}C-NMR spectrum of compound 1

Based on the ^{1}H-NMR spectrum in Figure 2, there are two chemical shifts at 2.13 and 2.31 ppm originating from protons from the methyl ester acetyl group, indicating that the esterification of two phenolic groups in artocarpin has occurred, while one unesterified phenolic -OH group is in the chemical shift of 13.7164 ppm, which is due to the steric hindrance of the prenyl group and the formation of hydrogen bonds between the phenolic -OH at C-5 and the carbonyl at C-4.

The ^{13}C-NMR spectrum of compound 1 shown in Figure 3 is characterized by the presence of chemical shifts at d 182.85, 168.94, and 169.23 ppm, which are associated with three carbonyl carbons, together with chemical shifts of two methyl carbons at d 20.70 and 20.96 ppm. These features of the spectrum confirm that esterification of artocarpin involves two hydroxyl groups of the A ring on C2' and 4'. This finding is in agreement with the result of esterification of artonin E with acetic anhydride, which shows that the hydroxyl groups experiencing esterification are those attached to the A ring [39].

The HSQC spectrum showing the binding of proton on specific carbon atoms in compound 1 is presented in Figure 4, and detailed data are compiled in Table 1.

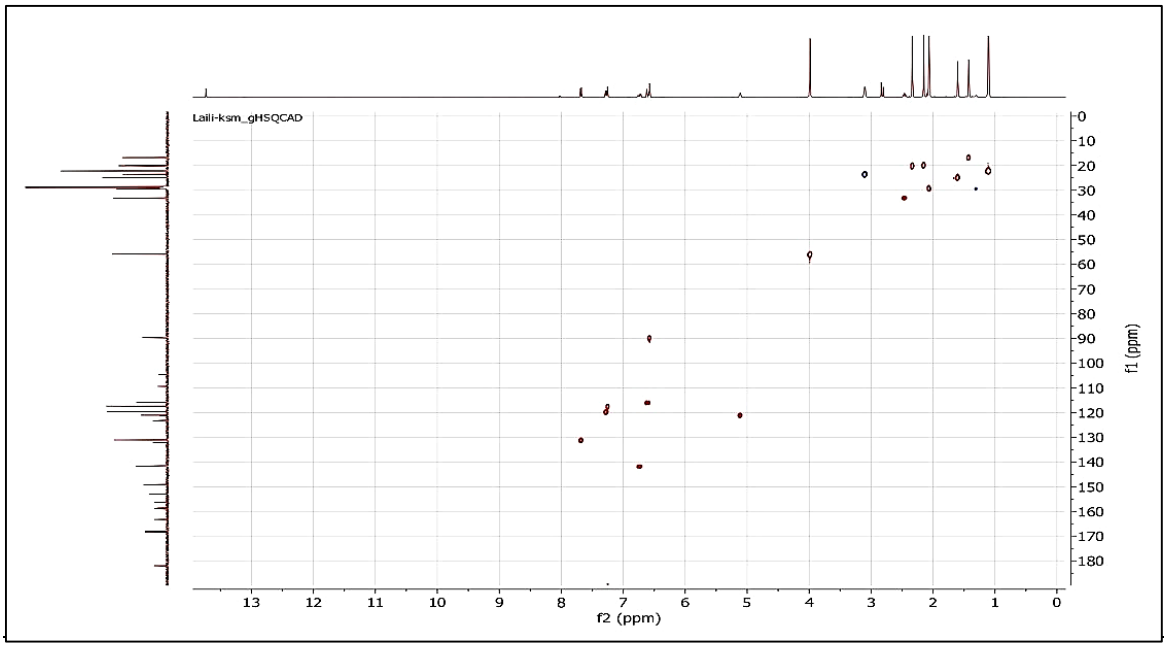

Figure 4. HSQC spectrum of compound 1

Table 1. HSQC spectrum data from compound 1

C Number	^{1}H-NMR	^{13}C-NMR
9	3.08	24.39
10	5.10	121.92
12	1.58	25.76
13	1.41	17.70
14	6.57	116.84
15	6.73	142.68
17	1.09	23.11
18	1.08	23.11
3’	7.23	118.28
6’	7.65	131.99
OCH_3	3.97	56.75
2’-CH_3	2.13	20.70
4’-CH_3	2.31	20.96

The HMBC spectrum which displays the position of hydrogens and carbons together with hydrogen-carbon in the compound 1 molecule is presented in Figure 5 and detailed data are compiled in Table 2.

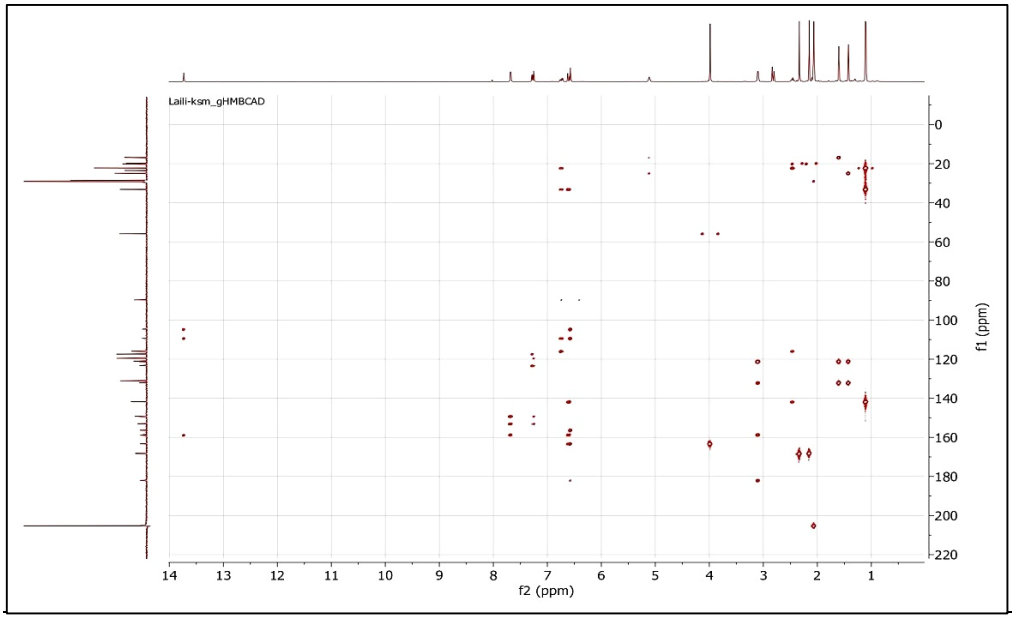

Figure 5. HMBC spectrum on compound 1

Table 2. Proton and carbon correlation spectrum data from HMBC spectrum on compound 1

C Number	δ (ppm)		HMBC Correlation
	^{1}H-NMR	^{13}C-NMR	
5	13.70 (-OH)	159.73	C-4a, C-5, C-6
8	6.61	90.57	C-4a, C-7, C-8a
9	3.08	24.39	C-3, C-4, C-10, C-11
12	1.58	25.76	C-10, C-11, C-13
13	1.41	17.70	C-10, C-11, C-12
14	6.57	116.84	C-5, C-6
15	6.73	142.68	C-14
16	2.43	34.00	C-14
17	1.09	23.11	C-15, C-16, C-18
18	1.09	23.11	C-15, C-16, C-17
3’	7.23	118.28	C-1’, C-2’, C-4’, C-5’
5’	7.27	120.47	C-4’
6’	7.65	131.99	C-2, C-1’, C-4’, C-5’
OCH_3	3.97	56.75	C-6, C-7
CH_3 2’- acetyl	2.13	20.70	2’-COO
CH_3 4’- acetyl	2.31	20.96	4’-COO

An important correlation obtained from the HMBC spectrum of compound **1** is shown in Figure 6. As can be seen, the hydroxyl group on C5 was not converted into ester, suggesting the existence of correlations between hydrogen atoms from the hydroxyl group on C5 with C4a and C6 carbon. This also means that the hydroxyl group on C-5 forms a hydrogen bond with the carbonyl group on C4, and because of its ortho position against the prenyl group on C6, this particular hydroxyl group is difficult to esterify due to its strong steric effect.

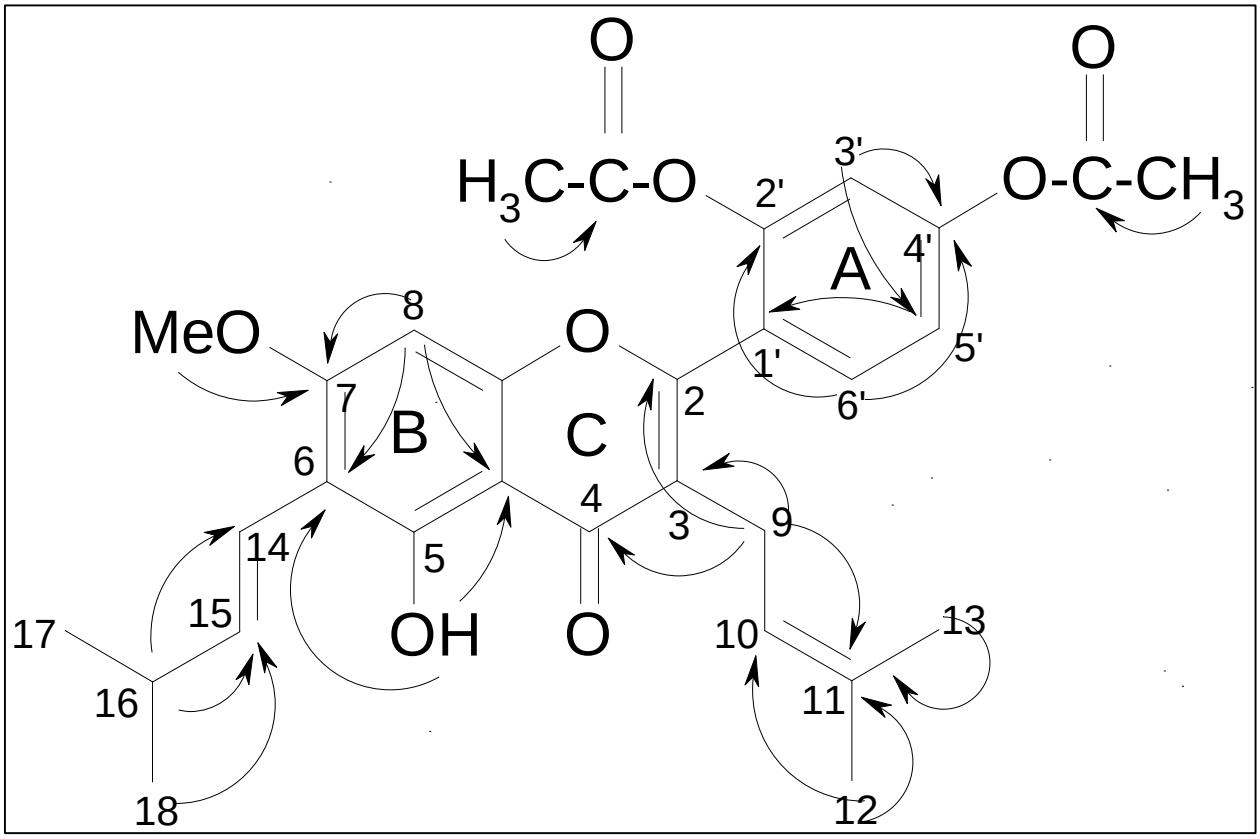

Figure 6. Important correlation from HMBC spectrum of compound 1

The successful modification of artocarpin to produce compound **1** is further supported by the HRMS spectrum (Figure 7), which confirms that the esterification only involved two hydroxyl groups. Its molecular formula was determined to be $C_{30}H_{32}O_8$ from the TOF MS ES+ ion at m/z 521.2175 ([M+H], calculated for C_{30} H_{33} O_8).

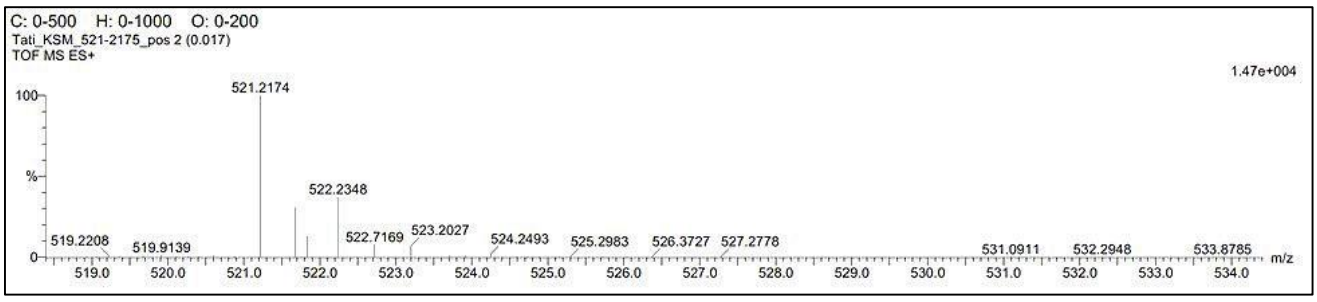

Figure 7. HRMS spectrum, TOF MS ES+ of compound 1

The chemical shifts of compound **1** provided by the ^{1}H-NMR and ^{13}C-NMR analyses and supported by HSQC and HMBC data compared to those of the artocarpin proton [19, 40] are compiled in Table 3.

3-2- Toxicity Test

The results of toxicity tests of compound 1 against murine leukemia P-388 cells, conducted using the colorimetric method [38], with triplicate measurements are shown in Table 4, and the results of IC_{50} determination performed using Origin 8.5 software are presented in Figure 8.

Table 3. **^{1}H-NMR chemical shift of artocarpin [19], ^{1}H- and ^{13}C-NMR of the modification result compound from artocarpin**

C Number	δ (ppm)		
	^{1}H-NMR, $(CD_3)_2CO$		^{13}C-NMR, $(CD_3)_2CO$ of Compound (1)
	Artocarpin [19]	Compound (1)	
2			159.53
3			122.24
4			182.85
4a			105.56
5		13.70 (-OH)	159.73
6			110.25
7			164.15
8	6.53 (1H, s)	6.58 (1H, *s*)	90.57
8a			157.22
1’			118.30
2’			157.2
3’	6.55 (1H, *d*, *J* = 2,2 Hz)	7.23 (1H, *d*, *J* = 2,3 Hz)	118.28
4’			153.91
5’	6.49 (1H, *dd*, *J* = 8,4 and 2.2 Hz)	7.25 (1H, *dd*, *J* = 8,4 and 2.3 Hz)	120.48
6’	7.18 (1H, *d*, *J* = 8.4 Hz)	7.65 (1H, *d*, *J* = 8.4 Hz)	131.99
9	3.06 (2H, *d*, *J* = 6.9 Hz)	3.08 (2H, *d*, *J* = 6.9 Hz)	24.39
10	5.1 (1H, m)	5.10 (1H, *t*, *J* = 7.15 Hz)	121.92
11			132.93
12	1.41 (3H, *s*)	1.41 (3H, *s*)	17.70
13	1.55 (3H, *s*)	1.59 (3H, *s*)	25.70
14	6.57 (1H, *d*, *J* = 16.1 Hz)	6.6 (1H, *d*, *J* =16.3 Hz)	116.84
15	6.70 (1H, dd, *J* = 16.1 and 7 Hz)	6.73 (1H, dd, *J* = 16.3 and 9.9 Hz)	142.68
16	2.41 (1H, *m*)	2.43 (1H, *m*)	34.00
17	1.07 (6H, *d*, *J* = 7 Hz)	1.09 (6H, *d*, *J* = 6.7 Hz)	23.11
18	1.07 (6H, *d*, *J* = 7 Hz)	1.09 (6H, *d*, *J* = 6.7 Hz)	23.11
7-OCH_3	3.94 (3H, *s*)	3.97 (3H, *s*)	56.75
2’-OCO			168.94
4’OCO			169.23
CH_3 2’-acetyl		2.13 (3H, *s*)	20.70
CH_3 4’-acetyl		2.31 (3H, *s*)	20.96

Table 4. **The results of toxicity test of compound 1 against murine leukemia P-388 cells**

Concentration, ppm	Optical density of compound 1			Average
100	0.236	0.237	0.24	0.237667
30	0.211	0.204	0.208	0.207667
10	0.171	0.178	0.177	0.175333
3	0.205	0.318	0.215	0.246
1	2.052	1.809	1.647	1.836
0.3	1.881	1.519	1.85	1.75
0.1	1.617	1.581	1.764	1.654

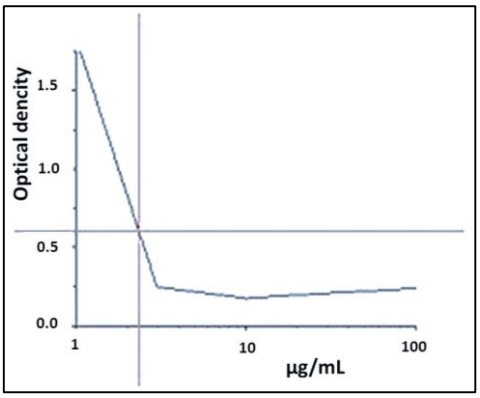

Figure 8. Cytotoxicity test of compound 1 using leukemia cells P-388 graph

A compound is categorized as an active anticancer compound for *in vitro* if the compound has the IC_{50} < 2 µg/mL (very active), IC_{50} 2-4 µg/mL (active), and IC_{50} > 4 µg/mL (inactive) [38]. The IC_{50} of compound **1** investigated in this study is 2.35 µg/mL, implying that the compound is categorized as an active anticancer compound. On the other hand, artocarpin was reported to have an IC_{50} of 1.9 µg/mL [21, 38] and was categorized as very active. This higher IC_{50} of the compound investigated is attributed to the OH group in B of artocarpin. Even though it has a higher IC_{50} than that of artocarpin, compound **1** is more resistant to discoloration during storage, as can be seen by the colors of the compounds presented in Figure 9. As can be seen, the color of the artocarpin displayed a significant change, while the color of the modified artocarpin is relatively the same as that of the fresh artocarpin. This difference in color suggests that modified artocarpin is more stable and therefore has a promising potential for further development.

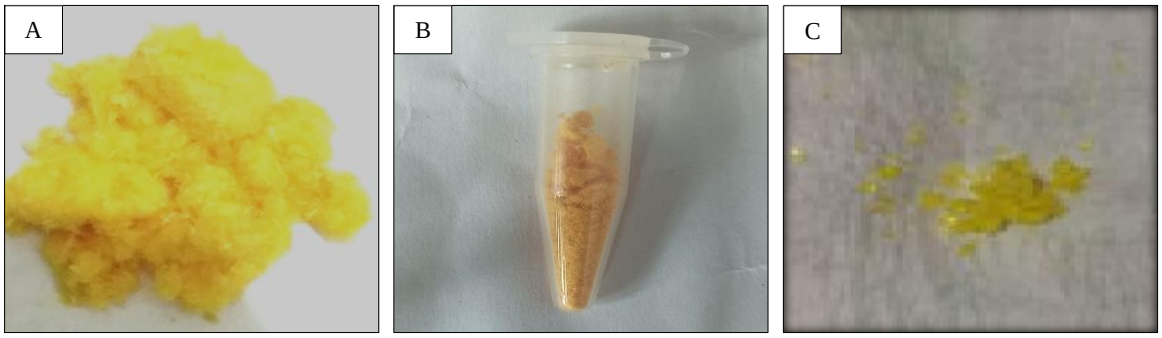

Figure 9. A) Isolated artocarpin compound, B) Isolated artocarpin compound during storage, and C) Modified artocarpin compound during storage

4- Conclusion

The results obtained in this investigation indicated that the esterification process to convert artocarpin isolated from the root of *A. kemando* Miq. into artocarpin acetate, specified as compound 1, was successfully achieved. The results of characterization of compound 1 using ^{1}H-NMR showed two new chemical shifts at 2.13 and 2.31 ppm originating from protons from the methyl ester acetyl group, indicating that the esterification of two phenolic groups in artocarpin has occurred, while the data of ^{13}C-NMR indicated the presence of chemical shifts at d 182.85, 168.94, and 169.23 ppm, which are associated with three carbonyl carbons, together with the chemical shift of two methyl carbons at d 20.70 and 20.96 ppm. These features of the spectra confirm that esterification of artocarpin involves two hydroxyl groups of the A ring on C2' and 4' at the artocarpin molecule to -$OOCH_3$ has occurred. Based on the MS analysis of compound 1, it was proposed to have a molecular formula of $C_{30}H_{32}O_8$. Another important feature of compound 1 that should be noted is the significant improvement in stability compared to the unmodified artocarpin. The improved stability of the modified compound (compound 1) is qualitatively displayed by the different color of the compound as compared to the color of the original artocarpin. Anticancer activity tests against P-388 murine leukemia cells revealed that compound 1 has an IC_{50} of 2.35 µg/mL, confirming that the compound is categorized as an active anticancer agent and suggesting that the compound has promising potential that deserves further investigations.

5- Declarations

5-1-Author Contributions

Conceptualization, T.S. and Y.Y.; methodology, T.S., Y.Y., and S.H.; software, S.T. and K.S.; validation, T.S., K.S., Y.Y., and S.H.; writing—original draft preparation, T.S. and K.S.; writing—review and editing, Y.Y. and S.H; visualization, K.S.; supervision, T.S., Y.Y., and S.H.; project administration, T.H. and S.H.; funding acquisition, T.S. All authors have read and agreed to the published version of the manuscript.

5-2- Data Availability Statement

The data presented in this study are available in the article.

5-3- Funding

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5-4- Acknowledgements

The authors acknowledged the Ministry of Education, Culture, Research, and Technology of Indonesia for assisting this study.

5-5- Institutional Review Board Statement

Not applicable.

5-6- Informed Consent Statement

Not applicable.

5-7- Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

6- References

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