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Efek Toksik Senyawa Flavon Prenilasi dari Fraksi Kloroform Kulit Batang Kaledang (Artocarpus Lanceifolius Roxb.) Secara In Vitro

Toxic Effects of Prenylated Flavone Compounds from the Chloroform Fraction of Kaledang (*Artocarpus Lanceifolius* Roxb.) Stem Bark In Vitro

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Abstrak
Artocarpus merupakan genus dalam famili Moraceae yang banyak mengandung senyawa flavonoid terprenilasi. Penelitian ini bertujuan untuk mengisolasi senyawa flavonoid terprenilasi dari fraksi kloroform kulit batang Artocarpus lanceifolius Roxb. Metode ekstraksi yang digunakan adalah maserasi bertingkat, dengan pelarut berbasis peningkatan polaritas. Fraksinasi dilakukan menggunakan Kromatografi Kolom Vakum (KVK), isolasi menggunakan Kromatografi Radial (KRK), dan elusidasi struktur menggunakan spektrofotometer UV-Vis, FTIR, 1H-NMR, 13C-NMR, dan NMR dua dimensi (HMBC dan HSQC). Uji toksisitas dilakukan menggunakan Brine Shirimp Lethality Test (BSLT) menggunakan Artemia salina Leach. Hasil isolasi menunjukkan adanya senyawa flavon terprenilasi, Artobilosanton. Uji toksisitas Artobilosanton menunjukkan nilai LC50 sebesar 3,36 µg/mL, sehingga sangat toksik.
Kata Kunci: *Toksisitas, Artocarpus Lanceifolius, Flavon terprenilasi, LC50*

Abstract
Artocarpus is a genus in the Moraceae family that contains many prenylated flavonoid compounds. This study aimed to isolate prenylated flavonoid compounds from the chloroform fraction of the stem bark of Artocarpus lanceifolius Roxb. The extraction method used was multistage maceration, using solvents based on increasing polarity. Fractionation was performed using Vacuum Column Chromatography (VCCh), isolation using Radial Chromatography (CR), and structure elucidation using UV-Vis spectrophotometers, FTIR, H-NMR, C-NMR, and two-dimensional NMR (HMBC and HSQC). Toxicity testing was carried out using the Brine Shirimp Lethality Test (BSLT) using Artemia salina Leach. The isolation results indicated the presence of a prenylated flavone compound, Artobilosanthone. The toxicity test for Artobilosanthone showed an LC50 value of 3.36 µg/mL, making it highly toxic.
Keywords: *Toksisitas, Artocarpus Lanceifolius, Flavon terprenilasi, LC50*

BACKGROUND

The search for new anticancer compounds from natural sources is a crucial research activity. This is driven by the fact that synthetic drugs used for chemotherapy in cancer treatment tend to be inefficient. Besides being very expensive, they also have side effects such as causing baldness, nausea, vomiting, and others. Therefore, efficient and affordable chemopreventive agents from natural sources are needed. One potential plant is the Moraceae family. This family consists of 60 genera and 1,400 species. Artocarpus is the largest genus containing flavonoid secondary metabolites. Chemotaxonomically, plants of the same genus and family tend to contain identical secondary metabolites. Artocarpus lanceifolius Roxb is one of the Artocarpus species, endemic to South Kalimantan

Based on data from the Global Cancer Observatory (GLOBOCAN), the International Agency for Research on Cancer (IARC) it is known that in 2022 there were 19.9 million new cases of cancer worldwide with a death toll of 9.7 million people (GLOBOCAN, 2022). Based on the 2018 basic health research of the Indonesian Ministry of Health, the prevalence of cancer in Indonesia increased from 1.4 per thousand population in 2013 to 1.79 per thousand population in 2018. The highest figure was in the Special Region of Yogyakarta Province with 4.86 per thousand population, followed by West Sumatra with 2.47 per thousand population, and Gorontalo with 2.44 per thousand population.

Medical cancer treatments such as chemotherapy, surgery, and radiation are extremely expensive. Cancer treatment still relies heavily on chemotherapy. Chemotherapy with anticancer drugs is hampered by side effects, such as inhibiting the growth of normal cells in the human body, such as hair growth, leading to baldness. Furthermore, it can cause nausea, vomiting, dizziness, and drug resistance. Therefore, the development of efficient and affordable

chemopreventive agents is essential. antibacterial activity (Soekamto, 2019), Search for antibacterial compounds of Artocarpus lanceifolius (Hasan, 2023). The use of Artocarpus plants as traditional medicine is related to the secondary metabolites they contain and chemotaxonomically, plants of the same family will contain identical chemical compounds

A literature search revealed no studies on the toxic effects of chemical compounds from the bark of Artocarpus lanceifolius Roxb. This study aimed to isolate chemical compounds from the bark of A. lanceifolius and then test their toxicity using the Brine Shrimp Lethality Test (BSLT). Testing for toxic effects with shrimp larvae is similar to the initial anticancer screening test.

METHODS

Tools and Materials

Masserator vessel, coarse and analytical balance, vials, evaporator, thin-layer chromatography plate (Merck Kiesel-Gel 60 GF254, 0.25 mm), vacuum liquid chromatography, UV-VIS spectrophotometer, FTIR spectrophotometer, NMR 500 MHz, radial chromatography, distillation, BSLT test vessel, aerator, UV lamps 254 nm and 366 nm, silica gel 60 GF254, n-hexane, ethyl acetate, chloroform, methanol. All solvents used were redistilled before use. Dimethylsulfoxide (DMSO), Artemia salina L. shrimp larvae, Artocarpus lanceifolius Roxb. bark powder.

Research Flow

This research follows a flowchart as shown in Figure 3. The stages include sample preparation, extraction using multistage maceration, fractionation to simplify the chemical composition, and purification to obtain pure compounds. The pure compounds were structurally elucidated using UV-Vis, FTIR, 1H-NMR, and 2C-NMR spectroscopy. Their toxic effects were then tested.

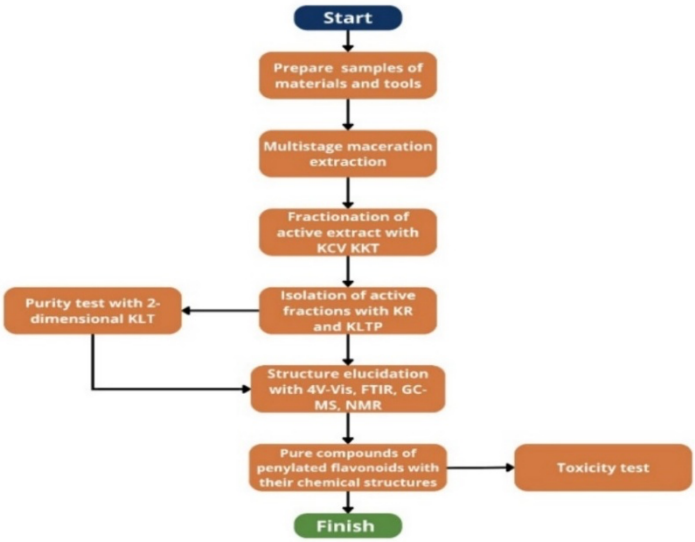

Figure 1. Research Flow

Toxicity Testing with Brine Shrimp Lethality Test (BSLT)

This toxicity test was based on Afriani's 2016 research, with slight modifications. The first stage involved hatching Artemia salina L. shrimp eggs into larvae after 48 hours. The second stage involved preparing test isolate solutions at varying concentrations of 5, 10, 20, 40, 80, and 160 µg/mL using DMSO as solvent. In the next stage, 10-15 larvae were placed in a vial containing 10 ml of seawater and extracted at each concentration. Seawater containing 10-15 shrimp larvae, without sample, was used as a control, and the larvae were left for 24 hours. The number of dead and surviving larvae was observed to determine the LC50 value.

RESULTS AND DISCUSSION

The extraction results using multistage maceration showed differences in the immersion of each fraction. The screening results can be seen in Table 1.

Table 1. Phytochemical screening results of n-hexane, chloroform, ethyl acetate, and methanol extracts of Artocarpus lanceifolius Roxb.

Extract	Flavonoids	Alkaloids	Terpen	Steroid	Tanin	Saponin
n-Heksan	-	+	+	-	-	-
Kloroform	+	+	-	-	-	-
Etil asetat	+	_	-	-	+	_

Methanol	+	+	+	_	+	-

+ : positive for the compound; - : does not contain compounds

The isolate was obtained as a brownish yellow powder. UV (MeOH) λmax (nm) (log ε): 388.5 and 269.5 (Figure 2). This isolate was obtained as a brownish yellow powder of 5.6 mg. Mg and HCl tests showed positive results as flavonoids. UV spectroscopic analysis measured at a wavelength (λ) of 200 - 700 nm in methanol solvent gave maximum absorption at wavelengths: 388 nm (band 1), 269 nm (band 2), which are characteristic of flavone compounds (Markham, 1988). Absorption at 269 nm (band 2) indicates the absorption of the benzoyl system. Absorption at 388 nm (band 1) shows the characteristics of the cinnamoyl system (Nomura, 1988).

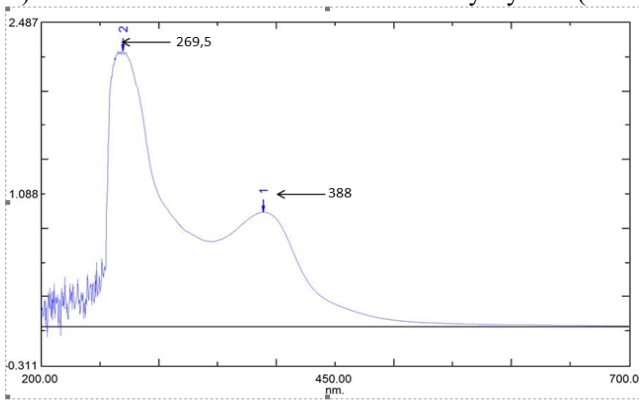

Figure 2. Spektrum UV-Vis

IR (KBr) υ_{max} (cm^{-1}): 3412 (OH), 2900, 2921, 2851 (CH alifatik), 1651 (C=O),1595,1560, (C=C aromatic) (Picture 3).

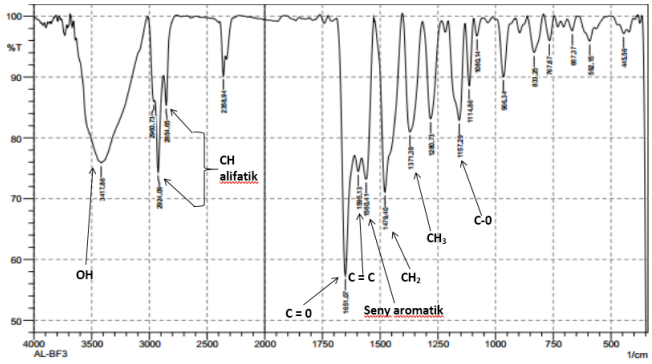

Figure 3. Spektrum FTIR

Spektrum **^{1}H-NMR** (500 MHz, aseton d_6) (ppm): 13,38 (OH, H-5); 6,13 (1H, s, H-6); 6,58 (1H, s, H-3'); 3,42 (1H, *d*, J=14,7 Hz, H-9); 2,43 (1H, *dd*, J=15,9; 6,5 Hz, H-10); 4,02 (1H, *d*, J=6,4 Hz, H-11); 4,30 (1H,s) dan 4,66 (1H, s) H-12; 1,78 (3H, s, H-13); 6,95 (1H*, d,* J=9,5 Hz, H-14); 5,67 (1H, *d*, J=10 Hz, H-15); 1,45 (3H, s, H-17); 1,49 (3H, s, H-18).

The 1H-NMR chemical shift (δ) data of the compound shows the presence of 22 protons. In the downfield region there is a chemical shift of 13.36 ppm (1H,s) which is a characteristic of protons from the chelated hydroxyl group at C-5 (Febriana and Ersam, 2016; Syah, 2016). The chemical shifts of 6.13 ppm (1H,s, H-6) and 6.58 ppm (1H,s, H-3') indicate the presence of 2 singlet proton signals in the aromatic region indicating that rings A and B have undergone pentasubstitution.

Proton 6.95 (1H, d, J=9.5 Hz, H-) has the same coupling as 5.67 (1H, d, J=10 Hz). This coupling value is suitable for cis coupling, which originates from isoprene bound to C-8 and cyclized with OH at C-7 to form a pyran ring (Syah, 2016). This is supported by the presence of signals 1.45 (3H, s, H-17) and 1.49 (3H, s, H-18.). Furthermore, chemical shifts of 3.42 (1H, dd, J=14.75 Hz, H-9) and 2.43 (1H, dd, J=15.9; 6.5 Hz, H-9) indicate a methylene group. Likewise, chemical shifts of 4.30 (1H, dd, H-12) and 4.66 (1H, dd, H-12) also indicate the presence of methylene groups and are characteristic for the dihydrobenzoxanthone framework. The presence of chemical shifts of 4.01 (1H, dd, J=6.4 Hz, H-10), 3.42 (1H, dd, J=14.75 Hz, H-9) and 2.43 (1H, dd, J=15.9; 6.5 Hz, H-9) show typical signals in the C-3 isoprene signal that has been cyclized to form a dihydrobenzoxanthone framework as a result of the C-C bond between C-10 and C-6'.

13C-NMR spectrum (125 MHz, acetone d6) (ppm): 162.1 (C, C-2); 110.9 (C, C-3); 180.7 (C, C-4); 105.0 (C, C-4a); 158.9 (C-OH, C-5); 6.13 (CH, C-6); 161.2 (C, C-7); 101.5 (C, C-8); 151.8 (C, C-8a); 106.0 (C, C-1'); 151.0 (C, C-2'); 103.2 (CH, C-3'); 150.4 (C, C-4'); 144.8 (C, C-5'); 129, 4 (C, C-6'); 21.7 (CH, C-9); 37.6 (CH, C-10); 136.2 (CH, C-11); 111.3 (CH2, C-12); 21.4 (CH3, C-13); 115.6 (CH, C-14); 127.3 (CH, C-15); 78.6 (C, C-16); 27.6 (CH3, C-17); 27.9 (CH3, C-18) Figure 5. 13C-NMR spectrum showing the presence of 25 carbon signal data consisting of 3 methyl carbons, 2 methylene carbons, 5 methine carbons and 15 quaternary carbons, which are consistent for a prenylated

flavone framework. This is supported by the carbon signal at a chemical shift of 181.2 ppm, typical for carbonyl in flavone framework compounds (Syah, 2016). Carbon signals with δC 27.6 (C-17); 27.9 (C-18); and 21.4 (C-13) are signals from methyl groups.

The carbon signals with δC 21.7 (C-9); and 111.3 µg/mL (C-12) are signals from the CH2 group. Five signals at δC 37.6 (C-10); 99.3 (C-6); 115.6 (C-14); 127.3 (C-15); and 103.2 µg/mL (C-3') show the typical characteristics of methine carbon. The presence of a carbon signal with δC 99.3 at C-6 indicates that the chromene ring formed in the structure is an angular chromene ring (Jayasinghe et al, 2008). Meanwhile, seventeen signals with δC 162.1 (C-2); 110.9 (C-3); 180.7 (C-4); 105.0 (C-4a); 158.9 (C-5); 150.4 (C-4'); 101.5 (C-8); 161.2 (C-7); 151.8 (C-8a); 106.0 (C-1'); 151.0 (C-2'); 144.8 (C-5'); 129.4 (C-6'); and 78.2 µg/mL (C-16) indicate quaternary carbon. In addition, there are also seven oxyaryl carbon signals. The seven signals are at δC 162.1 (C-2); 158.9 (C-5); 161.2 (C-7); 151.8 (C-8a); 151.0 (C-2'); 150.4 (C-4'); 144.8 ppm (C-5'). One carbonyl group 180.7 (C-4).

As additional evidence confirming the molecular structure of the compound based on a comparison of the chemical shift values of the compound with artobilosanton which has been previously reported (Sitularak, 2010). As shown in Table 2 below.

Table 2. Spektrum ^{1}H, ^{13}C NMR, and HMBC Artobilosanton

No	^{1}H NMR, $^{\delta}$ ppm (multiplisitas, J (HZ)		^{13}C NMR$^{\delta}$ ppm		HMBC
	1	1'	1	1'	
2	-		162,1	161,7	
3	-		110,9	111,4	
4	-		180,7	180,3	
4a	-		105,0	105,0	
5			158,9	159,0	
6	6.13 (1H.s)	6.13(1H.s)	99,3	99,7	C-5, C-7,C-8,C-4a
7	-		161,2	159,4	
8	-		101,5	99,8	
8a	-		151,8	152	
1'	-	-	106,0	105,5	
2'	-	-	151,0	149,8	
3'	6.58(1H.s)	6.60(1H,s)	103,2	103,5	C-1', C-5', C-4',C-6'
4'	-	-	150,4	144,5	
5'	-	-	144,8	136,2	
6'	-	-	129,4	127,8	
9	3,42(1H, *dd*, J =15,20 ; 6,5Hz) dan 2,43 (1H, *dd*, J=15,9; 6,5 Hz)	3,42(1H, d*d*, J =15,0);6,6 dan 2,44 (1H, dd, J=15,9; 6,5 Hz)	21,7	22,2	C-3, C-6', C-2, C-4 dan C-2, C-6', C-5', C-4
10	4,01 (1H, *dd*, J=15,19;6,4 Hz)	4.01 (1H,dd, J= 15,2; 6,4 Hz)	37,6	38,1	C-11, C-12, C-13
11	-	-	136,2	136,2	
12	4,30 (1H, s) dan 4,66 (1H,s)	4,66(1H, s) & 4,31 (1H, s)	111,3	111,4	C-13. C-10
13	1,78 (3H, s)	1,79 (3H, s)	21,4	22,2	
14	6,95(1H, d, J = 9,5	6,92(1H, d, J = 9,9 Hz)	115,6	116,1	C-8a, C-7, C-16
15	5,67 (1H, d, J =10 Hz)	5,67(1H, d, J = 9,9 Hz)	127,3	127,8	C-14, C-17, C-18, C-16
16	-	-	78,2	78,6	
17	1,45 (3H, s)	1,44(3H, s)	27,6	28,1	C-16
18	1,49 (3H, s)	1,47(3H, s)	27,9	28,4	C-16
	13,37 (OH,s)	13,37 (1H, s)			C-6, C-4a, C-5

1 ; Research result 1' ; Research result Sitularak 2010

The structure of the compound Artobilosanton is shown in Figure 3 below:

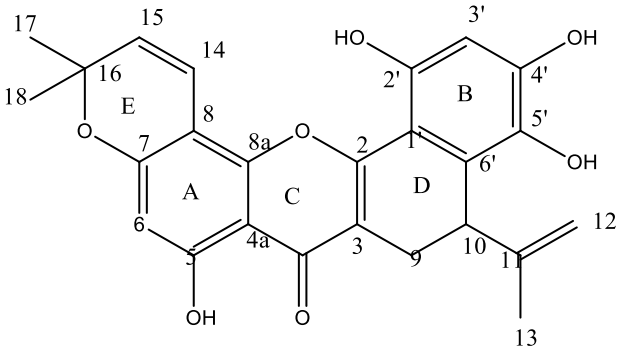

Figure 4. Structure of the compound Artobilosanton

The results of the toxicity test using the Brine Shrimp Lethality Test method are shown in Table 3 below:

Table 3. Nilai Toksisitas Artobolosanton (LC_{50})

Log (sampel)	Nilai Probit	LC_{50} (µg/mL)
-1,00	-	3,633
0,00	4,05	
1,00	5,95	

The test results of the Artocarpus lanceifolius Roxb isolate against Artemia salina showed an LC50 value of 3.36 µg/mL. This value is categorized as highly toxic. This toxicity is due to the presence of a prenyl group bound to the flavonoid structure. The prenyl group can increase the lipophilic properties of flavonoids, making them easier to enter and accumulate in cell membranes. Excessive accumulation can disrupt membrane integrity and cause ion/enzyme leakage, resulting in cytotoxic effects (Hatano, 2000).

CONCLUSION

The results of the isolation of prenylated flavone compounds from Artocarpus lanceifolius Roxb showed the presence of Artobilosanton compounds with an LC50 value of 3.36 µg/mL, a very toxic category.

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