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Tracing Antibacterial Compounds from Kaledang (*Artocarpus lanceifolius* Roxb.) Stem Bark

Hamsidar Hasan[1*], Teti S. Tuloli[1], Syamsul Bahri[2], La Ode Aman[3]
[1]Department of Pharmacy, Faculty of Sports and Health, Gorontalo State University, Indonesia
[2]Department of Animal Husbandry, Faculty of Agriculture, Gorontalo State University, Indonesia
[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Gorontalo State University, Indonesia

ABSTRACT

Artocarpus lanceifolius Roxb. or kaledang is a species of the mulberry family, Moraceae. Several extracts from its family have been reported to have antibacterial activity. Thus, it is necessary to determine compounds that have nature as antibacterial to be raw materials for antibacterial herbal medicines. The purpose of the study was to trace compounds that have antibacterial activity from *Artocarpus lanceifolius* stem bark. The study method was extraction by graded maceration, fractionation with column vacuum chromatography (CVC), isolation by radial chromatography, determination of antibacterial activity by agar diffusion method using the Gram positive and Gram negative bacteria. The characterization result of antibacterial compounds from *Artocarpus lanceifolius* chloroform extract stem bark showed the presence of prenylated flavonoid compound, namely 14-hydroxyartonin E (previously reported) with a diameter of inhibition zone at 5% concentration was 14.5 ± 0.1 mm against *S. aureus* (medium category), and 16.20 ± 0.2 mm against *S. mutan*s (strong category), 14.3 ± 0.2 mm against *E. coli* (medium category), and 16.8 ± 0.7 mm against *P. aeruginosa* (strong category). It is concluded that 14-hydroxyartonin E in *Artocarpus lanceifolius* showed potential antibacterial activity.

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**corresponding author*
Email: hamsidar.hasan@ung.ac.id

INTRODUCTION

Research on new drugs as an alternative to treating bacterial infections is mostly done by professionals. The study of antibacterial compounds is currently an urgent research activity, because many infectious diseases are caused by highly resistant bacteria (WHO, 2014;Ventola, 2015). In this decade, it was reported that 23.000 Americans died from untreatable bacterial infections due to antibiotic resistance (Xie et al., 2014). Various approaches have been taken to find new natural sources of antibacterial compounds, including plant materials.

Moraceae family is a high-level plant that is the target of the discovery of antibacterial compounds. This family generally contains prenylated flavonoids and some have been identified as antibacterial (Mawea, et al., 2019). Research about extracts of several species of *Artocarpus* showed *Artocarpus lanceifolius*, *Artocarpus elasticus*, and *Artocarpus odoratissimus* had potential as an antibacterial against Multidrug Resistance (MDR) *E. coli* isolated in Urinary Tract Infection (UTIs) (Prastitanto, 2021). Antibacterial activity on jackfruit (*Artocarpus heterophyllus*) leaves has also been reported with the zone of inhibition against *Staphylococcus aureus* is 10.8 mm, *Escherichia coli* is 9.2 mm, *Staphylococcus epidermis* is 9.6 mm and *Salmonella typhi* is 8.8 mm (Gurming et al., 2019). The extracts *Artocarpus altilis* were evaluated, the inhibitor diameters ranged from 8 mm to 16 mm (Araya et al., 2018). Phenyl group substitution with hydroxyl groups forming heterocyclics which may reduce activity (Xie et al., 2014). Prenylation can increase the hydrophobicity simplifying the partition into the bacterial cell membrane (Araya et al.,2018). *Artocarpus* plants are rich with phenolic compounds especially prenylated flavonoid and pyranoflavonoids (Jamil, et al., 2014). Research has shown that *Artocarpus lanceifolius* Roxb. contains isoprenylated flavonoids and a compound called 12-hydroxyartonin E (Setiawan et al., 2022). Thus, it is very important to explore compounds that have antibacterial activity from the bark of *Artocarpus lanceifolius.* According to Hakim (2011), *Artocarpus* genus compounds generally contain prenyl or isoprene groups. Plants of the same genus usually contain similar compounds. Based on this, searching for compounds that have antibacterial activity from *Artocarpus lanceifolius* bark is very important.

Artocarpus lanceifolius Roxb. is a species of genus *Artocarpus*. This plant is an endemic plant. Natives use the fruit as additional food, while the woodsare used as a building material for housing. Empirically, *A. lanceifolius* leaves are used reduce swelling and the bark is usually brewed to treat diarrhea (Heyne, 1987).

Diarrhea is associated with bacteria. The use as traditional medicine is based on hereditary experience and has not been proven by experimental so research on the activity of this plant is still needed.

Based on literature research, generally still refers to the antibacterial activity of the extract. Thus, the novelty of this research is there has been no research on tracing compounds that have antibacterial activity ranging from extracts to isolates of the stem bark of *Artocarpus lanceifolius* Roxb.

MATERIALS AND METHODS

Materials

Macerator, rotary evaporator, set of distillation apparatus, set of glass ware, petri dish, autoclave, laminar airflow, incubator, analytical balance, Thin Layer Chromatography (TLC) chamber, Column Vacuum Chromatography (CVC) was performed with silica gel (230-400 Mesh), Radial Chromatography (RC) (Cycoclograph, USA), Buchner funnel, the Agilent 8453 UV-Visible Spectrophotometer Hewlett Packard technologies, Fourier Transform Infrared (FTIR) Spectrophotometer (Prestige 21 Shimadzu, Japan) vernier caliper, magnetic stirrer, UV lamp 254 nm and 366 nm. The solvents used were normal-hexane 20 L (technical), ethyl acetate 20 L (technical), chloroform 20 L (p.a), methanol 20 L (technical), methanol 5 L (p.a), and technically redistilled before use,silicagel GF254 500 g, silica gel G60 500 g, NutrientAgar (Merck) 500 g, Nutrient Broth (Merck) 500 g, amoxicillin 500 mg (positive control), dimethyl sulfoxide (DMSO) 500 mL as negative control, discpaper, aquadest,TLC plate, 1H-NMR, 13C-NMR, Heteronuclear Multiple Bond Correlation (HMBC), Heteronuclear Single Quantum Coherence (HSQC).

Extraction and Fractionation of kaledang (*Artocarpus lanceifolius* Roxb) stem bark

Sampling was carried out in Paramasan Village, Banjar Regency, South Kalimantan Province and identified at Herbarium Bogoriense. The dry powder of the *A. lanceifolius* Roxb stem bark was extracted by staged maceration, starting with non-polar solvent (n-hexane), then chloroform (non-polar solvent), ethyl acetate (semi polar), and methanol (polar). Each maceration was carried out for 3 x 24 hours and the obtained maceration was concentrated with an evaporator until a thick extract was obtained. All extracts obtained were weighed to determine their total weight and their chemical components were monitored by TLC. Based on the TLC results, the extract that gave the largest spot and immersion was the chloroform extract. The four viscous extracts were tested for their chemical components content and antibacterial activity.

Table 1. Chemical component screening test result of the extract

Chemical component screening	Reagent	Extract			
		n-Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloid	Meyer	-	-	+	+
	Dragendorf	-	-	+	+
Flavonoid	HCl + Mg	-	+	+	+
Phenol	$FeCl_3$ + ethanol 70 %	-	+	+	+
Terpenoid	Lieberman Burchard	+	-	-	-

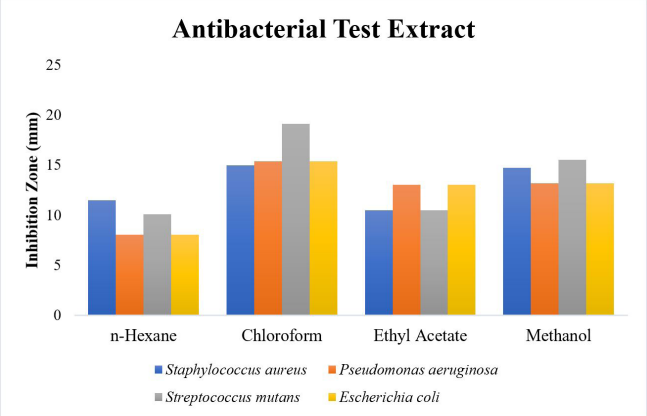

Figure 1. Antibacterial activity of *Artocarpus lanceifolius* Roxb extract

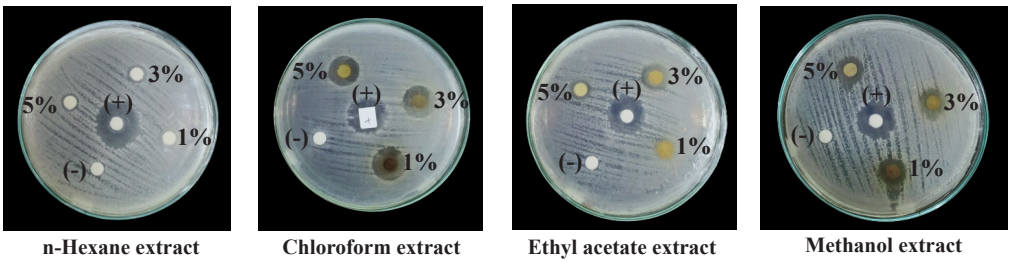

Figure 2. Antibacterial test results of n-hexane, chloroform, ethyl acetate, and methanol extract at several concentrations against *Streptococcus mutans*

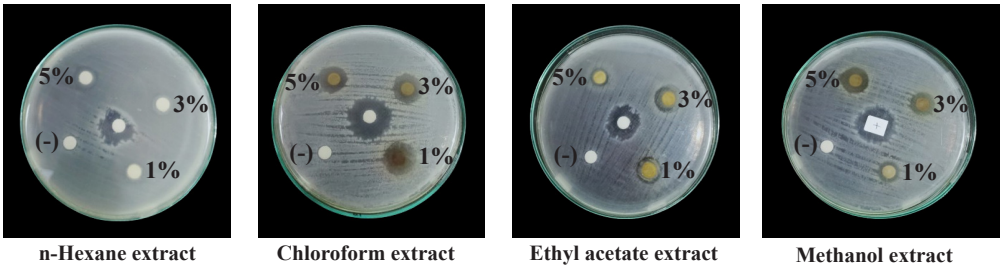

Figure 3. Antibacterial test results of n-hexane, chloroform, ethyl acetate, methanol extract at several concentrations against *Pseudomonas aeruginosa*

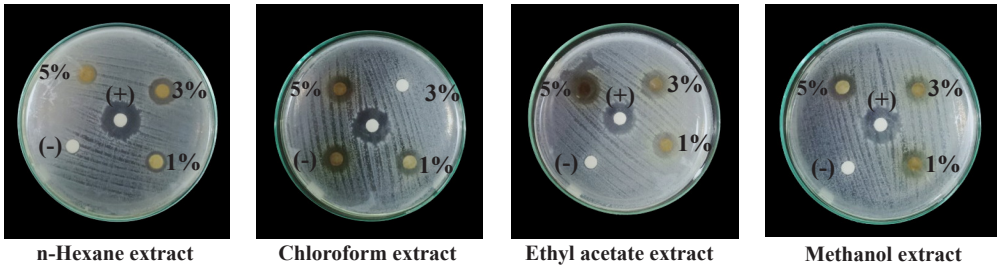

Figure 4. Antibacterial test results of n-hexane, chloroform, ethyl acetate, and methanol extract at several concentrations against *Escherichia coli*

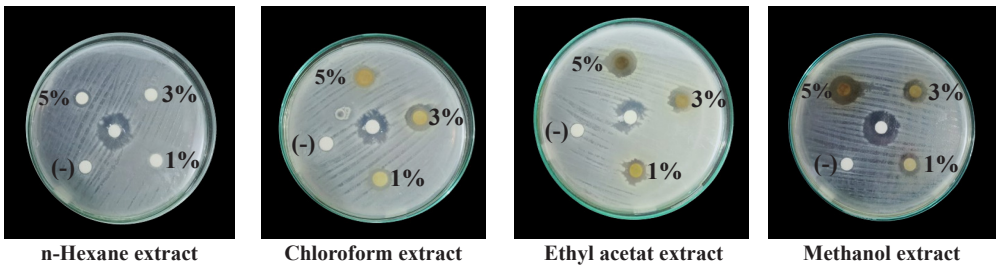

Figure 5. Antibacterial test results of n-hexane, chloroform, ethyl acetate, and methanol extract at several concentrations against *Staphylococcus aureus*

The chloroform extract was fractionated by column vacuum chromatography using n-hexane and ethyl acetate as eluent with a ratio based on increasing polarity of n-hexane, respectively, as follows: n-hexane:ethyl acetate 90:10, 80:20, 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90; ethyl acetate 100%. From the fractionation .we obtained 65 fractions. Based on TLC test, the same spots were combined and produced ten main fractions: A(4.83g), B(3.59g), C(2.19g), DEF(2.35 g), G(3.9 g), H(4.8 g), I(2.4 g), and J(1.98g). Ten main fractions were tested by TLC because of fractions D, E, and F had the same stain, so they were combined into DEF with a weight of 2.35 g.

Table 2. Antibacterial test results of 5% concentration of *Artocarpus lanceifolius* stem bark extract

Extract	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Streptococcus mutans*	*Escherichia coli*
n-Hexane	11.5 ± 0.09	8.05 ± 0.3	10.1 ± 1.4	8.05 ± 0.2
Chloroform	15 ± 0.04	15.35 ± 0.3	19.1 ± 1.33	15.35 ± 1.14
Ethyl acetate	10.5 ± 1.2	13.05 ± 0.9	10.5 ± 0.9	13.05 ± 0.9
Methanol	14.75 ± 1.06	13.18 ± 0.9	15.5 ± 0.25	13.16 ± 1.6

Data area mean ± standard deviation (n = 3)

Tabel 3. Antibacterial of *Artocarpus lanceifolius* stem bark fraction

Fraction	Diameter of Inhibition Zone (mm) at concentration 5%			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Streptococcus mutans*	*Escherichia coli*
A	10.3 ± 0.39	8.6 ± 0.49	10.2 ± 0.09	11.2 ± 0.2
B	9.0 ± 0.25	7.8 ± 0.29	8.5 ± 0.28	10.0 ± 0.1
C	7.0 ± 0.64	10.0 ± 0.7	9.0 ± 0.07	10.4 ± 1.82
DEF	14.05 ± 0.12	18.65 ± 0.14	16.45 ± 0.26	17.55 ± 0.9
G	10.3 ± 0.04	7.5 ± 0.25	8.1 ± 0.09	9.5 ± 0.04
H	8.4 ± 0.36	7.8 ± 0.25	8.3 ± 0.49	10.0 ± 0.31
I	8.9 ± 0.04	8.7 ± 0.09	11.0 ± 0.25	9.8 ± 0.01
J	6.9 ± 0.01	8.0 ± 0.19	7.0 ± 0.13	6.7 ± 0.28

Data area mean ± standard deviation (n = 3)

Separation, Purification, and Analysis of Pure Compounds of DEF Fraction

Separation and purification of the DEF fraction were carried out using a radial chromatography (RC) technique with the appropriate eluent from the TLC analysis. The purity test of the compounds obtained was carried out by TLC analysis using three eluent systems and analyzed by 1H-NMR. 13C-NMR, HSQC, HMBC, FTIR spectrophotometer and UV-vis spectrophotometer.

Preparation of Extract Test Solution

The viscous extracts of n-hexane, chloroform, ethyl acetate, and methanol were weighed 1 g, 3 g, and 5 g, respectively, then dissolved in 100 mL DMSO to obtain a concentration of 1%, 3%, and 5%. The A-J fraction (without DEF) were weighed into 50 mg, 150 mg, and 250 mg respectively, then dissolved in 5 mL DMSO to obtain a concentration of 1%, 3%, dan 5%.

The DEF fraction was weighed 10 mg, 30 mg, and 50 mg respectively, then dissolved in 1 ml of DMSO to obtain a concentration of 1%, 3%, and 5%. Antibacterial activity testing was carried out on all variations of extract concentration and subfraction using disc diffusion method for antibiotic sensitivity test.

Antibacterial Activity Test of Extracts, Fractions, and Subfractions

This test refers to previous studies (Zakaria et al., 2017; Suhartini et al. 2019; Hayun et al., 2014) through several stages such as preparation of Nutrient Agar (NA) solid media, preparation of Nutrient Broth (NB) liquid media, preparation of bacterial suspension, and inhibition test using the disc diffusion method. Antibacterial activity can be seen by observing the clear zone around the paper disc. The clear zone formed was measured using a caliper.

RESULTS AND DISCUSSION

The staged maceration of *A. lanceifolius* stem bark yielded the following amounts of extracts: 32.1 g of n-hexane extract, 36.5 g of chloroform extract, 21.4 g of ethyl acetate extract, and 24.5 g of methanol extract. Chemical components screening of the extracts was conducted using colorimetric tests, and the results are presented in Table 1.

The screening revealed that the chloroform, ethyl acetate, and methanol extracts contained flavonoids, alkaloids, and phenol while the n-hexane extract contained terpenoid. The presence of these chemical components is closely related to the pharmacological activities of the extracts (Hakim, 2011).

Table 4. DEF subfraction antibacterial activity test results at 5% concentration

Sample Name	Test Bacteria	Average Inhibition Diameter (mm) for 24 hours				
		DEF 2 (D2)	DEF 3 (D3)	DEF 4 (D4)	DEF 5 (D5)	Control +
DEF	*S. aureus*	9.16±0.04	10.4±0.72	10.2 ±0.72	14.5 ± 0.1	13.45±0.29
	S. mutans	10.34±0.36	11.55±0.4	13.3 ± 0.02	16.2± 0.2	17.65±0.25
	E. coli	17.55±0.78	9.55±0.3	12.1 ± 0.25	14.3 ± 0.2	17.00±0.25
	P. aeruginos	18.65±0.55	11.8±0.41	13.1 ± 0.38	16.8± 0.7	17.75±0.7

Data area mean ± standard deviation (n = 3)

Table 5. Wavenumbers and functional groups of FTIR spectrophotometer results

Functional Group	Wavenumber (Cm^{-1})	Functional Group	Wavenumber (Cm^{-1})
OH	3392	CH_2	1431
C-H	2970	CH_3	1362
C-H	2928	C-O	1153
C-H	2858		
C=O	1653		
C=C	1566		

All the extracts were subjected to TLC using n-hexane:ethyl acetate eluent with various ratios. This step aimed to determine the suitable eluent for the subsequent fractionation process. Fractionation is a process of separating chemical compounds within an extract based on their polarity. Column vacuum chromatography was employed for the fractionation process.

Among the extracts, the chloroform extract showed the largest spot and immersion on TLC. This finding is consistent with the results of antibacterial testing, where all the extracts demonstrated the largest zone of inhibition against the test bacteria (Figure 1, Figure 2, Figure 3, Figure 4, and Figure 5). Subsequently, fractionation and purification were focused on the chloroform extract, considering its prominent antibacterial activity and higher chemical content.

The results of the antibacterial test showed that there were different zones of inhibition in some extracts. Table 2 and Figure 1 showed the inhibition zone range was 10.1 - 19.1 mm against *Streptococcus mutans* bacteria, 10.5 - 15 mm against *Staphylococcus aureus* (Gram-positive). As for Gram-negative bacteria, the inhibition zone was 8.05 – 15.35 mm against *Pseudomonas aeruginosa* and 8.05 – 15.35 mm against *Escherichia coli*.

Chloroform extract showed the greatest inhibition at a 5% concentration of 19.10 mm against *Streptococcus mutans*, 15.35 mm against *Pseudomonas aeruginosa*. The inhibition zone of the chloroform extract was included in the strong category (Greenwood, 1995; Zakaria et al*,* 2017) and the highest value among the 4 extracts so that it was continued for fractionation.The antibacterial activity of the extract against both Gram positive and Gram negative bacteria is closely associated with its chemical composition. According to Jamil et al. (2014), *Artocarpus lanceifolius* is rich with phenolic compounds especially prenylated flavonoid and pyran flavonoids. Prenylation usually renders flavonoids with improved bioactivities. The mechanism of action is prenylation increases the lipophilicity of flavonoids, which results in a higher affinity to biological membranes and a better interaction with target proteins (Osorio et al, 2021).

Fractionation of chloroform extract by column vacuum chromatography yielded 65 fractions. After combining, the resulting 8 main fractions, namely A(4.83 g), B(3.59 g), C(2.19 g), DEF(2.35 g), G(3.9 g), H (4.8g), I(2.4g), J(1.98g). TLC monitoring showed that the D, E, F fractions produced the same stain, so they were combined into the DEF fraction. The antibacterial test results of fractions showed that the DEF fraction had the highest inhibition zone is shown in Table 3 and Figure 6.

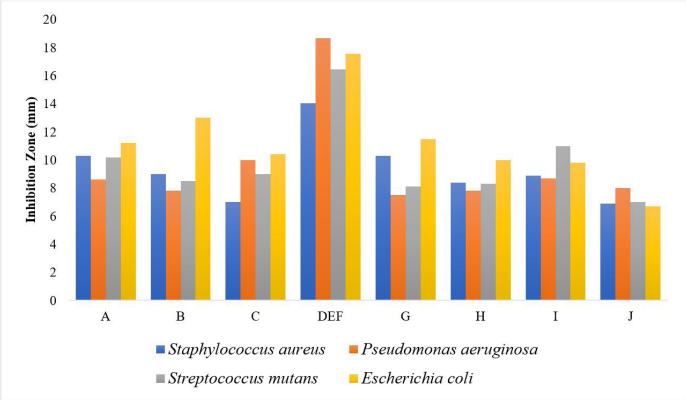

Figure 6. Antibacterial activity of *Artocarpus lanceifolius* according to the diameter of zone of inhibition

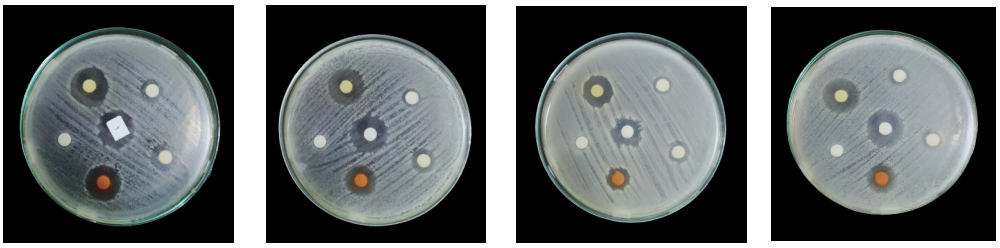

Figure 7. DEF subfraction antibacterial test results

Figure 6 shows the zone of inhibition for each fraction (A, B, C, DEF, G, H, I, and J). The antibacterial activity test of the DEF fraction showed the largest inhibition zone for Gram negative bacteria, which was 17.55 ± 0.36 against *Escherichia coli* and 18.65± 0.14 mm against *Pseudomonas aeruginosa*. In Gram positive bacteria, it was 14.05±0.12 mm against *Staphylococcus aureus*, and 16.45±0.26 mm against *Streptococcus mutans*. The DEF fraction was separated by radial chromatography, resulting in 5 subfractions, namely DEF_1, DEF_2, DEF_3, DEF_4, and DEF_5. DEF_1 subfraction was contaminated with fungi, rendering it unsuitable for further testing. The antibacterial evaluation of subfraction DEF_{2-5} is presented in Table 4 and Figure 7. Statistical analysis using SPSS 16 was conducted on *Staphylococcus aureus*, and subfractions DEF_2, DEF_3, and DEF_4 exhibited significant values of $p<0.05$ compared to the positive control. However, DEF_5 showed a value of $p>0.05$, indicating no significant difference. When tested against *Streptococcus* mutans, DEF_2, DEF_3, DEF_4, and DEF_5 all demonstrated significant values of $p<0.05$ compared to the positive control. This suggests that no group exceeded or was nearly equal to the inhibition produced by the positive control. In the case of *Escherichia coli*, DEF_3, DEF_4, and DEF_5 showed significant values of $p<0.05$. However, DEF_2 had a non-significant value of $p>0.05$ compared to the positive control, indicating no significant difference. Based on this analysis, it can be concluded that subfractions DEF_2 and DEF_5 are suitable for further purification and isolation. Among these two subfractions, DEF_5 was found to be a single spot-on two-dimensional TLC tracing and displayed consistent results on TLC tests with various eluents. DEF_5 was analyzed by FTIR, 13C-NMR and 1H-NMR, HMBC, HSQC, and UV-Vis Spectrophotometer.,

Analysis of DEF_5 Subfraction Compounds

Results of compound analysis with UV-VIS spectrophotometer

Spectroscopic analysis of UV light measured at the wavelength (λ) 200 - 700 nm in methanol solvent gave λ_{max} of 386 nm (band 1) and λ_{max} 283 nm (band 2). The absorption at 386 nm (band 1) it showed the characteristics of the cinnamoyl system (Nomura et al.,1998). On that absorption indicated the presence of π-π* excitation which indicated the presence of conjugation (-C=C-C=O). The absorption at 283 nm (band 2) indicated the absorption of the benzoyl system. The absorption indicated the excitation of π–π* which is a typical chromophore for conjugated double bond systems (-C=C-C=C-) or on aromatic rings (Markham,1988). Spectrum UV-Vis shown in Figure 8.

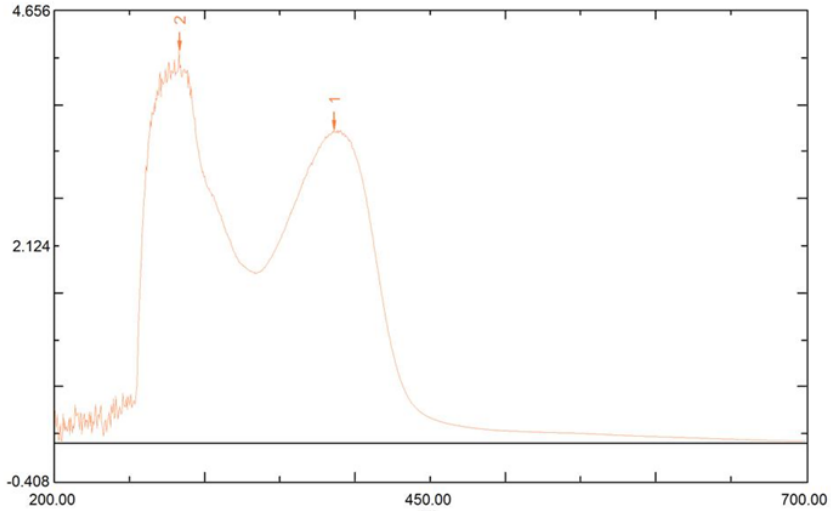

Figure 8. Spectrum UV-VIS DEF_5

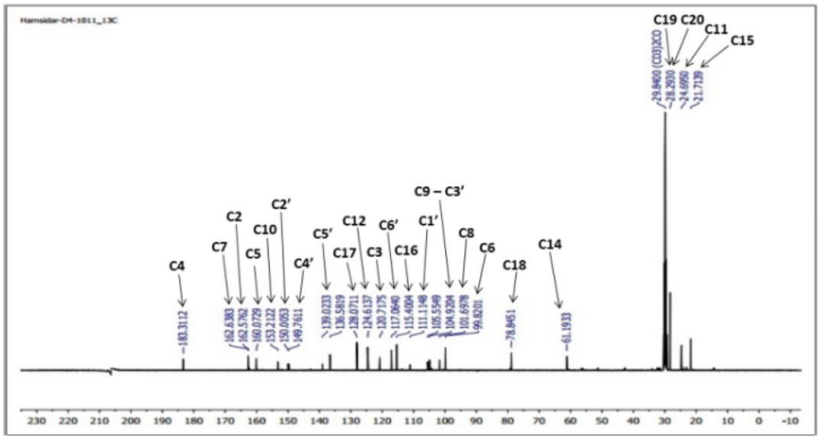

Figure 9. 13C-NMR spectrum of DEF_5 subfraction

Results of compound analysis with FTIR

Measurement of isolates with the FTIR spectrophotometer instrument showed DEF_5 subfraction contained several absorption bands at wavenumbers as a marker of the functional groups present in these compounds.

The wavenumbers and functional groups resulting from the FT-IR spectrophotometer are presented in Table 5. Table 5 showed the absorption band at wavenumber 1653 cm^{-1} identified as a conjugated carbonyl group (C=O). At wavenumbers 1566, 1521, and 1481 cm^{-1} indicated the presence of aromatic compounds.The absorption bands at wavenumbers 2970, 2928, 2858 cm^{-1} indicated aliphatic CH, which is supported by the presence of absorption bands at 1362 and 1431 cm^{-1} indicating CH_3 and CH_2 groups. in the 3392 cm^{-1} wave region indicated the presence of a hydroxy group (OH) which is supported by the presence of C-O at 1153 cm^{-1}.

Analysis results of DEF_5 subfraction compounds with 13C-NMR and 1H-NMR

Analysis of the DEF_5 subfraction compounds with 13C-NMR showed the presence of 25 carbon signals. The singlet signal at C-4 with a chemical shift of 183.3 showed the typical characteristics of flavones compound from the flavonoid group (Syah, 2016) as shown in Figure 9. This is supported by the results of the 1H-NMR spectrum that showed a chemical shift in the downfield region of 13.08 ppm (1H,s) which is a typical characteristic of protons from chelated OH at C-5 (Syafitri & Ersam, 2016) as shown in Figure 10.

Based on the HMBC and HSQC spectra, the proton signal at H 3.2 ppm (H-11) correlated with the carbon signal 162.5 (C-2); 120.7 (C-3); 183 (C-4); 127.5 (C-12); and 136.6 ppm (C-13), indicating that on H-11 there is a methylene group and an isoprene group attached to C-3.

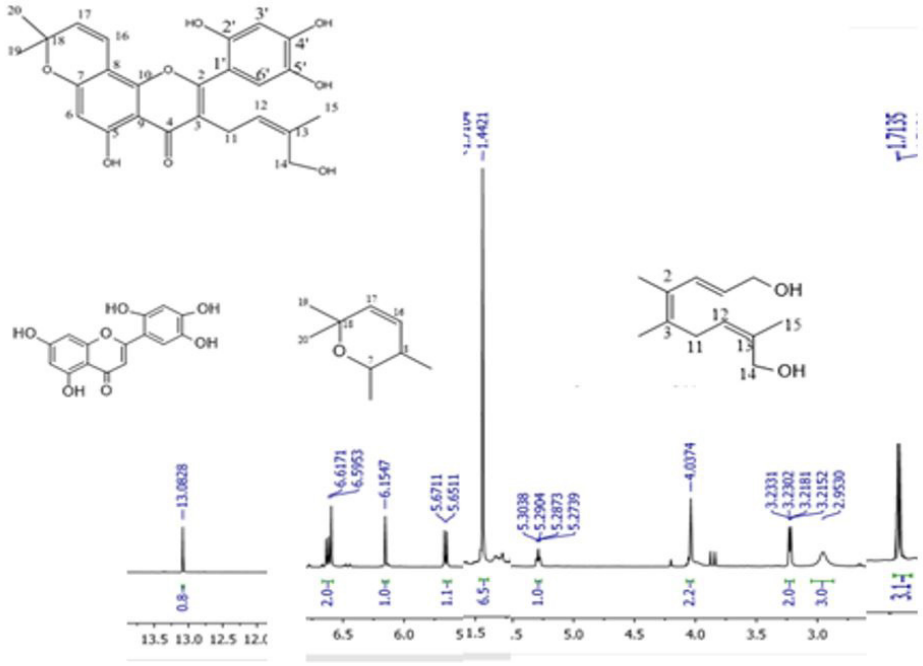

Figure 10. Spectrum of 1H-NMR DEF_5 subfraction

Furthermore, the chemical shift of 6.62 ppm (H-16) showed a long-range correlation with the carbon signal 101.7 (C-8); and 153.2 (C-10); 78.0 (C-18), and 160 (C-5) indicated the isoprene group bound to C-8. Based on this, it can be concluded that the isolate subfraction DEF_5 is a prenylated flavonoid compound that has been previously reported (Cao, 2003).The relationship between structure and activity is that the presence of a phenyl group in the structure will increase the lipophilicity of flavonoids (Yang et al., 2015) which causes flavonoids to become non-polar.

Non-polar compounds will more easily penetrate bacterial lipophilic membranes resulting in increased affinity for biological membranes and increased interactions with target proteins. In addition, polyhydroxy groups in ring A and ring B were identified as antibacterial activity of flavones (Hashim et al, 2012). The structure of the isolated compound as shown in Figure 11**.**

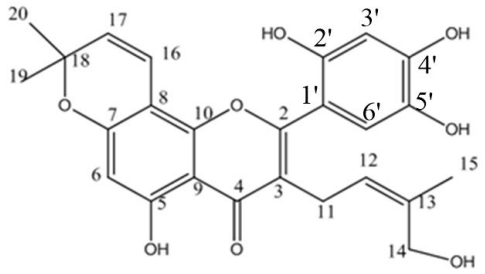

Figure 11. Structure of 14-hydroxyartonin E

CONCLUSION

It is concluded that 14-hydroxyartonin E in *Artocarpus lanceifolius* Roxb. stem bark showed potential antibacterial activity against *S. aureus* (medium category), *S. mutans* (strong category), *E. coli* (medium category), and *P. aeruginosa* (strong category).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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