Prenylated flavon and antibacterial activities of *Artocarpus lanceifolius* Roxb bark

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Abstract. Prenylated flavone compounds, 14-hydroxyartonin E have been isolated from CHCl3 extract of *Artocarpus lanceifolius* Roxb bark. The molecular structure compound was determined based on the UV-Vis, IR, 1H-NMR and 13C-NMR HSQC, HMBC spectra and compared to the previous data reported. 14-hydroxyartonin E compound provides antibacterial activity with a moderate category against *Streptococcus pneumonia* and *Staphylococcus aureus* but the category is weak against *Escherichia coli* and *Salmonella thyposa*.  

1. Introduction

Infectious diseases caused by bacteria, fungi, viruses, and parasites are a major threat to public health. Although remarkable progress has occurred in the human medical world, the impact is huge, especially in developing countries due to lack of sufficient drugs and the emergence of widespread drug resistance [1]. To date, the search effort of new antimicrobial compounds is an important research activity. Various approaches used in finding sources of new compounds including compounds from natural ingredients. This approach will be produced antimicrobial compounds targeting the bacterial metabolic system, thereby it will inhibit bacterial growth or even causing the death of the bacteria itself.

One of the plants able to grow in tropical and sub-tropical regions including in Indonesia with the potential as a source of bioactive chemicals in the family of Moraceae. Moraceae consists of 60 genera and 1400 species. The three main genera are Artocarpus, Ficus, and Morus. Artocarpus consists of 50 species and spread from South Asia, Southeast Asia to the Solomon Islands, Pacific Islands, North Australia, and Central America [2]. Ethnobotany, some parts of the Artocarpus plant by the community have long been used as traditional medicine. The extract of stem bark *A. elasticus* is used as a preventive agent, the gum used as dysentery and fever relief medicine [3]. The roots of *A. heterophyllus* are used to treat fever, dysentery, and malaria, the seeds for the treatment of diarrhea, the leaves are a remedy for boils, fever, wounds, and skin diseases [4]. The utilization of the Artocarpus plant as traditional medicine is related to the secondary metabolites contents.

The *Artocarpus lanceifolius* Roxb plant with the local name Kaledang is one of the Moraceae families. It's has been widely used as a producer of wood for heavy construction,
furniture, boat building, household utensils, as a coloring material, and the fruit can be consumed. Empirically the leaves of A. lanceifolius are used as anti-inflammatory drugs and steeping of the bark is believed to be a drug for diarrhea. Up to now the utilization of traditional medicine by the people still based on experience only. There is not supported by scientific data experimentally so that research on secondary metabolites and plant activity is still needed.

2. Experiments
2.1 General experimental procedure
Spectroscopy UV, FTIR spectrophotometer Shimadzu Prestige-21, Agilent NMR 500 MHz with DD2 console system that operates at 500 MHz (1H) and 125 MHz (13C) frequencies, using residual and deuterated solvent peaks as reference standards, VLC, and radial chromatography were carried out using Merck silica gel 60 GF254 and for TLC analysis, precoated silica gel plates (Merck Kiesel-gel 60 GF254, 0.25 mm) were used. All solvents were of technical grade and were distilled before use.

2.2 Plant material
The collected bark of Artocarpus lanceifolius Roxb from South Kalimantan Province on January 2018. It was identified by staff at the Herbarium Bogorience, Bogor Botanical Garden, Bogor Indonesia and, a voucher specimen has been deposited at the herbarium.

2.3 Extraction and isolation
The dried powdered bark of Artocarpus lanceifolius Roxb (6 Kg) was successively extracted with n-hexane, CHCl3. EtOAc and MeOH. The liquid extract CHCl3 was evaporated with a rotary evaporator to obtain thick chloroform extract (36.5 gr). All CHCl3 extracts were fractionated by VLC (Si-gel, n-hexane: EtOAc). The fractions eluted with n-hexane –EtOAc (1:1) was subjected to radial chromatography (Si-gel, n-hexane-EtOAc, 1:1) to yield, resulting in a compound (43.2 mg) in the form of yellow powder.

2.4 Antibacterial screening
The bacterial strains employed in screening were Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli, and Salmonella typhosa. The test method refers to reference [5]. Antibacterial activity of the compound was determinate by the disc diffusion method with slight modification in term of sample concentration, the bacteria were cultured at 37°C for overnight in nutrient broth. The compound to be tested were dissolved in dimethyl sulphoxide (DMSO) at concentration 100 ppm. For screening, 10 μL of each sample solution was loaded on Whatman No.1 filter paper disk ((Ø 6 mm). The disc was placed on the surface of the agar plate (Nutrient agar) previously inoculated with bacteria. The agar plate was then inverted and incubated for 24 h and 48 h at 37°C. The antibacterial activity was recorded by measuring the zone of inhibition (in mm) around each disc. Ampicillin was used as positive control and DMSO (Dimethylsulfoxide) as the negative control in assays.

3. Results and Discussion
3.1 Isolation
The results of prenylated flavones, 14-hydroxyartonin E isolated have the color of yellow powder, UV (MeOH) λmax nm (log e): 283 (4.23), 386 (3.38) show the structure of flavone [6],[7] The FTIR-Vmax (KBR) cm⁻¹ spectrum of Vmax 3392 cm⁻¹ shows the presence of
absorption bands for hydroxyl (OH) groups, a carbonyl (C=O) group conjugated at 1653 cm$^{-1}$, and a benzene ring at 1566 cm$^{-1}$, 1521 cm$^{-1}$, 1481 and 1431 cm$^{-1}$ (Figure 1) show the structure of flavone [7].

Figure 1. 14-hydroxyartonin E FTIR spectrum

Spectrum data are $^1$H-NMR (d$_6$-acetone, 500 MHz) and $^{13}$C-NMR (d$_6$-acetone, 125 MHz) (Table 1). In the $^1$H-NMR spectrum, $\delta$ 13.08 ppm (OH, s) shows chelate OH at C-5 [8] a characteristic of flavonoid. In the $^{13}$C-NMR spectrum, a quaternary carbon ($\delta_c$ = 183 ppm) shows a carbonyl group (C = O) conjugated to C-4 is a characteristic of a flavone [9]. These H and C signals can be explained in detail with the help of a two-dimensional NMR spectrum such as Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC). In the $^1$H-NMR(d$_6$-acetone, 500 MHz) spectrum shows the presence of signals for 2 methyl protons at $\delta$ 1.4 (6H, s), 1 methyl proton at $\delta$ 1.7 (3H, s) and $\delta$H 4.03 associated with 2 proton oxymethylene at C-14 [10]. This shows the presence of an isoprene group on the C-3 skeleton of flavones. It also supported by the presence of signals for 3 methyl groups ($\delta$ 28.3) with two methyl groups, $\delta$ 21.7 1 methyl, other signals on the $^1$H-NMR spectrum show the presence of 2 aromatic protons at $\delta$ 6.15 (IH, s, H-6) and $\delta$ 6.59 (1H, s, H-3'). The other methyl position is replaced by the presence of an oximetry group at $\delta$ 61.2 ppm. The two vinyl protons at $\delta$ 6.62 (1H, d, J = 10 Hz) and $\delta$ 5.67 (1H, d, J = 10) suggest the presence of a dimethylallyl group and this indicates a large coupling that matches the cis coupling so that the group might the side containing the signal in the form of isoprene which undergoes cyclization forms a Piran ring. $^{13}$C NMR spectrum of the compound showed resonance to 25 carbon atoms. Including three methyl groups, 1 oxymethylene group, 6 oxyaryl carbons, five carbon metines, 1 carbonyl group corresponding to 14-hydroxyartonin E (Figure 2). The above data suggests that the isolated compounds are 14 hydroxyartonin E. It is appropriate previous study [10] in Table 1.
Table 1. Data spectrum 1H-NMR and 13C-NMR, HMBC 14-hydroxyartonin E

| C atom number | 1H NMR, δ ppm (multiplicities, J in Hz unit) | 13C NMR δ ppm | HMBC           |
|---------------|---------------------------------------------|----------------|----------------|
| 1             | 6.15 (1H, s) 6.28 (1H, s)                   | 99.8 98.7 C-5, C-7, C-8, C-9 |
| 2             | 162.5 161.7                                    |                |                |
| 3             | 120.7 119.5                                   |                |                |
| 4             | 183 181                                        |                |                |
| 5             | 160 160.8                                      |                |                |
| 6             | 162.6 158.4                                   |                |                |
| 7             | 104.9 104.2                                   |                |                |
| 8             | 101.7 100.4                                   |                |                |
| 9             | 153.2 151.7                                   |                |                |
| 10            | 111.1 109.1                                   |                |                |
| 1'            | 6.15 (1H, s) 6.28 (1H, s)                   | 104.9 103.9 C-1', C-5', C-4', C-2 |
| 2'            | 149 148.4                                      |                |                |
| 3'            | 6.59 (1H, s) 6.62 (1H, s)                   | 104.9 103.9 C-1', C-5', C-4', C-2 |
| 4'            | -                                            |                |                |
| 5'            | -                                            |                |                |
| 6'            | 6.94 (1H, s) 7.04 (1H, s)                    | 117 116.1 C-2', C-5', C-2 |
| 11            | 3.2 (2H, d, J =6.05) 3.27 (2H, d, 7.7)        | 24.7 23.1 C-3, C-12, C-13, C2, C4 |
| 12            | 5.28 (1H, t, 9.7) 5.62 (1H, t, 7.7)           | 124.6 127.5 C-15, C-11 |
| 13            | -                                            |                |                |
| 14            | 4.03 (2H, s) 4.30 (2H, s)                    | 61.2 59.2       |
| 15            | 1.7 (3H, s) 1.89 (3H, s)                     | 21.7 21.1 C-3, C-12, C-13 |
| 16            | 6.62 (1H, d, 10) 6.62 (1H, d, 10)            | 115.4 114.1 C-10, C-8, C-18, C-5' |
| 17            | 5.67 (1H, d, 10) 5.56 (1H, d, 10)            | 128 127.6 C-18, C-8 |
| 18            | -                                            | 78.8 78.0       |
| 19            | 1.4 (3H, s) 1.47 (3H, s)                     | 28.3 27.6 C-17 |
| 20            | 1.4 (3H, s) 1.24 (3H, s)                     | 28.3 27.6 C-17 |
| 21            | 13.08 (OH, s)                                 |                |                |

1 Isolated compounds (1H NMR, 500 MHz, acetone-d6, 13C NMR, 125 MHz, acetone-d6)

Shugeng CaO et al 2014 ((1H NMR, 500 MHz, CDCl3, 13C NMR, 125 MHz, DMSO)

The HMBC spectrum (Figure 2) shows a long-distance correlation between singlet protons (1H, 6) and four quaternary carbons at δ 160 (C-5), δ 162.6 (C-7), δ 107.1 (C-8) and δ 104.9 (C-9). This shows the existence of a substituted isoprene group at C-8 which undergoes cyclization form a Piran ring. The singlet proton at δ 13.08 also shows the same long-distance correlation with the singlet proton (1H, 6) the doublet proton at δ 24.7 (2H, 11) shows a long-distance correlation and four quaternary carbon δ 120.7 (C-3), δ 183 (C-4), δ 162.5 (C-2), δ 136.6 (C-13). This shows the presence of an isoprene group on C-3.
3.2. Antimicrobial screening
Antibacterial activity tests of these compounds using sterile paper disc diffusion methods, agar nutrient media, using gram-positive bacteria (Staphylococcus aureus and Streptococcus pneumonia) and gram-negative bacteria (Escherichia coli and Salmonella typhosa) with inhibitory zones are shown in Table 2.

Table 2. Results of antibacterial screening of 14-hydroxyartonin E compounds

| Compounds               | The Diameter of the inhibition zone (mm) | Gram-positive | | Gram-negative | | |
|-------------------------|----------------------------------------|---------------|---------------|---------------|---------------|
|                         |                                        | S. aureus 24 hour | S. aureus 48 hour | S. pneumonia 24 hour | S. pneumonia 48 hour | E. coli 24 hour | E. coli 48 hour | S. typhosa 24 hour | S. typhosa 48 hour |
| 14-hydroxyartonin E     |                                        | 10.8          | 14.0          | 10.3          | 10.8          | 9.1           | 10.1           | 9.1           | 9.8           |
| Positive control Ampicillin |                                        | 9.5           | 9.8           | 13.2          | 9.2           | 7.3           | 14.2           | 7.4           | 8.0           |
| Negative control DMSO   |                                        | 6.2           | 6.4           | 6.7           | 6.5           | 6.3           | 6.3           | 6.5           | 6.5           |

Table 2 shows that the 14-hydroxyartonin E compound provides antibacterial activity with a moderate category against S. aureus and S. pneumonia with inhibition zones of 14 mm and 10.8 mm [11], but the category is weak against E. coli bacteria and S. thyphosa with a barrier zone of 10.1 mm and 9.8 mm respectively. However, the antibacterial activity of the compound was stronger than that of positive ampicillin control. The results of previous studies revealed that the antibacterial activity in these compounds is related to the presence of polyhydroxy groups in rings A and B, as well as the presence of aliphatic substitutions on ring A [11].

Figure 2. Structure and correlation of HMBC 14-hydroxyartonin E
4. Conclusion
This research has isolated a prenylated flavone compound, 14-hydroxyartonin E from the bark of *Artocarpus lanceifolius* Roxb. 14-hydroxyartonin E compound provides antibacterial activity with a moderate category against *Streptococcus pneumonia* and *Staphylococcus aureus* but the category is weak against *Escherichia coli* and *Salmonella thyphosa*.

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