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Original Research Article

Cytotoxic Activity of Two Compounds Extracted from *Artocarpus elasticus* Reinw. Ex Blume Leaves on Selected Cancer Cell Lines

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ABSTRACT

Artocarpus elasticus Reinw. Ex Blume is a genus in the Moraceae family and it contains an abundance of phenolics, such as flavonoids, stilbenoids, dihydrochalcones, dihydrobenzoxanthones, and prenylated flavonoids. This study was aimed at isolating chemical constituents from *Artocarpus elasticus* leaves and investigate the cytotoxic effect of the isolated compounds on selected cancer cell lines. Extract was prepared from *A. elasticus* leaves, which was followed by fractionation and isolation of chemical constituents via chromatographic technique. The chemical structures of the two compounds obtained from the leaf extract were established using several spectroscopic methods. Cytotoxic effect of both compounds against P388, MCF-7, and T47D cancer cells was investigated using the MTT assay. The results revealed that the two compounds isolated from the leaves of *A. elasticus* were apigenin and 6-prenyl apigenin. The *in vitro* cytotoxicity assay showed IC_{50} values of 14.13, 40.35, and 434.20 µg/mL and 6.65, 19.75, and 313.61 µg/mL for apigenin and 6-prenyl apigenin, respectively. This observation demonstrates that 6-prenyl apigenin displayed higher cytotoxic activity compared to apigenin. The finding of this research suggests that both compounds might be useful as anticancer agents.

***Keywords*:** *Artocarpus elasticus* Reinw. Ex Blume, apigenin, 6-prenyl apigenin, anticancer

Introduction

Moraceae is a large family of plants consisting of 1400 species and 60 genera. *Artocarpus* is one of the genera of the Moraceae family which is widespread throughout the Archipelago. Some species of the Moraceae family are endemic to South Sulawesi, Indonesia.[1] *Artocarpus* is known to contain prenylated phenolic compounds, especially flavonoids with a variety of diverse structures such as; flavanones, flavones, xanthones, chalcones, and stilbene.[2,3,4] In most flavonoids, the prenyl group is in position C-3 (ring C), while, ring B can be oxygenated at positions C-4' or C-2', C-4'or C-2', C-4', C-5'. Besides, prenylation can also occur in positions C-6, C-8, and C3'. This pattern is rarely found in plant families other than the Moraceae.[3,5] The uniqueness of the flavonoid structure in *Artocarpus* produces extensive physiological effects such as artonin E, artonin I, oxepinoflavone, and artobiloxanthone which inhibits the action of the 5-lipoxygenase arachidonate enzyme and also provides activity as an antitumor.[2,6,7] From previous research on phytochemical screening, the fractionation of *A. elasticus* leaf extract revealed the presence of flavonoids in the ethyl acetate, *n*-butanol, and aqueous fractions of the plants.[8] Evaluation of the antioxidant activity of the crude methanol extract of the plant, as well as, its *n*-hexane, ethyl acetate, *n*-butanol, and aqueous fractions using the DPPH method revealed IC_{50} values of 45.74, 50.96, 19.20, 23.90, and 257.71 µg/mL, respectively.[9] Preliminary BSLT screening for cytotoxic activity of the methanol extract of the plant and its fractions showed that both *n*-butanol and aqueous fractions were not toxic (LC_{50}> 1000 µg/mL), while, the methanol extract, hexane, and ethyl acetate fractions were 238.23, 302.69, and 796.16 µg/mL, respectively.[8]

Therefore, the significantly active ethyl acetate fraction was subjected to further fractionation, isolation, and characterization of some of its bioactive constituents using chromatographic and spectral techniques.

The present study was conducted to fractionate and isolate chemical constituents from the leaf extract of *Artocarpus elasticus* and establish the chemical structures of the isolated compounds. Also, the cytotoxic effect of the isolated compounds on selected cancer cell lines was investigated.

Materials and Methods

Plant material

Fresh leaves of *A. elasticus* were collected in November 2010, from Mekongga forest, South East Sulawesi, Indonesia. The plant was authenticated at the Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences with voucher specimen number: UHA-46.

Instrumentation

NMR spectra were recorded at ambient temperature with JEOL JNM ECA-500 spectrometer, operating at 500 MHz (^{1}H-NMR) and 125.76 MHz (^{13}C-NMR), using TMS (Tetra Methyl Silane) as an internal standard. LC-MS (Mariner Biospectrometry spectrometer) was used to determine the molecular weight of the isolate with ESI (Electrospray Ionisation) and positive ion mode system.

Preparation and fractionation of Artocarpus elasticus leaf extract

The dried leaves of *A. elasticus* (2.15 kg) were successively extracted with *n*-hexane (10 L) and methanol (10 L). The methanol extract (114.63 g) was further partitioned successively with *n*-hexane (1 L), ethyl acetate (1 L), and *n*-butanol (1 L), respectively. The ethyl acetate fraction (29.74 g) was further fractionated on a silica gel column chromatography using a gradient elution of *n*-hexane, ethyl acetate, and methanol to give ten (10) fractions. The third fraction on

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recrystallization with acetone and *n*-hexane gave rise to compound 1, while the fifth fraction was further purified over Sephadex LH-20 using dichloromethane: methanol (1:1) as eluting solvent to afford compound 2.

In vitro cytotoxicity testing

The *in vitro* cytotoxicity testing of compounds 1 and 2 against murine leukemia P-388 cells and human breast cancer cell lines (MCF-7 and T47D) were conducted using MTT assay according to the method previously described.[10-12] These experiments were analysed with linear regression.

Statistical Analysis

Statistical analysis was performed by linear regression method using Microsoft Excel software.

Results and Discussion

Spectral analysis of compound 1

Compound 1 was characterized as follows: ^{1}H-NMR (DMSO-d_6; 500 MHz; δ ppm): 6.78 (s, 1H, H-3), 6.17 (d, 1H, *J* = 1.95 Hz, H-6), 6.46 (d, 1H, *J* = 1.95 Hz, H-8), 7.92 (d, 2H, *J* = 9,08 Hz, H-2'/H-6'), 6.92 (d, 2H, *J* = 9,08 Hz, H-3'/H-5'), 12.96 (5-OH). ^{13}C-NMR (DMSO-d_6, δ ppm): 163.7 (C-2), 102.8 (C3), 181.7 (C-4), 161.4 (C-5), 98.9 (C-6), 164.6 (C7), 94.1 (C-8), 157.4 (C-9), 103.6 (C-10), 121.2 (C-1'), 128.5 (C-2'/C-6'), 116.0 (C-3'/C-5'), 161.2 (C-4'). ESI-MS (m/z) 271.2195 [M+H].

Spectral analysis of compound 2

Compound 2 was characterized as follows: ^{1}H-NMR (Acetone-d_6; 500 MHz; δ ppm): 6.63 (s, 1H, H-3), 6.61 (s, 1H, H-8), 7.91 (d, 2H, *J* = 9,05 Hz, H-2'/H-6'), 7.01 (d, 2H, *J* = 9,05 Hz, H-3'/H-5'), 3.35 (d, 2H, *J* =7.15 Hz, H-1"), 5.28 (tt, 1H, H-2"), 1.64 (s, 3H, H-4"), 1.78 (s, 3H, H-5"), 13.29 (5-OH). ^{13}C-NMR (Acetone-d_6; δ ppm): 164.8 (C-2), 94.1 (C3), 183.2 (C-4), 160.2 (C-5), 112.3 (C-6), 162.6 (C7), 104.0 (C-8), 156.6 (C-9), 105.2 (C-10), 123.3 (C-1'), 129.2 (C-2'/C-6'), 116.9 (C-3'/C-5'), 161.9 (C-4'), 22.0 (C-1"), 123.2 (C-2"), 131.6 (C-3"), 25.9 (C-4"), 17.9 (C-5"), ESI-MS (m/z) 338.5542 [M+H].

Compound 1 was obtained as yellow crystals (20.00 mg) with molecular weight $[M+H]^+$ 271.2195 with molecular formula $C_{15}H_{10}O_5$. ^{1}H-NMR of compound 1 revealed several de-shielded peaks, out of which peaks ranging from δ_H 6.17 – 7.92 ppm were assigned to 7 aromatic protons were shown in the chemical shift δ_H 6.17 ppm (1H, *d*, *J* = 1.95 Hz), 6.46 ppm (1H, *d*, *J* = 1.95 Hz), 6.78 ppm (1H, s), 6.92 ppm (2H, *d*, *J* = 9.08 Hz) and 7.92 ppm (2H, *d*, *J* = 9.08 Hz). From the constant coupling value, it can be seen that at 6.17 ppm, the chemical shift has a *meta* correlation to chemical shift at 6.46 ppm, and at chemical shift 6.92 ppm (2H) has an *ortho* correlation to chemical shift, at 7.92 ppm (2H) which indicated the presence of AA'XX' ring system. Besides, there was intramolecular hydrogen at a chemical shift of 12.96 ppm (1H, s). This data is strengthened by the results of the measurements of ^{13}C-NMR and DEPT 135 (125 MHz, DMSO-d_6). Peaks that appeared at δ_C 94.1, 98.9, 102.8, 116.0 x 2, and 128.5 x 2 ppm were assigned to 7 aromatic methine carbon atoms at positions 3, 6, 8, 2´, 3´, 5´, and 6´. A highly de-shielded peak at δ_C 181.7 ppm confirms the presence of a carbonyl group at position 4, while, peaks at δ_C 103.8, 121.2, 157.4, 161.2, 161.4, 163.7, 164.6 were assigned to other quaternary carbon atoms at position 10, 1´, 9, 4´, 5, 2, and 7. The ^{1}H and ^{13}C-NMR correlations can be supported by proton-carbon (H-C) correlation data in the HMQC and HMBC spectrum (Figure 1a). The HMBC correlation supported that compound 1 has a flavone skeleton. Ring A with a *meta* correlation; H-6 correlated with C-5, C-7, C-8, and C-10; and H-8 correlated with C-6, C-7, C-9, and C-10. The single proton at position 3 correlated with the carbonyl group at C-4, a quaternary oxygenated C-2, and a quaternary carbon C-10. The presence of the AA'XX' ring system was also confirmed in ring B. Based on the above spectral data and previous research,[13] compound 1 was determined to be apigenin (4´,5,7-trihydroxyflavone) as shown in Figure 1b.

Compound 2 was obtained as a yellow powder (15 mg) with molecular weight of $[M+H]^+$ 338.5542, an indication that it was $[M]^+$ 337.5542 ≈ 338.0 g/mol. Its ^{1}H-NMR (in acetone-d_6) displayed the presence of a signal at δ_H 13.29 ppm, indicative of a hydroxyl group at C-5. Six aromatic protons having signals (δ_H ppm), coupling constants (J, Hz) and multiplicities at 6.61 ppm (1H, s), 6.63 ppm (1H, s), 7.01 ppm (2H, d, J = 8.45 Hz) and 7.91 ppm (2H, d, J = 8.45 Hz) were also identified. From the constant coupling value, it can be seen that signal δ_H at 7.01 ppm (2H) has an *ortho* correlation to the signal at δ_H 7.91 ppm (2H). Though the proton signals of compound 2 were similar to that of compound 1, a different signal was observed at H-6 for compound 2. This was attributed to the presence of a prenyl group in compound 2.[14] The prenyl group appeared at δ_H 1.78 (3H, s); 1.64 (3H, s); 5.28 (1H, tt) and 3.35 ppm (2H, d, *J* = 7.15 Hz). The measurements of ^{13}C-NMR (125 MHz, acetone-d_6) displayed two (2) methyl carbons at δ_C 17.9 and 25.9 ppm; a (1) methylene carbon at δ_C 22.0 ppm; seven (7) sp^2 hybridized methine carbon atoms which appeared at δ_C 94.1; 104.0; 116.9 (2CH); 129.2 (2CH), and 123.2 ppm with ten (10) quaternary carbons at 105.2; 112.3; 123.3; 131,6; 156.6; 160.2; 161.9; 162,6; 164.8; and 183.2 ppm. The chemical shift at 183.2 ppm was assigned to a carbonyl group at C-4. Compound 2 was suspected to be a prenyl flavonoid. The correlation of HMQC and HMBC spectrum as seen in Figure 2 shows that proton H-1" correlates with a quaternary C-6, an olefinic C-2" and C-3" confirming the presence of a prenyl group at the position C-6. From the results of NMR measurements (^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC), in comparison with literature data,[14] compound 2 was identified as 6-prenyl apigenin (4´,5,7-trihydroxy-6-(3-methylbut-2-enylflavone).

Evaluation of compounds 1 and 2 for their cytotoxicity against the P-388 murine leukaemia cells, breast cancer cell lines (MCF-7 and T47D) revealed that compound 2 was more active than compound 1 (Table 1). The activity displayed by compound 2 could be attributed to the presence of the prenyl unit. These data suggest that the presence of an isoprenoid moiety in a flavonoid increases its cytotoxicity against cells.[15] However, more findings need to be carried out to ascertain the safety and mechanism of action of the isolated compounds.

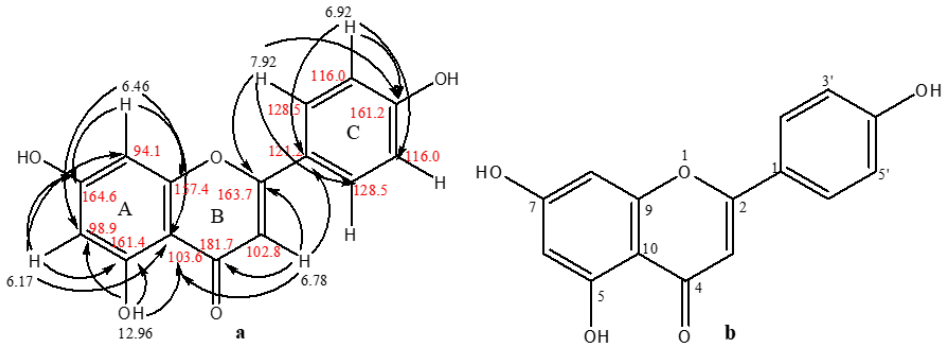

Figure 1: The HMQC and HMBC correlation (a) and chemical structure of apigenin (b) isolated from ethyl acetate fraction of *A. elasticus* leaves.

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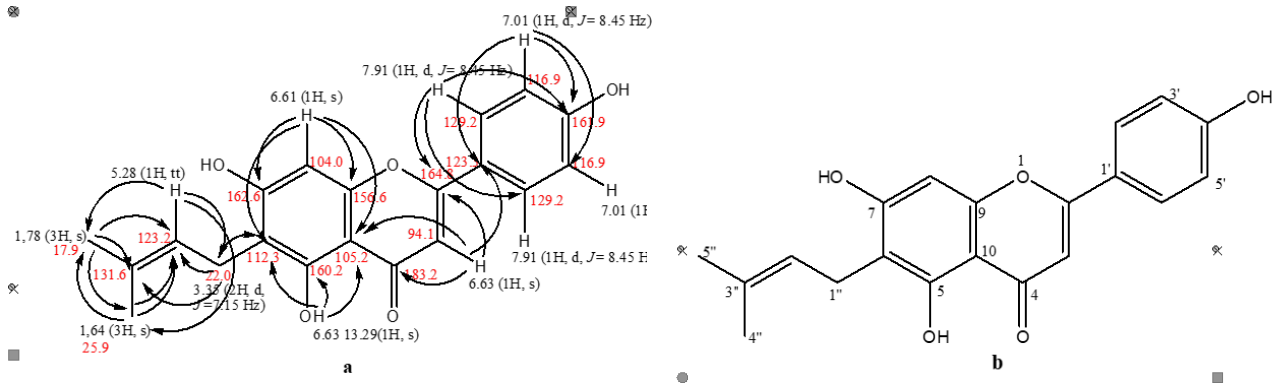

Figure 2: The HMQC and HMBC correlation (a) and chemical structure of 6-prenyl apigenin (b) isolated from ethyl acetate fraction of *A. elasticus* leaves.

Table 1: Cytotoxic activity (IC_{50}) of compounds 1 and 2 against selected cancer cells lines

Compound	IC_{50} (µg/mL)		
	P-388	MCF-7	T47D
Apigenin	14.13	40.35	434.20
6-prenyl apigenin	6.65	19.75	313.61

Conclusion

In the present study, the isolation of active compounds apigenin and 6-prenyl apigenin from the leaf extract of *Artocarpus elasticus* Reinw Ex Blume was described. The finding reveals that 6-prenyl apigenin showed significant cytotoxic effects against the P388, MCF-7, and T47D cancer cell lines.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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