

## Prenylated Flavonoids from *Artocarpus elasticus*: Structural Characterization and Molecular Docking toward Acetylcholinesterase

Iqbal Musthapa\*, Yolanda Sebastian, Vidia Afina Nurani  
Chemistry Study Program, Faculty of Mathematics and Natural Sciences Education,  
Universitas Pendidikan Indonesia, Jl. Dr. Setiabudhi 229, Bandung 40154, Indonesia  
\*E-mail: [iqbalm@upi.edu](mailto:iqbalm@upi.edu)

DOI: <https://doi.org/10.26874/jkk.v8i2.980>

Received: 23 Aug 2025, Revised: 6 March 2026, Accepted: 11 March 2026, Online: 19 March 2026

### Abstract

Prenylated flavonoids from *Artocarpus* spp. are a rich source of bioactive natural products, and several members have been reported to inhibit acetylcholinesterase (AChE), a therapeutic target for symptomatic management of cognitive impairment. However, reports focusing on AChE-related studies of prenylated flavonoids from Indonesian *Artocarpus elasticus* bark remain scarce. In this study, the ethyl acetate extract of *A. elasticus* stem bark collected in West Java, Indonesia, was fractionated by chromatographic techniques to afford two prenylated flavonoids. Compound 1 was tentatively assigned as artobiloxanthone based mainly on <sup>1</sup>H NMR comparison with literature data, whereas compound 2 was identified as cudraflavone C supported by UV, FTIR, <sup>1</sup>H NMR, and HR-ESIMS. A validated molecular docking protocol (re-docking RMSD 0.37 Å) using human AChE (PDB 4EY7) indicated low predicted binding energies for 1 and 2 (−4.1 and −3.9 kcal/mol) relative to donepezil (−12.0 kcal/mol). Both ligands mainly interacted with peripheral/mid-gorge residues and did not engage the catalytic triad, suggesting limited AChE inhibition potential *in silico*. These findings provide chemotaxonomic information on Indonesian *A. elasticus* bark and highlight the need for complementary *in vitro* AChE assays to confirm bioactivity.

**Keywords:** acetylcholinesterase, artobiloxanthone, *artocarpus elasticus*, cudraflavone C, prenylated flavonoids

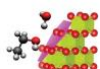
### 1 Introduction

Flavonoids are the largest phenolic compounds found in plants and act as natural coloring pigments. The flavonoid skeleton consists of 15 carbon atoms, consisting of two benzene rings (C<sub>6</sub>) and one propane chain (C<sub>3</sub>) in a C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> arrangement [1]. Flavonoids have been reported to have various biological activities, such as therapeutic effects against various degenerative and cardiovascular diseases and anticancer, antioxidant, anti-inflammatory, anti-aging, cytotoxic, and  $\alpha$ -glucosidase inhibitor properties [2] [3].

In the flavonoid group, there is a compound group called prenylated flavonoids, which are flavonoid compounds that undergo prenylation on their basic skeleton. Prenylation refers to alkyl group substituents, such as prenyl, geranyl, lavandulyl, and farnesyl groups [4] [5].

Prenylation makes flavonoids more soluble in fat, gives them a higher affinity for cell membranes, has greater potential, provides variations in molecular interactions with cellular targets, and has higher and stronger biological activity than non-prenylated flavonoids, so that they can have great potential in the drug development [5].

The type of plant that produces many prenylated flavonoids comes from the genus *Artocarpus*, which has 50 species and is spread across tropical regions in Asia [6]. *Artocarpus* trees are spread across Sumatra, Kalimantan, Java, and the Nusa Tenggara Islands. Besides Indonesia, *Artocarpus* trees are also found in Myanmar, Thailand, and the Philippines [7]. One of the *Artocarpus* plant species is *Artocarpus elasticus*, which is known to the public as the teureup tree (in Sundanese). In addition to prenylated flavonoids, there are other bioactive compounds,



such as flavonoids, alkaloids, tannins, terpenoids, xanthenes, triterpenes,  $\beta$ -sitosterol, stilbenes, arylbenzofurans, neolignans, and Diels-Alder adducts [8] [9]. Previous research reported that flavonoids found in *A. elasticus* include dihydrochalcones, flavanones, flavones, prenylated flavones, and flavononols, which have cytotoxic, antioxidant, antimicrobial, anticancer, antibacterial, anti-inflammatory, and neuroprotective properties and act as selective enzyme inhibitors [10] [11] [12] [13] [2] [9].

As previously described, flavonoids have been reported as therapeutic leads for degenerative diseases through multiple mechanisms, including antioxidant and anti-inflammatory effects. Acetylcholinesterase (AChE) hydrolyzes acetylcholine, and its inhibition remains a validated symptomatic strategy; nevertheless, current drugs can cause adverse effects and do not halt disease progression [33] [34]. Several prenylated flavonoids have been reported as AChE inhibitors in vitro and/or in silico, suggesting that increased lipophilicity and aromatic surface area may promote binding within the active-site gorge [14] [33]. Within Moraceae, *Artocarpus* species are particularly rich in prenylated flavonoids, and extracts/isolated metabolites from several *Artocarpus* spp. have shown AChE inhibitory activity [37]. However, systematic studies focusing on prenylated flavonoids from Indonesian *A. elasticus* bark and their interactions with human AChE remain limited.

Based on the considerations above, this work aimed to isolate prenylated flavonoids from the stem bark of *Artocarpus elasticus* growing in West Bandung Regency, West Java, Indonesia, and to evaluate their interaction with AChE by a validated molecular docking approach. Bark was selected because it functions as a protective tissue against environmental stress and pathogens, often resulting in the accumulation of defensive secondary metabolites.

## 2 Method

### 2.1 Sample Preparation

The *Artocarpus elasticus* plant was collected from Cisarua District, West Bandung Regency, West Java, Indonesia, in 2024. *A. elasticus* samples were identified in the Herbarium Bandungense SITH, Institut Teknologi Bandung. The *A. elasticus* stems were separated into bark and heartwood. The bark was cut into small pieces

and air-dried without exposure to sunlight. The dried samples were ground into powder.

### 2.2 Extraction

Powdered simplicia was extracted using the maceration method using distilled ethyl acetate for 24 hours at room temperature. The maceration mixture was filtered, and the filtrate was concentrated using a vacuum evaporator. The concentrated extract was separated and purified using various chromatographic methods.

### 2.3 Fractionation and Purification

The extracts were separated using vacuum liquid chromatography (VLC) and gravity column chromatography (GCC). Before being separated using VLC, the extracts were tested using thin-layer chromatography (TLC) to determine the eluent for separation using VLC. The eluent for each chromatography method was based on the TLC results.

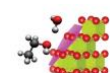
### 2.4 Characterization of Isolated Compounds

Compound characterization was performed using ultraviolet (UV) spectroscopy, Fourier Transform Infrared (FTIR), Nuclear Magnetic Resonance (NMR) ( $^1\text{H}$ -NMR), and Mass Spectroscopy (MS).

### 2.5 In silico Acetylcholinesterase Inhibition Assay

The AChE receptor (PDB ID: 4EY7) [36] that forms a complex with the ligand donepezil (ID: E20) was obtained from the Protein Data Bank (PDB) website (<https://www.rcsb.org/>). The AChE target protein (receptor) was separated from other molecules, such as water and its native ligand (donepezil), leaving only the protein chain. Ligand preparation was carried out using a similar method, but removing the protein and water molecules left only the native ligand. Next, the chemical structure of the isolate (test ligand) obtained from the PubChem Database (<https://pubchem.ncbi.nlm.nih.gov>) was energy minimized using the MM2 method in Chem3D software and converted into a .pdb file. Docking calculations were performed using AutoDock Vina, and binding energies are reported in kcal/mol [35].

The molecular docking method was first validated by re-docking the native ligand



(donepezil) of the AChE protein (PDB ID: 4EY7) until the RMSD was  $\leq 2$  Å. Grid box settings were performed to determine the interaction position between the ligand and the active site of the receptor. The grid box position refers to the native ligand bound to the receptor at the time of download. Three ligands (donepezil as a control and two isolates as test ligands) were docked to the previously prepared AChE protein. The docking results were visualized in 3D using BIOVIA Discovery Studio Visualizer, and analysis of protein-ligand binding interactions was performed.

### 3 Result and Discussion

#### 3.1 Sample Preparation

The stem of *A. elasticus* was separated into bark and heartwood. A total of 3 kg of wet bark was dried by airing without exposure to sunlight. Drying aims to reduce water content, stop enzymatic activity, and prevent deterioration or damage to the sample. In addition, drying can keep the sample from being easily damaged, and it can be stored for a long time [18]. The dried bark of *A. elasticus* was cut into small pieces and ground into powder to increase its surface area, thereby improving contact between the sample and the solvent used. During the extraction process, the chemical compounds contained in the sample can be extracted more quickly and optimally [19]. The resulting bark powder yielded 1.3 kg.

#### 3.2 Extraction

*A. elasticus* bark powder of 1.3 kg was extracted using the maceration method for 24 hours using 4.2 liters of technical ethyl distilled beforehand. Solvent distillation aims to remove impurities that can interfere with the extraction, separation, and purification processes and interfere with signals when analyzed using spectroscopy, so that data interpretation becomes less accurate. The choice of ethyl acetate as a solvent for maceration is due to its semi-polar nature, so it can be used to extract compounds with medium polarity, such as flavonoids, tannins, and alkaloids. Ethyl acetate can combine polar and non-polar groups, so it can extract compounds that are polar and non-polar [20].

After the maceration was completed, the maceration mixture was filtered with a Buchner funnel to speed up the filtration process, and the filtrate was concentrated using a vacuum evaporator to separate it from the solvent (ethyl acetate), so that a concentrated ethyl acetate

extract of *A. elasticus* stem bark was obtained in the form of a paste, blackish brown in color, with a distinctive odor of 35.2478 grams.

#### 3.3 Fractionation and Purification

The concentrated ethyl acetate extract of *A. elasticus* stem bark was subjected to TLC before separation using other chromatography methods. This aims to determine the appropriate eluent for subsequent separation and purification methods, resulting in optimal compound separation. The eluent used for TLC is a gradient eluent using a ratio of *n*-hexane to ethyl acetate (10:0; 9:1; 8:2; 7:3; 6:4; 5:5; and 4:6). Furthermore, the concentrated ethyl acetate extract of *A. elasticus* stem bark was separated using the Vacuum Liquid Chromatography (VLC) method with silica gel PF<sub>254</sub> as the stationary phase because this method can separate large quantities of samples in a short time because the process is assisted by a vacuum that can accelerate the eluent (mobile phase) to elute the compound components in the column which then come out into fractions with different polarities according to the eluent used [21]

The eluents used for VLC were 100% *n*-hexane, *n*-hexane:ethyl acetate (9:1; 8:2; 7:3), and 100% ethyl acetate. The separation process produced 20 fractions, and each fraction was tested by TLC eluting with *n*-hexane:ethyl acetate (9:1) to see the presence or absence of compounds in each fraction, and if there were similar spots on the TLC plate (retardation factor (R<sub>f</sub>) similarity), then the fractions could be combined. The first VLC result fractions were combined into four combined fractions (A-D) and analyzed by TLC eluting with *n*-hexane:ethyl acetate (9:1).

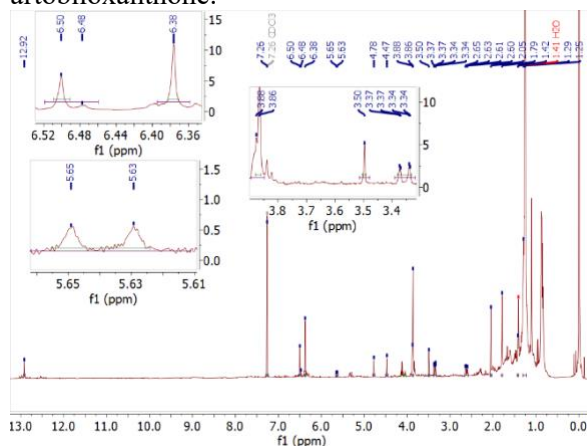
Fraction D (2.0937 grams) allows for further separation because it shows the presence of the target compound and has a larger mass than the other fractions. Separation using VLC eluting with *n*-hexane:ethyl acetate (7:3; 1:1) and 100% EtOAc. Separation produced 15 fractions, and each fraction was tested by TLC again eluting with *n*-hexane:ethyl acetate (6:4). Four combined fractions were obtained, namely fractions D<sub>1</sub>-D<sub>4</sub>, and analysed by TLC eluting with *n*-hexane:ethyl acetate (7:3).

Separation was carried out again for fraction D<sub>3</sub> (543.6 mg) using VLC eluting with *n*-hexane:ethyl acetate (7:3; 6:4; 5:5) and dichloromethane:methanol (9.5:0.5). The results of the VLC separation were continued with separation using GCC (silica gel 60 stationary phase (70-230 mesh)) eluting with dichloromethane:methanol:*n*-hexane (9.5:0.5:6)

and were obtained as isolate **1** in the form of a yellowish-brown solid (10.5 mg). Fraction D<sub>4</sub> (303.7 mg) was separated using a similar method and was obtained as isolate **2** in the form of a brown solid (13.4 mg).

### 3.4 Characterization of Isolated Compounds Structure Elucidation of Isolate Compound **1**

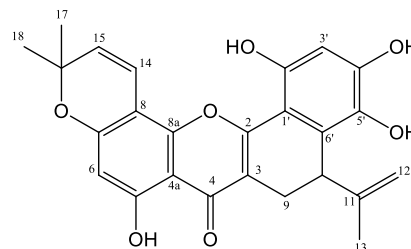
Isolate **1** was characterized using <sup>1</sup>H-NMR spectroscopy. The <sup>1</sup>H-NMR spectrum of isolate **1** is shown in **Fig. 1**. The <sup>1</sup>H-NMR data of isolate **1** (500 MHz, CDCl<sub>3</sub>) showed a chelated hydroxyl signal at δH 12.92 (1H, s, 5-OH), typical for a 5-hydroxyflavonoid with an intramolecular hydrogen bond to the C-4 carbonyl. A pentasubstituted aromatic proton on ring A appeared at δH 6.38 (1H, s, H-6). Signals at δH 3.87 (1H, br d, J = 5.65 Hz, H-10) together with diastereotopic methylene protons at δH 3.36 (1Hβ, dd, J = 16.6, 1.6 Hz) and 2.62 (1Hα, dd, J = 16.6, 7.55 Hz) (H-9) support a dihydrobenzoxanthone-type scaffold. An exomethylene group was indicated by δH 4.78 (1Hβ, br s) and 4.47 (1Hα, br s) (H-12), accompanied by an allylic methyl singlet at δH 1.79 (3H, s, H-13). A singlet at δH 2.05 (3H) was attributed to residual ethyl acetate (CH<sub>3</sub>COO-) as a common trace impurity in CDCl<sub>3</sub> [32] and was therefore not used for structural assignment. In addition, two methyl singlets at δH 1.47 and 1.46 (each 3H, s; H-17 and H-18) and an olefinic pair at δH 6.50 (1H, d, J = 10 Hz, H-14) and 5.64 (1H, d, J = 10 Hz, H-15) indicate a fused 2,2-dimethylchromene moiety. A second aromatic singlet at δH 6.48 (1H, s, H-3') is consistent with a highly substituted ring B. Collectively, these features are consistent with the prenylated dihydrobenzoxanthone artobioxanthone.



**Figure 1.** <sup>1</sup>H-NMR spectrum of isolate **1**

After comparing the <sup>1</sup>H-NMR data of isolate **1** with literature data, the overall chemical shift

pattern and diagnostic signals are highly consistent with artobioxanthone (**Fig. 2**). Minor deviations can arise from experimental factors (e.g., concentration and trace solvent residues). Based on the comparison in Table 1, isolate **1** was tentatively assigned as artobioxanthone; additional <sup>13</sup>C/2D NMR and/or HRMS data would further strengthen this assignment.



**Figure 2.** Structure of artobioxanthone (**1**)

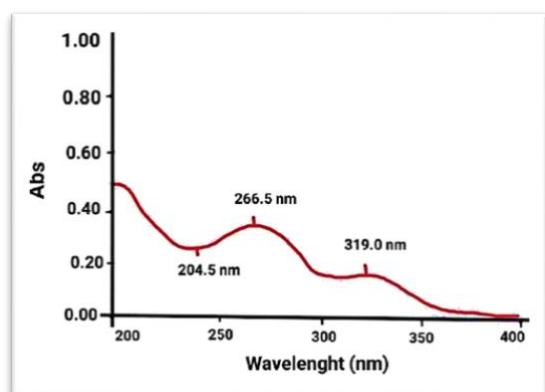
**Table 1.** Comparison of <sup>1</sup>H NMR data of isolate **1** and artobioxanthone

| C/H position | δ <sub>H</sub> (Int, m, J = Hz) |                                 |
|--------------|---------------------------------|---------------------------------|
|              | Isolate 1                       | Artobioxanthone                 |
|              | 500 MHz, CDCl <sub>3</sub>      | 500 MHz, CDCl <sub>3</sub> [22] |
| 6            | 6.38 (1H; s)                    | 6.39 (1H; s)                    |
| 9            | 3.36 (1Hβ; dd; 16.6; 1.6)       | 3.36 (1Hβ; dd; 16.6; 1.7)       |
|              | 2.62 (1Hα; dd; 16.6; 7.55)      | 2.63 (1Hα; dd; 16.6; 7.8)       |
| 10           | 3.87 (1H; br d; 5.65)           | 3.86 (1H; br d; 7.0)            |
| 12           | 4.78 (1Hβ; br s)                | 4.80 (1Hβ; br s)                |
|              | 4.47 (1Hα; br s)                | 4.51 (1Hα; br s)                |
| 13           | 1.79 (3H; s)                    | 1.79 (3H; s)                    |
| 14           | 6.50 (1H; d; 10)                | 6.54 (1H; d; 10.2)              |
| 15           | 5.64 (1H; d; 10)                | 5.64 (1H; d; 10.2)              |
| 17           | 1.47 (3H; s)                    | 1.46 (3H; s)                    |
| 18           | 1.46 (3H; s)                    | 1.48 (3H; s)                    |
| 3'           | 6.48 (1H; s)                    | 6.51 (1H; s)                    |
| 5-OH         | 12.92 (1H; s)                   | 13.01 (1H; s)                   |

### Structure Elucidation of Isolate Compound **2**

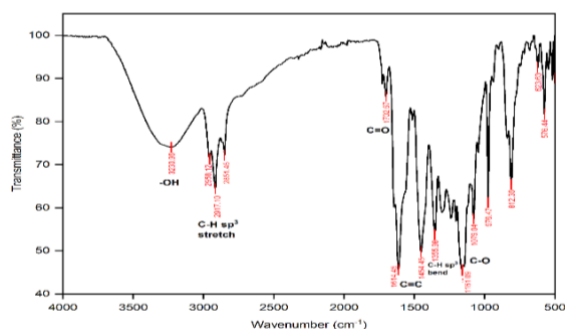
Isolate **2** was characterized using UV, FTIR, <sup>1</sup>H-NMR, and mass spectroscopy. UV spectroscopy testing was carried out at wavelengths in the range of 200-400 nm. The characterization results showed absorption at wavelengths of 319 nm, 266.5 nm, and 204.5 nm,

as shown in **Fig. 3**. The wavelength of 319 nm (band I, cinnamoyl) indicates the presence of a conjugation system typical for flavones or flavonols that have OH or isoprene substituents on their aromatic rings. The wavelength of 266.5 nm (band II, benzoyl) indicates the presence of a benzoyl group in the compound structure and is commonly found in terhydroxy flavonoids at the C-5 or C-7 position. The wavelength of 204.5 nm is an absorption associated with the electron transition from  $\pi \rightarrow \pi^*$  of the aromatic ring or conjugated double bond, and this supports the presence of an aromatic system in the structure of isolate 2.



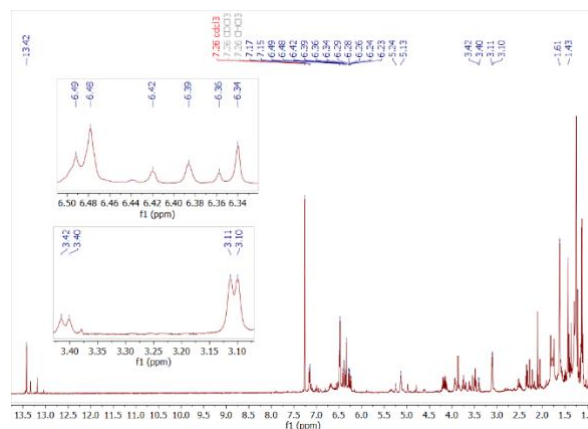
**Figure 3.** UV spectrum of isolate 2

Characterization was continued with FTIR spectrophotometry at wavenumbers 500 - 4000  $\text{cm}^{-1}$  (**Fig. 4**). The wavenumber of 3230.9  $\text{cm}^{-1}$  is characteristic of the hydroxyl group (-OH). There is absorption of substituted aliphatic groups in the flavonoid skeleton, indicated by absorptions of 2958.1  $\text{cm}^{-1}$ , 2917.1  $\text{cm}^{-1}$ , and 2851.4  $\text{cm}^{-1}$  for the -CH  $sp^3$  stretching group and 1356  $\text{cm}^{-1}$  for -CH  $sp^3$  bending. Absorption at 1702.7  $\text{cm}^{-1}$  indicates a carbonyl group (C=O) and 1614-1454  $\text{cm}^{-1}$  indicates a C=C double bond of the aromatic ring. Absorption at 1161.1  $\text{cm}^{-1}$  and 1079  $\text{cm}^{-1}$  indicates C-O-C and C-O vibrations, which are common to phenolic compounds and flavonoids.



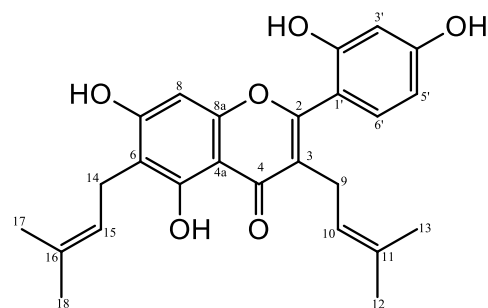
**Figure 4.** FTIR spectrum of isolate 2

The  $^1\text{H}$ -NMR data of isolate 2 (500 MHz,  $\text{CDCl}_3$ ) (**Fig. 5**) showed a chelated hydroxyl signal at  $\delta\text{H}$  13.42 (1H, s, 5-OH), typical for 5-hydroxyflavones. A pentasubstituted aromatic proton on ring A was observed at  $\delta\text{H}$  6.34 (1H, s, H-8). An ABX system on ring B was indicated by  $\delta\text{H}$  6.42 (1H, d,  $J = 2.0$  Hz, H-3'), 6.48 (1H, dd,  $J = 8.9, 2.0$  Hz, H-5'), and 7.16 (1H, d,  $J = 8.9$  Hz, H-6'), supporting a 2',4'-dioxygenated B ring. Two prenyl substituents were suggested by methylene signals at  $\delta\text{H}$  3.11 (2H, br d,  $J = 6.78$  Hz, H-9) and 3.41 (2H, br d,  $J = 7.22$  Hz, H-14), olefinic methines at  $\delta\text{H}$  5.13 (1H, t, H-10) and 5.24 (1H, t, H-15), and four allylic methyl singlets at  $\delta\text{H}$  1.43 (6H, br s; H-12/H-13) and 1.61 (6H, br s; H-17/H-18).

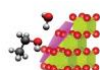


**Figure 5.**  $^1\text{H}$ -NMR spectrum of isolate 2

The  $^1\text{H}$ -NMR data of isolate 2 were compared with literature reports of prenylated flavones. Because some published  $^1\text{H}$ -NMR data for cudraflavone C are reported in acetone- $d_6$  [23], we emphasize the agreement in diagnostic signal patterns and coupling constants (e.g., ABX system on ring B and prenyl signals), while acknowledging that absolute chemical shifts may vary with solvent and concentration. Accordingly, isolate 2 was assigned as cudraflavone C (**Fig. 6**) (**Table 2**).



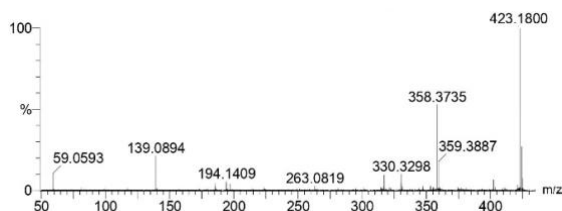
**Figure 6.** Structure of cudraflavone C (2)



**Table 2.** Comparison of  $^1\text{H}$  NMR data of isolate 2 and cudraflavone C

| C/H position | $\delta_{\text{H}}$ (Int, m, $J$ = Hz) |                              |
|--------------|----------------------------------------|------------------------------|
|              | Isolate 2                              | Cudraflavone C               |
|              | 500 MHz, $\text{CDCl}_3$               | 400 MHz, acetone- $d_6$ [23] |
| 8            | 6.34 (1H; s)                           | 6.40 (1H; s)                 |
| 9            | 3.11 (2H; br d; 6.78)                  | 3.12 (2H; br d; 7)           |
| 10           | 5.13 (1H; t)                           | 5.14 (1H; m)                 |
| 12           | 1.43 (3H; br s)                        | 1.43 (3H; br s)              |
| 13           | 1.43 (3H; br s)                        | 1.57 (3H; br s)              |
| 14           | 3.41 (2H; br d; 7.22)                  | 3.41 (2H; br d; 7)           |
| 15           | 5.24 (1H; t)                           | 5.29 (1H; m)                 |
| 17           | 1.61 (3H; br s)                        | 1.78 (3H; br s)              |
| 18           | 1.61 (3H; br s)                        | 1.65 (3H; br s)              |
| 3'           | 6.42 (1H; d; 2)                        | 6.57 (1H; d; 2)              |
| 5'           | 6.48 (1H; dd; 8.9; 2.0)                | 6.52 (1H; dd; 8; 2)          |
| 6'           | 7.16 (1H; d; 8.91)                     | 7.19 (1H; d; 8)              |
| 5-OH         | 13.42 (1H, s)                          | 13.43 (1H, s)                |

The characterization data for isolate 2 are supported by high-resolution mass spectrometry (**Fig. 7**). HR-ESIMS showed an ion at  $m/z$  423.1800  $[\text{M}+\text{H}]^+$ , consistent with the molecular formula  $\text{C}_{25}\text{H}_{26}\text{O}_6$  (calculated for  $\text{C}_{25}\text{H}_{27}\text{O}_6^+$   $[\text{M}+\text{H}]^+$ : 423.1808). No meaningful peak corresponding to  $m/z$  432 was observed; therefore,  $m/z$  423.1800 was taken as the molecular ion for the protonated molecule.

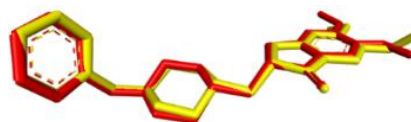
**Figure 7.** HR-ESIMS spectrum of isolate 2

### 3.5 *In silico* Acetylcholinesterase Inhibition Activity

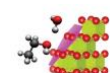
The AChE receptor in the PDB data still contains water molecules, native ligands, and other residues, so it is necessary to separate the receptor and its native ligand into separate files in .pdb format. This separation aims not to interfere with the docking process and ensure that only the ligand and receptor interact. During the preparation, hydrogen atoms were added in the AutoDockTools software to adjust the docking

atmosphere, so that it is closer to the physiological pH (normal and optimal pH level for biological processes in living organisms, around pH 7) [26]. The results of this preparation are saved in .pdbqt format. Preparation was also carried out on the test ligands (isolates 1 and 2) by minimizing the energy of the 3D structure using the MM2 method in the Chem3D software and converting it into a .pdb file. This aims to obtain the lowest energy, which can indicate the stability of the chemical structure of the ligand [27].

The molecular docking method was first validated by re-docking the native ligand (donepezil) of the AChE protein (PDB ID: 4EY7). A grid box was previously set, referring to the native ligand bound to the receptor when downloaded. The center of the grid box was at position  $x = -13.988$ ,  $y = -43.906$ , and  $z = 27.108$  with dimensions of  $x = 40 \text{ \AA}$ ,  $y = 40 \text{ \AA}$ , and  $z = 40 \text{ \AA}$ . The parameter for validation was the Root Mean Square Deviation (RMSD) value, which indicates the level of deviation or difference in position between the docked ligand and the initial ligand. The smaller the RMSD value, the smaller the error rate that occurs during docking. The docking method is valid if the RMSD value is  $\leq 2 \text{ \AA}$  [28]. The validation results in **Fig. 8** show that the ligands before and after docking overlap with an RMSD value is  $0.367 \text{ \AA}$ , so this method can be used for docking the test ligand.

**Figure 8.** Validation results of donepezil ligand docking method (red = native ligand, yellow = re-docked ligand)

The ligand donepezil (control) and the two isolates (test ligands) were docked to the AChE target protein, and the interactions were visualized using BIOVIA. A summary of the docking results is provided in Table 3, including predicted binding energy, hydrogen bonds, and hydrophobic contacts with amino acid residues. More negative binding energy values indicate stronger predicted binding within the docking scoring function [35]. Hydrogen bonding is a specific interaction between polar groups on the ligand and amino acid side chains, while hydrophobic and  $\pi$  interactions (alkyl,  $\pi$ -alkyl,  $\pi$ -cation,  $\pi$ - $\sigma$ , and  $\pi$ - $\pi$  stacking) help stabilize ligands along the aromatic gorge [30] [34].

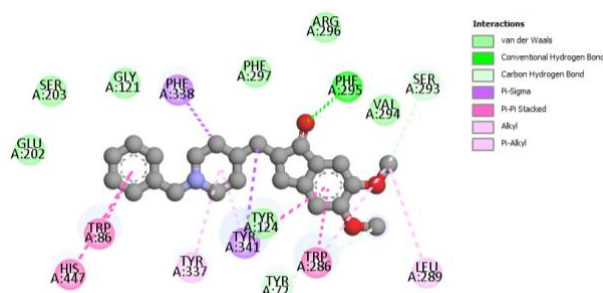


**Table 3.** Ligand interaction with AChE

| Ligand              | Binding Energy (kcal/mol) | Hydrogen Bonding                          | Hydrophobic Bonding                                                                                                                   | Total Bonds |
|---------------------|---------------------------|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Donepezil           | -12.0                     | <b>Phe295</b> , Ser393 (C-H), Tyr72 (C-H) | Glu202, Ser203, Gly121, <b>Phe338</b> , Phe297, <b>Arg296</b> , Val294, Leu289, Trp286, Tyr124, <b>Tyr341</b> , Tyr337, His447, Trp86 | 17          |
| Artobiloanthone (1) | -4.1                      | -                                         | Arg245, Met241, Asp266, Pro162, Asp304                                                                                                | 5           |
| Cudraflavone C (2)  | -3.9                      | Asp266, Pro162                            | Arg245, Met241, Asn265, Gly242                                                                                                        | 6           |

Red = key residue

Donepezil, the standard inhibitor (native ligand), has the lowest binding affinity, at -12.0 kcal/mol, indicating a strong interaction with the active site of the AChE enzyme. This interaction is shown in **Fig. 9**.

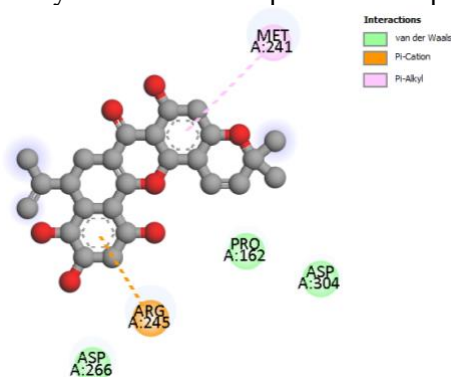
**Figure 9.** Interaction of donepezil with AChE receptor

The human AChE active-site gorge contains multiple functional subsites, including the catalytic triad (Ser203, Glu334, His447) at the base, the catalytic anionic site (CAS; e.g., Trp86), the acyl-binding pocket (Phe295/Phe297), and the peripheral anionic site (PAS; e.g., Trp286 and Tyr341) near the gorge entrance [33] [34]. In the donepezil-bound crystal structure (PDB 4EY7), donepezil engages aromatic residues along the gorge (including Trp86, Trp286, Phe295, and Tyr337/Tyr341), which stabilizes its high affinity binding [36].

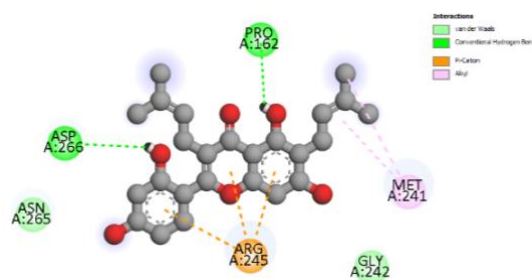
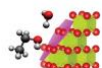
Artobiloanthone (1) showed a much less negative predicted binding energy (-4.1 kcal/mol) than donepezil (-12.0 kcal/mol), indicating substantially weaker binding in the docking model. This interaction is shown in **Fig. 10**.

The  $\pi$ -cation interaction with the Arg245 residue occurs between the aromatic ring of artobiloanthone (1), which has a high  $\pi$  electron density with a positive charge on the side chain of Arg245. The  $\pi$ -alkyl interaction with the Met241 residue occurs between the aromatic ring of

artobiloanthone (1) and the nonpolar group of the Met241 residue. In addition, there are weak Van der Waals interactions between residues Asp266, Asp304, and Pro162 with the area around artobiloanthone (1). The higher binding affinity value of artobiloanthone (1) than donepezil is also in line with the absence of interactions with key residues involved in AChE binding, namely Phe295, Tyr341, Phe338, and Arg296, so that artobiloanthone (1) has weak activity as an AChE enzyme inhibitor compared to donepezil.

**Figure 10.** Interaction of artobiloanthone (1) with AChE receptor

Cudraflavone C (2) similarly showed a less negative predicted binding energy (-3.9 kcal/mol) than donepezil (-12.0 kcal/mol), suggesting weaker binding. This interaction is shown in **Fig. 11**.

**Figure 11.** Interaction of cudraflavone C (2) with AChE receptor

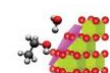
For cudraflavone C (2), hydrogen bonds were predicted between phenolic –OH groups and Asp266/Pro162, while additional hydrophobic/ $\pi$  interactions involved Arg245 and Met241 (Figure 11). Notably, neither 1 nor 2 formed interactions with the catalytic triad or the canonical CAS/PAS aromatic residues that dominate donepezil binding in 4EY7 [34] [36]. A plausible explanation for the weak predicted binding is that these prenylated flavonoids are neutral polyphenols lacking a cationic center, whereas potent AChE inhibitors typically exploit cation– $\pi$  interactions (especially with Trp86) and extensive aromatic stacking along the gorge [33] [34]. Therefore, the present docking results suggest limited AChE inhibition potential for 1 and 2, and experimental enzyme assays are required to confirm any inhibitory activity.

#### 4 Conclusion

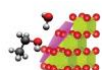
Two prenylated flavonoids were isolated from the stem bark of *Artocarpus elasticus*, namely artobiloxanthone (1) (tentative assignment based mainly on <sup>1</sup>H NMR comparison) and cudraflavone C (2) (supported by UV, FTIR, <sup>1</sup>H NMR, and HR-ESIMS). In a validated molecular docking protocol against human AChE (PDB 4EY7), both compounds displayed weak predicted binding energies (–4.1 and –3.9 kcal/mol) compared with donepezil (–12.0 kcal/mol) and did not engage key subsites (CAS/PAS) or the catalytic triad. These results indicate limited AChE inhibition potential in silico and underline the need for follow-up in vitro AChE assays and expanded spectroscopic characterization (especially for compound 1).

#### References

- [1] Heliawati, L., 2018, Kimia Organik Bahan Alam, Bogor: Universitas Pakuan Bogor.
- [2] Shah AB., Baiseitova A., Lee G., Kim JH., Park KH., 2024, Analogues of Dihydroflavonol and Flavone as Protein Tyrosine Phosphatase 1B Inhibitors from the Leaves of *Artocarpus elasticus*. *ACS omega*, 9(8): 9053–9062. Available from: <https://pubs.acs.org/doi/abs/10.1021/acsomega.3c07471>
- [3] Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B., 2022, Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chem.* 2022; 383 (February):132531. Available from: <https://doi.org/10.1016/j.foodchem.2022.132531>
- [4] Arung ET, Wicaksono DB, Sandra F., 2009, Prenylated Flavonoid sebagai Senyawa Anti Kanker yang Berpotensi, *Cermin Dunia Kedokt.*, 36(1):20–2.
- [5] Lv HW, Wang QL, Luo M, Zhu MD, Liang HM, Li WJ, Cai H, Zhou ZB, Wang H, Tong SQ, Li XN, 2023, Phytochemistry and Pharmacology of Natural Prenylated Flavonoids., *Arch Pharm Res.*, 46(4): 207–272. Available from: <https://doi.org/10.1007/s12272-023-01443-4>
- [6] Ramli F, Rahmani M, Ismail IS, Sukari MA, Abd Rahman M, Zajmi A, Akim AM, Hashim NM, Go R. A., 2016, A New Bioactive Secondary Metabolite from *Artocarpus elasticus*, *Nat Prod Commun.*, 11(8):1103–6.
- [7] Nabila MAS, Nurmilawati M, Primandiri PR, Santoso AM., 2022, Karakteristik Morfologi Bendo (*Artocarpus elasticus* Reinw.) di Kabupaten Kediri. In: Seminar Nasional Sains, Kesehatan, dan Pembelajaran, Kediri, p. 517–22.
- [8] Hakim A., 2011, Keanekaragaman metabolit sekunder Genus *Artocarpus* (Moraceae). *Bioteknologi*, 8(2):86–90.
- [9] Yamin Y, Kasmawati H, Allo LTL., 2020, Skrining Fitokimia dan Uji Toksisitas Ekstrak dan Fraksi Kulit Batang Kumbou (*Artocarpus elastica* Reinw. ex Bl) dengan Metode Brine Shrimp Lethality Test (BSLT). *Pharmauho J Farm Sains, dan Kesehat.* 6(1):15–9.
- [10] Etti I, Abdullah R, Kadir A., 2018, Apoptosis-Inducing Effect of Artonin E in Breast Cancer. *IntechOpen*.
- [11] Jenis J, Baiseitova A, Yoon SH, Park C, Kim JY, Li ZP, Lee KW, Park KH., 2019, Competitive  $\alpha$ -glucosidase Inhibitors, Dihydrobenzoxanthones, from the Barks of *Artocarpus elasticus*. *J Enzyme Inhib Med Chem.* 34(1):1623–32. Available from: <https://www.tandfonline.com/doi/abs/10.1080/014756366.2019.1660653>
- [12] Nurfadillah A, Miladiarsi, Irma A, Adri TA., 2023, Aktivitas Antibakteri Ekstrak Biji Tarra (*Artocarpus elasticus*) Terhadap Multidrug Resistant (Mdr) *Pseudomonas Aeruginosa*. *J Vocat Heal Sci.* 2(2):79–96.
- [13] Sazali SN, Jalil J, Arriffin NM, Abdullah SA, Jamil S., 2017, In vitro inhibitory effects of flavonoids from the extracts of *Artocarpus* species on prostaglandin E2 (PGE 2) production in human plasma. *J Innov Pharm Biol Sci.* 4(4):106–11.
- [14] Khan H, Marya, Amin S, Kamal MA, Patel S., 2018, Flavonoids as Acetylcholinesterase Inhibitors: Current Therapeutic Standing and Future Prospects. *Biomed Pharmacother.*



- 101:860–70. Available from: <https://doi.org/10.1016/j.bioph.2018.03.007>
- [15] Daus M, Chaithada P, Phongpaichit S, Watanapokasin R, Carroll AR, Mahabusarakam W., 2017, New prenylated dihydrochalcones from the leaves of *Artocarpus elasticus*. *Phytochem Lett.* 19:226–30. Available from: <https://www.sciencedirect.com/science/article/pii/S187439001730054X>
- [16] Ko HH, Lu YH, Yang SZ, Won SJ, Lin CN., 2005, Cytotoxic Prenylflavonoids from *Artocarpus elasticus*. *J Nat Prod.* 68(11):1692–5. Available from: <https://pubs.acs.org/doi/abs/10.1021/np050287j>
- [17] Musthapa I, Juliawaty LD, Syah YM, Hakim EH, Latip J, Ghisalberti EL., 2009, An oxepinoflavone from *Artocarpus elasticus* with cytotoxic activity against P-388 cells. *Arch Pharm Res.* 32(2):191–4. Available from: <https://link.springer.com/article/10.1007/s12272-009-1134-0>
- [18] Ariani N, Musiam S, Niah R, Febrianti DR., 2022, Pengaruh Metode Pengeringan Terhadap Kadar Flavonoid Ekstrak Etanolik Kulit Buah Alpukat (*Persea americana* Mill.) dengan Spektrofotometri. *J Pharmascience.* 9(1):40–7.
- [19] Yunus R, Alimuddin AH, Ardiningsih P., 2014, Uji Aktivitas Antibakteri Ekstrak Kulit Buah Tampoi (*Baccaurea macrocarpa*) terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*. *J Kim Khatulistiwa.* 3(3):19–24.
- [20] Kumalasari E, Septia A, Febrianti DR, Aisyah N., 2023, Penetapan Kadar Flavonoid Total Ekstrak Etanol dan Fraksi Etanol, Fraksi Kloroform, Fraksi N-Heksana, Fraksi Air, Fraksi Etil Asetat dari Daun Bawang Dayak (*Eleutherine palmifolia* (L.) Merr.). *J Ilm Manuntung Sains Farm Dan Kesehat.* 9(2):167–73.
- [21] Syafitri IF, Ersam T., 2016, Senyawa Sikloartobiloksanton dari Kulit Akar *Artocarpus elasticus*. *J Sains dan Seni ITS.* 5(2):80–4.
- [22] Jayasinghe ULB, Samarakoon TB, Kumarihamy BMM, Hara N, Fujimoto Y., 2008, Four new prenylated flavonoids and xanthenes from the root bark of *Artocarpus nobilis*. *Fitoterapi.* 79:37–41.
- [23] Hano Y, Matsumoto Y, Shinohara K, Sun J-Y, Nomura T., 1990, Cudraflavones C and D, Two New Prenylflavones from the Root Bark of *Cudrania tricuspidata* (Carr.) Bur. *Heterocycles.* 31(7):1339–44.
- [24] Musthapa I., 2009, Keanekaragaman metabolit sekunder turunan fenol dari beberapa spesies tumbuhan *Artocarpus* asal indonesia serta aktivitas biologinya. Institut teknologi Bandung.
- [25] Sultanbawa MUS, Surendrakumar S., 1989, Two Pyranodihydrobenzoxanthenes from *Artocarpus Nobilis*. *Phytochemistry.* 28(2):599–605.
- [26] Sari IW, Junaidin, Pratiwi D., 2020, Studi Molecular Docking Senyawa Flavonoid Herba Kumis Kucing (*Orthosiphon Stamineus* B.) Pada Reseptor A-Glukosidase Sebagai Antidiabetes Tipe 2. *J Farmagazine.* 7(2):54–60.
- [27] Pratama AB, Herowati R, Ansory HM., 2021, Studi Docking Molekuler Senyawa dalam Minyak Atsiri Pala (*Myristica fragrans* H.) dan Senyawa Turunan Miristisin terhadap Target Terapi Kanker Kulit. *MaJ Farm.* 17(2):233–42.
- [28] Frimayanti N, Lukman A, Nathania L., 2021, Studi Molecular Docking Senyawa 1,5-benzothiazepine sebagai Inhibitor Dengue DEN-2 NS2B/NS3 Serine Protease. *Chempublish J.* 6(1):54–62.
- [29] Alsedfy MY, Ebnalwaled AA, Moustafa M, Said AH., 2024, Investigating the Binding Affinity, Molecular Dynamics, and ADMET Properties of Curcumin-IONPS as a Mucoadhesive Bioavailable Oral Treatment for Iron Deficiency Anemia. *Sci Rep.* 14(1):1–12. Available from: <https://doi.org/10.1038/s41598-024-72577-8>
- [30] Motiejunas D, Wade RC., 2007, Structural, Energetic, and Dynamic Aspects of Ligand–Receptor Interactions. In: Taylor JB, Triggle DJ, editors. *Comprehensive Medicinal Chemistry II*. Elsevier; p. 193–213.
- [31] Önder FC., 2023, Discovery of Donepezil-Like Compounds as Potential Acetylcholinesterase Inhibitors Determined by Pharmacophore Mapping-Based Virtual. *Med J Süleyman Demirel Univ.* 30(2):143–53.
- [32] Gottlieb HE, Kotlyar V, Nudelman A., 1997, NMR chemical shifts of common laboratory solvents as trace impurities. *J Org Chem.* 62(21):7512–7515. doi:10.1021/jo971176v.
- [33] Peitzika S-C, Pontiki E., 2023, A Review on Recent Approaches on Molecular Docking Studies of Novel Compounds Targeting Acetylcholinesterase in Alzheimer Disease. *Molecules.* 28(3):1084. doi:10.3390/molecules28031084.
- [34] Hung L-W, Sanbonmatsu KY, Williams RF, Chen JC-H., 2025, Acetylcholinesterase:



- Structure, dynamics, and interactions with organophosphorus compounds. *Protein Sci.* 34(10):e70297. doi:10.1002/pro.70297.
- [35] Trott O, Olson AJ., 2010, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.* 31(2):455-461. doi:10.1002/jcc.21334.
- [36] Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, Franklin MC, Height JJ., 2012, Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *J Med Chem.* 55(22):10282-10286. doi:10.1021/jm300871x.
- [37] Abdullah SA, Abdul Lathiff SM, Jamil S., 2022, Acetylcholinesterase Activity of Crude Extracts and Flavonoids from *Artocarpus anisophyllus* Miq. and *Artocarpus lowii* King. *J Sci Math Lett.* 10(2):62-66. doi:10.37134/jsml.vol10.2.7.2022.

