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Biological Evaluation and Molecular Docking Study of Four Prenylated Flavonoids from *Artocarpus elasticus* Wood against Acetylcholinesterase

(Penilaian Biologi dan Kajian Dok Molekul Empat Flavonoid Terprenilasi daripada Kayu Artocarpus elasticus terhadap Asetilkolinesterase)

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ABSTRACT

Artocarpus elasticus is a plant notable for its high concentration of flavonoid compounds, specifically prenylated flavonoids. These flavonoids are recognized for their significant biological activities, including antioxidant, anti-inflammatory, and antidiabetic properties. Furthermore, flavonoids are known to function as acetylcholinesterase inhibitors, which are relevant in the context of neurodegenerative disorders such as Alzheimer's disease. To the best of our knowledge, the potential of prenylated flavonoids from *A. elasticus* has not been previously explored. Consequently, this study aims to investigate the potential of prenylated flavonoids from *A. elasticus* against acetylcholinesterase. The isolation process yielded four prenylated flavonoids, such as racemic cyclomourisin (**1**), cycloartocarpin (**2**), artocarpin (**3**), and cudraflavone C (**4**). Molecular docking results indicated binding affinity values of (*R*)-cyclomourisin –2.5 kcal/mol, (*S*)-cyclomourisin –4.8 kcal/mol, cycloartocarpin –2.8 kcal/mol, artocarpin –4.0 kcal/mol, and cudraflavone C –3.9 kcal/mol. Moreover, *in vitro* results demonstrated that the inhibition of the four prenylated flavonoids were 27.52% (racemic cyclomourisin), 0% (cycloartocarpin), 48.32% (artocarpin), and 37.19% (cudraflavone C). Based on these findings, artocarpin shows potential as an acetylcholinesterase inhibitor. Thus, further research of artocarpin as a lead compound targeting acetylcholinesterase can be conducted to obtain the optimized structure.

Keywords: Acetylcholinesterase; *Artocarpus elasticus*; *in vitro*; molecular docking; prenylated flavonoid

ABSTRAK

Artocarpus elasticus merupakan tumbuhan yang terkenal dengan kepekatan sebatian flavonoid yang tinggi, khususnya flavonoid terprenilasi. Flavonoid ini dikenali kerana aktiviti biologinya yang ketara, termasuk sifat antioksidan, anti-radang dan antidiabetes. Tambahan pula, flavonoid diketahui berfungsi sebagai perencat asetilkolinesterase yang relevan dalam konteks gangguan neurodegeneratif seperti penyakit Alzheimer. Sepanjang pengetahuan kami, potensi flavonoid terprenilasi daripada *A. elasticus* belum diterokai sebelum ini. Oleh itu, penyelidikan ini bertujuan untuk mengkaji potensi flavonoid terprenilasi daripada *A. elasticus* terhadap asetilkolinesterase. Proses pengasingan menghasilkan empat flavonoid terprenilasi seperti siklomourisin rasemik (**1**), sikloartokarpin (**2**), artokarpin (**3**), dan cudraflavone C (**4**). Keputusan dok molekul menunjukkan nilai afiniti pengikatan (R)-siklomurisin 2.5 kcal/mol, (S)-siklomurisin 4.8 kcal/mol, sikloartokarpin 2.8 kcal/mol, artokarpin 4.0 kcal/mol dan cudraflavone C 3.9 kcal/mol. Tambahan pula, keputusan *in vitro* menunjukkan bahawa perencatan empat flavonoid terprenilasi adalah 27.52% (siklomurisin rasemik), 0% (sikloartokarpin), 48.32% (artokarpin), dan 37.19% (cudraflavone C). Berdasarkan penemuan ini, artokarpin menunjukkan potensi sebagai perencat asetilkolinesterase. Oleh itu, kajian lanjut tentang artokarpin sebagai sebatian utama yang mensasarkan asetilkolinesterase boleh dijalankan untuk mendapatkan struktur yang dioptimumkan.

Kata kunci: *Artocarpus elasticus;* asetilkolinesterase; dok molekul; flavonoid terprenilasi*; in vitro*

INTRODUCTION

Alzheimer's disease (AD) is one of the neurodegenerative diseases that is the leading cause of dementia (Soreq & Seidman 2001; 2025 Alzheimer's disease facts and figures 2025). This condition is indicated by cognitive impairment, such as a decline in memory and difficulty

processing information (Singh & Gupta 2017). One of the key factors contributing to the onset of this disease is an imbalance in acetylcholine (ACh) levels, a neurotransmitter that plays a significant role in learning and memory processes (Danova et al. 2025; Vecchio et al. 2021). In the nervous system, the acetylcholinesterase (AChE) breaks down ACh to maintain the stability of neural activity (Danova et al. 2025; Peitzika & Pontiki 2023). However, in AD patients, excessive AChE activity causes ACh levels to decrease more rapidly, thereby exacerbating cognitive impairments (Marucci et al. 2021; Talesa 2001). Therefore, inhibiting AChE activity has become a primary approach in AD therapy, aiming to maintain ACh levels in the brain while slowing the progression of the disease (de Almeida et al. 2023).

The primary treatment strategy for Alzheimer's disease involves the use of acetylcholinesterase inhibitors (AChEIs), which function by increasing acetylcholine levels in the nervous system (Gao, Liu & Li 2024; Kim et al. 2025; Mehta, Adem & Sabbagh 2012). Among natural products, alkaloids have been widely utilized in Alzheimer's therapy (Cahlíková et al. 2021; Williams, Sorribas & Howes 2011; Zhang et al. 2023). Tacrine was the first inhibitor introduced for Alzheimer's treatment; however, its use was limited due to severe side effects, including hepatotoxicity and gastrointestinal disturbances (Mitra et al. 2022; Watkins et al. 1994). Consequently, synthetic drugs such as donepezil, rivastigmine, and galantamine were developed as alternative therapies, although these agents also present undesirable side effects (Marucci et al. 2021). Nevertheless, owing to such limitations, recent studies have increasingly focused on natural compounds derived from plants as safer and potentially effective therapeutic alternatives for Alzheimer's disease management (Houghton, Ren & Howes 2006; Peitzika & Pontiki 2023).

Flavonoid compounds have been reported to possess neuroprotective properties, offering benefits in addressing neurodegenerative disorders such as Alzheimer's disease (Gondokesumo et al. 2024). The role of flavonoid compounds in this neurological disorder is to inhibit the activity of the acetylcholinesterase (AChE) enzyme, preventing a decrease in acetylcholine levels in the nervous system by binding to the active site of the acetylcholinesterase (Cichon et al. 2025; Peitzika & Pontiki 2023; Uriarte-Pueyo & Calvo 2011). Several flavonoids have been reported to actively function as acetylcholinesterase inhibitors, including quercetin and genistein. Quercetin inhibits acetylcholinesterase by 79% compared to the galantamine control (Gajendra et al. 2024; Mukherjee et al. 2007; Smyrska-Wieleba & Mroczek 2023).

One of the plants rich in flavonoids is *Artocarpus elasticus*, a species belonging to the genus *Artocarpus* and the family *Moraceae* (Sreeja Devi, Kumar & Sabu 2021). Flavonoids are secondary metabolites of the phenolic group, consisting of 15 carbon atoms arranged in two aromatic rings connected by a three-carbon bridge (C_6–C_3–C_6) (Duarte et al. 2025; Roy et al. 2022; Samanta, Das & Das 2011). In *Artocarpus elasticus*, various flavonoid derivatives have been identified, including flavones, dihydrochalcones, and dihydrobenzoxanthones (Daus et al. 2017; Ko et al. 2005; Ramli et al. 2013; Shah et al. 2024). A characteristic feature of flavonoids in the *Artocarpus* genus is the presence of prenylated flavonoids. The prenyl group is typically bound to the flavonoid at positions C-3, C-6, and C-7 (Hakim et al. 2006; Morante-Carriel et al. 2024). The presence of the prenyl substituent in flavonoids enhances their activity, such as increased antioxidant, antibacterial, antidiabetic, and anticancer activity (Lin et al. 2009; Musthapa et al. 2009; Shen et al. 2022; Solichah et al. 2021).

Flavonoids without prenyl, like quercetin, are known for their strong antioxidant and can inhibit acetylcholinesterase (Kim et al. 2011; Sreeja Devi, Kumar & Sabu 2021; Uriarte-Pueyo & Calvo 2011). This result is in line with prenylated flavonoid compounds that have high antioxidant activity and are expected to have the potential as acetylcholinesterase inhibitors. Therefore, this research aims to evaluate the potential of prenylated flavonoids against acetylcholinesterase is highly intriguing and important to be conducted.

MATERIALS AND METHODS

PLANT MATERIAL

The *Artocarpus elasticus* wood was obtained from Cisarua District, West Bandung Regency, West Java in October 2024. The plant was identified at the School of Life Sciences and Technology, Institut Teknologi Bandung, West Java.

INSTRUMENTS

The instruments used in this research include glassware, rotary vacuum evaporator, vacuum liquid chromatography (VLC), thin-layer chromatography (TLC), 254 nm and 366 nm UV lamps, and capillary tubes. The characterization instruments used include a UV-Vis spectrophotometer Shimadzu Uvmini-1240, ATR-FTIR (ALPHA II, Bruker), NMR Varian Unity INOVA-500 MHz Agilent (1D and 2D), and Waters LCT Premier XE ESI-TOF mass spectrometry.

ISOLATION PROCEDURES

Dried wood bark (1.5 kg) was extracted using the maceration method for 24 h at room temperature (25 °C) using ethyl acetate as the solvent. The maceration product was concentrated using a rotary vacuum evaporator, yielding a concentrated ethyl acetate extract of dark green color weighing 11.1316 grams (0.74%). The ethyl acetate extract was fractionated using vacuum liquid chromatography (VLC) with silica gel 60 (70-230 mesh) in a gradient using n-hexane and ethyl acetate (10:0–1:1), yielding six main fractions (A–F).

Fraction B (268.6 mg) was purified by recrystallization with n-hexane as the solvent, yielding compound **1** (114.2 mg). Fraction C (293.5 mg) was separated by vacuum liquid chromatography using n-hexane and ethyl acetate (10:0 - 1:1) as mixture of solvents to obtain, yielding compound **2** (50.5 mg). Fraction E was separated by vacuum liquid chromatography using n-hexane and ethyl acetate (9:1-1:1) as eluent, to get three subfractions: E1 (60.7 mg), E2 as compound **3** (825 mg), and E3 (71 mg). Subfraction E3 was combined with Fraction F (1.4 g) and then separated by vacuum liquid chromatography using n-hexane and ethyl acetate (8:2-0:10) as eluents, to yield four subfractions (EF1-EF4). Next, subfraction EF3 was parted again using vacuum liquid chromatography with n-hexane and ethyl acetate (3:2) as eluents, yielding four subfractions (EF31-EF34). Subfraction EF33 was washed with chloroform to obtain pure compound **4** (78 mg).

MOLECULAR DOCKING ANALYSIS

The AChE receptor protein was obtained from the Protein Data Bank (rcsb.org) with PDB ID 4EY6 (Cheung et al. 2012). The AChE protein and galantamine ligand were separated using Discovery Studio 2021. The compounds resulting from the structure determination of the isolate were drawn using ChemDraw Ultra 12.0 and then optimized in Chem3D using minimize energy (MM2).

Each AChE receptor as a macromolecule, galantamine as a control ligand, and the isolate as a test ligand were prepared using AutoDockTools-1.5.7 by adding the protein and ligand to AutoDockTools-1.5.7. The grid box was setting using 'center on ligand'. For the grid box size is X = 40, Y = 40, Z = 40 with coordinates (-10.492; -43.526; and 29.454). Docking simulations were performed using Command Prompt with Grid in a .txt file adjusted based on the preparation results. Docking results were analyzed based on bond affinity energy values (kcal/mol) and bond interactions between the ligand and AChE protein, visualized using Discovery Studio 2021 in 2D and 3D formats.

In vitro ASSAY OF ACETYLCHOLINESTERASE INHIBITORS

Acetylcholinesterase inhibition assays were performed using a microplate reader modified from the Ellman colorimetric method (Danova et al. 2025). Briefly, 25 µL of sample in 50 mM Tris-HCl buffer A (pH 8.0), 50 µL of 50 mM Tris-HCl buffer A (pH 8.0), 125 µL of 3 mM DTNB in buffer C, and 25 µL of 1.5 mM ATCI in Milli-Q water were added to the wells. Then, 25 µL of 0.3 U/mL enzyme (AChE) in 50 mM Tris-HCl buffer B was added and incubated for 10 min at room temperature. The reaction was measured at 405 nm using a BIOBASE microplate reader in triplicate.

The percentage inhibition at each concentration was calculated by subtracting the observed enzyme activity (%) from 100%. Enzyme activity was calculated as the percentage of the sample rate relative to the negative control. The percentage inhibition of acetylcholinesterase was calculated using the following equation:

$$\text{Inhibition (\%)} = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100$$

where A sample is the reaction rate of the sample; A control is the reaction rate of the negative control; and IC_{50} is calculated using the percentage of inhibition versus concentration. Galantamine is used as a positive control.

RESULTS AND DISCUSSION

ISOLATION OF *Artocarpus elasticus* WOOD

Four isolates were obtained from the *Artocarpus elasticus* wood. The structural identification of compounds **1**–**4** was based on spectroscopic data analysis and compared with previously reported compounds, including the racemic mixture of cyclomorusin (**1**) which was first identified in *Artocarpus elasticus*, cycloartocarpin (**2**) artocarpin (**3**) and cudraflavone C (**4**) (Hakim et al. 1998; Hano et al. 1990; Lu & Lin 1994; Musthapa et al. 2009; Pu et al. 2024).

In prenylated flavonoids, the prenyl group is characterized by signals appearing at δ 1-2 ppm, with two singlet signals corresponding to the two methyl groups of the γ,γ-dimethylallyl [$(CH_3)_2C{=}CH{-}CH_2{-}$] and an additional singlet signal with an integration of 6H for the 3-methyl-1-butenyl moiety [$(CH_3)_2CH{-}CH{=}CH{-}$] (Hakim et al. 1998). The presence of a pyran ring formed by the prenyl group, as observed in racemic cyclomorusin (**1**) and cycloartocarpin (**2**), is indicated by two doublet signals at δ 5-6 ppm with *J* coupling values of 9-10 Hz. In a rasemic cyclomorusin (**1**), the presence of enantiomers of cyclomorusin is possible, as enantiomers in a compound cause changes in the NMR environment (Table 1). In this study, a double doublet signal appeared at δ 5.14 (2H, dd, *J* = 15.2 Hz, 8.6 Hz). The study by Guo et al. (2018) reported a racemic mixture (1:1) of cyclomorusin at the C-9 as a chiral center, and analysis using polarimeter revealed that (*R*)-cyclomorusin and (*S*)-cyclomorusin have rotations of +138.0 and -138.0, respectively. This confirms that the compound in isolate **1** contains a racemic mixtures of (*R*)-cyclomorusin and (*S*)-cyclomorusin.

SPECTROSCOPIC DATA

Racemic mixtures of cyclomorusin (**1**): UV (MeOH) λ_{max} 293 & 369 nm. IR: 3409, 2958, 2933, 1651, 1580, 1462, 1022 cm^{-1} ^{13}C NMR (125 MHz, $CDCL_3$) (Table 1). ^1H NMR (500 MHz, $CDCL_3$) (Table 1). ESI-MS m/z 419.1493 $[M+H]^+$ (calcd for $C_{25}H_{22}O_6$).

Cycloartocarpin (**2**): UV (MeOH) λ_{max} 291 & 366 nm. IR: 3376, 2958, 2848, 1620, 1577, 1325, 1205 cm^{-1}. ^{13}C

NMR (125 MHz, DMSO-d_6) (Table 2). ^{1}H NMR (500 MHz, DMSO-d_6) (Table 2). ESI-MS m/z 435.1811 $[M+H]^+$ (calcd for $C_{26}H_{26}O_6$).

Artocarpin (**3**): UV (MeOH) λ_{max} 277 & 318 nm. IR: 3280, 2957, 2866, 1650, 1582, 1317, 1202, 1094 cm^{-1}. ^{13}C NMR (125 MHz, $CDCL_3$) (Table 3). ^{1}H NMR (500 MHz, $CDCL_3$) (Table 3). ESI-MS m/z 437.1950 $[M+H]^+$ (calcd for $C_{26}H_{28}O_6$).

Cudraflavone C (**4**): UV (MeOH) λ_{max} 266, 319 nm. IR: 3280, 2957, 2866, 1614, 1514, 1356, 1205, 1046 cm^{-1}. ^{1}H NMR (500 MHz, $CDCL_3$) (Table 4). ESI-MS m/z 423.1800 $[M+H]^+$ (calcd for $C_{25}H_{26}O_6$).

MOLECULAR DOCKING STUDY

Molecular docking studies aim to predict the binding interactions of ligands to the active sites of target proteins (Danova et al. 2025; Silalahi, Shufyani & Siregar 2020). In the molecular docking study, isolated compounds from the bark of *Artocarpus elasticus* **1**-**4** were used, where isolate 1 is a racemic mixture of (*R*)-cyclomorusin and (*S*)-cyclomorusin. Table 5 shows the molecular docking results for the native ligand galantamine and compounds **1**-**4**, including the binding affinity energy and binding interactions between the ligands and the target protein.

The first step involved validating the docking simulation method using AutoDockTools-1.5.7. The docking method was validated by redocking the native ligand into the target protein to obtain the RMSD value of < 2 Å. In this redocking, the coordinates obtained were X = -10.492, Y = -43.526, and Z = 29.454. These values indicated that the ligand was bound to the active site of the enzyme. The redocking also yielded an RMSD value of 0.381 Å. The RMSD (Root Mean Square Deviation) value is a reference parameter used to evaluate the docking process and describe the ligand conformation before and after redocking. The redocking results are considered valid if the RMSD value is < 2 Å (Figure 2) (Gondokesumo et al. 2024).

The results of molecular docking showed a binding affinity value of the native ligand with the target protein of -10.3 kcal/mol. Meanwhile, the binding affinity values of the isolated compounds with the target protein were (*R*)-cyclomorusin -2.5 kcal/mol, (*S*)-cyclomorusin -4.8 kcal/mol, cycloartocarpin -2.8 kcal/mol, artocarpin -4.0 kcal/mol, and cudraflavone C -3.9 kcal/mol (Table 2). Based on these results, the prenylated flavonoids from *Artocarpus elasticus*, namely (*S*)-cyclomorusin and artocarpin, have lower binding affinity compared to the other compounds. Based on the molecular docking results, prenylated flavonoids exhibited a binding affinity of approximately 50% compared to the control, galantamine.

The binding interactions between galantamine and the target protein acetylcholinesterase involve seventeen binding interactions, including two hydrogen bonds with Glu202, Ser203, three carbon-hydrogen bonds with His447, Ser125, Tyr124, and seven van der Waals bonds with Tyr133, Gly448, Gly122, Tyr341, Asp74, Tyr337, Gly120, one pi-sigma bond with Trp86, one amide-π bond with Gly121, and three pi-alkyl bonds with Phe338, Phe297, Phe295 (Figure 3). However, the prenylated flavonoid compounds only have approximately

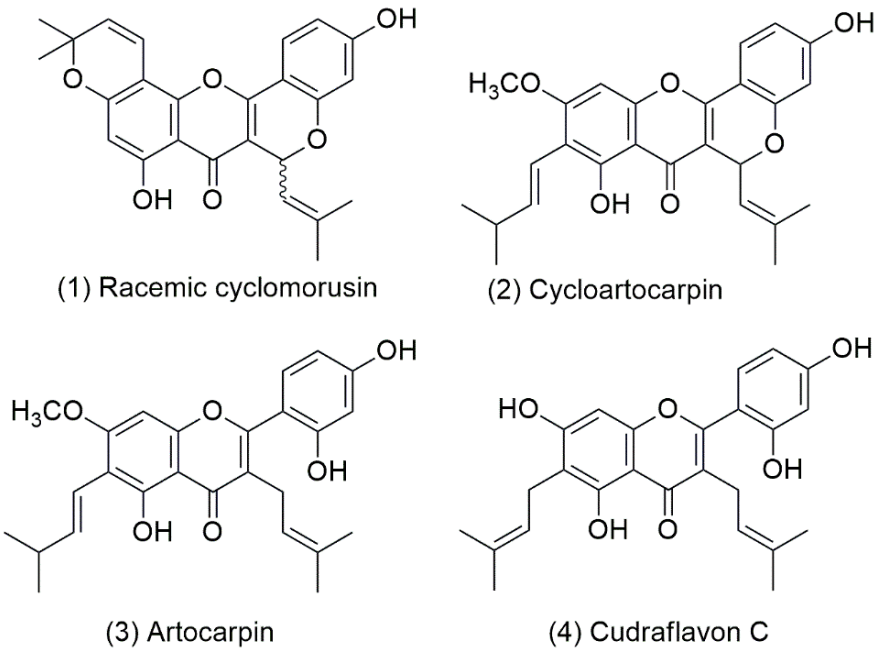

FIGURE 1. Chemical structures of the isolated compounds **1**-**4** from the wood of Artocarpus elasticus

TABLE 1. NMR data of isolated compounds (**1 - 4**)

No	**1**[a]			Cyclomorusin[b]		**2**[c]			Cycloartocarpin[d]		**3**[e]			Artocarpin[f]		**4**[g]	Cudraflavone C[h]
	δ_H (ΣH, mult, *J* in Hz, Int)	δ_C (ppm)	HMBC (H ⇌ C)	δ_H (mult, *J* in Hz, Int)	δ_C (ppm)	δ_H (mult, *J* in Hz)	δ_C	HMBC (H ⇌ C)	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	HMBC (H ⇌ C)	δ_H (mult, *J* in Hz)	δ_C	δ_H (ΣH, mult, *J* in Hz)	δ_H (ΣH, mult, *J* in Hz)
2	-	158.1	-	-	156.5	-	158.0	-	-	159.7	-	160.0	-	-	163,6	-	-
3	-	115.7	-	-	109.9	-	106.8	-	-	109	-	120.8	-	-	122,2	-	-
4	-	178.6	-	-	179.2	-	178.2	-	-	180	-	182.3	-	-	183,9	-	-
4a	-	104.5	-	-	106.2	-	104.9	-	-	106.7	-	104.9	-	-	106,0	-	-
5	13.05 (1H, s)	156.6	C-4a, C-6, C-8a	12.95 (1H, s)	156.0	13.54 (1H, s)	158.5	C-4a, C-5, C-6	13.65 (1H, s)	160	13.49 (1H, s)	158.3	C-4a, C-5, C-6	13.86 (1H, s)	159,7	13.42 (1H, s)	13.43 (1H, s)
6	6.36 (1H, s)	95.7	C-5, C-7	6.13 (1H, s)	100.4	-	108.9	-	-	110.8	-	109.7	-	-	110,5	-	-
7	-	159.1	-	-	159.9	-	163.9	-	-	164.8	-	162.9	-	-	164,3	-	-
8	-	109.8	-	-	102.2	6.35 (1H, s)	91.0	C-7, C-8a	6.39 (1H, s)	91.7	6.33 (1H, s)	89.6	C-4a, C-6, C-7, C-8a	6.50 (1H, s)	90,7	6.34 (1H, s)	6.40 (1H, s)
8a	-	155.5	-	-	151.9	-	155.1	-	-	156.9	-	156.1	-	-	158,0	-	-
1’	-	121.9	-	-	108.2	-	109.1	-	-	111.1	-	112.3	-	-	113,2	-	-
2’	-	156.4	-	-	159.1	-	155.8	-	-	157.2	-	155.1	-	-	157,8	-	-
3’	6.41 (1H, d, *J* = 2.4 Hz)	121.1	C-2’, C-5’	6.42 (1H, d, *J* = 2.4 Hz)	104.9	6.76 (1H, d, *J* = 2.5 Hz)	104.2	C-1’, C-2’, C-5’	6.43 (1H, d, *J* = 2.2 Hz)	105.6	6.48 (1H, d, *J* = 1.7 Hz)	103.8	C-2’, C-4’	6.55 (1H, d, *J* = 1.0 Hz)	103,7	6.42 (1H, d, *J* = 2 Hz)	6.57 (1H, d, *J* = 2 Hz)
4’	-	161.5	-	-	164.3	-	162.6	-	-	163.3	-	159.2	-	-	161,9	-	-
5’	6.54 (1H, dd, *J* = 8.6; 2.4 Hz)	140.8	C-1’, C-4’	6.62 (1H, dd, *J* = 8.0, 2.0 Hz)	111.0	6.56 (1H, dd, *J* = 8.6; 2.5 Hz)	116.1	C-1’, C-3’	6.63 (1H, dd, *J*= 8.6; 2.2 Hz)	117.5	6.49 (1H, dd, *J* = 8.2 Hz; 1.7 Hz)	108.3	C-3’, C-4’, C-5’	6.40 (1H, dd, *J* = 8.0, 2.0 Hz)	108,0	6.48 (1H, d, *J* = 8.9 Hz)	6.52 (1H, dd, *J* = 8.0, 2.0 Hz)
6’	7.63 (1H, d, *J* = 8.6 Hz)	129.3	C-1’, C-5’	7.75 (1H, d, J = 8.0 Hz)	126.4	7.66 (1H, d, *J* = 8.7 Hz)	125.8	C-2, C-2’, C-4’	7.72 (1H, d, *J* = 8.6 Hz)	126.9	7.16 (1H, d, *J*=8.2 Hz)	133.1	C-2’, C-4’	7.07 (1H, d, *J* = 8 Hz)	132,8	7.16 (1H, d, *J* = 8.9 Hz)	7.19 (1H, d, *J* = 8 Hz)
9	6.24 (1H, d, *J* = 9.4 Hz)	69.8	C-2, C2’, C10	6.19 (1H, d, *J* = 9.5 Hz)	70.3	6.12 (1H, d, *J* = 9.5 Hz)	69.3	C-2, C-4, C-1’, C-2’, C-10, C-11	6.21 (1H, d, *J* = 9.5 Hz)	71.1	3.11 (2H, d, *J*=6.8 Hz)	24.3	C-2, C-3, C-4, C-11	3.09 (2H, d, *J* = 7.0 Hz)	24,9	3.11 (2H, d, *J* = 7.22 Hz)	3.12 (2H, br d, *J* = 7 Hz)
10	5.42 (1H, d, *J* = 9.4 Hz)	121.9	C-3, C-9, C-11,	5.47 (1H, d, *J* = 9.5 Hz)	122.1	5.40 (1H, d, *J* = 9.4 Hz)	121.5	C-12, C-13	5.47 (1H, d, *J* = 9.5 Hz)	122.8	4.12 (1H, m, *J*=7.2 Hz)	121.4	C-9, C-11	5.09 (1H, t, *J* = 7.0 Hz)	122,8	5.13 (1H, t)	5.14 (1H, m)

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11	-	139.3	-	-	138.8	-	138.6	-	-	139.6	-	131.6	-	-	132,4	-	-
12	1.83 (6H, s)	26.1	C-11	1.93 (6H, s)	25.9	1.88 (3H, s)	25.8	C-10, C-11	1.95 (3H, s)	26.5	1.60 (3H, s)	25.6	C-10, C-11, C-13	1.57 (3H, s)	25,9	1.43 (6H, s)	1.43 (3H, br s)
13	1.83 (6H, s)	26.1	C-11	1.68 (6H, s)	18.6	1.65 (3H, s)	18.8	C-11, C-12,	1.69 (3H, s)	19.3	1.42 (3H, s)	17.6	C-11, C-12	1.38 (3H, s)	17,7	1.43 (6H, s)	1.57 (3H, br s)
14	6.70 (1H, d, *J* = 10.0 Hz)	128.1	C-4a, C-7, C-8, C-8a	d, *J* = 10.0, 6.8 Hz)	115.4	6.44 (1H, d, *J* = 16.3 Hz)	110.6	C-5, C-6, C-7, C-15, C-16	6.56 (1H, d, *J* = 17.0 Hz)	111.5	6.51 (1H, d, *J* = 16.3 Hz)	115.5	C-4a, C-5, C-7, C-15, C-16	6.38 (1H, d, *J* = 16 Hz)	117,2	3.41 (2H, d, *J* = 7.22 Hz)	3.41 (2H, br d, *J* = 7 Hz)
15	5.60 (1H, d, *J* = 10.0 Hz)	140.8	C-8, C-16	5.74 (1H, d, *J* = 10.0)	128.6	6.60 (1H, dd, *J* = 16.2; 7.0 Hz)	141.8	C-14, C-16, C17/C18	6.76 (1H, dd, *J* = 17.0; 6.7 Hz)	143.3	6.66 (1H, dd, *J* = 16.3, 7.0 Hz)	142.6	C-6, C-14, C-16, C-17/18	6.62 (1H, dd, *J*=16.0, 6 Hz)	142,8	5.24 (1H, t)	5.29 (1H, m)
16	-	72.0	-	-	78.8	2.39 (1H, m, *J* = 6.8 Hz)	32.9	C-15, C-17/18	2.43 (1H, m, *J* = 6.7 Hz)	34.6	1.26 (1H, m)	33.0	C-14, C-15	2.44 (1H, m)	34,5	-	-
17	1.46 (6H, s)	29.2	C-15, C-16	1,46 (6H, s)	28.2	1.04 (6H, d, *J* = 6.7 Hz)	23.0	C-15, C-16	1.08 (6H, d, *J* = 6.7 Hz)	23.8	1.09 (6H, d, *J* = 6.7 Hz)	22.6	C-15	1.08 (6H, d, *J* = 7.0 Hz)	23,2	1.61 (6H, s)	1,78 (3H, br s)
18	1.46 (6H, s)	29.2	C-15, C-20, C-21	1,46 (6H, s)	28.2	1.04 (6H, d, *J* = 6.7 Hz)	23.0	C-15, C-16	1.08 (6H, d, *J* = 6.7 Hz)	23.8	1.09 (6H, d, *J* = 6.7 Hz)	22.6	C-15	1.08 (6H, d, *J* = 7.0 Hz)	23,2	1.61 (6H, s)	1,65 (3H, br s)
OCH_3						3.91 (3H, s)	56.8	C-7	3.99 (3H, s)	57.3	3.85 (3H, s)	55.9	C-7	3.88 (3H, s)	56,6		

[1] ^{1}H NMR 500 MHz and ^{13}C NMR 125 MHz in $CDCL_3$

[2] ^{1}H NMR 400 MHz and ^{13}C NMR 100 MHz in acetone-d_6 (Guo et al. 2018)

[3] ^{1}H NMR 500 MHz and ^{13}C NMR 125 MHz in $CDCl_3$

[4] ^{1}H NMR and ^{13}C NMR in acetone-d_6 (Lu & Lin 1994)

[5] ^{1}H NMR 500 MHz and ^{13}C NMR 125 MHz in $CDCl_3$

[6] ^{1}H NMR 300 MHz and ^{13}C NMR 75,4 MHz in CD_3OD (Hakim et al. 1998)

[7] ^{1}H NMR 500 MHz in $CDCl_3$

[8] ^{1}H NMR in aseton-d_6 (Hano et al. 1990)

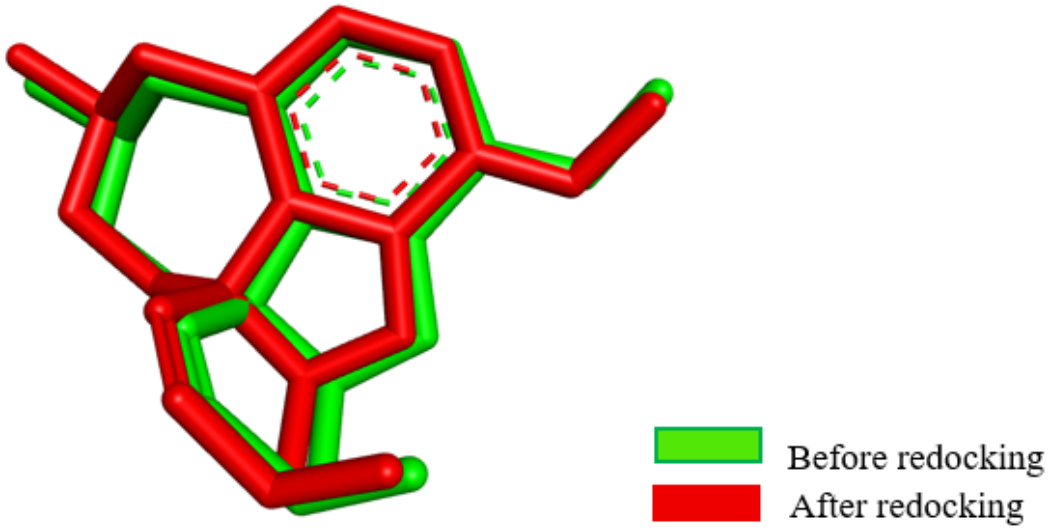


FIGURE 2. Superimposed of galantamine (before and after redocking) with RMSD value of 0.381 Å

TABLE 2. Binding affinity and interaction types of Compunds (R)-cyclomorusin, (S)-cyclomorusin, cycloartocarpin, artocarpin, cudraflavone C and galantamine against acetylcholinesterase (PDB ID: 4EY6)

No	Compound	Binding affinity (kcal/mol)	Interaction type
1a	(*R*)-Cyclomorusin	–2.5	van der Waals (3): Met241, Asp304, Gly305
1b	(*S*)-Cyclomorusin	–4.8	Hydrogen bond (1): Asn265
			van der Waals (4): Leu269, Gly163, Pro162, Met241
			Electrostatic (2): Asp266, Arg245
2	Cycloartocarpin	–2.8	van der Waals (4): Gly163, Asp304, Gly305, Ser309
			Alkyl (1): Met241
3	Artocarpin	–4.0	Hydrogen bond (1): Asp266
			Carbon hydrogen bond (1): Asn265
			van der Waals (2): Gly242, Gly163
			Pi-Alkyl (2): Pro162, Met241
			Electrostatic (2): Arg245, Asp266
4	Cudraflavone C	–3.9	Hydrogen bond (1): Arg245
			van der Waals (3): Asn265, Pro162, Gly242
			Alkyl (1): Arg245
			Pi-Sulfur (1): Met241
			Electrostatic (2): Asp266, Arg245
5	Galantamine (Native ligand)	–10.3	Hydrogen bond (2): Glu202, Ser203
			Carvon hydrogen bond (3): His447, Ser125, Tyr124
			van der Waals (7): Tyr133, Gly448, Gly122, Tyr341, Asp74, Tyr337, Gly120
			Amida-Pi (1): Gly121
			Pi-Alkyl (3): Phe338, Phe297, Phe295
			Pi-Sigma (1): Trp86

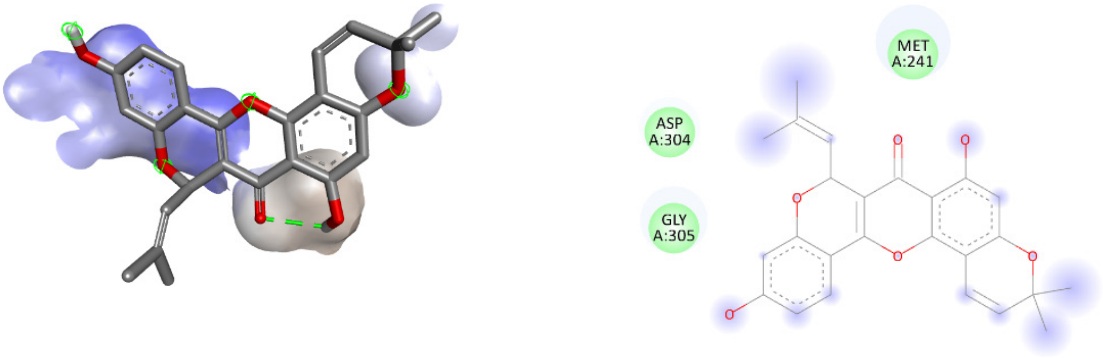
three to eight interactions, resulting in a larger complex formed between the test ligand and the target protein compared to the native ligand.

Interaction analysis was conducted to clarify the role of flavonoid structural features in acetylcholinesterase inhibition. Conformation in flavonoid compounds affects the stability of the complex formed through interactions with the target protein acetylcholinesterase (Das et al. 2017). Based on the molecular docking results, the A and C rings of flavonoids, such as (*S*)-cyclomorusin (**1b**), artocarpin (**3**), and cudraflavone C (**4**), were involved in electrostatic interactions with the Asp266 and Arg245 residues. In addition, structural modifications at specific positions also influenced binding affinity. A prenyl group forming a pyran ring at the C-3 position was found to decrease the stability of the flavonoid–protein complex. In cycloartocarpin (**2**), the pyran ring at C-3 did not show significant interactions with the residues, whereas an open-chain prenyl group at C-3, as observed in artocarpin (**3**) and cudraflavone C (**4**), generated pi–alkyl interactions with Arg245 and Met241. Substitution at the C-7 position also provided important contributions through additional hydrogen-bond formation. For instance, the methoxy group in artocarpin (**3**) formed carbon–hydrogen interactions with Asn265, while the oxygen atom at C-7 within the pyran ring of (*S*)-cyclomorusin (**1b**) established a strong hydrogen bond with Asn265, thereby enhancing the inhibitory potential of these flavonoids. Among the various interactions identified, hydrogen bonding emerged as the key factor in stabilizing the ligand–enzyme complex and played a central role in the inhibitory activity against acetylcholinesterase.

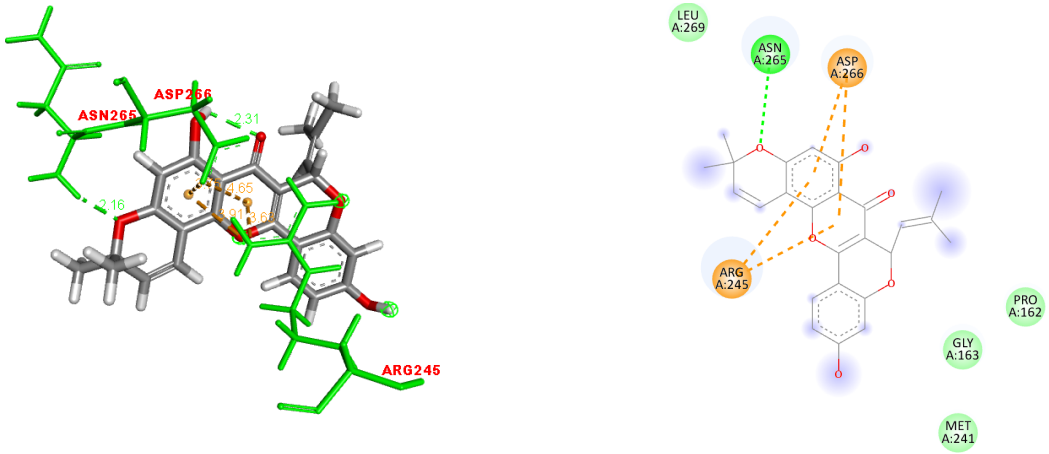
In vitro ACETYLCHOLINESTERASE INHIBITION ACTIVITY

In vitro assay of acetylcholinesterase inhibition using a modified Ellman calorimetry method (Danova et al. 2025). Based on the *in vitro* assay result, acetylcholinesterase (AChE) inhibition was tested at a concentration of 200 µg/mL (Table 3). The reference standard used was galantamine with an inhibition of 92.74 ± 2.20%.

Based on *in vitro* assay, artocarpin has a higher inhibition than the other three isolated compounds, with an inhibition of 48.32 ± 1.03%, followed by cudraflavone C at 37.19 ± 0.92%, the racemic cyclomorusin at 27.52 ± 10.05%, and cycloartocarpin with no inhibition. Functional groups in the flavonoid structure, such as hydroxyl groups, methoxy groups, aromatic rings, and prenyl, influence the ability of flavonoid compounds to inhibit acetylcholinesterase (de Almeida et al. 2023; Kim et al. 2011). Compound **1**,



(1a) (*R*)-cyclomorusin-AChE



(1b) (*S*)-cyclomorusin-AChE

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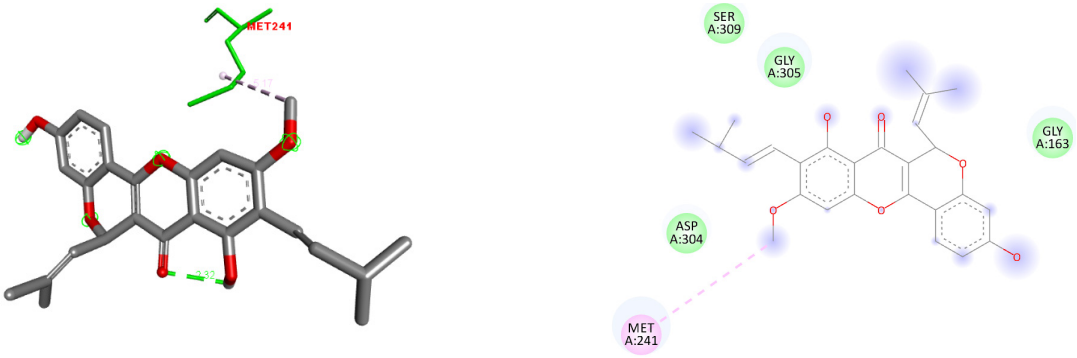
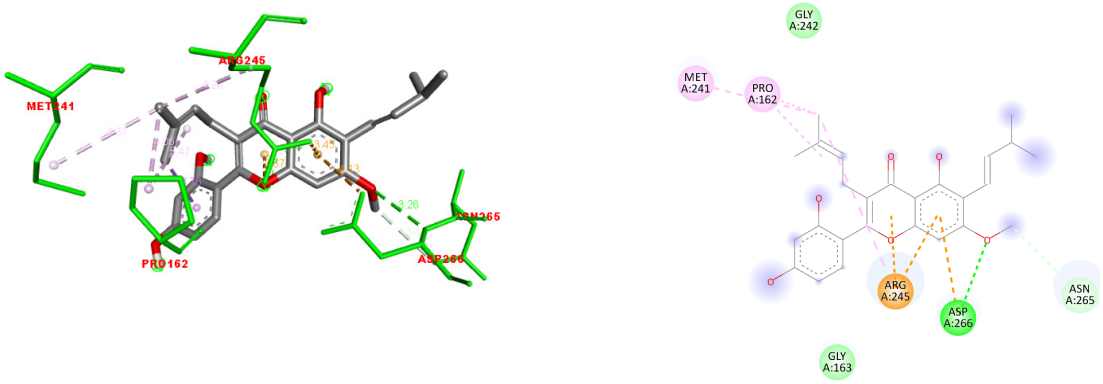
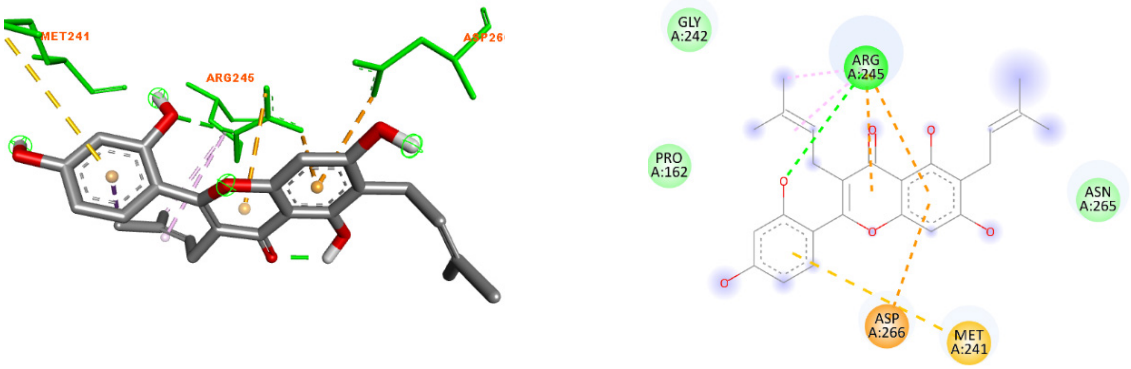


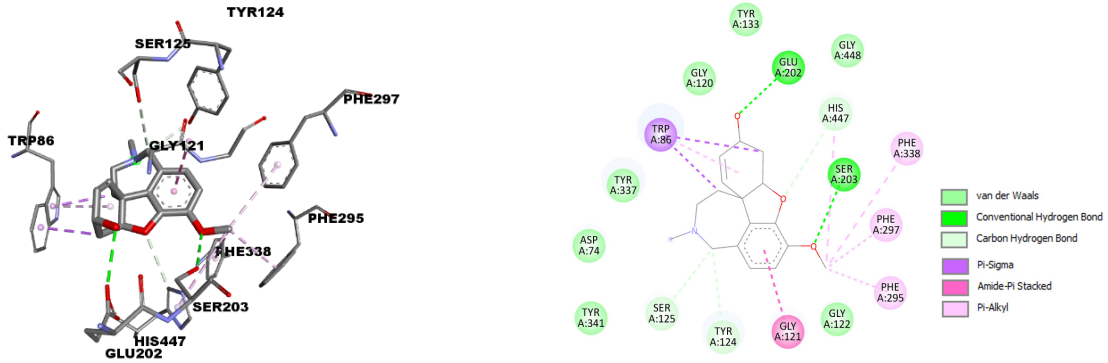
(2) Cycloartocarpin-AChE



(3) Artocarpin-AChE



(4) Cudraflavone C-AChE



(5) Galantamine-AChE

FIGURE 3. Interaction of prenylated flavonoid (**1a-4**) and galantamine (**5**) in the binding site of target protein

TABLE 3. Enzyme inhibition percentage of acetylcholinesterase from the control compound and penylated flavonoid compounds from Artocarpus elasticus

No	Compound	% Inhibition (200 µg/mL)	±SD
1	Cyclomorusin	27.52	10.05
2	Cycloartocarpin	No Inhibition	-
3	Artocarpin	48.32	1.03
4	Cudraflavone C	37.19	0.92
5	Galantamine	92.74	2.20

which is a racemic mixture of cyclomorusin, has a low inhibition percentage of 27.52 ± 10.05%. Enantiomers (*R*)-cyclomorusin and (*S*)-cyclomorusin has different activities, where (*R*)-cyclomorosin has high activity against phosphodiesterase-4, while (*S*)-cyclomorosin is inactive against phosphodiesterase-4 (Guo et al. 2018). This indicates that the enantiomers of a compound significantly influence the biological activity.

CONCLUSION

Prenylated flavonoids (racemic cyclomorusin, cycloartocarpin, artocarpin, and cudraflavone C) were isolated from *Artocarpus elasticus* wood and evaluated for acetylcholinesterase (AChE) inhibition. Docking to AChE (PDB: 4EY6) was used to propose binding poses and key interactions, whereas the Ellman assay quantified functional inhibition at 200 µg/mL. The docking trend supported the bioassay qualitatively. Artocarpin and cudraflavone C showed more favorable binding energies and stabilising contacts in the active-site gorge, consistent with measurable inhibition; artocarpin displayed the highest inhibition (48.32 ± 1.03%). Cycloartocarpin exhibited weaker predicted binding and was inactive under the assay condition. Docking scores were treated as mechanistic indicators rather than quantitative potency metrics. Variations may reflect solubility, enzyme dynamics beyond rigid docking, and potential enantioselective binding for racemic cyclomorusin.

This study is limited by single-concentration screening, the absence of IC_{50} values, and the lack of kinetic confirmation of inhibition mode. Selectivity against butyrylcholinesterase (BuChE) and other esterases was not assessed. Future work should include dose–response determination (IC_{50}), enzyme kinetics to define inhibition mechanism, and selectivity profiling versus BuChE. Enantiomeric separation and testing of cyclomorusin are recommended. Additional validation using molecular dynamics and free-energy refinement would further strengthen the docking–bioassay relationship and improve biological relevance.

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