A geranylated chalcone with antiplatelet activity from the leaves of breadfruit (Artocarpus altilis)

by Marce Bessi

Submission date: 10-May-2023 11:26AM (UTC+0700)
Submission ID: 2089199060
File name: Pharmacia.pdf (1.26M)
Word count: 5751
Character count: 30236

PHARMACIA

Pharmacia 67(4): 173–180
DOI 10.3897/pharmacia.67.e56788

Research Article

A geranylated chalcone with antiplatelet activity from the leaves of breadfruit (*Artocarpus altilis*)

Nanang Fakhrudin[1], Krisna Kharisma Pertiwi[3], Marce Inggrita Takubessi[4], Eka Fitri Susiani[5], Arief Nurrochmad[1], Sitarina Widyarini[2], Ari Sudarmanto[1], Arief Adi Nugroho[1], Subagus Wahyuono[1]

1 *Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia*
2 *Faculty of Veterinary Medicine, Universitas Gadjah Mada, Jl Fauna No. 2 Karangmalang, Yogyakarta 55281, Indonesia*
3 *Faculty of Pharmacy, Institut Ilmu Kesehatan Bhakti Wiyata, Kediri 64114, Indonesia*
4 *Department of Pharmacy, Politeknik Kesehatan Kemenkes Kupang, Kupang 85111, Indonesia*
5 *Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Borneo Lestari, Banjarbaru 70714, Indonesia*

Corresponding author: Nanang Fakhrudin (nanangf@ugm.ac.id)

Received 21 July 2020 ♦ **Accepted** 19 August 2020 ♦ **Published** 2 October 2020

Citation: Fakhrudin N, Pertiwi KK, Takubessi MI, Susiani EF, Nurrochmad A, Widyarini S, Sudarmanto A, Nugroho AA, Wahyuono S (2020) A geranylated chalcone with antiplatelet activity from the leaves of breadfruit (*Artocarpus altilis*). Pharmacia 67(4): 173–180. https://doi.org/10.3897/pharmacia.67.e56788

Abstract

Platelet plays a crucial role in cardiovascular diseases (CVDs) development. Abnormalities in platelet aggregation provokes thromboembolism, eventually leading to death. In Indonesia, breadfruit (*Artocarpus altilis*) leaf is traditionally used to treat CVDs. This study aimed to evaluate the antiplatelet activity of *A. altilis* leaf extract (AAE) and to identify its active compound. *A. altilis* leaves were extracted with ethanol, and the antiplatelet activity was assessed using ADP-induced platelet aggregation. The major compound was isolated with column chromatography followed by preparative TLC, and the structure was determined on the basis of UV, MS, IR, and NMR spectra. The binding mode of the active compound to platelet receptors was characterized in *in silico* study. AAE exhibited an antiplatelet activity (IC50 of 252.23 µg/mL). A geranylated chalcone, 2-geranyl-2′,3,3,4′-tetrahydroxydihydrochalcone (GTDC) was identified as the antiplatelet compound (IC50 of 9.09 µM). GTDC actions with P2Y12 platelet receptor involving three amino acid residues.

Keywords

Artocarpus communis, 2-geranyl-2′,3,3,4′-tetrahydroxydihydrochalcone, thrombosis, P2Y12, P2Y1

Introduction

The cardiovascular diseases remain the main cause of death globally (Benjamin et al. 2019). The development of cardiovascular diseases and blood hemostasis involves platelets, which are unnucleated blood components. During a vascular injury, platelets are recruited, and aggregated upon the binding of platelet surface receptors to their respective agonists. These agonists and receptors are ADP for P2Y1 and P2Y12 receptors, thrombin for PAR-1 and PAR-4 receptors, epinephrine for α2a receptor, thromboxane A2 for TPα and TPβ receptors, serotonin for 5HT-2A receptor, and collagen for GPIa/IIa, GP IIb/IIIa, and GP VI receptors. Their binding initiates the change in the shape of platelets and their aggregation, leading to blood coagulation and clotting (thrombus). Uncontrolled platelet aggregation can trigger circulatory disorders, including stroke, myocardial infarction, and thrombosis (Mackman 2008; Yeung et al. 2018).

Among agonists, ADP plays a significant role because it not only induces platelet aggregation through the activation of P2Y1 and P2Y12 receptors; but it is also released when platelets degranulate even when they are activated by other agonists. ADP induces the initial and late phases of platelet aggregation (Puri and Colman 1997; Murugappa and Kunapuli 2006) In the initial phase, the activation of the P2Y1 receptor by ADP causes the mobilization of intracellular calcium ions; as a result, platelet shape changes, thromboxane A2 forms, and platelets start to aggregate. Conversely, in the late phase of aggregation, the activation of the P2Y12 receptor stabilizes platelet aggregation and maintains platelets in their aggregated state by intensifying platelet degranulation and thromboxane A2 production (Puri and Colman 1997; Murugappa and Kunapuli 2006; Koupenova and Ravid 2018). With this advanced understanding, ADP receptors are considered potential therapeutic targets in cardiovascular diseases (Storey 2001) and important components of the development of antiplatelet drugs, such as prasugrel and clopidogrel, which target the P2Y12 receptor.

The discovery of antiplatelet agents is a promising approach to treat cardiovascular diseases (Mackman et al. 2020). Antiplatelet agents are beneficial to patients with a high risk of thromboembolism. These agents control the function of platelets by preventing platelet adhesion, activation, and aggregation (Lopes 2011). Although some antiplatelet agents, such as aspirin, thienopyridine derivatives (clopidogrel and ticagrelor), and GP IIb/IIIa receptor antagonists (abciximab and tirofiban), have been developed, their efficacy, and safety profiles should be improved. Furthermore, medicinal plants have inspired drug discovery and provided abundant structures of bioactive molecules (Thomford et al. 2018). *Artocarpus altilis* has been traditionally used by folks in the west part of Central Java, Indonesia, as a herbal remedy to treat cardiovascular diseases. It is composed of promising natural compounds beneficial to human health (Jagtap and Bapat 2010).

This work aimed to investigate the antiplatelet activity of *A. altilis* leaf extract and identify the active compound responsible for its antiplatelet activity. This study provided a scientific basis for promoting the traditional usage of *A. altilis* to treat cardiovascular diseases.

Materials and methods

Plant material

A. altilis leaves were collected from Mlati, Sleman District, Yogyakarta Province of Indonesia. The plant was identified by Dr. Djoko Santosa, a botanist at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia (number BF/192/Ident/Det/VI/2013).

Subject

Platelet-rich plasma (PRP) was obtained from 10 healthy participants who met the criteria described previously (Cattaneo et al. 2013). The inclusion criteria were as follows: aged 18–45 years and platelet number of $15 \times 10^4/\mu L$–$40 \times 10^4/\mu L$. The exclusion criteria were as follows: pregnant women, those who smoked at least 30 min before blood withdrawal, those who consumed caffeine at least 2 h before the experiment, those who consumed drugs affecting hemostasis (e.g., NSAIDs, anticoagulant, or antiplatelet agents) at least a week before the experiment, and participants with hemophilia or bleeding disorders. The protocol for this study was approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Universitas Gadjah Mada-DR. Sardjito General Hospital (Ref: KE/FK/0320/EC/2017.

Extraction, fractionation, and isolation

The dried leaf powders of *A. altilis* (2 kg) were extracted with 20 L of ethanol. The extract was concentrated using a vacuum rotary evaporator, left at room temperature until it was dry (262 g), and partitioned with a mixture of n-hexane : ethyl acetate : methanol : water (3:1:3:1) to yield the upper (94 g) and lower (169 g) fractions. The upper fraction containing chlorophylls and inert nonpolar components was separated, while the lower fraction was collected and dried. The lower fraction was subjected to silica vacuum chromatography (diameter of 7 cm and height of 5 cm) by using the gradient polarity of n-hexane, ethyl acetate, and methanol to yield 16 fractions. The fractions (F9 [12.9 g], F10 [27.5 g], F11 [30.6 g], F12 [25.1 g], and F13 [30.5 g]) were combined, subjected to Sephadex LH-20 column chromatography (height of 40 cm and diameter of 1.5 cm), and eluted with methanol : dichloromethane (9:1) to give 12 fractions. The fractions (F5' [1.9 g], F6' [7.0 g], F7' [9.6 g], F8 [8.0 g], F9 [4.3 g], and F10 [0.8 g]) were combined, further separated through silica gel column chromatography (diameter of 0.5 cm and height of 30 cm), and eluted with n-hexane : ethyl acetate in gradient polarity (10:0 to 0:10) to give 10 fractions. The fractions (F5"–7" [5.97 g]) were separated via preparative TLC and eluted with n-hexane : ethyl acetate (2:1) to yield 2.84 g of pure active compound.

Structural identification of the compound

The structure of 2-geranyl-2',3,4,4'-tetrahydroxydihydrochalcone (GTDC) was elucidated on the basis of its spectral data. Spectral UV and IR were recorded on a spectrophotometer (Hitachi U-2800) and an FTIR spectrometer (Perkin Elmer Spectrum 100). LC-MS data were obtained from a Mariner Biospectrometry (Cambridge Scientific, USA) connected to an HPLC instrument (Hitachi L 6200) by using a Q-TOF mass spectrometer (ESI). NMR spectra were obtained using a DELTA 2500M Hz spectrometer (Jeol) running at 500 M Hz (^{13}C-NMR) and 125.76 M Hz (^{1}H-NMR) by using $CDCl_3$ as a solvent and TMS as an internal standard.

Evaluation of antiplatelet activity

The platelet aggregation assay was done using turbidimetry-based method (Koltai et al. 2017) in an aggrego-

meter (Chrono-log 490-2D, Chrono-log Corporation, Pennsylvania, USA) in accordance with the protocol described previously (Fakhrudin et al. 2019). Blood was collected in a tube containing sodium citrate and centrifuged at 2000 rpm for 10 min at room temperature and further at 3500 rpm for 15 min to obtain platelet-rich plasma (PRP) and platelet-poor plasma, respectively. The tested samples were added to the siliconized cuvettes containing PRP and stirred for 2 min at 37 °C prior to the induction of platelet aggregation with 10 μM ADP (Sigma Aldrich; Missouri, USA). The percentage of platelet aggregation was compared with that of the solvent-treated group and determined as a decrease in the amplitude after 10 min of incubation with ADP. GraphPad Prism 8 was used to generate a dose–response curve and a nonlinear regression curve for IC50 calculation. Ticagrelor was utilized to confirm the responsiveness of the bioassay to the antiplatelet drug.

Molecular docking study

A docking study was conducted to examine the affinity of GTDC to the ADP receptor (P2Y12) by using MOE-Dock 2015.10 (Chemical Computing Group Inc.) on a computer with Intel Core i5-4460 3.20 GHz, 4.00 GB memory, and a Windows operating system. The crystal structures of the protein complex were taken from the Protein Data Bank (PDB id:4NTJ). Ticagrelor (a selective P2Y12 receptor agonist), ethyl 6-[4-(benzylsulfonylbamoyl) piperidin-1-yl]-5-cyano-2-methylpyridine-3-carboxylate or AZD 1283 (a native ligand), and ADP (a purinergic P2Y12 agonist) were used in this study. In addition, the Triangle Matcher function and London DG were used as the placement method and scoring function, respectively.

Results

Structure identification of the active compound

The isolated active compound was identified as a geranylated chalcone, 2-geranyl-2',3,4,4'-tetrahydroxydihydrochalcone (GTDC). GTDC appeared as yellowish oil with ESI-MS 433 m/z [M+Na$^+$] (calc. for $C_{25}H_{30}O_5$); UV:342 nm; IR vmax in cm^{-1}: 1450 (CH), 1496 (C-C), 1628 (C=O), 2360 (C=C). ^{1}H-NMR (400 M Hz, $CDCL_3$): δ 6.37 (1H, dd, 2.4 Hz, H-3'), 6.36 (1H, dd, 2.4 Hz, H-5′), 7.57 (1H, d, 8.4 Hz, H-6′), 3.10 (2H, t, H-α), 2.97 (2H, t, H-β), 6.73 (1H, dd, 8 Hz, H-5), 6.71 (1H, dd, 8 Hz, H-6), 3.41 (2H, d, H-1''), 5.17 (1H, t, H-2′′), 2.04 (2H, m, H-4′′), 2.06 (2H, m, H-5′′), 5.00 (1H, t, H-6′′), 1.65 (3H, s, H-8′′), 1.79 (3H, s, H-9′′), 1.57 (3H, s, H-10′′), 12.75 (1H, s, 2'-OH), 5.88 (1H, s, 4′-OH), 5.44 (1H, s, 3-OH), 5.33 (1H, s, 4-OH). ^{13}C-NMR (400 M Hz, $CDCL_3$): δ 113.86 (C-1'), 165.30 (C-2'), 103.66 (C-3′), 162.63 (C-4'), 107.81 (C-5′), 132.27 (C-6′), 203.87 (C=O), 39.81 (Cα), 27.78 (Cβ), 131.17 (C-1), 126.03 (C-2), 142.51 (C-3), 142.98 (C-4), 112.97 (C-5), 121.55 (C-6), 25.99 (C-1′′), 121.80 (C-2′′), 139.03 (C-3′′), 39.69 (C-4′′), 26.38 (C-5′′), 123.76 (C-6′′), 132.32 (C-7′′), 25.81 (C-8′′), 16.35 (C-9′′), 17.81 (C-10′′). The NMR spectra were compared with those of a previous study (Table 1). The data from the 2D HMBC and HMQC experiments are provided in Suppl. material 1. The obtained spectra were consistent with those in a previous study (McLean et al. 1996), and the chemical structure is presented in Fig. 1.

The antiplatelet activities of the extract and the compound were assessed using the ADP-induced platelet aggre-

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of GTDC in $CDCl_3$.

H No.	GTDC Data		GTDC (McLean et al. 1996)	
	^{1}H–NMR δ ppm (ΣH; m; J)	^{13}C–NMR Δ ppm	^{1}H–NMR δ ppm (ΣH; m; J)	^{13}C–NMR δ ppm
1'	–	113.86	–	113.61
2'	–	165.30	–	165.18
3'	6.37 (1H; dd; 2.4Hz)	103.66	6.38	103.57
4'	–	162.63	–	163.17
5'	6.36 (1H; dd; 2.4Hz)	107.81	6.36	108.01
6'	7.57 (1H; d; 8.4Hz)	132.27	7.57	132.25
C=O	–	203.87	–	204.02
A	3.10 (2H; t)	39.81	3.10	39.68
B	2.97 (2H; t)	27.78	2.97	27.81
1	–	131.17	–	132.09
2	–	126.03	–	126.12
3	–	142.51	–	142.52
4	–	142.98	–	142.74
5	6.73 (1H; dd; 8Hz)	112.97	6.71	112.92
6	6.71 (1H; dd; 8Hz)	121.55	6.65	121.34
1"	3.41 (2H; d)	25.99	3.40	25.84
2"	5.17 (1H; t)	121.80	5.17	121.86
3"	–	139.03	–	138.50
4"	2.04 (2H; m)	39.69	2.04	39.63
5"	2.06 (2H; m)	26.38	2.06	26.40
6"	5.00 (1H; t)	123.76	5.02	123.79
7"	–	132.32	–	131.25
8"	1.65 (3H; s)	25.81	1.65	25.69
9"	1.79 (3H; s)	16.35	1.78	16.30
10"	1.57 (3H; s)	17.81	1.57	17.71
2'–OH	12.75 (1H; s)	–	12.84	–
4'–OH	5.88 (1H; s)	–	5.81	–
3–OH	5.44 (1H; s)	–	5.81	–
4–OH	5.33 (1H; s)	–	5.57	–

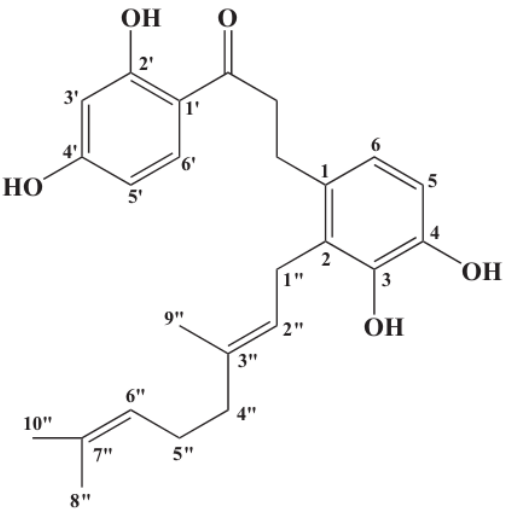


Figure 1. Structure of 2-geranyl-2′,3,3,4′-tetrahydroxydihydrochalcone (GTDC).

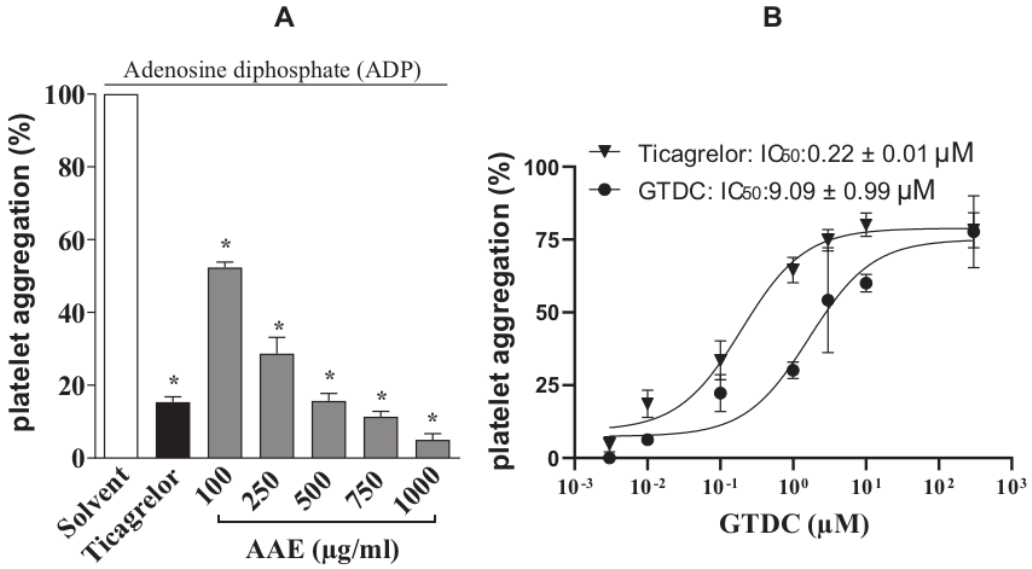


Figure 2. Antiplatelet activities of AAE and GDTC in ADP-induced platelet aggregation. Sigmoidal dose–response curves showing the antiplatelet activity of AAE (**A**), GTDC, and ticagrelor (**B**). The percentage of platelet aggregation was calculated on the basis of the decrease in the aggregation peak. Data were mean ± SD (n = 3); *p < 0.05 relative to the solvent-treated group (set as 100% aggregation).

gation assay. Fig. 2A indicates that AAE strongly inhibited platelet aggregation in a concentration-dependent manner with IC50 of 252.23 ± 6.46 µg/mL. The antiplatelet activity of AAE at a concentration of 500 µg/mL was equal to that of ticagrelor at 0.10 µM; while at 750 and 1000 µg/mL AAE demonstrated a stronger activity. This finding suggested that AAE might contain the antiplatelet compound. Further separation of the extract via liquid–liquid partition followed by Sephadex LH-20, silica gel column chromatography, and preparative TLC revealed GTDC as the active compound. This compound inhibited platelet aggregation in a dose-dependent manner in micromolar ranges with IC50 of 9.09 µM. By contrast, ticagrelor had IC50 of 0.22 µM (Fig. 2B), which is almost similar to that in a previous study (0.32 µM)(Ahn et al. 2016). This result indicated that the antiplatelet activity of GTDC was lower than that of ticagrelor.

In vitro antiplatelet activity

To obtain further details regarding the antiplatelet activity of GTDC, we assessed the platelet aggregation profile of GTDC in the late phase of aggregation (10 min). Fig. 3 shows the platelet aggregation profile of GTDC in ADP-induced platelet aggregation until 10 min of observation. GTDC moderately inhibited the initial platelet aggregation, as indicated by its lower aggregation peak (**black** and **red** line peaks) than that of the solvent-treated group (**blue** line peak). In the late phase of aggregation (10 min), the platelet aggregation level remained high in the absence of GTDC (**A**). The presence of GTDC lowered the aggregation peak in the initial phase (**red** and **black** lines in B compared with the **blue** line). However, GTDC strongly induced platelet disaggregation, as indicated by the regressed curves (**black** and **red** lines) after the aggregation peak. This result implied that GTDC not only inhibited platelet aggregation but also induced disaggregation to destabilize the aggregated platelets. The antiplatelet activity profile of GTDC mimicked that of ticagrelor (**green** line).

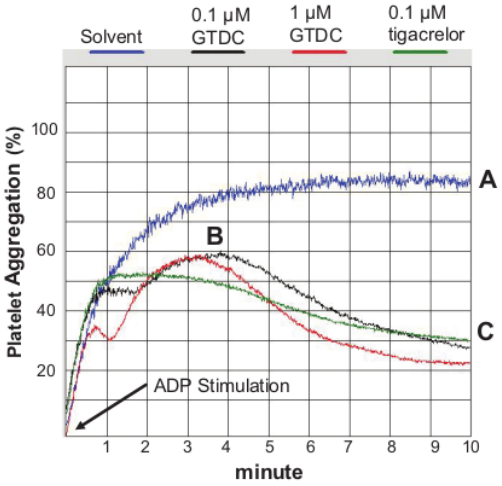
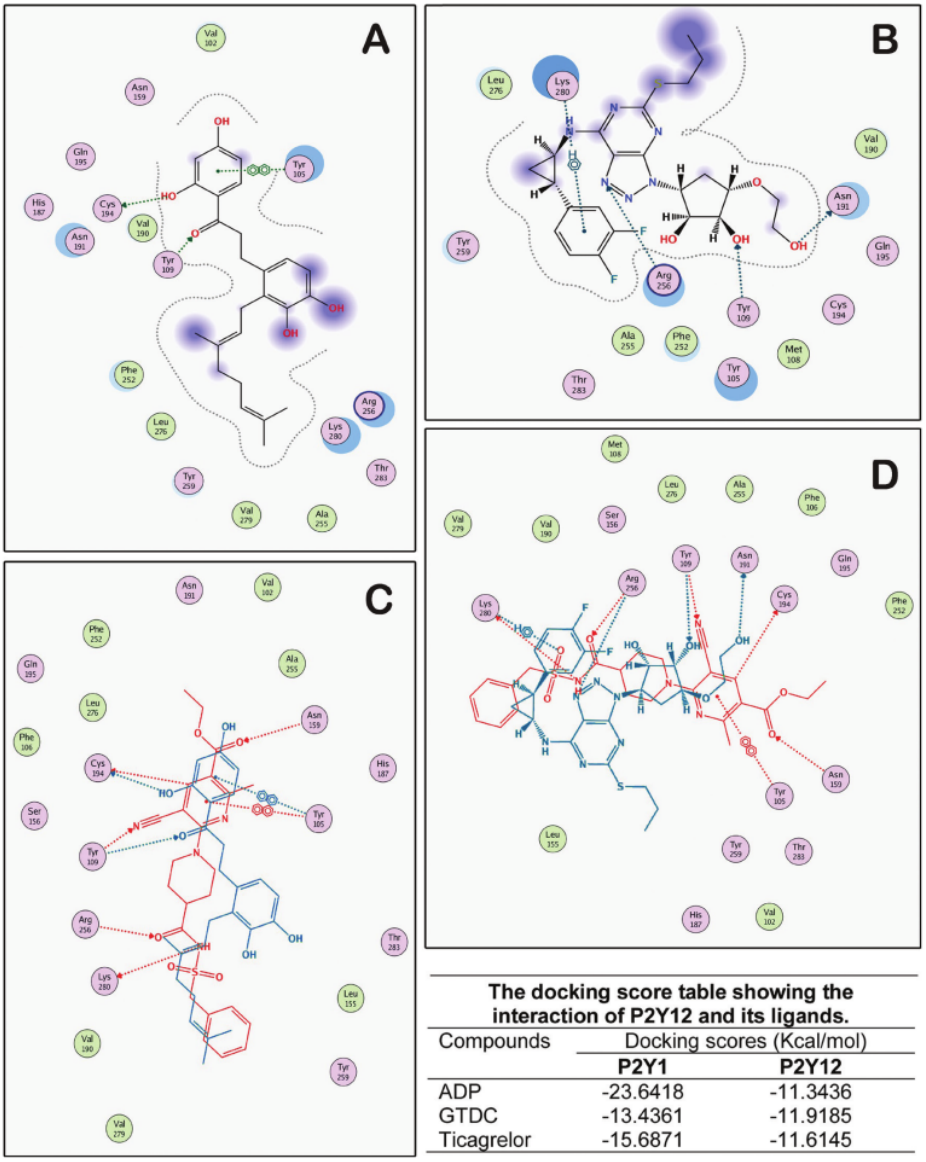


Figure 3. Curve showing the antiplatelet activity profiles of GTDC in ADP-induced platelet aggregation. **A** Baseline or peak of platelet aggregation (solvent treatment); **B** Peak of platelet aggregation in GTDC or ticagrelor treatments; **C** Platelet disaggregation in GTDC or ticagrelor treatments. **Blue line**, solvent; **black line**, 0.1 µM GTDC; **red line**, 1 µM GTDC; and **green line**, 0.1 µM tigacrelor.



The docking score table showing the interaction of P2Y12 and its ligands.

Compounds	Docking scores (Kcal/mol)	
	P2Y1	**P2Y12**
ADP	-23.6418	-11.3436
GTDC	-13.4361	-11.9185
Ticagrelor	-15.6871	-11.6145

Figure 4. Putative interactions of ligands and the binding pockets of P2Y12 receptor. The binding modes of GTDC (**A**), and ticagrelor (**B**) at P2Y12 receptor. (**C**) The overlay-complexes of GTDC (**blue**) and AZD 1283 (**red**); and (**D**) Ticagrelor (**blue**) and AZD 1283 (**red**) at the binding sites of P2Y12 receptor. The table shows the docking score of the compounds to P2Y12 receptor.

In silico experiment

A molecular docking study was performed to obtain insights into the interaction between GTDC and the ADP receptors responsible for platelet aggregation. The binding of GTDC to the platelet receptors (P2Y1 and P2Y12) was compared with that of ticagrelor. The table in Fig. 2 shows that in the P2Y1 receptor, the docking scores of GTDC and ticagrelor (−13.4361 and −15.6871, respectively) were higher than that of ADP (−23.6418). This result indicated that they did not effectively antagonize the P2Y1 receptor in the presence of ADP. Conversely, in the P2Y12 receptor, the docking scores of GTDC and ticagrelor (−11.9185, and −11.6145, respectively) were lower than that of ADP (−11.3436). This finding suggested that GTDC and ticagrelor could antagonize the P2Y12 receptor but not P2Y1 in the presence of ADP. The analysis of the binding mode (Fig. 4A, B) revealed that GTDC interacted with the P2Y12 receptor through three amino acid residues (Tyr105, Tyr109, and Cys194); while the interaction of ticagrelor with the receptor involving four

amino acid residues (Arg256, Tyr109, Asn191, and Lys280. This might explain the stronger activity of ticagrelor compared with GTDC. The overlays of GTDC and ticagrelor to the native ligand AZD 1283 demonstrated a similar interaction involving three amino acids (Tyr109, Arg256, and Lys280) as key residues in the interaction (Fig. 4C, D). These interactions included hydrogen bonding and π-π interactions.

Discussion

Preventing cardiovascular diseases by providing promising therapeutic agents is a challenge in biomedical sciences. *A. altilis* leaves have been traditionally used in Central Java, Indonesia, to treat cardiovascular-related diseases, which are correlated with platelet aggregation. In this study, an antiplatelet *in vitro* assay was conducted to assess the effectiveness of AAE, and the active compound was characterized. AAE inhibited platelet aggregation with IC50 of 9.09 μM. The active compound was identified as GTDC, which represented a major compound in the extract. Its chromatography profile is provided in Suppl. material 1: Fig. S2. Although the presence of GTDC in *A. altilis* leaves was described previously (McLean et al. 1996; Wang et al. 2007), the antiplatelet activity of GTDC has not been described in previous studies. Another study has identified three antiplatelet prenylflavonoid compounds (dihydroartomunoxanthone, artochamins B, and artocommunol CC) from *A. altilis* roots. These compounds demonstrate an antiplatelet activity in epinephrine-induced platelet aggregation (Weng et al. 2006). Additionally, Jantan et al. (Jantan et al. 2010) showed that prenylchalcones and prenylflavonoids (isobavachalcone, 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone, cycloartobiloxanthone, artonin E, and artonin E triacetate) from plants belonging to the genus of *Artocarpus* (*A. lowii*, *A. scortechinii*, and *A. teysmanii*) have antiplatelet activities in ADP-induced platelet aggregation. Here, we demonstrated that GTDC isolated from *A. altilis* leaves exerted an antiplatelet activity in ADP-induced platelet aggregation. This finding provided additional scientific evidence regarding the antiplatelet potential of geranylated or prenylated chalcone and flavonoid as typical compounds present in *Artocarpus* plants (Hakim et al. 2006).

In this study, we found that GTDC inhibited platelet aggregation and induced the disaggregation of aggregated platelets. This finding was interesting because the efficacy assessment of the antiplatelet agent in ADP-induced platelet aggregation might rely not only on the inhibition of platelet aggregation in the early phase (measured by a decrease in the aggregation peak) but also on the disaggregation activity after the aggregation peak (late phase) (Labarthe et al. 2005; Lordkipanidze et al. 2009). ADP-induced platelet aggregation involves two platelet surface receptors: a P2Y1 receptor, which mediates the initial phase of platelet aggregation, including platelet shape change, aggregation, and initial thrombi formation; and P2Y12, which participates in the late phase of platelet aggregation involving the stabilization of aggregates (Jarvis et al. 2000). Although the simultaneous activation of both receptors is required for normal platelet aggregation in the presence of ADP, the activation of the P2Y12 receptor is a key element for the stabilization of platelet aggregates and the amplification of other platelet responses that contribute to a major platelet aggregation leading to thrombosis (Cattaneo 2015; Benjamin et al. 2019).

In addition to the inhibitory effect on platelet aggregation, GTDC demonstrated a strong platelet disaggregation activity similar to that observed in ticagrelor in the late phase of platelet aggregation. Considering the antiplatelet curve profile of GTDC in the late phase of platelet aggregation as illustrated in Fig. 3, we found that GTDC showed a platelet disaggregation activity with IC50 of 0.34 μM (Suppl. material 1: Fig. S3). Previous studies also proposed this alternative way of assessing antiplatelet activities. The assessment of platelet disaggregation or aggregation in the late phase of aggregation following the treatment of an antiplatelet agent antagonist of ADP receptors should be considered as an antiplatelet measure because it matches with clinical efficacy (Labarthe et al. 2005; Lordkipanidze et al. 2009). Interestingly, the platelet disaggregation activity (measured in the late phase of aggregation) of GTDC was stronger than that of the inhibitory activity of platelet aggregation (measured in the initial phase of platelet aggregation or based on the peak of aggregation). As P2Y1 and P2Y12 receptors are responsible for the initial and late aggregation, respectively, GTDC may antagonize P2Y12 more strongly than the P2Y1 receptor. Nevertheless, further studies employing a receptor–ligand binding assay are needed to confirm this effectiveness.

The *in silico* approach revealed that the interaction between GTDC and P2Y12 receptor involved more amino acid residues compared with that of P2Y1. Thus, the binding of GTDC was more favorable to P2Y12 than to the P2Y1 receptor. The molecular docking study showed that the docking score of GTDC was lower (−11.9185) than that of ADP (−11.3436), suggesting that GTDC could prevent the binding of ADP to P2Y12. This finding explained the stronger binding affinity of GTDC to P2Y12 rather than to the P2Y1 receptor. The structure–activity relationship of chalcone-derived compounds demonstrated that the 4-OH phenolic group and the adjacent geranyl group are essential for the antiplatelet activity in ADP-induced platelet aggregation (Jantan et al. 2009; Jantan et al. 2010). Coincidentally, both functional groups are present in GTDC and may contribute to antiplatelet activities.

Recent studies and clinical evidence indicated that antagonizing the P2Y12 receptor remains the main target in coronary artery thrombosis as it shows a prolific efficacy (Nylander and Schulz 2016; Li et al. 2020; Mansour et al. 2020). Additionally, this receptor functions in platelet aggregation and aggregate stability. GTDC inhibited platelet aggregation and destabilized platelet aggregates, which might correspond to the antagonistic effect of the P2Y12 receptor. A similar effect was observed in ticagrelor used in this study. Similar to its predecessor clopidogrel, ticagrelor is an antiplatelet drug that shows clinical efficacy by selectively antagonizing the P2Y12 receptor (Anderson et al. 2010). However, both agents can cause serious bleeding and dyspnea as common adverse effects that limit their use (Nawarskas and Snowden 2011). Further investigation on the binding

mode and mechanistic characterization of P2Y12 ligands in platelet aggregation cascades is challenging because of the diverse chemical structure of the ligands with potentially distinct side effects. GTDC may lead the way for the discovery of a promising natural antiplatelet agent.

Conclusion

We found that GTDC, a geranylated chalcone isolated from *A. altilis* leaves demonstrated a promising antiplatelet activity. It exerts an antiplatelet activity by inhibiting platelet aggregation and inducing platelet disaggregation in the initial and late phases of aggregation, respectively. The docking study indicates that GTDC interacts with the P2Y12 receptor via three amino acid residues.

Acknowledgements

We thank Setiono and Miranda Pratiwi for excellent technical assistances. This work was used by KKP, MIT, EFS, and AAD for their thesis to obtain their academic degrees.

This work was financially supported by Deputi Bidang Penguatan Riset dan Pengembangan, Kementerian Riset dan Teknologi / Badan Riset dan Inovasi Nasional Republic of Indonesia (Hibah PDUPT: 2733/UN1.DITLIT/DITLIT/PT/2020).

References

Ahn YH, Lee JY, Park HD, Kim TH, Park MC, Choi G, Kim S (2016) Identification of a New Morpholine Scaffold as a P2Y12 Receptor Antagonist. Molecules 21(9): 1–1114. https://doi.org/10.3390/molecules21091114

Anderson SD, Shah NK, Yim J, Epstein BJ (2010) Efficacy and safety of ticagrelor: a reversible P2Y12 receptor antagonist. Annals of Pharmacotherapy 44(3): 524–537. https://doi.org/10.1345/aph.1M548

Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, Delling FN, Djousse L, Elkind MSV, Ferguson JF, Fornage M, Jordan LC, Khan SS, Kissela BM, Knutson KL, Kwan TW, Lackland DT, Lewis TT, Lichtman JH, Longenecker CT, Loop MS, Lutsey PL, Martin SS, Matsushita K, Moran AE, Mussolino ME, O'Flaherty M, Pandey A, Perak AM, Rosamond WD, Roth GA, Sampson UKA, Satou GM, Schroeder EB, Shah SH, Spartano NL, Stokes A, Tirschwell DL, Tsao CW, Turakhia MP, VanWagner LB, Wilkins JT, Wong SS, Virani SS (2019) Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation 139: e56–e528. https://doi.org/10.1161/CIR.0000000000000659

Cattaneo M (2015) P2Y12 receptors: structure and function. 13(S1): S10–S16. https://doi.org/10.1111/jth.12952

Cattaneo M, Cerletti C, Harrison P, Hayward CP, Kenny D, Nugent D, Nurden P, Rao AK, Schmaier AH, Watson SP, Lussana F, Pugliano MT, Michelson AD (2013) Recommendations for the Standardization of Light Transmission Aggregometry: A Consensus of the Working Party from the Platelet Physiology Subcommittee of SSC/ISTH. Journal of Thrombosis and Haemostasis 11(6): 1183–1189. https://doi.org/10.1111/jth.12231

Fakhrudin N, Wiyono T, Putra A, Nurrochmad A, Widyarini S (2019) The evaluation on anti-platelet and antithrombosis activities of Cinnamomum sintoc bark extract. Thai Journal of Pharmaceutical Sciences 43: 219–226. https://doi.org/10.1063/1.5062741

Hakim EH, Achmad SA, Juliawaty LD, Makmur L, Syah YM, Aimi N, Kitajima M, Takayama H, Ghisalberti EL (2006) Prenylated flavonoids and related compounds of the Indonesian Artocarpus (Moraceae). Journal of Natural Medicines 60: 161–184. https://doi.org/10.1007/s11418-006-0048-0

Jagtap UB, Bapat VA (2010) Artocarpus: a review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology 129(2): 142–166. https://doi.org/10.1016/j.jep.2010.03.031

Jantan I, Mohd Yasin YH, Jalil J, Murad S, Idris MS (2009) Antiplatelet aggregation activity of compounds isolated from Guttiferae species in human whole blood. Pharmaceutical Biology 47(11): 1090–1095. https://doi.org/10.3109/13880200903008641

Jantan I, Mohd Yasin YH, Jamil S, Sirat H, Basar N (2010) Effect of prenylated flavonoids and chalcones isolated from Artocarpus species on platelet aggregation in human whole blood. Journal of Natural Medicines 64(3): 365–369. https://doi.org/10.1007/s11418-010-0410-0

Jarvis GE, Humphries RG, Robertson MJ, Leff P (2000) ADP can induce aggregation of human platelets via both P2Y(1) and P(2T) receptors. British Journal of Pharmacology 129: 275–282. https://doi.org/10.1038/sj.bjp.0703046

Koltai K, Kesmarky G, Feher G, Tibold A, Toth K (2017) Platelet Aggregometry Testing: Molecular Mechanisms, Techniques and Clinical Implications. International Journal of Molecular Sciences 18(8): 1–1803. https://doi.org/10.3390/ijms18081803

Koupenova M, Ravid K (2018) Biology of Platelet Purinergic Receptors and Implications for Platelet Heterogeneity. Frontiers in Pharmacology 9. https://doi.org/10.3389/fphar.2018.00037

Labarthe B, Théroux P, Angioï M, Ghitescu M (2005) Matching the evaluation of the clinical efficacy of clopidogrel to platelet function tests relevant to the biological properties of the drug. Journal of the American College of Cardiology 46(4): 638–645. https://doi.org/10.1016/j.jacc.2005.02.092

Li F, Xu D, Hou K, Gou X, Li Y (2020) The role of P2Y12 receptor inhibition in ischemic stroke on microglia, platelets and vascular smooth muscle cells. Journal of Thrombosis and Thrombolysis. https://doi.org/10.1007/s11239-020-02098-4

Lopes RD (2011) Antiplatelet agents in cardiovascular disease. Journal of Thrombosis and Thrombolysis 31: 306–309. https://doi.org/10.1007/s11239-011-0558-9

Lordkipanidze M, Pharand C, Palisaitis DA, Schampaert E, Diodati JG (2009) Insights into the interpretation of light transmission aggregometry for evaluation of platelet aggregation inhibition by clopidogrel. Thrombosis Research 124(5): 546–553. https://doi.org/10.1016/j.thromres.2009.04.003

Mackman N (2008) Triggers, targets and treatments for thrombosis. Nature 451: 914–918. https://doi.org/10.1038/nature06797

Mackman N, Bergmeier W, Stouffer GA, Weitz JI (2020) Therapeutic strategies for thrombosis: new targets and approaches. Nature Reviews Drug Discovery 19: 333–352. https://doi.org/10.1038/s41573-020-0061-0

Mansour A, Bachelot-Loza C, Nesseler N, Gaussem P, Gouin-Thibault I (2020) P2Y(12) Inhibition beyond Thrombosis: Effects on Inflam-

mation. International Journal of Molecular Sciences 21: 1–1391. https://doi.org/10.3390/ijms21041391

McLean S, Reynolds WF, Tinto WF, Chan WR, Shepherd V (1996) Complete 13C and 1H Spectral Assignments of Prenylated Flavonoids and a Hydroxy Fatty Acid from the Leaves of Caribbean *Artocarpus communis*. Magnetic Resonance in Chemistry 34(9): 719–722. https://doi.org/10.1002/(SICI)1097-458X(199609)34:9%3C719::AID-OMR948%3E3.0.CO;2-Q

Murugappa S, Kunapuli SP (2006) The role of ADP receptors in platelet function. Frontiers in Bioscience 11: 1977–1986. https://doi.org/10.2741/1939

Nawarskas JJ, Snowden SS (2011) Critical appraisal of ticagrelor in the management of acute coronary syndrome. Therapeutics and clinical risk management 7: 473–488. https://doi.org/10.2147/TCRM.S19835

Nylander S, Schulz R (2016) Effects of P2Y12 receptor antagonists beyond platelet inhibition – comparison of ticagrelor with thienopyridines. British Journal of Pharmacology 173(7): 1163–1178. https://doi.org/10.1111/bph.13429

Puri RN, Colman RW, Liberman MA (1997) ADP-induced platelet activation. Critical Reviews in Biochemistry and Molecular Biology 32(6): 437–502. https://doi.org/10.3109/10409239709082000

Rubenstein DA, Yin W (2018) Platelet-Activation Mechanisms and Vascular Remodeling. In: Pollock DM (Ed.) Comprehensive Physiology. American Physiological Society, 1117–1156. https://doi.org/10.1002/cphy.c170049

Storey RF (2001) The P2Y 12 receptor as a therapeutic target in cardiovascular disease. Platelets 12(4): 197–209. https://doi.org/10.1080/09537100120058739

Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, Dzobo K (2018) Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. International Journal of Molecular Sciences 19(6): 1–1578. https://doi.org/10.3390/ijms19061578

Wang Y, Xu K, Lin L, Pan Y, Zheng X (2007) Geranyl flavonoids from the leaves of Artocarpus altilis. Phytochemistry 68: 1300–1306. https://doi.org/10.1016/j.phytochem.2007.01.009

Weng J-R, Chan S-C, Lu Y-H, Lin H-C, Ko H-H, Lin C-N (2006) Antiplatelet prenylflavonoids from Artocarpus communis. Phytochemistry 67: 824–829. https://doi.org/10.1016/j.phytochem.2006.01.030

Yeung J, Li W, Holinstat M (2018) Platelet Signaling and Disease: Targeted Therapy for Thrombosis and Other Related Diseases. Pharmacological Reviews 70(3): 526–548. https://doi.org/10.1124/pr.117.014530

Supplementary material 1

Supplementary Figures

Authors: Nanang Fakhrudin, Krisna Kharisma Pertiwi, Marce Inggrita Takubessi, Eka Fitri Susiani, Arif Nurrochmad, Sitarina Widyarini, Ari Sudarmanto, Arief Adi Nugroho, Subagus Wahyuono

Data type: **Figure S1.** 2D HMBC and HMQC observation of the correlations in the NMR spectral experiments of GTDC. **Figure S2.** TLC profile of *A. altilis* extract and the isolated compound GTDC. **Figure S3.** Sigmoidal dose–response curve showing the platelet disaggregation activity of GDTC in ADP-induced platelet aggregation.

Link: https://doi.org/10.3897/PlantSociology.67.56788.suppl1

A geranylated chalcone with antiplatelet activity from the leaves of breadfruit (Artocarpus altilis)

ORIGINALITY REPORT

16%	9%	13%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

1%

★ synapse.koreamed.org

Internet Source

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

A geranylated chalcone with antiplatelet activity from the leaves of breadfruit (Artocarpus altilis)

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8