Screening of selected Indonesian plants for antiplatelet activity

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Abstract. Fakhrudin N, Mufinnah FF, Husni MF, Wardana AE, Wulandari EI, Putra AR, Santoso D, Nurrochmad A, Wahyunono S. 2021. Screening of selected Indonesian plants for antiplatelet activity. Biodiversitas 22: 5268-5273. Cardiovascular diseases remain the leading cause of death worldwide. Platelet aggregation plays a crucial role in the development of cardiovascular diseases. Thus, the discovery of bioactive compounds targeting platelet aggregation is a promising approach for combating cardiovascular diseases. Indonesia has plants diversity with the potential to be developed as antiplatelet agents. Thus, this study was aimed to identify the antiplatelet potential of selected Indonesian plant extracts. The plant extracts (100 µg mL−1) were evaluated for their antiplatelet activity in CaCl2-induced platelet aggregation in 96-well plates. In an aggregometer, the active extracts were further evaluated in adenosine diphosphate (ADP)-induced platelet aggregation. The antiplatelet agents, aspirin, and ticagrelor were used as reference drugs in the respective screening assays. Of the 139 tested extracts, ten demonstrated antiplatelet activity in CaCl2-induced platelet aggregation. The evaluation of the ten active extracts in ADP-induced platelet aggregation revealed four active extracts. The most active extract was the methanol extract of Cinnamomum sintoc bark, followed by the methanol extract of Lelea aequata leaf, and the dichloromethane and methanol extracts of Physalis angulata petal with moderate activities. This study provides essential scientific evidence for the development of antiplatelet agents from plant origins.

Keywords: Adenosine diphosphate, cardiovascular diseases, herbal medicine, platelet aggregation, thrombosis

INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide. In 2019, approximately 17.9 million people died from this disease, and the number of suffered patients rises annually (Virani et al. 2021; WHO 2021). The main feature of cardiovascular disease is atherothrombosis, in which platelet aggregation plays a crucial role. Therefore, inhibition of platelet aggregation is the main target in cardiovascular disease pharmacotherapy (Yeung et al. 2018; Nording et al. 2020). Rester platelets with a biconvex discoid shape change into a fully spread star-like form that tends to aggregate due to an increase in intracellular calcium level. Further, the platelets immediately aggregate in response to the binding of fibrinogen with glycoprotein IIb/IIIa receptors (Ghoshal and Bhattacharyya 2014; Chu et al. 2021). The aggregated platelets release platelet activators, such as thromboxane A₂, adenosine diphosphate (ADP), and serotonin, which intensify platelet aggregation, leading to clot formation, thrombus, and vascular blockage (Yun et al. 2016; Rubenstein and Yin 2018). Therefore, inhibition of platelet aggregation serves as a promising therapeutic target in treating cardiovascular diseases (Pimentel et al. 2003; Toyoda et al. 2018).

Currently, the most favorable drug inhibitors of platelet aggregation (antiplatelet) are aspirin and thienopyridines (prasugrel, clopidogrel, and ticlopidine). However, the long-term use of those drugs is limited by the risk of bleeding, drug resistance, and response variability (Di Minno et al. 2011; Ray 2014; Hidayati et al. 2017). Therefore, the search for new antiplatelet agents is a promising approach for combating cardiovascular diseases (Nording et al. 2020; Thomas and Nicolson 2021). For decades, plants have inspired the development of many drugs benefitting human health (Atanasov et al. 2021; Yumni et al. 2021). Indonesia has a vast plant diversity, providing abundant natural compounds that can be explored as antiplatelet agents. This study was aimed to test the antiplatelet activity of 139 selected Indonesian plant extracts. The active extracts can be further developed as antiplatelet agents for the treatment of cardiovascular diseases.

MATERIALS AND METHODS

Procedures

Plant extracts

The extracts evaluated in this study were obtained from the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. The origin plant materials of the extracts were
authenticated by a botanist (Dr. Djoko Santosa), and the certificate of authentication was documented in the library of the Faculty of Pharmacy, UGM. All extracts were produced by maceration of the plant materials using ethanol, methanol, dichloromethane, chloroform, water, and ethyl acetate, and the extracts were preserved in the deposit fridge (-20°C) in the Research Laboratory of the Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM.

Chemicals

Sodium citrate, aspirin, ticagrelor, bovine serum albumin, Hepes, Tyrode’s buffer, Giemsa dye, and adenosine diphosphate were purchased from Sigma–Aldrich (USA). DMSO, dichloromethane, chloroform, and calcium chloride were obtained from Merck (Germany). Methanol, ethanol, and aquadest were procured from Brataco (Indonesia).

Human participants and platelet source

Human platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained from healthy donors who met the inclusion and exclusion criteria (Cattaneo et al. 2013) as follows: men/women aged 18-40 years having a platelet number of (15-40) *10^10* μL⁻¹, not pregnant, no caffeine consumption, and smoking at least 120 and 30 min before blood withdrawal, respectively, no consumption of drugs affecting hemostasis (e.g., NSAIDs, anticoagulant, or antiplatelet agents) at least 7 days before blood withdrawal, and no hemophilia or bleeding disorders. In addition, all participants received informed consent, and the institutional ethics committee approved the method of the Faculty of Medicine, Public Health, and Nursing, UGM, Yogyakarta (number KE/FK/622/EC/2015).

Platelet preparation

Human platelets from healthy individuals were prepared as previously described (Fakhrudin et al. 2019; Hastuti et al. 2021). Blood from the donors was added with sodium citrate solution (10%) and centrifuged (1000 rpm, 15 min). The upper phase supernatant (PRP) was separated, and the lower phase was further centrifuged (3500 rpm, 15 min). Next, the upper supernatant (PPP) was separated. The number of platelets in the PRP was counted, and only the platelet number of (15-40) *10^10* μL⁻¹ was used for the experiment.

CaCl₂-induced platelet aggregation assay

The antiplatelet screening assay was performed as previously described (Pimentel et al. 2003) in a 96-well plate. The extracts were tested at 0.5 and 2 mg mL⁻¹. PRP was resuspended (1:5 v/v) in a Hepes–Tyrode buffer containing 1% bovine serum albumin and then incubated at 37°C for 5 min. The extracts (2.5 μL in DMSO) were added to the platelet suspension (192 μL) and incubated for 30 min. Aspirin and DMSO were used as positive and negative controls, respectively. Platelet aggregation was induced by adding 250 mM CaCl₂ (4 μL) followed by 30 min incubation. Each well was added with Giemsa stain (4 μL) and incubated for 5 min. The solution was gently discarded, and platelet aggregation was characterized by the formation of violet gel in the well. The extract that was able to inhibit violet gel formation was considered active. This experiment was performed in a triplicate.

ADP-induced platelet aggregation assay

The second bioassay for confirming the antiplatelet activity of the extracts was conducted using ADP-induced platelet aggregation in an aggregometer instrument (Chrono-Log 490 2D; USA) (Fakhrudin et al. 2020). The extracts were tested at a concentration of 100 μg mL⁻¹. DMSO was used as a solvent vehicle or negative control, whereas ticagrelor (10 μg mL⁻¹) was used as a reference drug or positive control. PRP (495 μL) was added with the extracts (100 μg mL⁻¹ in DMSO) and then incubated (37°C for 3 min) with constant stirring (1200 rpm). In brief, 2.5 μL of 10 μM ADP was added to induce platelet aggregation, and the aggregation was recorded for 6 min. The degree of platelet aggregation was normalized to the PPP and calculated based on the amplitude of the curve generated in the aggregometer (Wiyono et al. 2018).

Data analysis

The data obtained from the ADP-induced platelet aggregation assay were analyzed in GraphPad Prism 8 software, and the statistical analysis was conducted using ANOVA followed by Dunnet post-hoc test.

RESULTS AND DISCUSSION

This exploratory study aimed to find antiplatelet agents from plant origins. Platelet aggregation has a complex pathway with many proteins involved. Thus, two bioassays were employed to screen active plant extracts. The CaCl₂-induced platelet aggregation, recommended by Pimentel et al. (2003) is a fast and general method to evaluate antiplatelet activity. The active extracts obtained from the first method were evaluated in the second method utilizing ADP-induced platelet aggregation (Fakhrudin et al. 2020). ADP induces platelet aggregation by binding to P2Y12 receptor. Using these two bioassays, two pathways of platelet aggregation were targeted.

Results from the first antiplatelet screening assay showed that 10 out of the 139 plant extracts were active by inhibiting the platelet aggregation at 2 and 10 mg mL⁻¹. The ethanol extract of Averrhoa bilimbi leaf (12), methanol extract of Cinnamomum sintoc bark (26), ethanol extract of Garcinia mangostana rind (50), methanol extract of Leea aequata leaf (68), ethanol and dichloromethane extracts of Physalis angulata petal (85 and 86), ethanol extract of Piper cubeba fruit (93), methanol extract of Rubus chrysophyllus leaf and stem (104), and methanol and dichloromethane extracts of Tetracera mainaengyi leaf and stem (136 and 137) inhibited platelet aggregation in CaCl₂-induced platelet aggregation. As expected, aspirin, a clinically used antiplatelet drug, showed potent activity. This result suggested that the method is suitable for the detection of antiplatelet agents.
| Local name       | Plant species               | Family                  | Part of plant | Solvent | 10 mg mL⁻¹ | 2 mg mL⁻¹ |
|------------------|-----------------------------|-------------------------|----------------|---------|------------|-----------|
| Bawang putih     | Allium sativum L.           | Amaryllidaceae          | t              | DCM     | +          | +         |
| Bawang putih     | Allium sativum L.           | Amaryllidaceae          | t              | DCM     | +          | +         |
| Bawang putih     | Allium sativum L.           | Amaryllidaceae          | l              | EtOH    | +          | +         |
| Bawang putih     | Allium sativum L.           | Amaryllidaceae          | l              | EtOH    | +          | +         |
| Sambiloto        | Andrographis paniculata (Burm.f.) Nees | Acanthaceae          | l, s            | EtOH    | +          | +         |
| Seledri           | Apium graveolens L.         | Apiaceae                | l              | EtOH    | -          | -         |
| Akar kuning      | Arcangelisia flavia (L.) Merr. | Menispermaceae          | s              | MeOH    | -          | -         |
| Sukun            | Artocarpus altulis (Parkinon ex F.A. Zorn) Fosberg | Moraceae             | l              | EtOH    | +          | +         |
| Sukun            | Artocarpus altulis (Parkinon ex F.A. Zorn) Fosberg | Moraceae             | l              | Aq      | +          | +         |
| Belimbing wuluh  | Averrhoa bilimbi L.         | Oxalidaceae             | l              | DCM     | +          | +         |
| Belimbing wuluh  | Averrhoa bilimbi L.         | Oxalidaceae             | l              | EtOH    | -          | -         |
| Belimbing wuluh  | Averrhoa bilimbi L.         | Oxalidaceae             | f              | EtOH    | -          | -         |
| Belimbing wuluh  | Averrhoa bilimbi L.         | Oxalidaceae             | f              | DCM     | +          | +         |
| Belimbing wuluh  | Averrhoa carambolae L.      | Oxalidaceae             | l              | DCM     | +          | +         |
| Belimbing wuluh  | Averrhoa carambolae L.      | Oxalidaceae             | l              | EtOH    | -          | -         |
| Belimbing wuluh  | Averrhoa carambolae L.      | Oxalidaceae             | f              | DCM     | +          | +         |
| Belimbing manis  | Averrhoa carambolae L.      | Oxalidaceae             | f              | EtOH    | +          | +         |
| Asam riang       | Begonia coriacea Hassk.     | Begoniaceae             | s, f, fl       | CHCl₁   | +          | +         |
| Asam riang       | Begonia coriacea Hassk.     | Begoniaceae             | s, f, fl       | MeOH    | +          | +         |
| Rotan sega       | Calamus caesius Bl.         | Areceae                 | h              | CHCl₁   | +          | +         |
| Rotan sega       | Calamus caesius Bl.         | Areceae                 | l              | MeOH    | +          | +         |
| Daun teh         | Camellia sinensis (L.) O.K. | Theaceae                | l              | EtOH    | +          | +         |
| Tali putri       | Cassytha filiformis L.      | Lauraceae               | l, s, f        | MeOH    | +          | +         |
| Tali putri       | Cassytha filiformis L.      | Lauraceae               | l, s, f        | MeOH    | +          | +         |
| Sintok           | Cinnamomum sintoc Bl.       | Lauraceae               | c              | MeOH    | +          | +         |
| Sintok           | Cinnamomum sintoc Bl.       | Lauraceae               | c              | CHCl₁   | +          | +         |
| Kayu manis Sri Lanka | Cinnamomum zeylanicum Bl. | Lauraceae               | c              | EtOH    | +          | +         |
| Galing kerbau    | Cissus adnata Roxb.         | Vitaceae                | l, s, f, fl    | MeOH    | +          | +         |
| Galing kerbau    | Cissus adnata Roxb.         | Vitaceae                | l, s, f, fl    | DCM     | +          | +         |
| Galing kerbau    | Cissus adnata Roxb.         | Vitaceae                | l, s, f, fl    | MeOH    | +          | +         |
| Irah-irah        | Cissus javana DC.           | Vitaceae                | l, s            | MeOH    | -          | -         |
| Senggugu         | Clerodendron serratum (L.) Moon | Lamiaceae             | r              | EtOH    | +          | +         |
| Kunyit           | Curcuma longa L.            | Zingiberaceae           | rh             | EtOH    | +          | +         |
| Kunyit           | Curcuma longa L.            | Zingiberaceae           | rh             | Aq      | -          | -         |
| Temu putih       | Curcuma zedoaria Roxb.      | Zingiberaceae           | t              | EtOH    | +          | +         |
| Temu putih       | Curcuma zedoaria Roxb.      | Zingiberaceae           | t              | EtOH    | +          | +         |
| Temu putih       | Curcuma zedoaria Roxb.      | Zingiberaceae           | t              | EtOH    | +          | +         |
| Temu putih       | Curcuma zedoaria Roxb.      | Zingiberaceae           | t              | EtOH    | +          | +         |
| Wortel           | Daucus carota L.            | Apiceae                 | t              | EtOH    | +          | +         |
| Rigu ripung      | Erechtites minima (Poir.) DC. | Compositae             | l, s, fl       | MeOH    | +          | +         |
| Rigu ripung      | Erechtites minima (Poir.) DC. | Compositae             | l, s, fl       | MeOH    | +          | +         |
| Daun dadap serep | Erythrina subumbrae (Hassk.) Merr. | Leguminosae           | r, s, f        | CHCl₁   | +          | +         |
| Daun dadap serep | Erythrina subumbrae (Hassk.) Merr. | Leguminosae           | r, s, f        | MeOH    | +          | +         |
| Teklan           | Equatorium riparium Reg.    | Compositae             | l, s            | CHCl₁   | +          | +         |
| Teklan           | Equatorium riparium Reg.    | Compositae             | l, s            | MeOH    | +          | +         |
| Jirak            | Eurya nitida Korth.         | Penthialyaceae         | l, s            | MeOH    | -          | -         |
| Pasak bumi       | Eurycoma longiseta Jack.    | Simaroubaceae          | r              | MeOH    | +          | +         |
| Awar-awar        | Ficus septica Burm.f.       | Moraceae                | l              | EtOH    | +          | +         |
| Akar karamaha    | Ficus sagittata Vahl.       | Moraceae                | l, s            | CHCl₁   | +          | +         |
| Akar karamaha    | Ficus sagittata Vahl.       | Moraceae                | l, s            | MeOH    | +          | +         |
| Manggis          | Garcinia mangostana L.      | Clusiaceae             | ri             | EtOH    | +          | +         |
| Kapulaga ambon   | Globba marantina L.         | Zingiberaceae           | h              | MeOH    | +          | +         |
| Kapulaga ambon   | Globba marantina L.         | Zingiberaceae           | h              | MeOH    | +          | +         |
| Daun dewa        | Gymnura procumbens (Lour.) Merr. | Compositae             | l              | EtOH    | +          | +         |
| Daun dewa        | Gymnura procumbens (Lour.) Merr. | Compositae             | l              | DCM     | -          | -         |
| Sirihan          | Heckeria peltata (L.) Kunth. | Piperaceae              | l, s            | CHCl₁   | +          | +         |
| Sirihan          | Heckeria peltata (L.) Kunth. | Piperaceae              | l, s            | MeOH    | +          | +         |
| Tumpak bumi      | Horstmania mollis (Bl.) Val. | Zingiberaceae           | rh             | MeOH    | +          | +         |
| Tumpak bumi      | Horstmania mollis (Bl.) Val. | Zingiberaceae           | rh             | CHCl₁   | +          | +         |
| Buah naga        | Hylocereus costaricensis (F.A.C.Weber) Briton & Rose Cactaceae | f              | EtOH    | +          | +         |
| Bunga sapa       | Impatiens platypetala Lindl. | Balsaminaceae          | ae              | CHCl₁   | +          | +         |
| Bunga sapa       | Impatiens platypetala Lindl. | Balsaminaceae          | ae              | MeOH    | -          | -         |
| Ubi jalar        | Ipomoea batata (L.) Lam.    | Convolvulaceae         | l, s, r        | CHCl₁   | +          | +         |
| Daun gatal       | Laportea aequans (L.) Chew. | Urticaceae             | l, s, r        | MeOH    | +          | +         |
| Daun gatal       | Laportea aequans (L.) Chew. | Urticaceae             | l, s, r        | MeOH    | +          | +         |
| Daun gatal       | Laportea stimulans (L.f.) Miq. | Urticaceae             | l, s, r        | CHCl₁   | +          | +         |
| Daun gatal       | Laportea stimulans (L.f.) Miq. | Urticaceae             | l, s, r        | MeOH    | -          | -         |
| Mali-mali putih  | Lea aequata L.              | Vitaceae               | l, s            | CHCl₁   | +          | +         |
| Mali-mali putih  | Lea aequata L.              | Vitaceae               | l, s            | MeOH    | -          | -         |
| Plant Name | Scientific Name | Family | Extraction Method | Availability |
|------------|-----------------|--------|-------------------|--------------|
| Ambulia    | Linnophila rugosa (Roth.) Merr. | Plantaginaceae | l.s. DCM | + + |
| Ambulia    | Linnophila rugosa (Roth.) Merr. | Plantaginaceae | l.s. MeOH | + + |
| Sanrego    | Lanasia amara Blanco | Rutaceae | l.s. MeOH | + + |
| Apel       | Malus domestica Borkh. | Rosaceae | f EtOH | + + |
| Mangga kasturi | Manggera castari Kosterm. | Anacardiaceae | f MeOH | - + |
| Senggani   | Marunia affinis Korth. | Melastomaceae | l.s. DCM | + + |
| Senggani   | Marunia affinis Korth. | Melastomaceae | l.s. MeOH | + + |
| Mengkudu   | Morinda citrifolia L. | Rubiaceae | f EtOH | + + |
| Kemuning   | Murraya paniculata (L.) Jack. | Rutaceae | t EtOH | + + |
| Kemangi    | Ocimum basilicum L. | Lamiaceae | l EtOH | + + |
| Daun kumis kucing | Orthosiphon aristatus (Bl.) Miq. | Lamiaceae | l EtOH | + + |
| Ginseng    | Panax notoginseng (Burkill) F.H.Chen | Araliaceae | r EtOH | + + |
| Meniran    | Phyllanthus niruri L. | Phyllanthaceae | l.s. DCM | + + |
| Cipulkan   | Phyllanthus angulata L. | Solanaceae | p EtOH | - - |
| Poh-pohan  | Pilea melastomoides (Poir) Bl. | Urticaceae | l.s, r, fl. CHCl3 | + + |
| Sirhit hitam | Piper acre Blume | Piperaceae | l.s. CHCl3 | + + |
| Sirhit hitam | Piper acre Blume | Piperaceae | l.s. MeOH | - + |
| Kemukus    | Piper cubeba L.f. | Piperaceae | f EtOH | - - |
| Cabe jawa  | Piper retrofractum Vahl. | Piperaceae | f EtOH | + + |
| Jering     | Archidendron ellipticum (Blanco) Lc.Nielsen | Leguminosae | l.s. CHCl3 | - + |
| Jering     | Archidendron ellipticum (Blanco) Lc.Nielsen | Leguminosae | l.s. MeOH | - - |
| Daun sendok | Plantago lanceolata L. | Plantaginaceae | l.DCM | + + |
| Daun sendok | Plantago lanceolata L. | Plantaginaceae | l.EtOH | + + |
| Daun sendok | Plantago lanceolata L. | Plantaginaceae | l.EtOH | + + |
| Matoa      | Pomeia pinnata J.R.Forst. & G.Forst. | Sapindaceae | l.DCM | + + |
| Matoa      | Pomeia pinnata J.R.Forst. & G.Forst. | Sapindaceae | l.EtOH | + + |
| Buas-bus, Singkil | Premna cordifolia Roxb. | Lamiaceae | l.EtOH | + + |
| Jambu biji | Myrciu guajava L. | Myrtaceae | f EtOH | + + |
| Beberetan, Arben | Rubus chrysophyllus Reinh. Ex Miq. | Rosaceae | l.s. MeOH | - - |
| Beberetan, Arben | Rubus chrysophyllus Reinh. Ex Miq. | Rosaceae | l.s. CHCl3 | + + |
| Akar kuay ruang | Schefflera scandens (Bl.) R. Vig. | Araliaceae | l.s. CHCl3 | - + |
| Akar kuay ruang | Schefflera scandens (Bl.) R. Vig. | Araliaceae | l.s. MeOH | - + |
| Jakatuwa   | Scoparia dulcis L. | Scrophulariaceae | l.s. MeOH | - + |
| Jakatuwa   | Scoparia dulcis L. | Scrophulariaceae | l.s. DCM | + + |
| Daun cakay ayam | Selaginella plana Hieron | Selaginellaceae | l.s, r. CHCl3 | + + |
| Daun cakay ayam | Selaginella plana Hieron | Selaginellaceae | l.s. t. MeOH | - + |
| Sidadori, Saliguri | Sida myoresens Wigt & Arn. | Malvaceae | l.s, fl. MeOH | - + |
| Tomat      | Solanum lycopersicum L. | Solanaceae | l EtOH | + + |
| Tempuyung | Sonchus arvensis L. | Compositae | l EtOH | + + |
| Kemangi cina | Spigelia anthelina L. | Loganiaceae | l.s. DCM | + + |
| Kemangi cina | Spigelia anthelina L. | Loganiaceae | l.s. MeOH | + + |
| Sedonong   | Spondias dulcis Parkinson | Anacardiaceae | l DCM | + + |
| Sedonong   | Spondias dulcis Parkinson | Anacardiaceae | l EtOH | - + |
| Kepel      | Stelechocarpus burahol (Blume) Hook.f. & Thomson | Annonaceae | l.DCM | + + |
| Kepel      | Stelechocarpus burahol (Blume) Hook.f. & Thomson | Annonaceae | l.EtOH | + + |
| Daun hantap | Sterculia oblongata R. Br. | Sterculiaceae | l MeOH | + + |
| Keji beling | Sericoclyx crusps (L.) Brenek. | Acanthaceae | l EtOH | + + |
| Mahoni     | Svetienia macrophylla King | Meliaceae | se EtOH | + + |
| Jambu air  | Syzygium aquum (Burm.f.) Alston | Myrtaceae | f DCM | + + |
| Jambu air  | Syzygium aquum (Burm.f.) Alston | Myrtaceae | l DCM | + + |
| Jambu air  | Syzygium aquum (Burm.f.) Alston | Myrtaceae | f EtOH | - + |
| Jambu air  | Syzygium aquum (Burm.f.) Alston | Myrtaceae | l EtOH | + + |
| Jambu air  | Syzygium aquum (Burm.f.) Alston | Myrtaceae | l EtOH | + + |
| Jambu air  | Syzygium cuminii (L) Skeats | Myrtaceae | se EtOH | + + |
| Jambulang  | Syzygium cuminii (L) Skeats | Myrtaceae | se DCM | + + |
| Jambulang  | Syzygium cuminii (L) Skeats | Myrtaceae | l EtOH | + + |
| Jambulang  | Syzygium cuminii (L) Skeats | Myrtaceae | l EtOH | + + |
| Jambulang  | Syzygium cuminii (L) Skeats | Myrtaceae | l EtOH | + + |
| Jambulang  | Syzygium cuminii (L) Skeats | Myrtaceae | l EtOH | + + |
| Jambulang  | Syzygium cuminii (L) Skeats | Myrtaceae | f EtOH | - + |
| Jambulang  | Syzygium cuminii (L) Skeats | Myrtaceae | f DCM | + + |
| Som Jawa   | Talinum paniculatum (Jacq.) Gaertn. | Portulacaceae | r DCM | + + |
| Som Jawa   | Talinum paniculatum (Jacq.) Gaertn. | Portulacaceae | r EtOH | + + |
| Mempezas   | Tetraera maingayi Hoogland | Dilleniacae | l.s. CHCl3 | - - |
| Bratawali  | Tinospora crispa (L.) Hook. f. & Thomson | Menispermacae | f EtOH | + + |
| Bangle     | Zingiber montanum (J.Koenig) Link ex A.Dietr. | Zingiberaceae | rh EtOH | + + |
| Asparagus  | | | | |
Note: Part of plant. b: bark; f: fruit; fl: flower; h: herb; l: leaf; t: tuber; p: petal; r: root; rh: rhizome; ri: rind; s: stem; se: seed. Solvent. CHCl₃: chloroform; MeOH: methanol, EtOH: ethanol; DCM: dichloromethane; Aq: aquadest; EtOAc: ethyl acetate. Results, + : violet gel presents (aggregation); - : no violet gel (no aggregation). The bold letters indicated the active plant extracts.

Platelet aggregation is also triggered by the activation of platelet receptors, such as P2Y12, PAR2, PAR4, TPα, α2, and glycoprotein receptors by their respective ligands. Among these receptors, P2Y12 is the most studied and represents a promising target for antiplatelet agents. Activation of P2Y12 by its ligand ADP not only induces platelet aggregation but also provokes platelet degranulation, leading to additional ADP release that intensifies aggregation (Murugappa and Kunapuli 2006; Yeung et al. 2018). The use of prasugrel and ticagrelor as potent P2Y12 antagonists is effective for atherothrombosis (Collet et al. 2021). Thus, we evaluated the effectiveness of the active plant extracts to inhibit platelet aggregation induced by ADP (Figure 1). Ticagrelor inhibited platelet aggregation induced by ADP, indicating that this bioassay was appropriate for the screening of P2Y12 antagonists. The methanol extract of C. sintoc cortex (27), methanol extract of L. aequata leaf (68), ethanol and dichloromethane extracts of P. angulata petal (85 and 86) demonstrated antiplatelet activity.

Figure 1 shows that the methanol extract of C. sintoc cortex showed the strongest effect among the extracts. The bark of C. sintoc contains tannins, 3-allyl-6-methoxyphenol, methyl eugenol, cis-methyl isoeugenol, p-acetamidophenol, methyl myristate, methyl palmitate, methyl linoleate, methyl octadec-9-enoate, methyl stearate, and methyl 11-eicosenoate (Jantan et al. 2005; Fakhrudin et al. 2019). Interestingly, none of these compounds were reported to have antiplatelet activity. Although a previous study demonstrated that the tannin content of the methanol extract of C. sintoc cortex is linearly correlated with the antiplatelet activity induced by epinephrine (Fakhrudin et al. 2019), the compound responsible for the antiplatelet activity remains unknown. Thus, further research on isolating and identifying the antiplatelet compound from C. sintoc bark is a vast prospect. Cinnamomum tenuifolium (Dong et al. 2013) and C. philippinense (Yu et al. 1994) contain the antiplatelet agents isoumenolide and cinnamophilin, respectively. However, no study reported the presence of these compounds in C. sintoc.

The other plant extracts with a moderate activity are the methanol extract of L. aequata leaf (68) and the ethanol and dichloromethane extracts of P. angulata petal (85 and 86). Lelea aequata is a wild shrub traditionally used because of its antiinflammarial, antifever, antiantelmentic, antiajaundice, and astringent effects (Sinaga et al. 2018). A recent study has reported the presence of new compounds, including (7S,8R)9′-O-acetylcedrusin (a lignan) and (3S,4S)-4-chloro-3-hydroxyperipederin-2-one (a lactam), and several known compounds (Tun et al. 2019). However, none of them have antiplatelet activity. Apr from L. aequata, P. angulata contains physalin B as an antiplatelet compound isolated from the aerial parts (Mangwala et al. 2013; Hsu et al. 2014; Yang et al. 2018). In line with the previous finding, we found that the petal extracts of P. angulata showed antiplatelet activity. Therefore, it merits further study because it is underutilized apart from the edible fruit.

In summary, we identified ten plant extracts showing antiplatelet activity by using CaCl₂-induced platelet aggregation. Four out of ten active extracts demonstrated significant antiplatelet activity in ADP-induced platelet aggregation. The methanol extract of C. sintoc bark is the most potent extract followed by the methanol extract of L. aequata leaf, the dichloromethane and methanol extracts of P. angulata petal. These extracts are promising to be developed further as antiplatelet agents to combat cardiovascular diseases. This study provides a scientific basis for the development of antiplatelet agents from natural plant products.

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