

INTISARI

Platelet memainkan peran penting dalam *Cardiovascular Disease* (CVD). Agregasi platelet dapat memicu pembentukan trombus dan dapat diinduksi oleh berbagai agonis, yang dapat mengaktifkan agregasi melalui berbagai jalur aktivasi. Agonis platelet, seperti trombin, ADP (adenosin difosfat), TXA₂ (tromboksan A₂) atau epinefrin, merangsang reseptor permukaan tipe GPCR, sementara agonis yang lain, seperti kolagen dan vWF (faktor von Willebrand), merangsang reseptor imunoglobulin. Pada uji pendahuluan telah dilakukan skrining terhadap 139 tanaman asli Indonesia dan 10 diantaranya berpotensi mempunyai efek antiplatelet salah satunya yaitu buah kemukus (*Piper cubeba* L.f.). Golongan utama obat antiplatelet adalah inhibitor Cyclooxygenase (Aspirin), antagonis reseptor P2Y₁₂ (turunan Thienopyridine), inhibitor Phosphodiesterase (Cilostazol, Dipyridamole), Glycoprotein IIb/IIIa *receptor blocker* (Abciximab), dan Protease-activated 1 *receptor inhibitor* (Vorapaxar). Namun pemberian inhibitor aktivasi platelet sering mengakibatkan komplikasi perdarahan dan resistensi obat. Sehingga diperlukan senyawa komplementer atau alternatif dengan efek samping yang relatif lebih sedikit. Penelitian ini bertujuan untuk mengetahui senyawa aktif pada buah kemukus yang mempunyai efek antiplatelet. Serta untuk mengetahui pada jalur mana penghambatan agregasi platelet oleh isolat dan mengetahui aktivitas antitrombosisnya.

Isolasi diawali dengan proses ekstraksi serbuk buah kemukus dengan maserasi menggunakan pelarut etanol 96%, kemudian menggunakan kromatografi kolom cair vakum (KCV) dan diuji fraksi yang diperoleh terhadap efek antiplateletnya secara *in vitro* dengan berbagai agonis yaitu epinefrin, Asam Arakhidonat (AA), trombin, *Adenosine Diphosphat* (ADP), dan ristosetin sehingga didapatkan fraksi aktif. Pemisahan selanjutnya menggunakan kromatografi kolom dan kromatografi lapis tipis preparatif (KLTP). Senyawa aktif kemudian diisolasi dan diuji kemurniannya dengan KLT dan HPLC. Uji aktivitas secara *in vitro* dengan uji aggregometer menggunakan platelet dari darah manusia, serta uji *in vivo* yaitu uji antitrombosis digunakan mencit jantan galur Balb/c, organ paru, otak, dan jantung untuk analisis histopatologi dengan pewarnaan *hematoxyllin eosin* (HE).

Senyawa aktif dari buah kemukus yang mempunyai aktivitas antiplatelet secara *in vitro* adalah cubebin. Pada uji antiagregasi platelet, cubebin terbukti mempunyai efek antiplatelet pada platelet yang diinduksi epinefrin dan trombin dengan masing-masing nilai IC₅₀ 52,40±0,42 µM dan 14,62±1,16 µM. Cubebin dari buah kemukus berdasar analisis statistika jika dibandingkan dengan kelompok kontrol negatif, terbukti bahwa cubebin semua dosis tidak mampu memproteksi hewan uji terhadap kematian, cubebin dosis 2,4; 5,85; 11,7 mg/Kg BB mampu memperpanjang waktu waktu hidup hewan uji, dan semua kelompok perlakuan dengan cubebin dosis 2,4; 5,85; 11,7; dan 23,4 mg/Kg mampu menghambat terbentuknya trombus jika dibandingkan dengan kelompok kontrol negatif.

Kata kunci : antiplatelet, buah kemukus, cubebin

ABSTRACT

Antiplatelet agents play crucial role in the therapy of cardiovascular and cerebrovascular diseases. Antiplatelet drugs are important for antithrombotic treatment in patients at high risk of arterial thrombosis. Antiplatelet agents prevent the formation of second messengers, by interacting with intracellular signaling pathways, blocking membrane receptors, or inhibiting platelet aggregation. Aspirin is the most widely used antiplatelet agent because aspirin is effective in reducing the incidence of cardiovascular diseases such as ischemic stroke. Despite the considerable benefit, about 15% - 25% of patients have reported resistance to aspirin. This aspirin resistance can increase the risk of recurrent ischemic stroke and even death in patients. In addition, aspirin which is classified as a Non-Steroidal Anti-Inflammatory Drug (NSAID) has known for its side effect (stomach irritation and bleeding). The search for a new generation of antiplatelet bioactive compound from natural resources with high efficacy and favorable side effects is a promising approach for combating cardiovascular diseases. This study aims to determine the active compounds in cubeb fruit that have antiplatelet effects. As well as to find out which pathway inhibits platelet aggregation by isolates and determines their antithrombotic activity.

Isolation began with the process of extracting cubeb fruit powder by maceration using 96% ethanol solvent, then using vacuum liquid column chromatography (KCV) and testing the fractions obtained for their antiplatelet effect in vitro with various agonists namely epinephrine, Arachidonic Acid (AA), thrombin, Adenosine Diphosphate (ADP), and rissettin to obtain the active fraction. Further separation using column chromatography and preparative thin layer chromatography (KLTP). The active compound was then isolated and tested for purity by TLC and HPLC. The in vitro activity test was carried out using an aggregometer test using platelets from human blood, as well as an in vivo test, namely the antithrombosis test, using Balb/c strain male mice, lung, brain, and heart organs for histopathological analysis with hematoxylin eosin (HE) staining.

The active compound from cubeb fruit which has antiplatelet activity in vitro is cubebin. In the platelet anti-aggregation test, cubebin was shown to have an antiplatelet effect on epinephrine and thrombin-induced platelets with IC₅₀ values of $52.40 \pm 0.42 \mu\text{M}$ and $14.62 \pm 1.16 \mu\text{M}$, respectively. Cubebin from cubeb based on statistical analysis when compared to the negative control group, it was evident that all doses of cubebin were not able to protect test animals against death, cubebin dose of 2.4; 5.85; 11.7 mg/Kg BW was able to extend the life time of the test animals, and all groups were treated with cubebin doses of 2.4; 5.85; 11.7; and 23.4 mg/Kg was able to inhibit thrombus formation when compared to the negative control group.

Keywords : antiplatelet, cubeb, cubebin