

## Conference Paper

# Water Hyacinth (*Eichhorniacrassipes*) Ethanollic Extract Anti Platelet Activity

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**ORCID:**Perdina Nursidika: <https://orcid.org/0000-0003-0102-615X>**Abstract**

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. CVDs are responsible for 37% of deaths in Indonesia. Thrombosis is the main factor causing blood vessel clog in CVDs. Blood vessel clots then affect narrowed blood vessel. This causes strokes. The aim of this study is to determine the antithrombotic effect of water hyacinth ethanollic extract, the effective dose, and the potential to become anticoagulant agent. The water hyacinth was extracted by ethanol 96%. The extract was tested on five groups of mice, each group containing five mice. Each group was tested for fourteen days. Group I and II are fed by extract suspension with varied dose 1 mg/BW and 2 mg/BW. Group III is fed by warfarin as standard. Group IV and V are the positive and negative group, the mice were fed by NaCMC. On the 7<sup>th</sup> and 14<sup>th</sup> days, prothrombin time (PT) and activated partial thromboplastin time (aPTT) were conducted. The plasma of group I-IV mixed with adenosine diphosphate (ADP) as inducer, except plasma of group V used for negative control, did not mixed with ADP. Data analysed by ANOVA and LSD to obtain effective dose. The results showed the difference between PTT and aPTT among the tested groups. Group I and II showed prolonged PT which is 22 and 32 seconds respectively. The control positive showed PT 9.48 seconds on day 7<sup>th</sup> and 8.22 seconds on day 14<sup>th</sup>. LSD analysis showed that there is the significant difference between positive control and the other groups. It showed platelet aggregation performed due to the addition of ADP as inducer. There was a significant difference between dose 2 and the negative control group, and there is no significant difference of dose 1. In conclusion, water hyacinth has the antithrombotic activity, the effective dose is 1 mg/BW, and it potential to further developed as anticoagulant agent.

**Keywords:** antiplatelet, cardiovascular diseases, water hyacinth.

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## 1. Introduction

Cardiovascular diseases (CVDs) are group of disorders of the heart and blood vessels (Mishra & Monica, 2019). CVDs are responsible for 37% of deaths in Indonesia. Thrombosis is the main factor of blood vessel clot happened in CVDs. Blood vessel clot then affected on narrowed blood vessel. Coronary atherothrombotic diseases including

peripheral vascular disease, coronary artery disease, cerebrovascular disease, and heart failure. Arterial thrombosis is caused by atherosclerosis (Leys, 2001). The process of atherosclerosis itself consists of plaque rupture and platelet thrombus formation (Bentzon, Jacob Fog, Otsuka Fumiyuki, Virmani Renu, & Falk Erling, 2014).

Many of these mediators form clots and activate coagulation pathways. Thrombus formation can occur in the flow of blood that passes through the mooring with which it increases, which is why most of the thrombus is formed in the amplified vein (Garmo & Burns, 2019). Thrombus can form in any part of the vascular system. By narrowing the diameter of the vessels, blood flow can be obstructed (reduced or completely blocked). As a result, blood vessels become blocked causing interference in the form of stroke (Surgeons, 2016). Drugs for handling anticoagulation are still limited. Anticoagulant drugs currently used also have many side effects, for example on clopidogrel. Clopidogrel is reported by researchers in Germany that there are two cases of drug users who feel age (loss of taste) (Harter, Levine, & Henderson, 2015). While other conventional acetosal drugs 100 mg / day can cause stomach pain, burning, nausea and vomiting. But in patients who have a history of stroke, the drug must be used routinely and for a long period of time (Huang, Strate, Ho, Lee, & Chan, 2011).

For this reason, it is necessary to develop natural medicines derived from natural ingredients which have the efficacy as anticoagulants. Of the many medicinal plants spread throughout Indonesia, water hyacinth plants are plants that can grow well and have many uses empirically for example used to cure sore throat, difficulty urinating and boils (Honlah, Segbefia, Appiah, Mensah, & Atakora, 2019). The polysaccharides identified from the methanol extract of the water hyacinth leaves exhibit an anticoagulant effect by influencing the intrinsic pathway of coagulation factors. But the use of methanol can cause toxic effects to be used as oral preparations. Therefore in this study ethanol will be used as an extractor because it is safer than methanol as an extractor, also to test the most effective extract dosage as an anticoagulant.

Through research aimed at testing the anticoagulant activity of the water hyacinth leaf extract (*Eichhornia crassipes* Mart), it is hoped that benefits can be obtained in the form of effective dosage information from compounds that have the potential as anticoagulants. Further more it can be used as a functional composition or medicine to prevent various diseases caused by clogged arteries.

## 2. Methods

The test animals used were 20 Swiss Webster male mice aged 2-3 months and body weight 20-30 g obtained from the Animal Laboratory of the School of Life Sciences, Bandung Institute of Technology.

### 2.1. Collection, Determination, and Processing of Test Materials

The plants were obtained from the Lembang area, West Java. Then it was determined at Bandungense Herbarium School of Life Sciences and Technology, Bandung Institute of Technology. The plants is then extracted by maceration method. The solvent used is 96% ethanol(Nursidika, Saptarini, & Rafiqua, 2014). A total of 50 g of water hyacinth to be tested was macerated with 500 ml of 96% ethanol for three times 24 hours. Every 24 hours, the filtrate is taken and the residue is immersed again with 96 ml of ethanol as much as 500 ml. The filtrate obtained every 24 hours is put together then concentrated using a rotary evaporator. The extract was concentrated over a 50°C water bath to obtain a thick extract. Characterization of the test material was carried out by phytochemical screening against water hyacinth simplicia. The screening includes examining the content of alkaloids, flavonoids, tannins, saponins, quinones, steroids and terpenoids.

### 2.2. Dose orientation

Dose orientation is done before testing by using one mouse for each test material to get the test dose to be used in subsequent tests. In this dose orientation the measured parameters are Prothrombin time (PT) and activated partial trthromboplastine time (aPTT).

Dosage orientation begins with the division of groups of mice based on the test material that will be given include: group I dose I by administering an orientation dose of 1 mg / 20 g bb water hyacinth extract. Group II dose II with orientation dose 2 mg / 20 g BW water hyacinth extract. Group III given warfarin as a comparison with a conversion dose of 0.0052 mg / 20 g bw. Groups IV and V were positive and negative control groups which were both given a 0.5% CMCNa suspension.

### 2.3. Provision of Test Preparations

The test preparation consisted of water hyacinth extract suspension, warfarin suspension and 0.5% CMCNa suspension. A 0.5% CMCNa suspension was made by mixing 0.5 mg of CMCNa powder into 100 ml of warm water and crushed until homogeneous and a thick mass was formed.

The water hyacinth extract suspension is made of its stock solution by adding the water hyacinth extract into a 0.5% CMCNa suspension that was made previously. Then crushed until homogeneous. Warfarin suspension is made by adding warfarin powder to a 0.5% CMCNa suspension and crushed until homogeneous. All parts of the suspension are made daily during administration to mice to maintain suspension stability.

The preparations were given for 14 consecutive days with the following dose sharing: Group I was given a suspension of water hyacinth extract at a dose of 1 mg / 20 g bw. Group II was given a suspension of water hyacinth extract at a dose of 2 mg / 20g bw.

Group III was given warfarin suspension 2 mg / kg, converted to 0.0052 mg / 20 g body weight of mice. Group IV and V were only given a 0.5% CMCNa suspension with a dose adjusted to the dose group I which was 1mg / 20 g bb mice.

The parameters measured in this test were Prothrombin time (PT) and activated partialthromboplastine time (aPTT) which were carried out on the 7th and 14th days. After obtaining the PT and aPTT test results for each group on the 7th and 14th days, statistical data analysis was then performed.

The test results were processed statistically using the ANOVA test with a 95% confidence principle to find a significant difference between the test group and the control group on the lengthening of PT and aPTT. The test was carried out using SPSS for Windows Release 16.0 software.

ANOVA test begins with a test of normality to determine the distribution of test data. ANOVA can be done if the data has a normal distribution. Then carried out further analysis to determine the comparison of significance between groups using the LSD method.

## 3. Results and Discussion

Phytochemical screening results show that water hyacinth contains several classes of compounds, including: alkaloids, flavonoids, saponins, phenols. Steroids and triterpenoids in this study were declared undetectable. While in the literature declared to be detected. This can be caused by plants originating from different regions and conditions

from plants tested in the literature. Some other data are in accordance with the literature, one of which is flavonoids. Water hyacinth contains tannins, phlobatin, steroids, terpenoids, alkaloids, flavonoids, phenols, quinones, anthraquinones and cardiac glycosides (Rorong, Sudiarmo, Prasetya, Polii-Mandang, & Suryanto, 2012). Flavonoid in water hyacinth can significantly extend the activated partial thromboplastin time (aPTT) (Stainer dkk., 2019). Flavonoids are associated with a reduced risk of CVD, because of its ability to interact with platelet (Wang, Ouyang, Liu, & Zhao, 2014). Flavonoids can inhibit platelet aggregation based on *ex vivo* research due to the interaction of anticoagulant drugs such as warfarin (coumadin), and antiplatelet drugs, such as clopidogrel (Plavix), dipyridamole (Persantine), non-steroidal anti-inflammatory drugs (NSAID) and high intake of flavonoids so that it can increase the risk of bleeding (García Rodríguez, Martín-Pérez, Hennekens, Rothwell, & Lanan, 2016). Flavonoid metabolites such as isorhamnetin and tamarixetin can inhibit platelet function and thrombus formation in the initial activation process including calcium mobilization, granule secretion, and integrin activation. The development of flavonoid metabolites can offer new insights on how to treat CVD (Stainer dkk., 2019). Medicinal plants can be a new and supportive treatment resource. The World Health Organization (WHO) has declared medicinal plants to be used effectively in the public health care system (WHO, 2002).

Other compounds in water hyacinth that can inhibit platelet clott are alkaloids. The mechanism of action of alkaloids is different from aspirin, aspirin inhibits cyclooxygenase while alkaloids work by inhibiting synthesis of thromboxane A<sub>2</sub> induced by adenine diphosphate, arachidonic acid, collagen and inhibited PLCγ<sub>2</sub> and protein tyrosine phosphorylation with sequential suppression of cytosolic calcium mobilization and arachidonic acid liberation (Ain, Khan, Mubarak, & Pervaiz, 2016). Saponins are compounds found in water hyacinth extract. Saponins can inhibit platelet freezing by inhibiting the agonists-induced intracellular calcium mobilization (Qi dkk., 2016). Phenol compounds also have the ability as an antiplatelet (Maleš, Antolić, Babić, Jurić, & Bojić, 2017).

Dose orientation is carried out before testing to get the test dose that provides the most effective therapeutic effect. Tests carried out on the 7th and 14th day after giving test materials 14 days in a row. Orientation results using one mouse for each group stated that there were differences in the average PT (prothrombin time) and aPTT (activated partial thromboplastin time) tested as test parameters.

The result shows there are differences in the average prothrombin time (PT) and activated partial thromboplastin time (aPTT) between each group. The extract dose

used is based on the results of a fixed orientation of 1 mg / 20 g BW and 2 mg / g BW. The dose is then used also for the administration of positive and negative controls. As for the comparative dose, the initial dose is 0.0052 mg / 20 g BW.

### 3.1. Testing the anticoagulation effect of ethanol extract of water hyacinth leaves.

The test begins with the administration of test material for 14 consecutive days to describe the use of drugs in animals with subchronic conditions, because treatment using anticoagulant drugs is indeed used by patients with a relatively long period of time.

The testing time is carried out in two periods with the test schedule on the 7th and 14th day after giving test material. The 7th day was chosen because the test material was a solid extract whose pharmacokinetic profile was unknown, so to get effective results, the standard time for oral administration was 7 days. After blood sampling, platelets which function in stopping bleeding and repairing torn blood vessels take 1-2 weeks to form new connective tissue. Therefore, to get a good blood sample, a range of days 7 and 14 is chosen. After administering the test material, the blood sample is then processed into platelet poor plasma (PPP) because in PPP the platelet count is only small. So it will not interfere with the measurement of prothrombin time (PT) and activated partial thromboplastin time (aPTT) because the more platelets there are, the greater the platelet opportunity to shorten the plasma recalcification period so that the clotting time will be shorter. Furthermore, group I-IV PPP is given adenosine diphosphate (ADP) inductor because ADP can trigger platelet activation. ADP causes platelets to swell and push platelet membranes on adjacent platelets to cling to each other. Simultaneously, a further release reaction occurs that releases more ADP and thromboxane A<sub>2</sub> which causes secondary platelet aggregation. This process causes the formation of a platelet mass large enough to cause coagulation in endothelial damage areas.

The longer the gift, the longer the value of PT. This is also shown on the positive control PT value obtained is 9.48 seconds on the 7th day and 8.22 seconds on the 14th day the time is lower than the normal PT price obtained from the acquisition of the negative control PT value that is 21- 22.29 seconds. Then LSD continued testing with  $P < 0.05$ . These results indicate that there are significant differences between the positive control group and all other groups. The difference in the significance of the negative control indicates that platelet aggregation has been formed in the sample due to the administration of ADP as an inductor.

On the 7th and 14th day of testing, it was shown that the value of PT in dose II had a higher increase compared to dose I. This shows that at larger doses, the test extract gave a greater effect on decreasing factor activity factors that affect the testing of PT. Even so, dose II did not have as much activity as the comparison group as indicated by the difference in meaning between the comparison group and Dosage II. The comparative group's PT value is warfarin, which does work in the extrinsic pathway which shows a difference of meaningfulness to the positive control group. It also shows that the method used is a valid method.

Furthermore, the difference between dose I and dose II which states that dose II has a significant difference when compared with negative controls, while dose I did not show any significant difference.

This shows that in the dose I group that has been given an inductor, the extract can work as an anticoagulant until it reaches its normal function which is marked by the absence of meaningful differences with negative controls that represent normal conditions of the body. So it can be concluded that dose I is the most effective dose both in terms of treatment and in terms of side effects as anticoagulants that work in the extrinsic pathway.

### **3.2. Analysis of Data Activated partial thromboplastine time (aPTT)**

Measurement of activated partial thromboplastine time (aPTT) was carried out to determine the intrinsic pathway blood clotting system. The aPTT results obtained in this test have a synergistic value with PT. After giving for 14 consecutive days, the positive control group aPTT value obtained was 24, 04 seconds on the 7th day and 28.41 seconds on the 14th day of the time is lower than the normal price of the aPTT obtained from the acquisition of the aPTT value negative control that is 39,59-52.50 seconds.

Furthermore, LSD continued testing with  $P < 0.05$ . Based on the data, these results indicate that there are significant differences between the positive control group and all other groups. These results indicate that platelet aggregation was formed in the sample due to the administration of ADP as an inductor. Similar to the PT test, on the 7th and 14th day of testing, it showed that the aPTT value at dose II had a higher increase compared to dose I. This shows that at larger doses, the test extract also had an effect greater anticoagulation to decrease the activity of intrinsic factors that influence aPTT testing. This can also be proven by looking at the comparative group

aPTT value, namely warfarin, which shows a difference of significance to the positive control group. This shows that the method used is a valid method.

The results of aPTT compared to negative controls, warfarin showed differences in meaningfulness.

Warfarin has a very high aPTT value of 103.37-139.07 seconds while the negative control value is 39.59 - 52.50 seconds. This can be because the aPTT test is a test for drugs that work in the intrinsic pathway, while warfarin works in the extrinsic pathway, so the results obtained are very different from the negative controls that assume normal coagulation conditions. A significant difference was also demonstrated by the dose II group with aPTT 76.61-84.86 seconds. Although there is a difference in meaning between the comparison group and dose II, reviewing the difference in value that is quite far from normal conditions shows the extract dose is not good for the body's physiological system.

In contrast to dose II which shows a significant difference. The Dose I group showed no significant differences. This shows that after giving an inductor, the extract can work as an anticoagulant until it reaches its normal function which is marked by the absence of meaningful difference with negative control. So it can be concluded that extract dose I is also the most effective dose as an anticoagulant that works on the intrinsic pathway.

## 4. Conclusion

The water hyacinth extract at doses of 1 and 2 mg / kg body weight can prolong the prothrombin time (PT) and activated partial thromboplastin time aPTT significantly compared to positive controls ( $p < 0.00$ ). Compared with normal conditions An increase in dose of 1 mg / 20 g BW gives comparable PT and aPTT values. The duration of administration for 14 days raises the value of PT and aPTT longer than the administration of samples for 7 days. For further research, it can be done by developing drugs from water hyacinth extract, by conducting toxicity tests and clinical trials in humans

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