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Membrane stabilization activity as anti-inflammatory mechanisms of *Vernonia amygdalina* leaves extracts

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Abstract. Inflammation is a normal process in the human body as a response to injury from healing process. Meanwhile, chronic inflammation will cause new health problems to patients. Anti-inflammatory agents generally used for those conditions, have several side effects to patients. The aim of this research was to find alternative anti-inflammatory agents, especially from natural sources. *Vernonia amygdalina* knew locally as “daun afrika” belong to family *Apiaceae* is one of those potential natural sources for alternative anti-inflammatory agents. This plant is known as traditional medicine from East Kalimantan for health problems caused by the muscle stiffness and used as material in this research. The experimental method of anti-inflammatory measurement using membrane stabilization activity for *V. amygdalina* leaves extracts. The results showed that significant differences of EC_{50} ($p < 0.05$) achieved between indomethacin as the positive control ($26.39 \pm 2.91 \mu\text{g/mL}$) with *V. amygdalina* leaves extracts for concentration 1% ($131.81 \pm 2.95 \mu\text{g/mL}$) and 10% ($62.54 \pm 2.05 \mu\text{g/mL}$). EC_{50} of *V. amygdalina* leaves extracts showed the potential anti-inflammatory activities. It could be concluded that *V. amygdalina* leaves extracts to have anti-inflammatory activities, which could be further developed as a new natural source of the anti-inflammatory agents.

1. Introduction

Inflammation is a physiological process in the body in response to a lesion on the body, such as on work-related fatigue. Acute inflammation can be triggered by various stimuli and characterized by a rapid response of hosts to the site of infection or trauma tissue, i.e. delivery of leukocytes and plasma proteins such as antibodies, to the site of inflammation. Chronic inflammation may progress after acute inflammatory processes, which last for several weeks, months and even years [1].

During an acute and chronic inflammatory process, a number of chemical mediators released. A large number of inflammatory mediators released via arachidonic acid pathways, including prostaglandins, as a result of the breakdown of arachidonic acid by cyclooxygenase enzymes. Although this process is a physiological process in the body, if this process is excessive, it will appear adverse impact on patients. Anti-inflammatory drugs used to overcome this process, with a number of side effects related to the use of these drugs. Some of the most prominent side effects of using anti-inflammatory drugs are side effects on the gastrointestinal system that increase the risk of gastric ulcers; as well as the cardiovascular system that increases the risk of blood vessel occlusion due to



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blood clots. This is what makes incessant efforts to find alternative anti-inflammatory drugs, especially those derived from natural materials [2].

Indonesia is a country famous for its biodiversity including rich medicinal plants, with ethnic and cultural diversity. Each ethnic group has the cultural repertoire. Each ethnic community has local wisdom, including the use of plants for traditional medicine. Knowledge of the use of medicinal plants by indigenous ethnicity is very important for the traditional and modern medicine because many plant extracts for modern medicine are found through local knowledge approach. Utilization of medicinal plant data from ethnobotany research is an effective way of finding new chemicals used for treatment [3].

One of the medicinal plants suspected of having potential anti-inflammatory activity is *Vernonia amygdalina* Delile. These shrubs included in the *Apiaceae* family. This plant is commonly named as *daun afrika* in Indonesia or bitter leaf in English. A preliminary observation of the use of *V. amygdalina* is that these plants are commonly consumed by wild chimpanzees that look sick and at some later look healthy and do normally again [4]. The traditional use of this drug is for anti-worms, anti-malaria, and constipation [5]. Based on the above, this research intends to see the mechanism of action of anti-inflammatory extract of *V. amygdalina* leaves by using membrane stabilization test.

2. Material and methods

2.1. Medicinal plant sampling

The study conducted in September 2017. Sampling place of medicinal plants conducted in Kutai Kartanegara Regency, East Kalimantan. Processing of medicinal plant samples to the membrane stabilization test conducted at the Research Laboratory, Faculty of Medicine Mulawarman University. The study protocol approved by the Research Ethics Committee on Faculty of Medicine Mulawarman University.

2.2. Medicinal plant extraction

The crushed simplicia was then macerated with ethanol solvent. In this study used absolute ethanol solvent. For extract preparation, one part of the dry powder of the simplicia put into the macerator (weighed in the maceration bottle before insertion of simplicia and recalculated after insertion of simplicia), added 10 parts of the solvent (measuring volume before inserting). Then soaked for 6 hours while stirring occasionally (using an orbital shaker at 20 rpm for 10 minutes) at room temperature, then leave for 18 hours. The filtrate was separated by filtration using Whatman filter paper. The separation process repeated twice with the same type and number of solvents. All the filtrate was collected, then evaporated with rotavapor vacuum at 50°C until the viscous extract obtained. The resulting extract was then further dried by being placed in the desiccator containing blue silica gel inside an oven at 50°C temperature. After obtained dry extract, the calculated yield obtained, the percentage of weight (b/b) between yield with powder weight of simplicia used by weighing. Dry extracts stored in refrigerators -20°C before the further test [6].

2.3. Membrane stabilization test

Fresh blood samples were taken with the addition of anticoagulants. The blood samples were centrifuged at room temperature. Supernatants (plasma and leukocytes) are carefully removed, while red blood cells washed with normal saline. The washing and centrifugation process were repeated up to 5 times until the supernatant is clear. Then the erythrocyte suspension ready for the membrane stabilization test. This test using erythrocyte suspension with indomethacin as the positive control. The mixture consists of a hyposaline solution of sodium chloride, sodium phosphate buffer, erythrocyte suspension, test material (positive control standard and plant extract) and the last mixture added to normal saline. The test material was not added to the blood control, while the control of the drug was not added to the erythrocyte suspension. The solution mixture incubated in a water bath, followed by

centrifugation at room temperature. The discharged hemoglobin is read in the spectrophotometer at a wavelength of 560 nm. Percentage of membrane stability calculated by the following formula:

$$\text{Membrane Stability} = [100 - (\text{Absorbance Treatment} - \text{Absorbance Control})] \times 100\% \quad [7][8].$$

2.4. Data analysis

Membrane stabilization activity was tabulated in mean \pm SD, then calculated the EC₅₀ value. Differences to controls analyzed with the ANOVA test and said significant if $p < 0.05$.

3. Results and discussion

Based on Figure 1, the lowest absorbance of *V. amygdalina* 1% was found in dose of 100 mg/ml with its absorbance is 0.383 ± 0.001 . Based on Figure 2, the lowest absorbance of *V. amygdalina* 10% was found in dose of 100 mg/mL with its absorbance is 0.190 ± 0.010 . The half maximal effective concentration (EC₅₀) value of membrane stabilization activity of *V. amygdalina* 1% is 131.81 ± 2.95 mg/mL and 10% 62.54 ± 2.05 mg/mL when compared with indomethacin 26.39 ± 2.99 mg/mL, shown in Figure 3.

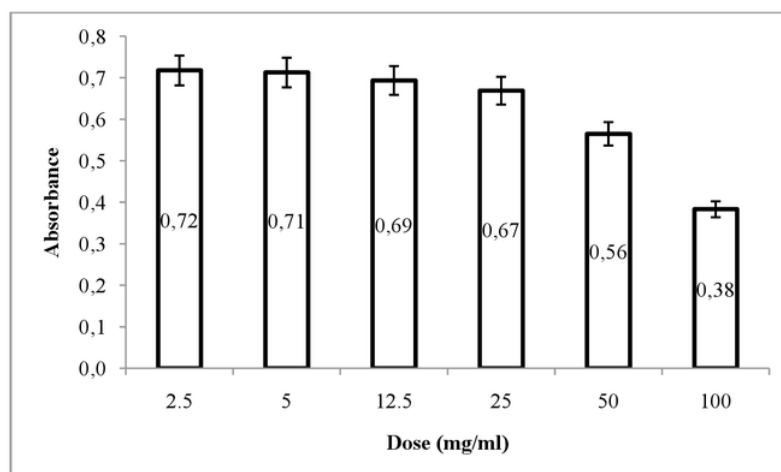


Figure 1. Absorbance of *V. amygdalina* 1% in different concentration

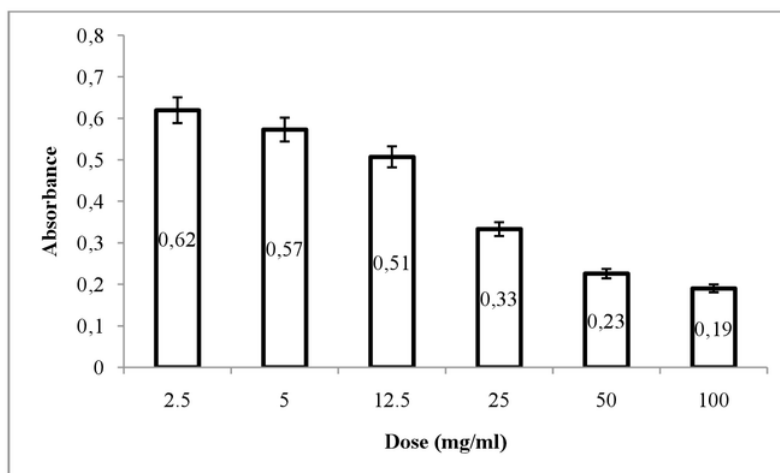


Figure 2. Absorbance of *V. amygdalina* 10% in different concentration

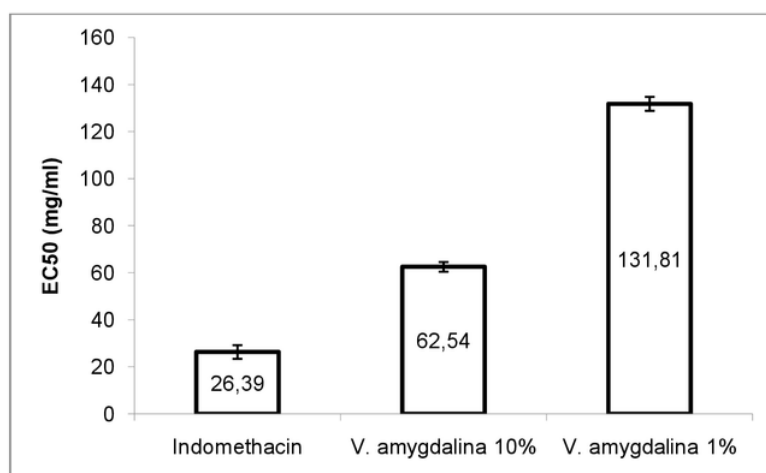


Figure 3. Differences of EC₅₀ results in membrane stabilization test between *V. amygdalina* 1%, 10%, and indomethacin as positive control (ANOVA p=0.000)

The smaller of EC₅₀ value the better the anti-inflammatory activity as membrane stabilization. The results showed that indomethacin as a positive control had better anti-inflammatory activity than *V. amygdalina* on membrane stabilization activity. This suggests that membrane stabilization activity is a possible mechanism of action for the anti-inflammatory activity of *V. amygdalina*.

This plant synonym is *Bracheilema paniculatum* R.Br., *Cacalia amygdalina* Kuntze, *Chelusia abyssinica* Sch.Bip.ex A.Rich., *Decaneurum amygdalinum* DC., *Vernonia adenosticta* Fenzl ex Walp., *Vernonia eritreana* Klatt, *Vernonia giorgii* De Wild., *Vernonia randii* S.Moore, *Vernonia* Benth., and *Vernonia weisseana* Muschl. This plant can grow as high as 2-5 m in the tropics, has a hard stem with a blackish color, and leaves are oval-shaped green with a distinctive odor and bitter taste. The active components of *V. amygdalina* are saponins, alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthenes, anthraquinones, edotid and sesquiterpenes [9]. The biological activity of *V. amygdalina* that shows antibacterial, antifungal, antiparasitic, antimalarial, antiviral,

pesticides, antimutagenic, anticancer, anticoagulant, antioxidant, antidiabetic and hypolipidemic effects [10].

There have been studies that have demonstrated central and peripheral analgesic effects of *V. amygdalina* extract, by testing of experimental animals with formalin test and induced writhing assay [11]. Other studies have also found that *V. amygdalina* extract is capable of inhibiting rat paw edema induced by carrageenan and xylene-induced rat ear edema. The study showed that *V. amygdalina* extract had in vivo anti-inflammatory activity [12].

Throughout the researcher's knowledge, this is the first study to investigate the *in vitro* anti-inflammatory activity of *V. amygdalina* leaves extracts, using membrane stability test. The mechanism of *V. amygdalina* leaves extracts as an anti-inflammatory agent suspected through the flavonoid content. An important mechanism for anti-inflammatory activity is inhibition of enzymes that produce eicosanoid, including A2 phospholipase, cyclooxygenase, and lipoxygenase, thereby lowering prostanoïd and leukotriene concentrations. Other mechanisms included inhibition of histamine release, phosphodiesterase, protein kinase and transcriptase activation [13].

4. Conclusion

The results of this study show the anti-inflammatory activity of *V. amygdalina* through membrane stabilization mechanism. Further research needed to allow *V. amygdalina* developed as a new anti-inflammatory agent based on medicinal plants.

Acknowledgement

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