Antibacterial Activity of Bajakah Stem (Spatholobus littoralis Hassk.) Ethanolic Extract in Carrageenan-Induced Paw Edema Mice

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INTRODUCTION

The immune system works by responding to exposure and infection of pathogens in the body. There are various kinds of inflammatory mediators such as cytokines, histamine, prostaglandins, and leukotrienes which will be released by leukocytes or inflamed tissues. The compound of the inflammatory mediator will cause inflammation or local inflammation of body tissues. Inflammation is characterized by redness, heat, pain and swelling of tissue (Ricciotti & Fitzgerald, 2011). The excessive inflammatory response will cause injury to blood vessels and other tissues. Hence, anti-inflammatory drugs are required.

Citation

to inhibit the synthesis of inflammatory mediators. Synthetic drugs which are commonly used to treat inflammation come from a medicine group of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and corticosteroids (Altman et al., 2015). The use of synthetic anti-inflammatory drugs can cause unwanted drug side effects, such as disorders of gastrointestinal tract, cardiovascular and kidney function (Altman et al., 2015; Salvo et al, 2011). Therefore, it is necessary to conduct research to find anti-inflammatory from natural ingredients with minimal side effects.

One of the plants which have potential medicinal ingredient is from the genus *Spatholobus* belongs to the Leguminosae family. Genus *Spatholobus*, known by its local name Bajakah plant, consists of 29 species spread across tropical and subtropical Asia (Ninkaew & Chantaranothai, 2014). Bajakah plant of genus *Spatholobus*, particularly from *S. suberectus* and *S. littoralis* species, has been known by the inland communities of Kalimantan, especially the Dayak tribe who have been using them as traditional medicine, both empirically and scientifically (Wardah & Sundari, 2019). One of them has anti-inflammatory activity, yet it still requires investigation both in vitro and in vivo.

The secondary metabolites which have been identified from *S. suberectus* include 3,7-trihydroxyflone, eriodictyol, plathymenin, dihydroquercetin, butin, neoisoliquiritigenin, dihydrokaempferol, liquiritigenin, and 6-methoxyeriodictyol. In addition, the bioactive compounds in *S. suberectus* showed cytotoxicity activities and inhibit estrogen receptors in the human breast cancer cells (Peng et al., 2014; Sun et al., 2016), anti-mutagenic (Inami et al., 2019) and have anti-inflammatory activity (Li et al., 2003).

The research on bajakah tampala extract (*Spatholobus littoralis*) as an anti-inflammatory is still limited. Therefore, it is necessary to figure out the anti-inflammatory potential of ethanol extract of the Bajakah plant stem (*S. littoralis*). The tests were performed in vivo using the carrageenan-induced mouse animal model. The inflammatory response was observed from the decrease in the volume of mouse paw edema (*Mus musculus* L.).

**MATERIALS AND METHODS**

**Preparation of Materials**

Bajakah stem (*Spatholobus littoralis* Hassk.) were obtained from the forest in the Sungai Ambawang, Kubu Raya District, West Kalimantan. Sample collection was carried out in July 2020. The identification of plant species was determined in Laboratory of Biology, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura. The laboratory animals used were male Swiss mice weighing 25-35 grams, aged ±3 months. Mice were acclimatized for 2 weeks, given food and drink *ad libitum*.

**Preparation of Bajakah Ethanol Extract**

Bajakah stems were washed with running tap water and dried for 7 days. After drying, the stems were chopped, powdered with a grinder, and sieved using a 25-mesh sieve. Then the powder was ready to be extracted using the maceration method (Wardoyo, 2006). The powder of the bajakah stem was weighed as much as 150 g, added 2.5 L of ethanol 96%, then allowed to stand for 24 hours. The extract was separated by filtering and the process was repeated twice with the same type and amount of solvent. All the filtrate obtained was mixed and concentrated using a rotary evaporator at a temperature of 60°C with a rotating speed of 30 rpm. Then, the filtrate was evaporated over a water bath at a temperature of 50°C to obtain a thick extract.
Preparation of Sodium Diclofenac and Carrageenan Solution 2%

The dose of diclofenac sodium was obtained from the conversion of a human dose of 2.5 mg kg⁻¹ BW to a mice dose of 0.0308 mg g⁻¹ BW or 30.8 mg kg⁻¹ BW. Diclofenac sodium powder dissolved in 0.5% CMC until homogeneous. The preparation of the carrageenan solution was carried out by dissolving 0.2 grams of lambda carrageenan powder (Sigma-Aldrich) in 10 ml of 0.9% sterile sodium chloride. The carrageenan solution was used as an irritant that causes edema.

Anti-inflammatory Method

This study used a Completely Randomized Design (CRD) with 5 treatment groups, each group consisting of five mice as replicates. The treatments consisted of: negative control, positive control and extract of Bajakah stem at a dose of 2.5; 250; 1250 mg kg⁻¹. The negative control was treated with inflammation induction by injecting 2% carrageenan and 0.5% CMC solution orally. Positive control was treated with inflammation induction and inflammatory drug sodium diclofenac orally. The treatment of bajakah extract was carried out by inflammation induction and followed with the bajakah extract orally.

The in vivo anti-inflammatory test method used was the Winter method using lambda carrageenan injection (Winter et al., 1992). Before testing, mice fasted for 18 hours. Then the test material was administered orally according to the experiment design. One hour later, the mice were subjected to edema induction by injecting 0.15 ml of 2% lambda carrageenan suspension subplantarly into the soles of the mice's hind legs. The diameter of the thickness of the edema of the feet of the mice was measured using a digital caliper. The anti-inflammatory activity of the test drug was shown by its ability to reduce the volume of induced edema of the soles of the feet. The edema diameter of the feet of mice after carrageenan injection was measured at the 30th, 60th, 90th, 120th, 150th, 180th, and 210th minutes using a digital caliper.

The edema volume (Ve) is the result of volume reduction after carrageenan injection (Vt) with the initial paw volume (Vi) (Eq.1). The area under the curve (AUC) was calculated by (Eq.2). The percentage of edema inhibition was calculated by the following formula (Sahlan et al., 2019):

\[ V_e = V_t - V_i \]  
\[ AUC = \frac{V_{en} + V_e (n-1)}{2} \times (tn - t(n-1)) \]  
\[ \text{Inflammatory Inhibition (%) = } 1 - \left( \frac{AUC_{test}}{AUC_{control}} \right) \times 100\% \]

Statistical Analysis

The results of the measurement of edema diameter, area under the curve (AUC) and the percentage of inflammation inhibition were statistically analyzed using one-way ANOVA with 95% confidence level.

RESULTS AND DISCUSSION

Based on the results of the study, the mouse paw diameter showed a significant increase in all groups after the carrageenan injection. Gradually, the edema volume will decrease over time. The negative control which was not treated with anti-inflammatory drugs showed a slower decrease in edema volume than the positive control and the extract of Bajakah (Table 1).

Carrageenan is a sulfate polysaccharide with a negative molecule, hence it has the ability to bind to inorganic molecules (Necas & Bartosikova, 2013). Carrageenan is a
polysaccharide, an extraction product of red algae (Rhodophyceae), especially the genera Chondrus, Euchema, Gigartina, Fucus, Furcellaria, Hypnea and Iridae. Based on the gel formation, carrageenan can be divided into lambda carrageenan, kappa carrageenan and iota carrageenan (Pacheco-Quito et al., 2020). The irritant nature of carrageenan administered by injection caused the edema (inflammation) formation in local tissues.

After carrageenan was injected, the pro-inflammatory mediators such as cytokines, prostaglandins and leukotrienes released by inflammation tissues. Induction of carrageenan will increase interleukin-8 (IL-8) and stimulate the TLR4 pathway which then initiates inflammation (Myers et al., 2019). These inflammatory mediators then causes vasodilation of blood vessels aiming to collect more leukocytes going towards the site of inflammation. Carrageenan is then phagocytosed by neutrophils so that the edema response gradually decreases.

<table>
<thead>
<tr>
<th>Test groups</th>
<th>Ve₀</th>
<th>Ve₁</th>
<th>Ve₂</th>
<th>Ve₃</th>
<th>Ve₄</th>
<th>Ve₅</th>
<th>Ve₆</th>
<th>Ve₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>2.28±0.1ᵃ</td>
<td>1.89±0.2ᵃ</td>
<td>1.52±0.2ᵃ</td>
<td>1.29±0.2ᵃ</td>
<td>1.21±0.2ᵃ</td>
<td>1.11±1.7ᵃ</td>
<td>1.02±0.4ᵃ</td>
<td>0.99±0.4ᵃ</td>
</tr>
<tr>
<td>Positive control</td>
<td>2.01±0.2ᵇ</td>
<td>1.72±0.3ᵃ</td>
<td>1.46±0.3ᵇ</td>
<td>1.19±0.2ᵇ</td>
<td>0.89±0.2ᵇ</td>
<td>0.69±0.1ᵇ</td>
<td>0.62±0.1ᵇ</td>
<td>0.56±0.1ᵇ</td>
</tr>
<tr>
<td>Bajakah 2.5 mg kg⁻¹</td>
<td>1.85±0.1ᵇ</td>
<td>1.59±0.2ᵇ</td>
<td>1.25±0.2ᵇ</td>
<td>1.08±0.3ᵇ</td>
<td>0.96±0.3ᵇ</td>
<td>0.91±0.3ᵇ</td>
<td>0.86±0.2ᵇ</td>
<td>0.79±0.2ᵇ</td>
</tr>
<tr>
<td>Bajakah 250 mg kg⁻¹</td>
<td>2.02±0.1ᵇ</td>
<td>1.77±0.1ᵇ</td>
<td>1.45±0.2ᵇ</td>
<td>1.22±0.2ᵇ</td>
<td>0.99±0.1ᵇ</td>
<td>0.98±0.1ᵇ</td>
<td>0.90±0.1ᵇ</td>
<td>0.76±0.1ᵇ</td>
</tr>
<tr>
<td>Bajakah 1250 mg kg⁻¹</td>
<td>2.19±0.2ᵇ</td>
<td>1.77±0.2ᵇ</td>
<td>1.28±0.1ᵇ</td>
<td>1.06±0.1ᵇ</td>
<td>1.03±0.2ᵇ</td>
<td>0.87±0.2ᵇ</td>
<td>0.76±0.2ᵇ</td>
<td>0.66±0.2ᵇ</td>
</tr>
</tbody>
</table>

Note: Ve₀ = edema paw volume in 0 minute. Ve₁ = edema paw volume in 30 minute. Ve₂ = edema paw volume in 60 minute. Ve₃ = edema paw volume in 90 minute. Ve₄ = edema paw volume in 120 minute. Ve₅ = edema paw volume in 150 minute. Ve₆ = edema paw volume in 180 minute. Ve₇ = edema paw volume in 210 minute. Data are average±standard deviation. Different value in the same column showed significant difference (P<0.05).

The Area Under the Curve (AUC) provides information regarding the decrease in edema diameter. The larger the AUC value, the smaller the effect of edema volume reduction, and the smaller the AUC value, meaning the larger the effect of edema volume reduction (Apridamayanti et al., 2018). The inflammatory response revealed a different decline pattern from the 30th minute to the 210th minute (Table 2). The AUC value of positive control was the smallest compared to the Bajakah extract group, indicating the biggest inhibition of the inflammation by diclofenac sodium compounds. The negative control treatment showed the largest AUC value from the 30th minute to the 210th minute. The slow decrease in AUC was because there was no drug substance treatment inhibiting the inflammatory reaction in the negative control. All doses of Bajakah extract from 150th minute to 210th minute did not show significant differences to one another, yet were significantly different from the positive control. The administration of ethanol extract of Bajakah stem gave an inflammatory response on the experimental animals, but was not equal to the anti-inflammatory response of diclofenac sodium as positive control.

The inhibition percentage of inflammation is opposite to the AUC value. The smaller the AUC value, the largest the inhibition of inflammation. Positive control had the smallest AUC value, showing the largest inflammation inhibition of 21.53%. Bajakah ethanol extract
at 2.5 mg kg\(^{-1}\) dose also revealed inhibition of inflammation (19.21%) nearly the same as the positive control (21.53%). The highest dose of bajakah extract 1250 mg kg\(^{-1}\) showed the smallest inhibition of inflammation of 12.69% (Figure 1).

Table 2. Area under the Curve (AUC) values

<table>
<thead>
<tr>
<th>Test group</th>
<th>AUC (mm.minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Negative control</td>
<td>62.67±0.1(^{a})</td>
</tr>
<tr>
<td>Positive control</td>
<td>56.10±7.1(^{bc})</td>
</tr>
<tr>
<td>Bajakah 2.5 mg kg(^{-1})</td>
<td>51.78±2.8(^{c})</td>
</tr>
<tr>
<td>Bajakah 250 mg kg(^{-1})</td>
<td>56.91±3.8(^{abc})</td>
</tr>
<tr>
<td>Bajakah 1250 mg kg(^{-1})</td>
<td>59.52±4.9(^{abc})</td>
</tr>
</tbody>
</table>

Data are average±standart deviation. Different value in the same column showed significant difference (P<0.05)

Diclofenac sodium is a Non-Steroidal Anti-Inflammatory Drug (NSAID). Diclofenac sodium works by inhibiting the cyclooxygenase enzyme (COX-2) so that the formation process of prostaglandins, thromboxanes and prostacyclins is inhibited (Altman et al., 2015). However, this class of drugs does not inhibit the formation of leukotrienes which also play a role in the process where inflammation happens. Diclofenac sodium is rapidly absorbed after oral administration and has a short half-life. Diclofenac sodium 30.8 mg kg\(^{-1}\) BW was used as the standard anti-inflammatory drug. Diclofenac sodium dose is higher than the ethanol extract of *Spatholobus* 2.5 mg kg\(^{-1}\) BW which gives the optimal inflammation inhibition percentage. Therefore, the ethanol extract of Bajakah stem at a low dose (2.5 mg kg\(^{-1}\) BW) is very potential to be used as an anti-inflammatory agent.

Bajakah plant has members of other species which are still included in the genus *Spatholobus*. One of them is *S. suberectus* which has antimutagenic and antitumor potential. *Spatholobus suberectus* has also been reported to have anti-inflammatory potential (Inami et al., 2019). A study on Bajakah tampala *S. littoralis* reported by Saputra et al.
(2019), at a concentration of 6.25% showed an inhibition response to the growth of *Escherichia coli* bacteria. The diameter of the strongest bacterial inhibition was produced by a concentration of 50%.

Inflammatory mediators are the primary target of anti-inflammatory treatment. In the conditions of injury, cell damage due to bacterial infection or free radical exposure, the body will release inflammatory mediators as a form of communication among cells in defending themselves from damage. These inflammatory mediators include a group of pro-inflammatory cytokines such as interleukin 1β (II-1β), interleukin 6 (IL-6), tumor necrosis factor (TNF-α), histamine released by leukocytes, as well as inflammatory mediators released by damaged tissues such as prostaglandins and leukotrienes.

The content of secondary metabolites in the stem of *S. littoralis* includes flavonoid, saponin and tannin compounds (Saputera et al., 2019). Liu et al. (2019) found that the secondary metabolites in the aqueous extract of *S. suberectus* mostly belonged to the group of flavones, isoflavones and anthocyanins. The names of the compounds found were 2,6-dimethoxy-1,4-benzoquinone, 4,7,20-trihydroxy-40-methoxy-isoflavanol, liquiritigenin, formononetin sodium, genistein, formononetin, daidzein, isoliquiritigenin, and genistin. In addition, five new isoflavone compounds were found, namely spasuberol A, homovanillyl-4- oxo-nonanoate, spusuberol C, spusubeside A, and spusubeside B. Huang et al. (2013) founded that *Spatholobus suberectus* contained 2% of total phenolic compound and was able to inhibit the enzyme elastase in neutrophil cells.

Liu et al. (2019) also figured out that the compounds of aglycone flavonoid have extremely high anti-inflammatory activity. Flavonoid compounds are antioxidants that work to neutralize oxygen radicals (ROS). The prevention of ROS formation by flavonoids is performed in several ways. The flavonoid quercetin works to reduce oxidative damage caused by ROS by increasing intracellular antioxidants such as glutathione, as a metal chelator, inhibiting the enzyme xanthine oxidase and increasing the activity of Superoxide Dismutase (SOD) (Brunetti et al., 2013). Choi & Kim (2013) reported that the flavonoid daidzein plays a role to stimulate catalase and superoxide dismutase (SOD) activities (Choi & Kim, 2013). Daidzein is a flavonoid that was found in *S. suberectus* plant. The ability to neutralize free radicals is played by the catechol group on the B ring of the flavonoid framework (Banjarnahor & Artanti, 2014).

The neutralization of free radicals by flavonoid compounds can increase the growth factor of tissues experiencing inflammation and is thought to modulate anti-inflammatory cytokines. Anti-inflammatory cytokines consist of interleukin-4 and interleukin-10 which play a role as regulators of pro-inflammatory cytokine secretion. The flavonoid compounds in *S. suberectus* play a role in inhibiting the mRNA expression of several pro-inflammatory cytokines such as interleukin 1β (II-1β), Tumor Necrosis Factor (TNF-α), nitric oxide synthase (iNOS) and cyclooxygenase enzymes (COX -2) (Chen et al., 2004; Liu et al., 2019).

Tannins are secondary metabolites which strongly interact with proteins. High tannin compounds from kesum leaves (*Bixa orellana* L.) showed anti-inflammatory activity in carrageenan-induced mice (Yusuf et al., 2012). Similar to flavonoids, tannins work to reduce the levels of ROS and modulate the immune system. Saponin compounds are glycosides that are soluble in water and have the characteristics of being able to make foam when shaken. Saponins steroids have mem-
brane toxicity and are able to cause rupture of red blood cells. Aglycon part of saponin is mainly responsible for the membrane stability of inflamed tissue (de Groot & Muller-Goy mann, 2016). High doses of ethanol extract of bajakah stem (250 mg kg−1 and 1250 mg kg−1) did not really show the effect of the inhibition of inflammation, possibly due to the high content of saponin compounds.

The results of the in vivo anti-inflammatory test also revealed that the highest dose of Bajakah S. littoralis extract (1250 mg kg−1) did not really provide inhibition to the formation of edema. Administration of herbal extract is thought to be dose-dependent in inhibiting the release of inflammatory mediators, such as synthesis of prostaglandins (PGE2) and nitric oxide (NO) (Kaur et al., 2004). High doses of Spatholobus extract may cause increased free radical damage, but this requires further investigation.

CONCLUSION

The conclusion from this research is that the ethanol extract of Bajakah tampala stem (Spatholobus littoralis Hassk.) shows anti-inflammatory potential. Where the dose of ethanol extract of S. littoralis 2.5 mg kg-1 was not significantly different from the inhibitory value of positive control.

AUTHOR CONTRIBUTION

D.W. performs anti-inflammatory tests and laboratory animal handling. E.R. contributed in the process of extraction bajakah stem. S.I. contributed to the preparation of the experimental design and data analysis. All authors contributed to the writing and revision of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest about the publication of this paper.

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