ANTIMALARIAL ACTIVITY OF CRUDE EXTRACTS OF ARTOCARPUS HETEROPHYLLUS, ARTOCARPUS ALTIILIS, AND ARTOCARPUS CAMANSI

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ABSTRACT

Objective: This research investigated in vitro and in vivo antimalarial activity of leaves and stem bark crude extract of Artocarpus species, focusing on Artocarpus heterophyllus, Artocarpus altilis, and Artocarpus camansi against Plasmodium falciparum and Plasmodium berghei.

Methods: Leaves and stem bark extracts of three Artocarpus species were tested for their antimalarial activities. The antimalarial in vitro test was conducted using P. falciparum (3D7 strain) culture in RPMI-1640 medium, while the antimalarial in vivo test was performed based on Peter’s test (The 4 days suppressive test) that using P. berghei (strain ANKA) infected mice.

Results: From total 6 extracts of Artocarpus leaves and stem bark, 2 extracts showed good antimalarial activities against P. falciparum and P. berghei. A. heterophyllus leaves extract (AHL) and A. altilis leaves extract (AAL) were classified as good to moderately active against P. berghei with inhibition concentration (IC50) value of 9.35 µg/ml and 1.32 µg/ml, respectively. In vivo antimalarial activity showed that AHL and AAL were very active against P. berghei with effective dose (ED50) value of 0.33 mg/kg body weight and 0.82 mg/kg body weight, respectively.

Conclusion: AAL has shown as the most active antimalarial activity with IC50 value of 1.32 µg/ml and ED50 value of 0.82 mg/kg body weight. AAL may become a potential candidate of the antimalarial drug from Artocarpus species.

Keywords: Artocarpus heterophyllus, Artocarpus altilis, Artocarpus camansi, Antimalarial activity.

INTRODUCTION

Malaria has been caused by five species of parasite that affect humans, and all of these species belongs to the genus Plasmodium: Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi. The WHO estimates that 207 million cases of malaria occurred globally in 2012 and 627,000 were fatal. Most cases (80%) and deaths (90%) occurred in Africa, and most deaths (77%) occurred in children under 5 years of age [1]. Resistance to some classes of antimalarial drugs has been responsible for a recent increase in malaria-related mortality [2,3]. The spread of P. falciparum resistance encourages the search for a new antimalarial drug. Natural products particularly used in the traditional medicine contain a great variety of chemical structures and have been screened for antimalarial activities as potential sources of new antimalarial drugs. Ethnopharmacological approaches seem to be promising in finding new antimalarial candidates [4-6].

The Artocarpus genus (Moraceae family) comprises about 50 species that are widely used in folk medicines. Artocarpus species are rich in phenolic compounds. The extract and metabolites of Artocarpus, particularly from leaves, bark, stem, and fruits, possess several useful bioactive compounds. Several pharmacological studies of Artocarpus have conclusively established their mode of action in a treatment of various diseases such as inflammation, malarial fever, diarrhea, diabetes, and tapeworm infection [7-9].

Artocarpus champeden is one of the plant species of Moraceae. It is locally known as cempedak. It is widely spread in Indonesia and has been traditionally used for malarial remedies [10]. In our previous studies, several prenylated flavones were isolated from A. champeden stem bark extract and showed potent antimalarial activity, and among the compounds isolated, hetero flavanone C had the most potent inhibitory activity against the growth of P. falciparum 3D7 strain with an inhibition concentration (IC50) value of 1 nmol/L [11]. A new isoprenylated flavone, artopeden A was isolated from the barks of A. champeden and showed potent antimalarial activity with IC50 value of 0.045 µg/ml [12]. Hafid et al. isolated active marker compound, morachalcone A (IC50 value of 0.18 µg/ml) from A. champeden that can be used as a marker compound in the standardization of ethanol extract of A. champeden stem bark as antimalarial phyto medicine product [13]. Another study reported that a prenylated stilbene compound was isolated from aerial parts of Artocarpus integer which has antimalarial activity with IC50 value of 1.7 µg/ml [14].

Considering the antimalarial properties of A. champeden as one of Artocarpus species that has phytochemical constituent similar to some other Artocarpus species, such as Artocarpus heterophyllus (jackfruit), Artocarpus altilis (breadfruit), and Artocarpus camansi (breadnut), then it is possible that these Artocarpus species also have antimalarial activity. This consideration encourages deeper examination on these three Artocarpus species as a promising antimalarial candidate. The present study aims to examine the antimalarial activity of A. heterophyllus, A. altilis, and A. camansi against P. falciparum and P. berghei.

METHODS

Plants material

Leaves and stem bark of A. heterophyllus were obtained from Gesik, East Java; A. altilis was obtained from Kediri, East Java, and A. camansi was obtained from Tabanah, Bali. Authentication and identification of plants were carried out at Purwodadi Botanical Garden, East Java.

P. falciparum strain and in vitro culture

P. falciparum strain 3D7 was obtained from Malaria Laboratory, Bijkman Institute of Molecular Biology, Jakarta, and was maintained
In vitro antimalarial test

Antimalarial in vitro test was performed based on Budimulya et al. [16]. A 10 mg sample was diluted into 100 ml DMSO. The sample was further diluted in RPMI-1640 medium and prepared in serial dilution at concentrations of 0.01, 0.1, 1, 10, and 100 mg/ml in microwells. Each microwell was added to 500 ml parasite culture (1% parasitemia, 5% hematocrit) and incubated at 37°C for 48 hrs at 37°C. After incubation, thin blood smears were made and stained using 20% Giemsa dye. The percentage of parasitemia was determined by counting infected erythrocytes per 1000 total erythrocytes under a microscope. The percentage of inhibition growth of \( P. falciparum \) was calculated using the following formula:

\[
100\% - \left( \frac{Xe}{Xk} \times 100\% \right)
\]

Xe: % parasitemia growth of experimental group
Xk: % parasitemia growth of negative control

In vivo antimalarial test

Antimalarial in vivo test was performed based on Pete's test (The 4 days suppressive test) [17]. Each plant extract was tested using 30 mice which were divided into six groups. Four groups were treated using extract at a dose of 100 mg/kg body weight, 10 mg/kg body weight, 1 mg/kg body weight, and 0.1 mg/kg body weight, respectively. Meanwhile, the other two groups were treated using CMC-Na 0.5% (as a negative control) and artesunate at a dose of 36.4 mg/kg body weight (as a positive control). Artesunate used as a positive control was taken from Arsumaooon\textsuperscript{®} tablet (Guilin Pharmaceutical Co., Ltd.). The tablets contain artesunate 50 mg/tablet. Each mouse was infected intraperitoneally with 0.2 ml \( P. berghei \) (5% parasitemia) at day 0. Treatment began when parasite infection occurred. Treatment of extract and control was given orally at day 0 until day 3. Thin blood smears were made every day for 7 days (day 0 until day 6) and stained using 20% Giemsa dye. Percentage of parasitemia and that of inhibition growth of \( P. berghei \) were calculated using the same formula as in vitro test. \( IC_{50} \) and effective dose (ED\textsubscript{50}) were analyzed using probit analysis.

RESULTS

In vitro antimalarial test

In vitro antimalarial test was conducted using 5 serial concentrations. Each concentration was observed in term of its each parasitemia percentage and the percentage of parasite growth inhibition was further calculated. The result is shown in Table 1. After 48 hrs incubations, \( A. heterophyllus \) stem (AHS), \( A. altilis \) leaves (AAL), \( A. camansi \) leaves (ACL), and \( A. camansi \) stem (ACS) afforded to inhibit \( P. falciparum \) growth more than 90% at a concentration of 100 µg/ml.

Rosaanoivo et al. (2004) classified that the extract with IC\textsubscript{50} value <0.1 µg/ml is very active, 0.1-1.0 µg/ml is active, 1.1-10 µg/ml is moderate to actively, 11-25 µg/ml is weak, 26-50 µg/ml is very weak while more than 100 µg/ml is inactive [18]. Another study classified active extract showing IC\textsubscript{50} < 10 µg/ml should be selected for further bioassay-guided fractionation [19].

The study indicated that out of 6 extracts used in the study, 4 extracts, AHL, AAL, ACL, and ACS showed good to moderate active antimalarial activities to \( P. falciparum \) 3D7 strain in vitro with an IC\textsubscript{50} value ranging from 1.32 to 9.35 µg/ml. AAL showed the best activity with IC\textsubscript{50} value of 1.32 µg/ml. Bhoonphong et al. (2007) reported eight prenylated flavones compounds isolated from \( A. altilis \) roots extract exhibited moderate antimalarial activity with IC\textsubscript{50} values ranging from 1.9 to 4.3 µg/ml [20]. Flavonoid compounds from \( A. camansi \) species might be representing the antimalarial activity of this species. AAL was potential to be selected for further investigation.

In vivo antimalarial test

The observation of the percentage of parasite growth's inhibition of extracts is shown in Table 2. An extract that performed percentage parasitemia suppression ≥50% at a dose of 500, 250, and 100 mg/kg body weight per day in its vivo antimalarial activity can be classified as moderate, good, and very good, respectively [21]. Based on this classification, all samples showed very good activity.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>% Inhibition at a dose (µg/ml)</th>
<th>IC\textsubscript{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHS</td>
<td>79.31</td>
<td>46.67</td>
</tr>
<tr>
<td>AAL</td>
<td>93.35</td>
<td>22.32</td>
</tr>
<tr>
<td>AAS</td>
<td>99.86</td>
<td>84.20</td>
</tr>
<tr>
<td>ACL</td>
<td>85.93</td>
<td>33.09</td>
</tr>
<tr>
<td>ACS</td>
<td>99.50</td>
<td>49.44</td>
</tr>
</tbody>
</table>

Table 1: In vitro antimalarial activity of extracts against \( P. falciparum \)

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>% Inhibition at a dose (mg/kg b.w.)</th>
<th>ED\textsubscript{50} (mg/kg b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHS</td>
<td>65.99</td>
<td>53.83</td>
</tr>
<tr>
<td>AAL</td>
<td>67.64</td>
<td>46.13</td>
</tr>
<tr>
<td>AAS</td>
<td>82.26</td>
<td>63.18</td>
</tr>
<tr>
<td>ACL</td>
<td>73.53</td>
<td>67.26</td>
</tr>
<tr>
<td>ACS</td>
<td>52.82</td>
<td>35.75</td>
</tr>
</tbody>
</table>

Table 2: In vivo antimalarial activity of extracts against \( P. berghei \)

**REFERENCES**

Based on Trager and Jensen modified method [15], \( P. falciparum \) strain 3D7 was maintained at 5% hematocrit (human type O-positive red blood cell) in complete RPMI-1640 medium supplemented with 5% human type O-positive serum, HEPES, hypoxanthine, and gentamicin. Incubation was done at 37°C in a modified candle jar.

\( P. berghei \) strain ANKA was obtained from Animal Laboratory, Faculty of Pharmacy, Universitas Airlangga. The parasite has been maintained by a combination of passage in male mice BALB/C strain.

Animals

Male mice BALB/C strains were obtained from Pusat Veterinaria Farma (Pusvetma) Surabaya. Mice used for this study had 20-30 g body weight and were maintained on standard animal pellets and water ad libitum at Faculty of Pharmacy, Universitas Airlangga. Permission and approval for animal studies were obtained from the Faculty of Veterinary Medicine, Universitas Airlangga.

Extraction of leaves and stem bark of \( A. sp \).

Each part of the dried plants was ground and weighted as much as 100 g. Maceration was carried out using 500 ml ethanol 80% for 2 hrs and extract was then filtered. The process was repeated for 3 times, and the total ethanol 80% used was 2 liters. The ethanol extract was dried using a rotary evaporator and weighed afterward. All the extracts were kept in airtight containers and were stored at 4°C for antimalarial bioassay.

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DISCUSSION

A. heterophyllus was one of the important plants in the various folk and traditional medicine system in Asia [9]. Leaves and stem barks have been used to treat anemia, asthma, dermatitis, diarrhea, and cough [7]. In this study, AHL exhibited antimalarial activity against P. falciparum and P. berghei with IC₅₀ value of 9.35 µg/ml and ED₅₀ value of 8.33 mg/kg body weight. AHS inhibited P. falciparum growth with an IC₅₀ value of 12.18 µg/ml and had shown better inhibition against P. berghei with ED₅₀ value of 10.35 mg/kg body weight.

The next results revealed that an excellent in vivo antimalarial activity was shown by both AAL and AAS with ED₅₀ value <2 mg/kg body weight. AAL performed linear results between in vitro and in vivo activity shown by its IC₅₀ value of 1.32 µg/ml and ED₅₀ value of 0.82 mg/kg body weight. AAL indicates a promising antimalarial activity. Meanwhile, AAS displayed contradictive results between in vitro and in vivo activity test. Vestegaard et al. (2007) considered that in vitro tests do not include host factors and the correlation between the results of in vitro and in vivo tests was inconsistent and was not well-understood [22]. This study shows that AAS showed a weak activity in vitro with IC₅₀ value of 13.02 µg/ml but had a very good in vivo activity with ED₅₀ value of 1.48 mg/kg body weight. Although another study stated that extract with IC₅₀ value < 50 µg/ml was still considered active [23], the in vitro and in vivo results seemed to be not linear. This phenomenon may occur because the compounds were metabolized to active metabolites.

Another set of experiment of ACL and ACS showed some contradictive results in which they were active in in vitro against P. falciparum with IC₅₀ value of 5.31 µg/ml and 5.65 µg/ml, respectively, but had no in vivo activity against P. berghei with ED₅₀ value of 87.43 mg/kg body weight and 67.57 mg/kg body weight, respectively. The results of the in vivo antimalarial activity did not correlate with the in vitro antimalarial activity, which may be due to poor bioavailability of the active compounds in the in vivo system [24].

CONCLUSION

Artocarpus altillis leaves extract showed the best antimalarial activity with IC₅₀ value of 1.32 µg/ml and ED₅₀ value of 0.82 mg/kg body weight. A. altillis leaves extract is a promising candidate of a new antimalarial drug.

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