ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 9, Issue 1, 2016

ISSN - 0974-2441

Research Article

ANTIMALARIAL ACTIVITY OF CRUDE EXTRACTS OF *ARTOCARPUS HETEROPHYLLUS*, *ARTOCARPUS ALTILIS*, AND *ARTOCARPUS CAMANSI*

ACHMAD FUAD HAFID[1,2]*, RIA PUTRI SEPTIANI[1], LINTANG HUDHA FABRIANA[1], NINIET FEBRIANTY[1], DIMAS RANGGADITYA[1], ATY WIDYAWARUYANTI[1,2]

[1]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya - 60826, Indonesia. [2]Division of Natural Product Medicine Research & Development, Institute of Tropical Disease, Universitas Airlangga, Surabaya - 60115, Indonesia. Email: achmadfuad@ff.unair.ac.id, achmadfuad@yahoo.com

Received: 30 September 2015, Revised and Accepted: 03 December 2015

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 stembark, 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. falciparum* with inhibition concentration (IC_{50}) 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 (ED_{50}) value of 8.33 mg/kg body weight and 0.82 mg/kg body weight, respectively.

Conclusion: AAL has shown as the most active antimalarial activity with IC_{50} value of 1.32 µg/ml and ED_{50} 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 antiplasmodial 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 (IC_{50}) 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 IC_{50} value of 0.045 µg/ml [12]. Hafid *et al.* isolated active marker compound, morachalcone A (IC_{50} 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 phytomedicine product [13]. Another study reported that a prenylated stilbene compound was isolated from aerial parts of *Artocarpus integer* which has antimalarial activity with IC_{50} 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 Gresik, East Java; *A. altilis* was obtained from Kediri, East Java, and *A. camansi* was obtained from Tabanan, Bali. Authentification 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, Eijkman Institute of Molecular Biology, Jakarta, and was maintained

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

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 the 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 *Artocarpus* sp.

Each part of the dried plants was grinded 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.

***In vitro* antimalarial test**

Antimalarial *in vitro* test was performed based on Budimulya *et al.* [16]. A 10 mg sample was diluted in 100 ml DMSO. The sample was further diluted in RPMI-1640 medium and prepared in serial dilution at concentration 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 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 Peter'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 Arsuamoon® 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_{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.

Rosoanaivo *et al*. (2004) classified that the extract with IC_{50} value <0.1 µg/ml is very active, 0.1-1.0 µg/ml is active, 1.1-10 µg/ml is good to moderately active, 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_{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_{50} value ranging from 1.32 to 9.35 µg/ml. AAL showed the best activity with IC_{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_{50} values ranging from 1.9 to 4.3 µg/ml [20]. Flavonoid compounds from *Artocarpus* 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* antiplasmodial activity can be classified as moderate, good, and very good, respectively [21]. Based on this classification, all samples showed very good activity.

Table 1: *In vitro* antimalarial activity of extracts against *P. falciparum*

Plant extract	% Inhibition at a dose (µg/ml)					IC_{50} (µg/ml)
	100	10	1	0.1	0.01	
AHL	79.31	46.57	27.41	6.36	0.21	9.35
AHS	93.35	22.32	19.90	6.55	1.56	12.18
AAL	99.86	84.20	40.34	12.4	0.07	1.32
AAS	85.93	33.09	17.25	5.42	0	13.02
ACL	99.50	49.44	17.14	8.32	0.61	5.31
ACS	91.85	58.80	16.53	7.55	0	5.65

ACS: *Artocarpus camansi* stem, ACL: *Artocarpus camansi* leaves, AAS: *Artocarpus altilis* stem, AAL: *Artocarpus altilis* leaves, AHS: *Artocarpus heterophyllus* stem, AHL: *Artocarpus heterophyllus* leaves, b.w.: Body weight *P. falciparum: Plasmodium falciparum*

Table 2: *In vivo* antimalarial activity of extracts against *P. berghei*

Plant extract	% Inhibition at a dose (mg/kg b.w.)				ED_{50} (mg/kg b.w.)
	100	10	1	0.1	
AHL	65.99	53.83	31.50	25.00	8.33
AHS	67.64	48.13	32.67	19.99	10.35
AAL	82.26	63.18	51.54	36.72	0.82
AAS	73.53	67.26	46.64	31.28	1.48
ACL	52.82	35.75	28.73	20.16	87.43
ACS	55.56	28.00	22.44	6.30	67.51

ACS: *Artocarpus camansi* stem, ACL: *Artocarpus camansi* leaves, AAS: *Artocarpus altilis* stem, AAL: *Artocarpus altilis* leaves, AHS: *Artocarpus heterophyllus* stem, AHL: *Artocarpus heterophyllus* leaves, b.w.: Body weight, *P. berghei: Plasmodium berghei*

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 IC_{50} value of 9.35 µg/ml and ED_{50} value of 8.33 mg/kg body weight. AHS inhibited *P. falciparum* growth with an IC_{50} value of 12.18 µg/ml and had shown better inhibition against *P. berghei* with ED_{50} 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 ED_{50} value <2 mg/kg body weight. AAL performed linear results between *in vitro* and *in vivo* activity shown by its IC_{50} value of 1.32 µg/ml and ED_{50} 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 IC_{50} value of 13.02 µg/ml but had a very good *in vivo* activity with ED_{50} value of 1.48 mg/kg body weight. Although another study stated that extract with IC_{50} 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 IC_{50} value of 5.31 µg/ml and 5.65 µg/ml, respectively, but had no *in vivo* activity against *P. berghei* with ED_{50} 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 altilis leaves extract showed the best antimalarial activity with IC_{50} value of 1.32 µg/ml and ED_{50} value of 0.82 mg/kg body weight. *A. altilis* leaves extract is a promising candidate of a new antimalarial drug.

ACKNOWLEDGMENT

This research was funded by the Project Grand of the Faculty of Pharmacy, Universitas Airlangga.

REFERENCES

1. WHO. World Malaria Report: 2013. Geneva: WHO Press; 2013.
2. White NJ. Antimalarial drug resistance. J Clin Invest 2004;113(8):1084-92.
3. Kim Y, Schneider KA. Evolution of drug resistance in malaria parasite population. Nat Educ Knowl 2013;4(8):6.
4. Bero J, Quetin-Leclercq J. Natural products published in 2009 from plants traditionally used to treat malaria. Plant Med 2011;77:631-40.
5. Nogueira CR, Lopes ML. Antiplasmodial natural products. Molecules 2011;16:2146-90.
6. Saxena S, Pant N, Jain DC, Bhakuni RS. Antimalarial agent from plant source. Curr Sci 2003;85:1314-29.
7. Jagtap UB, Bapat VA. Artocarpus: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 2010;129(2):142-66.
8. Chen CY, Cheng MJ, Kuo SH, Kuo SY, Lo WL. Secondary metabolites from stems of *Artocarpus heterophyllus*. Chem Nat Compounds 2010;46(4):638-40.
9. Baliga MS, Shivashankara AR, Haniadka R, Dsouza J, Bhat HP. Phytochemistry, nutritional and pharmacological properties of *Artocarpus heterophyllus* Lam (Jackfruit): A review. Food Res Int 2011;44(7):1800-11.
10. Hakim EH, Achmad SA, Juliawaty LD, Makmur L, Syah YM, Aimi N, *et al*. Prenylated flavonoids and related compounds of the Indonesian Artocarpus (Moraceae). J Nat Med 2006;60:161-84.
11. Widyawaruyanti A, Subehan, Kalauni SK, Awale S, Nindatu M, Zaini NC, *et al*. New prenylated flavones from *Artocarpus champeden*, and their antimalarial activity *in vitro*. J Nat Med 2007;61:410-3.
12. Wahyuni TS, Ekasari W, Widyawariyanti A, Hirasawa Y, Morita H, Zaini NC. Artopeden A, a new antiplasmodial isoprenylated flavone from *Artocarpus champeden*. Heterocycles 2009;79:1121-6.
13. Hafid AF, Ariantari NP, Tumewu L, Hidayati AR, Widyawaruyanti A. The active marker compound identification of *Artocarpus champeden* spreng stembark extract, morachacone a as antimalarial. Int J Pharm PharmSci 2012;4 Suppl 5:246-9.
14. Boonlaksiri C, Oonanant W, Kongsaeree P, Kittakoop P, Tanticharoen M, Thebtaranonth Y. An antimalarial stilbene from Artocarpus integer. Phytochemistry 2000;54(4):415-7.
15. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science 1976;193(4254):673-5.
16. Budimulja AS, Syafruddin, Tapchaisri P, Wilairat P, Marzuki S. The sensitivity of Plasmodium protein synthesis to prokaryotic ribosomal inhibitors. Mol Biochem Parasitol 1997;84(1):137-41.
17. Philipson JD. Assays for antimalarial and amoebicidal activities. In: Day PM, Harborne JB, editors. Methods in Plant Biochemistry. Vol. 6. London: Academic Press; 1991. p. 135-52.
18. Rosoanaivo P, Deharo E, Ratsimamanga-Urverg S, Frappier F. Guidelines for the nonclinical evaluation of the efficacy of traditional antimalarials. In: Merlin W, Gerald B, Philippe R, editors. Traditional Medicinal Plants and Malaria. USA: CRC Press; 2004. p. 256-68.
19. Valdés AF, Martínez JM, Lizama RS, Gaitén YG, Rodríguez DA, Payrol JA. *In vitro* antimalarial activity and cytotoxicity of some selected Cuban medicinal plants. Rev Inst Med Trop Sao Paulo 2010;52(4):197-201.
20. Boonphong S, Baramee A, Kittakoop P, Puangsombat P. Antitubercular and antiplasmodial prenylated flavones from the roots of *Artocarpus altilis*. Chiang Mai J Sci 2007;34(3):399-44.
21. Bantie L, Assefa S, Teklehaimanot T, Engidawork E. *In vivo* antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice. BMC Complement Altern Med 2014;14:79.
22. Vestergaard LS, Ringwald P. Responding to the challenge of antimalarial drug resistance by routine monitoring to update national malaria treatment policies. Am J Trop Med Hyg 2007;77 6 Suppl:153-9.
23. Köhler I, Jenett-Siems K, Siems K, Hernández MA, Ibarra RA, Berendsohn WG, *et al*. *In vitro* antiplasmodial investigation of medicinal plants from El Salvador. Z Naturforsch C 2002;57(3-4):277-81.
24. Ramazani A, Zakeri S, Sardari S, Khodakarim N, Djadidt ND. *In vitro* and *in vivo* anti-malarial activity of *Boerhavia elegans* and *Solanum surattense*. Malar J 2010;9:124.