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In vitro and *in vivo* antiplasmodial activity of oxyresveratrol and artonine isolated from two *Artocarpus* plants in Indonesia

TATI SUHARTATI[1], YANDRI[1], JHONS F. SUWANDI[2] and SUTOPO HADI[1]*

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences,
University of Lampung, Bandar Lampung 35145 (Indonesia)
[2]Study Program of Medicine, University of Lampung, Bandar Lampung 35145 (Indonesia).

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ABSTRACT

Antiplasmodial activity of two compounds, oxyresveratrol and artonin E isolated from *A. dadah* and *A. rigida,* respectively, were evaluated. The *in vitro* antiplasmodial activity was done on the chloroquine-resistant strain of *P. falciparum* 3D7, while the *in vivo* antiplasmodial assay was done on *Swiss mice* breed which were infected by *P. berghei*. The two compounds tested showed very high *in vitro* activity with IC_{50} value of 0.05 and 0.2 µg/mL for oxyresveratrol and artonin E, respectively. However, by contrast in the *in vivo* activity assay, oxyresveratrol has an ED_{50} of 10.26 mg/kg, while the artonin E was not able to be determined.

Key words: *A. dadah*, *A. rigida*, antiplasmodial activity, oxyresveratrol, artonin E.

INTRODUCTION

Malaria, a disease caused by *Plasmodium*, has been known since a century ago and continues to be a major public health problem in Indonesia and other tropical countries. Due to the wider effect caused by malaria, WHO pays attention to this disease by a program called Roll Back Malaria (RBM) where a few points of this program were immediate diagnoses and exact treatment to eradicate malaria[1].

The malaria cases in Indonesia between the periods of 1997 – 2001 increased sharply, including in the Provinces of Java and Bali by ten times, while outside these Provinces were increased 4-5 times. These cases were also followed by resistance cases toward standard drugs used in the malaria treatments, the chloroquine and the sulfadoxine-pirimetamine. In some provinces, there were more than 25% resistance cases which cause the use of these standard drugs to be much more limited, therefore efforts to find new potent antimalarial drugs are urgently required[2].

The genus *Artocarpus* (Moraceae) are indigenous to the region of Southeast Asia including Indonesia. This genus consists of approximately 50 species and it is an extremely rich source of prenylated flavonoid compounds. Different compounds isolated from some species of *Artocarpus* have been known to exhibit some interesting biological properties[3-10].

Boonphong *et al*. have isolated some prenylated flavanoids which include artonin E, cycloartobiloxanton, artocarpin, cycloartocarpin from *A. altilis* which had cytotoxic activity against *P. falciparum*, thus is potentially used as an antimalarial drug[6]. We have also previously published the results of our investigation on *Artocarpus* plants from Indonesia where we were

able to isolate artonin E, artocarpin, cyclomulberochromen, norartocarpetin, cycloartobiloxanton, artoindonesianin D, artonin O, oxyresveratrol, and artoindonesianin L which also have some interesting biological activities[7-10].

In this paper, we reported the results of *in vitro* and *in vivo* antiplasmodial activity assays of two pure compounds oxyresveratrol and artonin E, isolated from *A. dadah* and *A. rigida*, respectively. These compounds were well characterised and have previously been published[9,10].

EXPERIMENTAL

Plant Materials

Root wood samples of *A. dadah* were collected from Wonosari Village, North Metro, Lampung, Indonesia, while the stem bark samples of *A. rigida* were collected from Keputran Village, Sukoharjo, Tanggamus, South Lampung, Indonesia. They were identified by the staff at the Herbarium Bogoriense, Research Centre for Biology, Indonesian Institute of Sciences, Bogor, Indonesia and a voucher specimen has been deposited at the herbarium. The isolation, extraction and characterisation of the compounds used for the bioactivity assays have previously been published[9,10].

Bioactivity assays

In vitro antiplasmodial assays

The *in vitro* antiplasmodial assays were performed in the Laboratory of Malaria, Eijkman Institute for Molecular Biology, Jakarta. The malaria parasite *P. falciparum* 3D7 clone was essentially propagated according to the previously published procedure[11]. Briefly, parasite cultures were propagated in tissue culture flasks containing RPMI-1640 medium supplemented with 25 µg/mL gentamycin, 50 µg/mL hypoxanthine, 25 mM Hepes buffer, 25 mM sodium bicarbonate, 10% AB+ human serum, 5% haematocrit and human erythrocytes with the pH maintained at 7.4.

Each compound tested was first dissolved in DMSO and diluted to different concentration by adding complete malaria medium. Chloroquine was used as a positive control. To determine the antiplasmodial activity of each isolated compound, parasites were placed in a 24-well culture plate in the presence of a wide concentration range of each compound. The parasite growth was monitored by making a blood smear that was fixed with methanol and stained with Giemsa. Total parasitaemia was calculated as the number of parasites-observed, divided by the total erythrocyte multiplied by 100%. The concentration response parasite growth data were calculated by a linear regression provided by SYSTAT Sigma Plot, using the 50% inhibitory concentration (IC_{50}). The IC_{50} value is defined as that concentration of compound producing 50% growth inhibition relative to untreated control.

In vivo antiplasmodial assays

The *in vivo* antiplasmodial assays were performed in the Laboratory of Parasitology, The Medical Faculty**,** Gadjah Mada University, Yogyakarta, Indonesia. The age of the mice used were 6-8 weeks old with average weight of 25 grams.

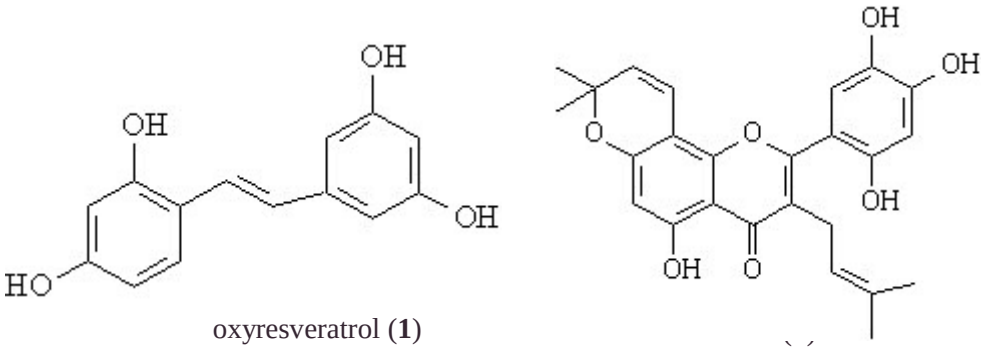
The *in vivo* antiplasmodial assays were done on Swiss mice breed. The mice were infected by *P. berghei*. The donor mice with parasitaemia degree of 30-40% had their heart blood taken and were diluted to get the inoculums of *P. berghei* 1×10^7 for every mouse. The intraperitoneally inoculation process was done by injecting inoculums 0.2 mL. Janse and Waters[12] stated that the normal dosage used in the inoculation of *P. berghei* on mouse was between 1×10^5 to 1×10^8. Ajaiyeoba et al. have used *P. berghei* 1×10^7 in the *in vivo* activity test[13].

The mice were then grouped into 21 groups of male and female and placed in the cage based on the group of dose and sex. Each cage contained three mice. The 4-day suppressive test method was used based on the known procedure[14]. The aquadest or the solvent used in the test were used as the negative control. The parasitaemia degree was the parameter observed. The ED_{50} value was calculated using probit analysis with SPSS 13.

RESULTS AND DISCUSSION

In vitro antiplasmodial assays

The *in vitro* antiplasmodial assay of oxyresveratrol, the structure shown in Fig. 1, showed very high activity toward Plasmodium with an IC_{50} value of 0.05 µg/mL (5×10^{-5} mg/mL), while artonin E showed less activity with an IC_{50} value of only 0.2



oxyresveratrol (**1**)

Fig. 1: The structure of the compounds used in the assays

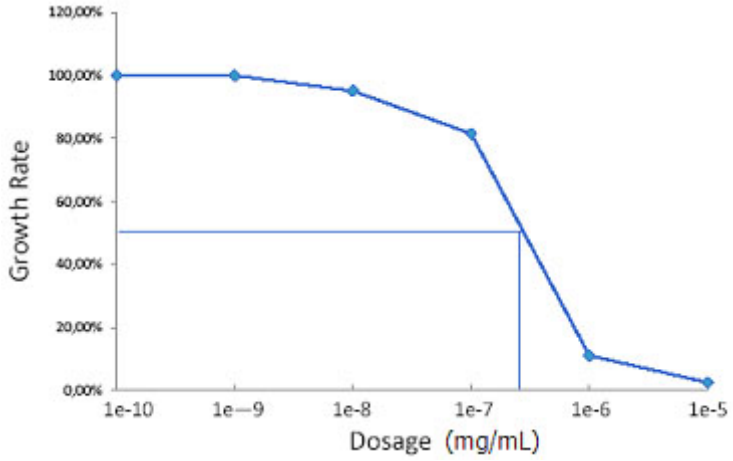


Fig. 2: *In vitro* antiplasmodial assay of oxyresveratrol (1)

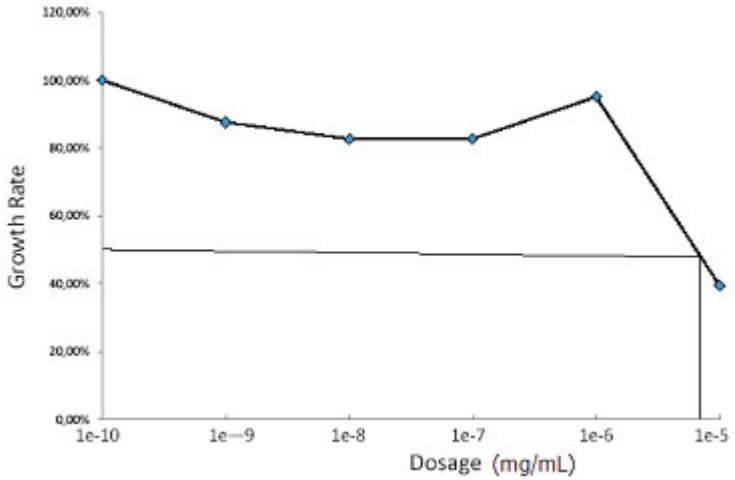


Fig. 3: *In vitro* antiplasmodial assaya of artonin E (2)

ìg/mL (2 x 10^{-4} mg/mL). Therefore, based on the IC_{50} values, oxyresveratrol was by far more active than the standard compound used, chloroquine with an IC_{50} value of 3 x 10^{-7} M (0.096 µg/mL or 9.6 x 10^{-5} mg/mL), and artonin E. From the data obtained, it is quite clear that oxyresveratrol shows a very promising result and it has potential as an antimalarial drug.

Fig. 2, 3 and 4 show the results of activity assay of oxyresveratrol, artonin E and chloroquine, respectively.

***In vivo* antiplasmodial assays**

The application of the compound tested will cause the inhibition of Plasmodium growth. The higher the dosage of compound used, the smaller the average of the parasitaemia and the higher the percentage of growth inhibition. The highest inhibition was observed for a group of mice that was given oxyresveratrol, i.e. 78.37 ± 0.17%, whereas for artonin E, the inhibition was much less than oxyresveratrol, and it was also less than 50% (Fig. 4) which meant its ED_{50} value was unable to

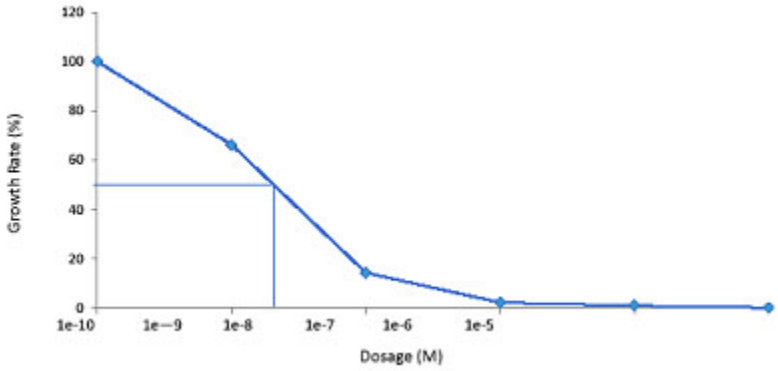


Fig. 4: *In vitro* antiplasmodial assay of standard compound, chloroquine

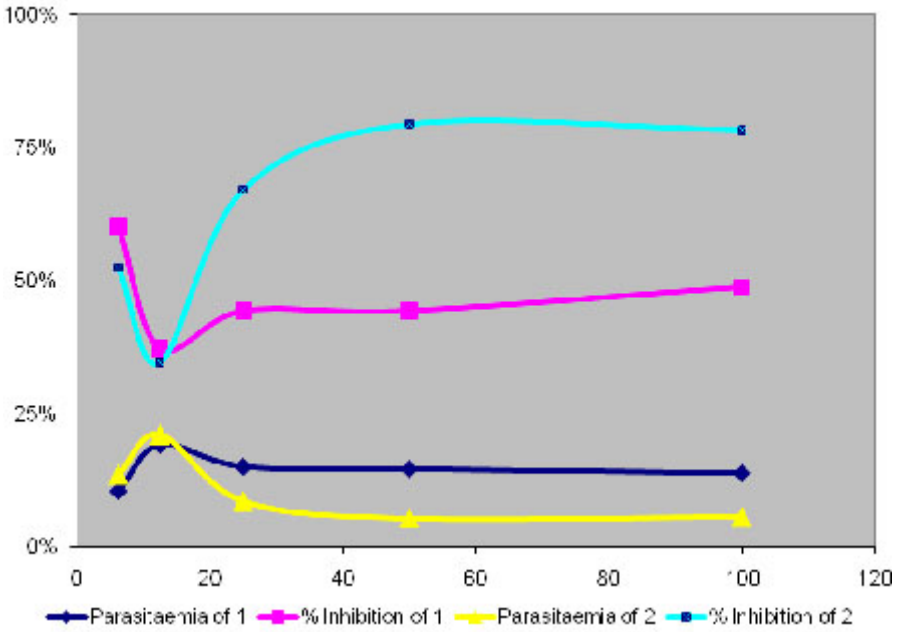


Fig. 4: The comparison of *in vivo* antiplasmodial assays of oxyresveratrol (1) and artonin E (2)

Table 1: The category of *in vivo* antiplasmodial activity

ED_{50} value (mg/kg/day)	Category
≤ 100	Excellent
101-250	Good
251-500	Fair
> 500	Bad/not active

be determined. The ED_{50} value for oxyresveratrol has been found to be 10.26 mg/kg.

According to Munoz et al. the *in vivo* antiplasmodial activities are categorised into 4 categories[15] as presented in Table 1. Based on the criteria in Table 1, oxyresveratrol is categorised as an excellent drug, as its ED_{50} was by far much smaller than the criteria for the excellent category, on the other hand, artonin E is categorized as an inactive compound as its ED_{50} value was unable to be determined, as the inhibition value did not exceed 50%.

In the *in vivo* activity assay, there are many individual factors of the mice which affect the growth of *P. berghei,* which then can give a variation in the parasitaemia[16]. The hospes (mice) and Plasmodium factors are also very important in the growth speed of *P. berghei* in mice[17]. The character of *P. berghei* which cannot be synchronized also affects the growth, as sometimes only 10% of *P*. *berghei* inoculated might grow. Due to the presence of erythrocyte schyzogony cycle in *P. berghei* at viscera organ, the phases of ring and trophozoite will mostly be taken as the inoculums. The characteristic of *P. berghei* will affect its growth rate in the host cell. These conditions may cause the variation of parasitaemia value in artonin E, as a result the ED_{50} was unable to be counted[16].

The mechanism of the compound tested as antiplasmodial might be *via* the inhibition of heme polymerisation. This is because the compounds tested have phenolic groups which can bind with electronic system in heme or iron ion in heme[18,19].

CONCLUSIONS

This study is believed to report the first antiplasmodial activity of two compounds, oxyresveratrol and artonin E isolated from Artocarpus plants, *A. dadah* and *A. rigida,* respectively, from Indonesia. It has clearly been shown that oxyresveratrol is a very promising and interesting new material for use as an antimalarial drug, as it is highly active both *in vitro* and *in vivo* activity assays. Therefore, the plants of *A. dadah* and *A. rigida* are considered as natural resources that are a rich source of compounds for antimalarial drugs and their future application will be very useful in attempts to reduce the malaria disease not only in Indonesia, but also in other tropical countries.

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