



Antioxidant and Antimalarial Potential of Methanolic Extract from Leaves of Titi Tree (*Alstonia sp*)

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Abstract: *Alstonia sp* leaves extract has previously been reported as a potential antimalarial drug as traditional. This study aims to analyze the antimalarial activity and determine the antioxidant potential of titi tree leaves (*Alstonia sp*). The method used in this research is a modified method of "4 Days Suppressive Test" which was originally described by Peter with different doses (0.55 g/kg, 0.65 g/kg and 0.75 g/kg body weight) of methanol extract of leaves of Titi tree (*Alstonia sp*) was administered orally to albino mice. Antioxidants were analyzed using a spectrophotometer. The Leaves of *Alstonia sp* has antimalarial activity and contains potential antioxidant activity with an IC₅₀ value of 70.12 ppm for leaves (strong category). The dose of 0.75 g has a better effect compared to the other effects because it lowers the level of parasitemia more strongly. So that the leaves of *Alstonia sp*. pressured to be developed as a nutraceutical.

Keywords: *Alstonia sp. leaves*; Antimalarial; Antioxidants

Introduction

Malaria is a potentially fatal disease transmitted through the bite of a female Anopheles mosquito and affects more than 106 countries worldwide. Mortality rates of malaria have increased over the past 20 years, primarily due to the resistance of *P. falciparum* to antimalarial drugs, especially chloroquine, artemisinin and its derivatives. Therefore, efforts are needed to develop new antimalarials that are effective, safe, and have fewer side effects as well as cheap and easy to obtain. One potential source is a compound derived from plants. Flavonoids are secondary metabolites found in plants, and many reports of this class of antimalarial activity have been reported.

The rapid emergence and widespread resistance to all currently available antimalarials, coupled with the fact that the chloroquine resistance transporter gene has also been associated with increased susceptibility to artemisinin, quinine, and amodiaquine calls for the development of new potent antimalarial (Idowu et al., 2016). Tajbakhsh et al. (2021) showed that, with the

rising resistance to frontline drugs (artemisinin-based combinations), there is a need to accelerate the discovery and development of newer antimalarials drugs. Therefore, high efforts to develop alternative antimalarial drugs are underway worldwide. Efforts to increase the discovery of antimalarial drugs are urgently needed. The goal must be to develop safe and affordable new drugs to counter the spread of malaria parasites that are resistant to existing agents.

Plants of the Titi tree (*Alstonia sp*) are widely used traditionally for treating malaria, fever, and diabetes mellitus. Local people in Southwest Maluku Province refer to it as Titi tree and Milk tree. Previous research found the bark of *Alstonia sp* to be a well-known drug for the treatment of various types of disorders in the plant medicine system. It has been reported to have antimalarial (Adotey et al., 2012; Dhruti et al., 2016; Tepongning et al., 2011), free radical scavenger and antioxidant (Ramachandran et al., 2012), antimicrobial activity (Amole et al., 2010), antidiabetic (Jong-Anurakkun et al., 2007; Otuu et al., 2021), antifertility

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(Choudhary et al., 2017) and wound healing capacity (Tan et al., 2019).

The chemical constituents of these plants are Alkaloids, phlorotannins, simple phenolics, steroids, saponins, and tannins have been reported in all parts of *Alstonia scholaris* (Khyade et al., 2014; Otuu et al., 2021). This provides an opportunity to develop this plant as a nutraceutical that is beneficial for health and has economic value for the community. However, scientific data for the development of this plant related to the levels of macronutrients and antioxidant activity is still very lacking. Therefore, it is necessary to conduct an in-depth study of the potential for macronutrients and antioxidant activity in the bark and leaves of *Alstonia sp* so that it can provide basic information on the utilization of the bark and leaves of *Alstonia sp* in industrial fields such as health drinks (Nutraceutical).

The human body has a limited amount of antioxidant reserves, so in a state of oxidative stress, in which the body's ability to ward off free radicals is smaller than the number of free radicals present in our body, the body will need an intake of antioxidants from the outside source (Zuraida et al., 2017). Plants containing large amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β -carotene, lycopene, lutein, and other carotenoids, can neutralize free radicals, quench singlet and triplet oxygen, or decompose peroxides (Dhruti et al., 2016; Djeridane et al., 2006). The content of antioxidants is very beneficial for health in preventing aging and degenerative diseases. Antioxidants can fight free radicals in the body.

Several phytochemical studies of *Alstonia sp* have been carried out, namely the phytochemical levels of leaves and bark of *Alstonia sp*. Qualitative phytochemical screening has revealed the presence of alkaloids, glycosides, flavonoids, saponins, terpenoids, gums, and mucilage as well as oils and fats in the extracts of the bark and leaves of *Alstonia sp* (Antony et al., 2011; Dhruti et al., 2016).

The purpose of this study was to determine antimalarial activity from the stem bark of *Alstonia sp* and determine the potency of macronutrients and antioxidants so that it can become a complete database for the development of titi trees (*Alstonia sp.*) as a nutraceutical.

Method

Tools and Materials

The tools used in the research include an Analytical balance, oven, desiccator, porcelain cup, ash oven, ashing cup, soxhlet, hotplate, measuring cup, nitrogen distillation set, burette and stative, destroyer tool,

Kjeldhal flask, Erlenmeyer, volume pipette, clamps, basin, Cross-section Container Mice, object glass, scissors, blender, sonde, syringe, microscope, Handcouter, 1 Ml Syringe and Surgical Board, evaporator rotary tweezers, surgical scissors, surgical needles, and digital camera.

The materials used in the research include Leaves of the *Alstonia sp* plant were sampled (1 kg), H₂SO₄, selenium, NaOH, HCl, acetone, ether, chloroform, filter paper, distilled water (aqua dest), 96% Ethanol, *Plasmodium berghei*, Giemsa dye, chloroquine sulfate, methanol absolute and alcohol 70%. The plant materials used in the research include the fresh leaves of *Alstonia sp* were collected from Moa Island, Maluku, Indonesia in the dry season, around September-December 2022, and cut into small pieces, washed, and air dried for two weeks under room temperature. This raw materials is stored at the Herbarium of the Parasitology laboratory, Pattimura University, Maluku, Indonesia.

Preparation of Ethanol Extract

The leaves were taken from the titi tree, then cleaned with distilled water, and dried in an oven at 50°C for five days. After drying, *Alstonia sp.* leaves are ground using a blender until they become powder. Coarsely powdered dry leaves (1 kg) were successively extracted in cold with 95% methanol as solvent, before maceration for 24 hours. The leaves mixture was filtered using Whatman no. 1 filter paper to obtain a clear filtrate extract solution. The filtrate was then freeze-dried to obtain the powder and stored at -20 °C until further use (Rahim et al., 2022).

Antimalarial Assay

Preparation of Test Animals (Mus musculus). Obtained experimental animals must obtain approval from the Tropical Disease Center, Airlangga University. Mice (*Mus musculus*) BALB/c mice between 20 and 22g were used for this study. Twenty mice were used for this study, of which five infected donor mice and fifteen test mice. The prepared test animals will be acclimatized. Fifteen mice were randomly divided into five groups, where one group consisted of three mice. On the first day (D0), the mice were each infected with 107 *Plasmodium berghei*. After 24 hours, the infected mice were subjected to extract or 10 mg -1 body weight of chloroquine. The reduction in parasitemia in the treated animals was quantified using a Giemsa Smear, from two replicate experiments.

Mice Infected Donors. *Plasmodium* was thawed first to infect the five donor mice interactively. Furthermore, on the fourth day after the infection is carried out.

Antimalarial Assay. Antimalarial *in vivo* test was performed based on Peter's test (The 4-day suppressive

test) that used *P. berghei* (strain ANKA) infected mice. Treatment for assay the antimalarial activity of the ethanolic extract of leaves of titi tree at doses of 0.55, 0.65, and 0.75 g/kg body weight/day and also treatment using chloroquine sulfate as a positive control and without treatment.

Parasitemia was monitored by standard methods, thin Blood smears were made on glass slides, fixed using ethanol, and stained using Giemsa stain, and parasitemia was counted using a microscope and was calculated as a percentage of infected red blood cells (RBCs) relative to the total number of cells in a microscopic field at $\times 100$ magnification as given below (Atanu et al., 2021; Tepongning et al., 2011):

$$\% \text{ Parasitemia} = \frac{\Sigma \text{Total number parasites} / \text{RBCs}}{\Sigma \text{Total number of RBCs}} \times 100 \quad (1)$$

Antioxidant analysis. Antioxidant activity was analyzed by first making a solution of DPPH (1,1-diphenyl-2-phikril-hydrazil) by dissolving the DPPH crystals into methanol at a concentration of 0.01 M. Then, 1 ml of 0.01 M DPPH was taken and methanol was added until it reached a volume of 5 ml. The spectrophotometer was used to measure the absorbance at 517 nm. The next process was the measurement of sample absorbance from extraction with n-hexane solvent. The absorbance of the sample was measured by taking 200 mg of the sample and dissolving it in 5 ml of methanol while vortexing it for 1 hour. Then, 1 ml of the mixture was taken and 1 ml of 0.01 M DPPH and methanol were added until it reached the volume of 5

ml. Then the absorbance of the sample was measured at a wavelength of 517 nm. The inhibition ratio was calculated as the percentage of inhibition using the following formula:

$$\% I = \frac{A_C - A_S}{A_C} \times 100\% \quad (2)$$

Where $\% I$ = percentage inhibition (%), A_C = absorbance of control, and A_S = absorbance of the test sample. Inhibition Concentration (IC) (%) is the concentration of an antioxidant substance that causes 50% of DPPH to lose its radical character (Peter, 2004).

Data Analysis

A one-way analysis of variance (ANOVA) was performed on the data, followed by Tukey's multiple comparison test ($p \leq 0.05$). The results are reported as the average of three measurements with the standard deviation.

Result and Discussion

Result

The Results of the Study of the Antimalarial Activity of the Ethanolic Extract of Titi Tree Leaves (Alstonia sp.)

The total from the beginning of the treatment to the end of the treatment for 14 days showed that in each treatment there was a change in the level of parasitemia depending on the treatment given. To see the development of parasitemia levels from time to time can be seen in table 1.

Table 1. The Level of Parasitemia of Mice on Blood Cells after Administration of Methanol Extract of Leaves *Alstonia* sp.

Treatment	After Giving Plasmodium (%)	After Treatment (%)	Treatment CQ (%)	No Treatment (%)	Suppression (%)
Control (-)	23.2 \pm 0.1b	-	-	17.1 \pm 0.4d	0.0 \pm 0.0b
Control (+)	23.5 \pm 0.5b	-	5.0 \pm 0.1a	-	69.7 \pm 0.2c
Dose 0.55	22.9 \pm 0.4c	7.1 \pm 0.3c	-	-	20.2 \pm 0.3a
Dose 0.65	23.5 \pm 0.3b	6.0 \pm 0.1b	-	-	23.51 \pm 0.2b
Dose 0.75	33.6 \pm 0.3b	4.9 \pm 0.2b	-	-	43.6 \pm 0.3b

Note: Superscripts with different letters are not significantly different in a particular column. The sign (-) has no value in the table group.

The data presented in Table 1 and Figure 1 provide an overview of how parasitemia develops in each condition. In all treatments before administration of *Plasmodium berghei*, the average of all mice was 0.0%, then each treatment was infected with *Plasmodium berghei*.

On the fourth day after infection, it was found that the average percentage of parasitemia in all mice was increased. In mice treated with methanolic extract of titi stem bark at a dosage of 0.55 g/kg/day, parasitemia values of 7.1% \pm 0.3, a dosage of 0.65 g/kg/day, parasitemia values of 6.0% \pm 0.1 and dosage of 0.75

g/kg/day, parasitemia values of 4.9% \pm 0.2 were recorded, compared to 17.1% \pm 0.4 in negative controls ($p < 0.05$).

The antimalarial assay revealed that there was a significant reduction ($p < 0.05$) in RBCs at all treatment doses. This suggests that extract of stem bark from *Alstonia* sp could have significant antimalarial activity in vivo. Doubling the dosage of the extracts significantly increases parasite suppression. The extract showed substantial dose-dependent antimalarial activity as indicated by the recorded suppressive (Figure 1).

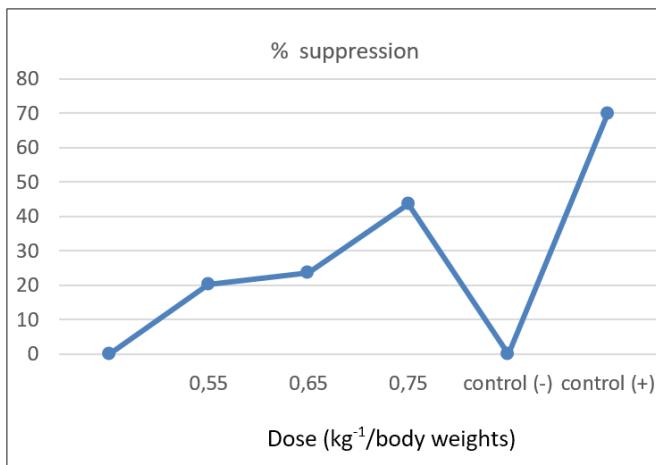


Figure 1. Percentage suppression of methanol extract of *Alstonia sp*

The Composition of Antioxidants in the Leaves of *Alstonia sp.*

Based data of DPPH protocol evaluation and regression linear graphic (Figure 1), show that methanol extracts of *Alstonia sp* were found to be effective free radical scavengers given that the IC₅₀ value was 70.12±1.59 ppm.

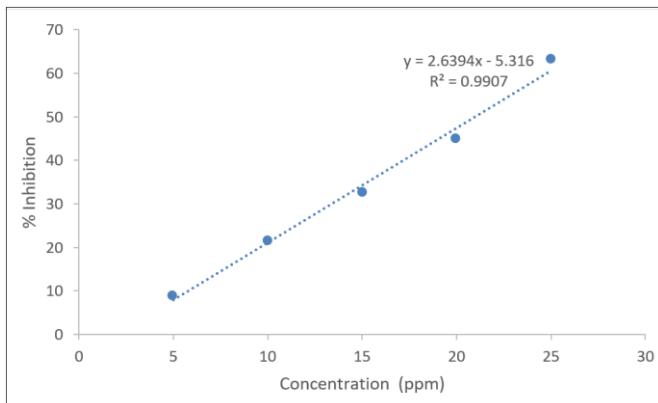


Figure 2. Free radical scavenging analysis of *Alstonia sp.*

As shown in Figure 2 and Table 2, the antioxidant activity of different formulations revealed almost the same free radical scavenging activity for all the formulations studied.

Table 2. Antioxidant Activity on the Leaves of *Alstonia sp.*

Extract/Standard	Abs 517 nm	(%) Inhibition	IC ₅₀ (ppm)
Leaves	0.14 ± 0.04	75.88 ± 0.3	70.12 ± 1.59
Ascorbic acid	0.08 ± 0.00	93.50 ± 0.5	9.03 ± 0.51

Based on the data in Table 2, the average antioxidant value of *Alstonia sp.* is 70.12 ppm (50-100 ppm), categorized as having a high antioxidant activity (Abu et al., 2016; Peter, 2004). They are antioxidants capable of capturing free radicals thereby preventing disease (Olafadehan et al., 2020).

Discussion

The result of the antimalarial tests of this study, of dose 0.65 and 0.75 g/kg body weight/day and above for in vivo studies and antimalarial ≥ 0.65 g/kg body weight/day for in vivo studies, were considered active. Table 1 shows a dose of 0.65 g of the ethanol extract of *Alstonia sp.* The effect is better than the other effects because it can reduce high parasitemia levels, but not high positive control. As reported by several related studies, the higher the dose given, the level of parasitemia can decrease (Cimanga et al., 2019; Intan et al., 2016; Otuu et al., 2021). The results from this study indicate that the ethanolic extract of leaves of *Alstonia boonei* has a good safety.

However, the percentage suppression achieved at 0.55g/kg dosage was lower than the percentage suppression achieved by the methanolic extract of *A. boonei* alone as reported by some workers (Iyiola et al., 2011). Methanol is a well-known solvent with high polarity properties and can extract substantial amounts of polyphenols compared to water and ethanol. Greater antioxidant activity correlated with high polyphenolic compounds through the synergistic effect of multiple polyphenols and hydrogen atom donation.

Pulai bark contains alkaloids which can act as antimalarials. Satyaningrum et al. (2021) revealed that saponins have antimalarial activity because they can inhibit heme polymerization. Saponins themselves are glycoside compounds with a relatively high molecular weight of sugars connected to triterpenoids/steroid aglycones. The triterpenoid-steroid compound inhibits heme polymerization by forming a heme carboxylate ring complex so that heme will remain a dimeric ring (Gnoatto et al., 2008).

Based on research on the average fat content of the leaves of *Alstonia boonei* (Fagbahun et al., 2019), members of the same genus have an average lipid content of 6.00%, almost the same as that found in *Alstonia sp.* on Moa Island. Compared to research conducted by Abu et al. (2016), the fat content of *A. boonei* leaves obtained was 0.84%.

The human body needs a different amount of fat. The functions of fat for the body are to serve as a building block for vitamins and hormones, produce the highest energy, and protect the body from low temperatures. Fat is the most important form of energy storage in the body as well as a source of essential nutrients (Almatsier, 2009). The results of the ash content of the bark of *Alstonia sp.* in Nyama Village, Moa Island is the same as the study by Hussain et al. (2013) who researched *Alstonia scholaris* stem bark with an ash content of 24.73%. Hidayat (2014) suggested that the differences in the nutritional levels of the stem bark of *Alstonia sp.* are presumably due to differences in

habitat conditions, soil type, and soil pH. Due to differences in the concentration of inorganic matter between one type of soil and another, the nutrients absorbed by plants are also different (Havlin et al., 2017).

Based on the research results of Abu et al. (2016), the ash level in the leaves of *Alstonia boonei* was 2.16%. According to the research conducted by Ifon and Bassir (1980) in Gbadamosi et al. (2011), plants with ash levels above 8.8% were good for health. Research on ash levels was also carried out by Gbadamosi et al. (2011), who argued that high ash levels had good nutritional value. According to Jensen (2010), mineral or ash levels were influenced by soil pH which could change the type of nutrients available in the soil solution; plants usually grew above pH 5.5 and as a rule, 6.5 was the right pH level for optimal nutrient absorption. A high pH promoted the availability of macronutrients rather than neutral or alkaline soils that supported plant growth (Loncaric et al., 2018). *Alstonia* sp. had the potential to be used as a source of nutritious drinks and for traditional medicines as well. The use of synthesized macronutrients played an important role in producing high quality antioxidant bioactive ingredients and functions in increasing secondary metabolites (Zubairi et al., 2014).

The results of antioxidants on the leaves of *Alstonia* sp. in Moa Village, Nyama Island, following the opinion of Peter (2004) who divided antioxidant activity into 4 (four) categories based on the IC₅₀ value, namely weak activity (150-200 ppm), moderate (100-150 ppm, strong (50-100 ppm) and very strong (<50 ppm), thus the IC₅₀ of *Alstonia* sp. bark has very strong antioxidant activity, this is also following the study of Antony et al. (2011), which showed that stem bark and leaf extracts were found respectively 1.21 mg/mL and 2.83 mg/mL in the DPPH assay.

Alstonia sp. has a high level of antioxidants so people on the island of Moa use it for the treatment of malaria, and diabetes and to increase immunity. This is to the results of research by Khyade et al. (2014), regarding *A. scholaris* and *A. macrophylla* for the treatment of malaria, jaundice, digestive disorders, and cancer. Due to the leaf morphology of *Alstonia* sp. on Moa Island have different leaf sizes and shapes from the genus *Alstonia* sp. According to Marjenah (2001), plants experience morphological adaptations to respond to the low light intensity if they have a larger leaf area.

Conclusion

Based on the results of the study it can be concluded that the leaves of *Alstonia* sp. contain antioxidant activity through the IC₅₀ value produced by the bark of 70.12 ppm (strong category of antioxidant activity) and

antimalarial with a dose of 0.75 g administration of ethanol extract of titi bark is more effective.

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Author Contributions

Authors listed in this article contributed to the research and development of the article.

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Conflicts of Interest

The author publishes this article for the needs of research output in the form of publication in scientific journals as proof of the required performance. The authors declare no conflict of interest.

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