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Antiplasmodial activity of faloak bark (*Sterculia quadrifida*, RBr.) extract from East Nusa Tenggara, Indonesia

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ABSTRACT

The current development of antimalarial drug resistance encourages researchers to discover and develop novel antimalarials. One of its alternatives for antimalarial discovery is using medicinal plants remembering the success of artemisinin. *Sterculia quardrifida* R. Br. bark, locally name as faloak, is an endemic medicinal plant from East Nusa Tenggara that has been used traditionally to treat malaria. However, its antimalarial activity has not been investigated, yet. This study was aimed to evaluate the antiplasmodial activity of ethanolic extract of faloak bark against *Plasmodium falciparum in vitro*. Using FCR-3 *P. falciparum* strain, the ethanolic extract was evaluated on various concentration (1, 10, 50, and 100 µg/mL, respectively). The IC_{50} value was determined by the relationship between concentration and percentage of growth inhibition. The result showed that the percentage of inhibition of *P. falciparum* was concentration dependent, higher concentration resulting on higher percentage of inhibition with the IC_{50} 42.399 ± 9.517 µg/mL. It can be concluded that the ethanolic extract of faloak bark have moderate antiplasmodial activity against *P. falciparum in vitro*.

ABSTRAK

Perkembangan resistensi terhadap obat antimalaria saat ini telah mendorong peneliti untuk menemukan dan mengembangkan antimalaria baru. Mengingat keberhasilan penemuan artemisinin sebagai malaria, tumbuhan obat menjadi salah satu alternatif sumber penemuan antimalaria baru. Kulit batang *Sterculia quardrifida* R. Br., dengan nama daerah faloak, adalah salah satu tanaman endemik di Nusa Tenggara Timur yang secara tradisional digunakan untuk mengobati malaria. Namun demikian, aktivitas antimalarianya belum pernah diteliti. Penelitian ini bertujuan untuk mengkaji aktivitas ekstrak kulit batang faloak terhadap pertumbuhan *Plasmodum falciparum* secara *in vitro*. Ekstrak etanol kulit batang faloak dengan berbagai konsentrasi (1, 10, 50, dan 100 µg/mL) diuji aktivitasnya terhadap penghambatan pertumbuhan *P. falciparum* galur FCR3. Nilai IC_{50} ditentukan dengan menghubungkan konsentrasi dan prosentase penghambatan pertumbuhan parasit. Didapatkan hasil berupa penghambatan pertumbuhan *P. falciparum* berkorelasi erat dengan konsentrasi ekstrak etanol kulit batang faloak yang dipaparkan. Konsentrasi yang lebih tinggi dapat menghambat pertumbuhan lebih besar dengan nilai IC_{50} yang didapat adalah sebesar 42.399 ± 9.517 µg/mL. Dapat disimpulkan bahwa ekstrak etanol kulit batang faloak memiliki aktivitas antiplasmodium sedang terhadap *P. falciparum* galur FCR-3 secara *in vitro*.

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INTRODUCTION

Malaria, one of tropical infectious disease that remains a global problem. Based on World Health organization (WHO) data, Africa is the most endemic region followed by South East Asia, including Indonesia.[1] Supporting the data, Indonesia ministry of health stated that several provinces have higher annual parasite incidence (API) including Papua, East Nusa Tenggara, Maluku, Bengkulu and Bangka-Belitung Islands.[2] Even though eliminating malaria program are conducted continuously, malaria outbreak persists annually. Varied problems create an obstacle in combating malaria including the emergence of *Plamodium falciparum* resistance to artemisinin-based combination therapy (ACT), the latest and recommended drugs by WHO[3-6] that also implemented in Indonesia. Since there is no new drug substitute the artemisinin, hence, it is important to discover new antimalarial drug to overcome that problem. Several sources can be used to explore new antimalarial drug, one of it is traditional medicinal plants that locally used by peoples in endemic areas.

Remembering successful story of chloroquine and artemisinin, exploration of medicinal plants is promising. In endemic area that generally consist of developing or undeveloped countries, medicinal plants are the most affordable treatment as Karou *et al.*[7] stated, along with traditional uses of its for various ailment since ancient time. Faloak (*Sterqulia quadrifida* R. Br.) is the endemic medicinal plant in East Nusa Tenggara (Kupang), Indonesia. Its bark widely uses by local people for treating hepatitis and malaria (decoction). A study of Saragih *et al.*[8] found its antioxidant activity with the active compounds are flavonoid, terpenoid, and alkaloid.[9] Moreover, it also correlated to macrophage activity[10] and active against hepatitis C virus.[11] To date, there is no antiplasmodial study using faloak bark has been reported. Although its active compounds have been identified as potent antimalaria in separate research. Thus, this study was aimed to evaluate the antiplasmodial activity of faloak bark against *P. falciparum* malaria parasite.

MATERIAL AND METHODS

Material and tools

Materials in this study were *S. guadrifida* bark, ethanol 70% (technical grade), RPMI 1640 (Sigma), HEPES (Sigma), NaHCO3 (technical grade), gentamycin (Merck), sterile aquadest, DMSO (Merck), Giemsa (Merck), *P. falciparum* FCR-3 strain, O^+ type human erythrocyte and serum.

While the tools utilized in this study were maceration chamber, glass apparatus, analytical scale, CO2 incubator, LAF, flask culture, centrifuge, Eppendorf, conical tube, micropipette, object glass, and microplate.

Extraction of faloak (*S. guadrifida* R. Br.) bark

The faloak bark extraction was conducted in the Department of Pharmacy, Poltekkes Kemenkes Kupang, East Nusa Tenggara. As the traditional uses, the parts of the plants taken for the extraction was the bark. As much as 150 g powdered faloak bark were extracted by maceration method in 70% of methanol for 5 days (1.125 L of solvent). This process was repeated for two days consecutively using fresh 70% ethanol. Following 3 days re-maceration in ethanol, the extracts were filtered, and the filtrates were evaporated to attain ethanol extract.

Characterization of active compound of faloak bark

In order to identify the ethanolic extract of faloak bark compounds, the characterization was performed using reagent-tube method. Positive flavonoid compound was identified by color alteration after NaOH, H_2SO_4 and Mg-HCl addition consecutively. Furthermore, using Mayer and Wagner reagents, the alkaloid compound was detected. While

foam formation after shake, display the saponin content. Others compound identification, steroid, was detected by brown color arrangement after addition of H_2SO_4 6 M meanwhile red color pattern after H_2SO_4 addition may detect terpenoid compound.

Antiplasmodial assay

The antiplasmodial activity assay was conducted in the Department of Pharmacology and Therapy, Faculty of Medicine, Publick Health and Nursing, Universitas Gadjah Mada, Yogyakarta. *Plasmodium falciparum* FCR-3 chloroquine-sensitive strain was cultured continuously using Trager and Jensen[12] method in in a 5% CO_2 atmosphere at 37°C by considering minor modifications. In brief, the parasites were kept *in vitro* in human red blood cells and diluted in RPMI 1640 medium, added by 25 mM HEPES, L-glutamine and completed with 5% human serum. The antiplasmodial activity was assessed similarly to what was previously reported by Desjardins *et al.*[13] and modified as follows. Extract dilutions and reference compounds were tested in triplicate, in 96-well plates with cultures at 1% parasitaemia and 2% haematocrit. The plates of parasite culture were incubated with extracts or reference compounds for 72 h and the parasite growth was estimated by thin smear-Giemsa stained for each well and observed under light microscope. The control parasite culture (without drug and with 2% of DMSO) was referred to as 100% growth. The IC_{50} values (50% inhibitory concentration) were determined as concentrations versus percentage of parasite growth using probit analysis.

RESULTS

In this present study, the maceration process of faloak bark yielded 28.5 % of crude extract. It was dark brown with typical smell and taste of extract. The detection of active compound was drawn on Table 1.

TABLE 1. Characterization of active compound of faloak bark ethanolic extract

Compounds	Result
Flavonoid	+
Alkaloid	+
Saponin	+
Terpenoid	-
Steroid	-

Following the detection of active compounds, the extract then tested against *P. falciparum*. The plasmodium growth inhibition after 72 h incubation with the faloak bark extract or chloroquine in various concentrations is presented in FIGURE 1, whereas the antiplasmodial activity is presented in Table 2.

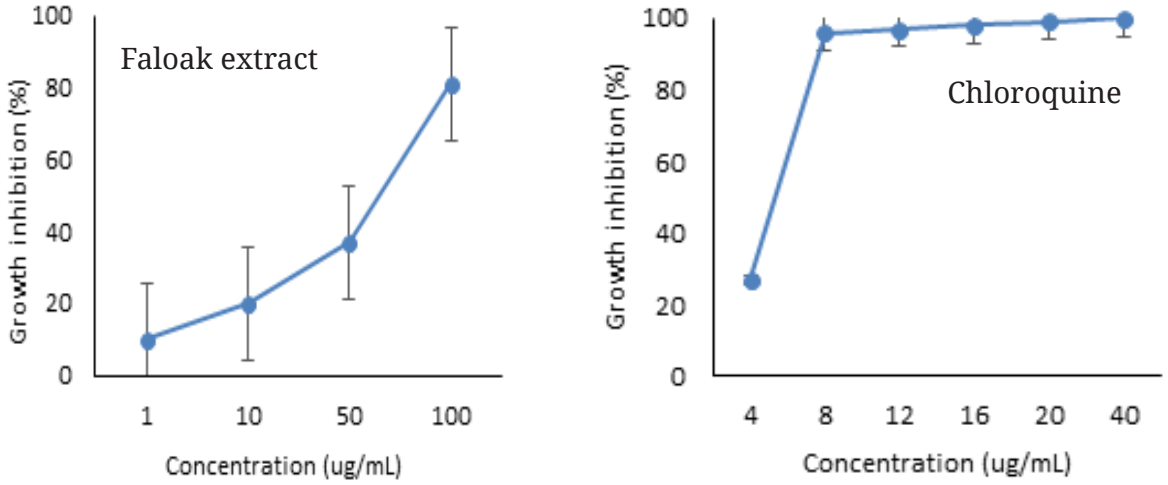

FIGURE 1. Parasite growth inhibition after incubation with A) faloak bark ethanolic extract and B) chloroquine.

TABLE 2. Antiplasmodial activity of faloak bark ethanolic extract

Sample	Concentration (μg/mL)	Inhibition of parasite growth (%)	IC_{50} (μg/mL)
Faloak bark ethanolic extract (FBEE)	1	10.14 ± 3.96	42.399 ± 9.517
	10	19.95 ± 17.65	
	50	36.86 ± 35.08	
	100	81.15 ± 31.96	
Chloroquine (CQ)	4	26.61 ± 6.56	4.808 ± 0.096
	8	96.26 ± 4.22	
	12	97.48 ± 3.51	
	16	98.02 ± 3.43	
	20	99.45 ± 0.95	
	40	100 ± 0.00	

The antiplasmodial activity was determined by the inhibition percentage of parasite growth. Based on the result, the inhibition percentage of FBEE was concentration dependent. Higher concentration exhibited higher % of parasite growth inhibition. It also showed that the FBEE having ability to kill the parasite thus inhibit its growth. For further evaluation, the % of parasite inhibition then calculated using probit to specify the level of IC_{50} (the concentration that inhibit 50% of parasite growth). The level IC_{50} of FBEE was 42.399 ± 9.517 μg/mL.

DISCUSSION

Sterculia quadrifida which locally known as faloak is one of endemic medicinal plant in Kupang, East Nusa Tenggara. It is traditionally used for various disease such as hepatitis and malaria, since East Nusa Tenggara is one of Indonesia Province with high incidence of malaria cases.[2] Local people commonly use faloak by drinking its bark decoction. Respecting the traditional uses, this study evaluates the activity of faloak bark by maceration method using ethanol 70%, the easiest and simple method.[14]

As a solvent, ethanol 70% is expected to attract polar active compounds resemble as it uses in community as well as exhibit its activity. Moreover, extraction process using ethanol 70% minimize bacterial contamination compared to water.[15] Tube-reagent method for active compounds characterization was written in Table 1. In this study active compound that has been identified were flavonoid, alkaloid and saponin. It supported previous finding that faloak bark contains flavonoid, alkaloid and saponin[9,16] that make it having potential for various activities.

This study focused on antiplasmodial activity in order to discover new antimalarial drug. *Plasmodium falciparum* is the deadliest malaria parasite in human and commonly found in Indonesia.[17] Understanding its life cycle and adapted *P. falciparum* into cell culture make a possibility to asses antiplasmodial activity *in vitro* using direct human parasite.

Based on the IC_{50} result, it was classified as moderate activity.[18,19] Although it level still far from positive control (CQ; 4.808 ± 0.096 μg/mL), the FBEE in a form of crude extract showed promising for further investigation. The antiplasmodial activity of FBEE are strongly corelated with its active compound; alkaloid, flavonoid and saponin. Alkaloid as well as quinine from *Chinchona bark* was well known its antiplasmodial activity. Several classes of alkaloid has been published on its activity including terpenoidal, indole, bisindole, quinoline, and isoquinoline.[20] While flavonoid compound also reported on antiplasmodial activity with the most active compound was quercertine analogue (IC_{50} 7.10 ± 10.32 μM) against K1 parasite strain.[21] For saponin, a study investigated it's *in vivo* antiplasmodial activity using mice and resulted that the activity was dose related.[22]

Even though there were no study of faloak bark on antiplasmodial activity yet, but other study on it may lead the comprehension of its potential antiplasmodial activity. Its capability on cytotoxic assay against T47D (breast cancer cell line) with potent IC_{50} (9.88 μg/mL) figure out its effectivity on inhibit cell growth. Moreover, its high selectivity index (30.23) showed that faloak bark are selective and do did not kill normal cell.[23]

Although previous findings stated the promising activity of faloak bark, the moderate activity resulted in this study was possibly due to the sample still in crude extract form. The fractionation and further isolation of active compound using bioassay guided may become a choice to explore its greater antimalarial activity. It is also interesting to investigate the IC_{50} level of artemisinin as positive control for direct comparison with the faloak bark. Moreover, using resistance strain of *P. falciparum* may also figure out the potential activity of faloak bark as an alternative therapy related to the antimalarial resistance problem. For better understanding on its mechanism, specific active compound identification is important to establish for further research.

Furthermore, this finding with all limitations demonstrated the antiplasmodial activity of faloak bark as well as a proof of its traditional uses among local people. Additionally, this results also beneficial for locals to cultivate the faloak bark for both treatment and economic purposes.

CONCLUSION

The faloak bark ethanolic extract possess moderate antiplasmodial activity against *P. falciparum* FCR-3 chloroquine sensitive strain *in vitro*.

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REFERENCES

1. WHO. World Malaria Report 2019.

Geneva: World Health Organization; 2019.
2. MoH. Profil Kesehatan Indonesia tahun 2015. Jakarta: Ministry of Health Republic of Indonesia; 2016.
3. Dondorp AM. New genetic marker for piperaquine resistance in *Plasmodium falciparum*. Lancet Infec Dis 2017; 17(2):119-21.
4. Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, *et al.* Dihydroartemisinin-piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: a multisite prospective cohort study. Lancet Infec Dis 2016; 16(3):357-65. https://doi.org/10.1016/S1473-3099(15)00487-9.
5. Chaorattanakawee S, Lon C, Jongsakul K, Gawee J, Sok S, Sundrakes S, *et al. Ex vivo* piperaquine resistance developed rapidly in *Plasmodium falciparum* isolates in northern Cambodia compared to Thailand. Malaria J 2016; 15(1):519. https://doi.org/10.1186/s12936-016-1569-
6. Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, *et al.* A surrogate marker of piperaquine-resistant *Plasmodium falciparum* malaria: a phenotype-genotype association study. Lancet Infec Dis 2017; 17(2):174-83. https://doi.org/10.1016/S1473-3099(16)30415-7
7. Karou D, Nadembega WMC, Ouattara L, Ilboudo DP, Canini A, Nikiema JB, *et al.* African ethnopharmacology and new drug discovery. Med Aromat Plant Sci Biotechnol 2007;1(1):1-7.
8. Saragih G, Siswadi S. Antioxidant activity of plant parts extracts from *Sterculia quadrifida* R. Br. Asian J Pharm Clin Res 2019; 12:143-8. https://doi.org/10.22159/ajpcr.2019.v12i7.33261
9. Munawaroh R, Siswadi S, Setyowati EP, Murwanti R, Hertiani T. Correlation between total flavonoid contents and macrophage phagocytosis activity of fractions from faloak (*Sterculia quadrifida* R.Br.) barks ethanolic extract *in vitro*. Trad Med J 2018; 23(1):47-55. https://doi.org/10.22146/mot.30882
10. Winanta A, Hertiani T, Purwantiningsih P, Siswadi S. *In vivo* immunomodulatory activity of faloak bar extract (*Sterculia quadrifida* R. Br.). Pakistan J Biol Sci 2019;22(12):590-6. https://doi.org/10.3923/pjbs.2019.590.596
11. Dean M, Handajani R, Khotib J. Faloak (*Sterculia quadrifida* R.Br) Stem bark extract inhibits hepatitis C virus JFH1. Orient J Chem 2019; 35:430-5. http://dx.doi.org/10.13005/ojc/350155
12. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science 1976; 193(4254):673-5. https://doi.org/10.1126/science.781840
13. Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob Agents Chemother 1979; 16:710-8. https://doi.org/10.1128/aac.16.6.710
14. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants 2015; 4(3). https://doi.org/10.4172/2167-0412.1000196
15. Ghasemzadeh A, Jaafar HZE, Juraimi AS, Tayebi-Meigooni A. Comparative evaluation of different extraction techniques and solvents for the assay of phytochemicals and antioxidant activity of hashemi rice bran. Molecules 2015; 20(17):10822-38. https://doi.org/10.3390/molecules200610822
16. Siswadi S, Rianawati H, Hadi D, Saragih G, editors. The potency of faloak's (*Sterculia quadrifida*, R.Br.) active compounds as natural remedy. International Seminar Proceeding "Forests & Medicinal Plants for Better Human Welfare" 10-12 September 2013; 2018:73-9. https://doi.org/10.5281/zenodo.3353788

17. Anandan JV. Parasitic disease. In: Burns MA, editor. Pharmacotherapy Principle and Practise. New York: Mc Graw Hill; 2008. p.1186-9.
18. Basco LK, Le Bras J. *In vitro* susceptibility of Cambodian isolates of *Plasmodium falciparum* to halofantrine, pyronaridine and artemisinin derivatives. Ann Trop Med Parasitol 1994; 88(2):137-44. https://doi.org/10.1080/00034983.1994.11812851.
19. Dolabela MF, Oliveira SG, Nascimento JM, Peres JM, Wagner H, Povoa MM, *et al. In vitro* antiplasmodial activity of extract and constituents from *Esenbeckia febrifuga*, a plant traditionally used to treat malaria in the Brazilian Amazon. Phytomedicine 2008; 15(5):367-72. https://doi.org/10.1016/j.phymed.2008.02.001.
20. Uzor PF. Alkaloids from plants with antimalarial activity: a review of recent studies. Evid Based Complement Alternat Med 2020; 2020:8749083. https://doi.org/10.1155/2020/8749083
21. Ganesh D, Fuehrer HP, Starzengrüber P, Swoboda P, Khan WA, Reismann JA, *et al.* Antiplasmodial activity of flavonol quercetin and its analogues in *Plasmodium falciparum*: evidence from clinical isolates in Bangladesh and standardized parasite clones. Parasitol Res 2012; 110(6):2289-95. https://doi.org/10.1007/s00436-011-2763-z.
22. Matthew AO, Olusola E, Ademola O, Aderotimi A, Adebola J. Anti-malarial activity of total saponins from *Terminalia avicennioides* and its effect on liver and haematological of infected mice. Drug Designing 2018; 7(2):2169-0138.1000161
23. Rollando R, Alfanaar R. Cytotoxic effect of 2,3-dihydro-6-hydroxy-2-methylenenaphtho[1,2-b] furan-4,5-dione compound from the bark of faloak (*Sterculia quadrifida* R.Br) in breast cancer cells T47D. Pharmaciana 2017; 7(2):289-94. https://doi.org/10.12928/pharmaciana.v7i2.6699