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Original Article

Phytochemical Screening and Haem Polymerization Inhibitory Activity of Root Extract and Fractions from *Strychnos lucida* R. Br.

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ABSTRACT

Background: *Strychnos lucida* R. Br. is used empirically by people in Eastern Indonesia to treat malaria symptoms. The Indonesian government has set a decrease in malaria morbidity as a long-term target. This study aimed to determine haem polymerization inhibitory activity and screen secondary metabolites of root extract and fractions from *S. lucida*. **Materials and Methods:** Screening of secondary metabolites of root extract and fractions were performed using TLC. Crude extract was fractionated using dichloromethane (DCM), ethyl acetate (EA), and N-butanol as solvents. Measurement of haem polymerization inhibition was carried out *in vitro* using β-haematin. **Results:** The DCM fraction showed the most effective haem polymerization inhibitory activity (IC_{50} of 8.96 mg/ml), followed by the n-butanol, aquadest, ethyl acetate, and crude extract. The IC_{50} values for the DCM, n-butanol, aquadest, and ethyl acetate fractions were smaller than the chloroquine diphosphate. Based on the results of secondary metabolite screening, the DCM fraction of *S. lucida* root contains terpenoids, alkaloids, and tannins. **Conclusion:** The content of secondary metabolites influenced the inhibitory activity of haem polymerization in each extract and fraction.

Key words: *Strychnos lucida* R. Br., β-haematin, Phytochemical screening, Hemozoin, Fraction, Antimalarial

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INTRODUCTION

Indonesia has the third-highest number of malaria cases in Southeast Asia, with 569396 malaria cases annually.[1] Based on the results of previous studies, several malaria-endemic areas in Indonesia use plants as medicine to prevent and cure malaria.[2] One of the plants used empirically for malaria infection is *Strychnos lucida* R. Br. in West Nusa Tenggara, Bali, and Irian Jaya.[3] Previous studies showed that the ethanolic extract of *Strychnos lucida* wood has antimalarial activity *in vitro* and *in vivo*.[4-6] The secondary metabolites in *Strychnos lucida* wood suspected of having an antimalarial effect are strychnine alkaloids, brucine, flavonoids, terpenoids, and quinic acid esters.[4]

Plasmodium falciparum has two life cycles: Sexual and asexual. Parasites digest hemoglobin in erythrocytes to obtain amino acids. The result of hemoglobin degradation is free haem (ferriprotoporphyrin IX- FePPIX), which is toxic to parasites. The parasite converts haem into hemozoin through haem polymerization reactions to prevent toxicity.[7-9] Therefore, one of the therapeutic targets of antimalarial drugs is haem polymerization inhibition.[7] Antimalarial drugs that have a site of action in hemozoin formation are chloroquine and artemisinin.[9]

This study aimed to determine the inhibitory activity of haem polymerization and identify secondary metabolites groups in the root extract and fractions of *Strychnos lucida*. Haem polymerization inhibition was studied *in vitro* using a hematin compound.

MATERIALS AND METHODS

Materials

The roots of *Strychnos lucida* were obtained from Dompu village, Bima district, West Nusa Tenggara Province, Indonesia. Purwodadi Botanical Gardens identified and determined the specimen as *Strychnos lucida R.Br.*. Measurement of haem polymerization inhibitory activity using β-hematin pro analysis Sigma, NaOH, DMSO. Chloroquine pro-analysis was obtained from Sigma and used as a standard medicine for testing the inhibitory activity of haem polymerization.

Extraction and fractionation

The roots were weighed 200 mg, then macerated using 80% ethanol for 72 hr. The filtrate was evaporated using a rotary evaporator and an oven until a steady weight was obtained. The ethanol extract was separated using a liquid-liquid fractionation method with dichloromethane (DCM), ethyl acetate (EA), and n-butanol as solvents. The extracts and fractions were weighed and stored at a 2°C-6°C temperature for further testing.

Secondary metabolites screening

Each extract and fraction weighed 10 mg and dissolved in ethanol. The sample solution was spotted on the TLC plate using silica GF_{254nm} as stationary phase and mobile phase, according to Table 1. TLC chromatograms were observed at UV light 254 nm, 366 nm, and visible light.

Inhibition of haem polymerization assay

The haem polymerization inhibition test was performed by the method of Basilico *et al*., with modification.[10] The stock solution was prepared from standard β-hematin and was diluted to a concentration of 1.2; 1.25; 0.63; 0.31; 0.16 mM. One hundred microliters of hematin solution were pipetted and added 50 µL of glacial acetic acid (quadruplicate), then incubated for 24 hr.

Haem polymerization inhibition activity from extract and fractions was carried out by reacting 100 µL of 1 mM β-hematin solution with 50 µL of the sample solution and 50 µL of glacial acetic acid. The sample extract and fraction were weighed 25 mg, dissolved in 2.5 ml aquadest, then diluted to a concentration of 10000; 5000; 2500; 1250; 625 ppm. The solution mixture was incubated for 24 hr at 37°C. Extract/fractions solution were substituted by aquadest to serve as control negative.

After incubation for 24 hr, each solution was centrifuged for 10 min at 800 rpm and washed using DMSO 3 times. The hematin crystal precipitate was dissolved in 200 µL of 0.1 M NaOH. A total of 100 µL of

Table 1: Mobile phases and reagents for secondary metabolite screening.

Secondary metabolite groups	Mobile phase (v/v)	Reagent
Flavonoid	n-butanol: glasial acetic acid: aquadest (2:1:4)	$AlCl_3$
Terpenoid	Chloroform: acetone (5:5)	Anisaldehid-H_2SO_4 10%
Alkaloid	Toluene: ethyl acetate: diethylamine (7:2:1)	Dragendroft
Antraquinone	Ethyl acetate: methanol: aquadest (100:13.5:10)	KOH 10%
Tannin	Chloroform: acetone (5:5)	$FeCl_3$ 10%

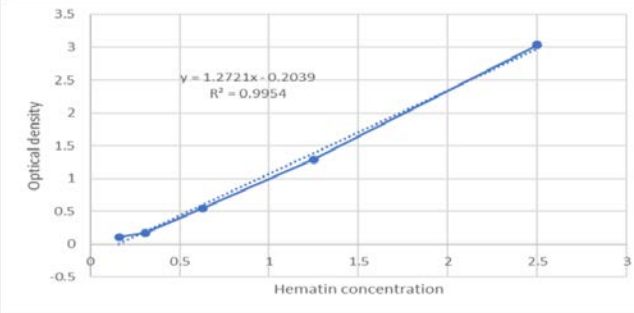

Figure 2: Callibration curve of haematin concentration vs optical density.

hematin crystal solution was transferred to a 96-well and measured on an ELISA Reader Biotrak II with a wavelength of 405 nm. The standard curve equation was made from the regression results between the hematin concentration and the optical density value. The standard curve equation was used to calculate the concentration of hematin after being reacted with the extract and fractions solution for 24 hr. The following formula calculated the haem polymerization inhibition activity:

$$\text{Inhibition}\,(\%) = \frac{A - B}{B} \times 100\%$$

A: hematin concentration of control negative

B: hematin concentration of each extract and fractions

The IC_{50} value was calculated as a concentration that could inhibit the formation of haem polymerization by 50%.

RESULTS

Extraction, Fractionation, and Secondary metabolite screening

The percentage yields from the extraction of the root powder were 8.47%. Liquid-liquid fractionation produces a dichloromethane (DCM); ethyl acetate (EA); n-butanol (NB); and aquadest fraction with a weight of 0.34; 0.08; 2.42; 1.91 g. Phytochemistry screening showed that the DCM fraction contained terpenoids, alkaloids, anthraquinones and tannins (Table 2). Meanwhile, the EA fraction in roots showed more flavonoid, antraquinone, and tannin compounds (Table 2).

Inhibition of heme polymerization

Figure 2 shows the regression equation between hematin concentration and optical density. The DCM fraction was the most effective at inhibiting haem polymerization, followed by NB fraction, water fraction, EA fraction, and ethanol crude extract (Table 3). All fractions show haem polymerization inhibitory activity more potential than standard chloroquine diphosphate.

Table 3: Haem polymerization inhibitory activity from crude extract and fractions of *S. lucida*.

Extract	IC_{50} (mg/mL)
Ethanol crude extract	71.45±11.99
Fraction DCM	8.96±0.34
Fraction EA	21.48±5.13
Fraction n-butanol	12.73±1.63
Fraction aquadest	13.51±1.42
Standard chloroquine diphosphate	24.90±7.48

DISCUSSION

Medicinal plants exhibit antimalarial activity through several mechanisms: 1) inhibition of folate metabolism in the DNA synthesis process; 2) inhibition of hemozoin formation in food vacuoles; 3) inhibition of isoprenoid synthesis that contributes to ubiquinone formation; 4) inhibition of dihydroorotate dehydrogenase; and 5) inhibition of the formation of parasitic membranes.[11-14] In the intraerythrocytic cycle, *Plasmodium* spp. digest hemoglobin and produce ferriprotophorpyrin (FP IX). Ferriprotophorphyrin IX is lipophilic, so it can bind to cell membranes and interfere with membrane permeability, induce lipid peroxidase, and cause cell lysis and parasite death.[7,15] Several antimalarial drugs that exhibit heme polymerization inhibitory activity include chloroquine and artemisinin.[10,16]

Strychnos lucida root samples showed the most significant haem polymerization inhibition in the DCM fraction, followed by the NB fraction, water fraction, EA fraction, and crude extract. Terpenoids and alkaloids dominated the secondary metabolite content of the DCM fraction. Meanwhile, the NB, water, and EA fractions contained more flavonoid and anthraquinone compounds. Terpenoids inhibit the formation of hemozoin through the mechanism of blockade of the heme biocrystalization process.[17] Meanwhile, Alkaloids will accumulate in the plasmodium vacuole and prevent hemozoin formation.[15] Previous

Table 2: Phytochemistry screening of crude extract and fractions.

Sample	Flavonoid	Terpenoid	Alkaloid	Antraquinone	Tannin
Crude extract	R_f = 0.42	R_f = 0.48; 0.63	R_f = 0.25	R_f = 0.05; 0.15; 0.3	Not detected
DCM Fraction	R_f = 0.26	R_f = 0.49; 0.61; 0.75; 0.8; 0.86	R_f = 0.28; 0.35	R_f = 0.05; 0.3	R_f = 0.03
EA Fraction	R_f = 0.44; 0.62; 0.78; 0.86	R_f = 0.15	Not detected	R_f = 0.08; 0.16; 0.3	R_f = 0.03; 0.09; 0.22
NB Fraction	R_f = 0.44	Not detected	R_f = 0.25	R_f = 0.05; 0.29	Not detected
Aquadest Fraction	R_f = 0.4	Not detected	Not detected	R_f = 0.05	Not detected

studies have shown that flavonoids have a strong bond with haem. The bonds between haem-flavonoids are hydrophobic, so that able to destabilize the bilayer membrane and ultimately cause the death of plasmodium.[18] Some biflavanone isolated from the stem bark of *Garcinia buchananii* Baker also inhibited *Plasmodium falciparum* growth.[19,20] However, in another study, it was stated that there was no correlation between the ability to inhibit hemozoin formation and antimalarial activity in some flavonoid compounds.[21] Based on the results of our study, the IC_{50} value of the DCM, EA, NB, and water fractions was smaller than the IC_{50} of chloroquine, so it may inhibit haem polymerization more potently, although this is yet to be verified.

CONCLUSION

Dichloromethane fraction of *S. lucida* root contains alkaloids and terpenoids, which may inhibit the formation of heme polymerization and future studies are required to verify this. The DCM fraction of *S. lucida* root has the most significant potential to be developed as an antimalarial herbal drug candidate with haem polymerization inhibitory activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DCM: Dichloromethane; **NB:** n-butanol; **FP IX:** Ferriprotophorpyrin IX.

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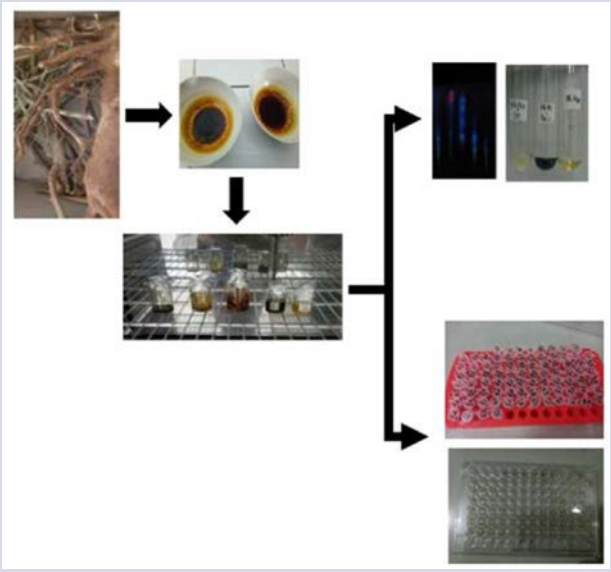
PICTORIAL ABSTRACT



SUMMARY

- Hemozoin polymerization inhibitory activity was performed for crude extract and fractions
- Phytochemical screening showed each fraction contained different secondary metabolites
- All fractions exhibit hemozoin polymerization inhibition more potential than chloroquine diphosphate
- Fraction DCM showed most potential hemozoin polymerization inhibition activity with IC_{50} 8.96 mg/mL

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