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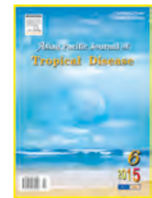

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Phytochemical screening and antimalarial activity of some plants traditionally used in Indonesia

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ABSTRACT

Objective: To evaluate ethanolic extracts of phytochemical screening, *in vitro* and *in vivo* antiplasmodial activities of 15 plants used as antimalarial in Sei Kepayang, North Sumatra.

Methods: Extraction was done through maceration with 70% ethanol and screened against chemical content, *in vitro* test anti-plasmodium against *Plasmodium falciparum* 3D7 strain and *in vivo* test in mice infected *Plasmodium berghei*.

Results: The results showed that the plant extract contained a group of saponins, flavonoids, alkaloids, quinone, sterols, triterpene, tannins and cumarine. However, extract of *Momordica charantia*, *Carica papaya*, *Garcinia atroviridis, Alstonia scholaris, Smallanthus sonchifolia* and *Cassia siamea* had strong anti-plasmodium activity both *in vitro* and *in vivo*.

Conclusions: *In vitro* and *in vivo* antiplasmodial activities of 15 plants are used as antimalarial in Sei Kepayang, North Sumatra. All the plants have *in vitro* and *in vivo* anti-plasmodium activity except *Orthosiphon stamineus* and *Luffa cylindrica* (ED_{50} > 1 000 mg/kg body weight and IC_{50} > 100 μg/mL, respectively).

1. Introduction

Malaria is a public health issue that can result in death, especially in high-risk groups such as infants, toddlers and pregnant women. In Indonesia, 1.25 to 2.5 million people had malaria cases with 45% to 50% being cases of *Plasmodium falciparum* (*P. falciparum*). As reported every year, approximately 350 000 people were confirmed to have malaria while around 500 persons died from malaria[1].

The efforts of finding out novel malaria had been intensively conducted during the last few decades by the world's researchers. Some novel antimalarial compounds, which had been successfully isolated from plants, include 17-O-acetyl,10-hydroxycorynantheol, an indole alkaloid isolated from *Strychnos usambarensis*[2], Cryptolepine derivatives isolated from the stems, roots and root bark of *Cryptolepis sanguinolenta* (Periplocaceae) growing in diverse regions in Africa, have also exhibited potent antimalarial properties[3]. Jansen *et al*. identified urospermal A-15-O-acetate as the main active compound responsible for the antiplasmodial activity of *Dicoma tomentosa* (Asteraceae) from Burkina Faso[4]. Based on their results, the IC_{50} of the compound was < 1 μg/mL against both 3D7 and W2 strains[4]. Some potent antimalaria compounds were derived from the isolation of herbal plants, which were traditionally used for malaria. They include artemycin derived from *Artemisia annua*, and quinine derived from *Cinchona succirubra*. A research conducted in Sei Kepayang, North Sumatra, found that 15 herbal

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plants from 6 families had been used for malaria[5]. Therefore, this paper establishes a basis for the use of some of these plants, and the evaluation of the potential constituents as a possible new antimalarial agent that will result in new antimalarial compounds.

2. Materials and methods

2.1. Collection and extraction of plant materials

Based on ethnomedical data, different plant parts (leaf, stem bark, root bark, seed, whole plant) of 15 plants species representing 6 families were collected in May-June 2014 from Sei Kepayang, North Sumatera (Figure 1). The plants collected were identified by a taxonomic botanist from North Sumatra University, where voucher specimens were deposited. Then, the extracts were prepared by maceration of the powder (1.5 kg) with 7 L methanol at room temperature for 24 h. The process was repeated three times, and the filtrates were combined and evaporated under vacuum to dryness.

2.2. Phytochemical screening

Preliminary screening of secondary metabolites such as alkaloids, flavonoids, saponins, coumarins, anthraquinones, terpenoids, steroids and sterols was carried out according to the common phytochemical methods[6].

2.3. In vitro antimalarial assay

The culture procedure was based on the method proposed by Trager and Jensen[7]. This procedure was conducted using a Petri dish in an aseptic way. The strain 3D7 of *P. falciparum* parasite, which was sensitive to chloroquine, was derived from frozen storage that had been thrown. Tube that contained frozen parasite was diluted under the temperature of 37 °C, then 3.5% NaCl was added in the same volume, and was transferred to a centrifuge tube using Pasteur pipette while being mixed softly. The culture was centrifuged in 1 500 r/min for 5 min under a temperature of 4 °C. Then, the supernatant was removed; the deposition was suspended using 5 mL of incomplete medium, mixed softly using Pasteur pipette, and finally centrifuged in 1 500 r/min for 5 min under a temperature of 4 °C[7].

The resulting supernatant was removed. The procedure was repeated twice. The resulting deposition was washed, added with complete medium and 50% erythrocyte, and mixed softly using Pasteur pipette (5% hematocrite). The culture was moved to Petri dish, put into candle jar, and then stored in an incubator under a temperature of 37 °C. The next step was replaced the medium on daily basis. However, sub-culture procedure might follow, when the parasitemia level was found to be more than 5%. After 48 h of incubation, the culture was harvested and made into thin-layer blood smear using 10% Giemsa stain. Thin-layer blood smear was left for 15 min, washed using tap water, and then desiccated. Then, the percentages of parasitemia, growth and inhibition of *P. falciparum* were calculated. This was done by calculating the infected erythrocyte per 1 000 under microscope. The formula used to determine parasitemia, growth and inhibition of *P. falciparum* is shown below. Each extract was evaluated in triplicate, and the mean was calculated as follows:

$$\text{a. Maturation percentage} = \frac{\text{No. of developed schizonts for test}}{\text{No. of developed schizonts for control}} \times 100$$

b. Inhibition percentage = 100 - maturation percentage

2.4. In vivo antimalarial activity

The *in vivo* antiplasmodial activity of the extracts was determined by the classic 4-day suppressive test against *Plasmodium berghei*, NK 65 strain in mice[8]. Thirty Swiss male mice weighing (20 ± 2) g were inoculated with 1×10^6 infected red blood cells intraperitoneally on Day 0. After inoculation for 2 h, mice were injected intraperitoneally with 0.1 mL of extracts tested in water

Table 1

Results of the phytochemical screening of the extracts of some plants traditionally used in Sei Kepayang.

Extracts	Major tested phytoconstituents							
	Alkaloid	Flavonoid	Saponin	Tannin	Quinone	Steroid	Triterpenoid	Cumarine
M. charantia (Momordicacea)	+	+	+	+	+	+	+	+
T. crispa (Menispermaceae)	-	+	-	+	+	+	+	+
O. stamineus (Lamiaceae)	+	+	+	+	-	+	+	+
G. mangostana (Clusiaceae)	-	+	+	+	+	+	+	+
P. niruri (Euphorbiacea)	-	+	+	+	+	+	-	+
C. papaya (Caricaceae)	+	+	+	+	+	+	+	+
G. atroviridis (Clusiaceae)	-	+	-	+	-	+	+	+
P. guajava (Myrtaceae)	+	+	-	+	-	+	+	-
A. scholaris (Apocynaceae)	+	+	-	+	+	+	+	+
B. balsamifera (Asteraceae)	-	+	-	-	+	+	+	+
S. sonchifolia (Asteraceae)	-	+	-	+	+	+	+	+
A. muricata (Annonaceae)	+	+	+	+	+	+	+	+
L. cylindrica (Cucurbitaceae)	-	-	-	-	-	+	+	-
S. mahagoni (Meliaceae)	+	+	+	-	-	-	+	+
C. siamea (Fabaceae)	+	+	-	+	+	+	+	+

M. charantia: *Momordica charantia*; *T. crispa*: *Tinospora crispa*; *O. stamineus*: *Orthosiphon stamineus*; *G. mangostana*: *Garcinia mangostana* L.; *P. niruri*: *Phyllanthus niruri* L.; *C. papaya*: *Carica papaya* L.; *G. atroviridis*: *Garcinia atroviridis* Griff. ex T. Anders; *P. guajava*: *Psidium guajava* L.; *A. scholaris*: *Alstonia scholaris* L.; *B. balsamifera*: *Blumea balsamifera* L.; *S. sonchifolia*: *Smallanthus sonchifolia*; *A. muricata*: *Annona muricata*; *L. cylindrica*: *Luffa cylindrica*; *S. mahagoni*: *Swietenia mahagoni* Jacq; *C. siamea*: *Cassia siamea*. +: Present; -: Absent.

solution at different concentrations, given doses ranging from 50 to 400 mg/kg of body weight/day. Eight animals were tested at each dose level. The injection of tested extracts was repeated daily for 3 consecutive days from Day 0 of parasite injection. The control group treated with distilled water was used in all extracts testing. On the 5th day after parasite inoculation, blood films were taken from the tail blood and the level of parasitemia was determined on Giemsa-stained smears by counting 2 000 erythrocytes. Parasite growth inhibition by extracts was determined by comparison with the level of parasitemia in the control group. For the 4-day suppressive method, ED_{50} values were determined through probit analysis.

3. Results

Phytoconstituents detected in plant samples such as sterols, triterpenes, alkaloids, flavonoids, tannins, saponins, quinone and cumarine were shown in Table 1. All studied plant samples had shown the presence of triterpene except *P. niruri.* Alkaloids were detected in 8 plant samples, while tannins were found to occur in 12 plant samples. Also, saponine were detected in trace amounts in 7 plant samples, while flavonoids were evidenced in 14 plant samples.

In vitro and *in vivo* anti-plasmodium assay of the 15 plants were shown in Table 2. From Table 2, it appeared that all the plants had *in vitro* and *in vivo* anti-plasmodium activity except *O. stamineus* and *L. cylindrica* (ED_{50} values > 1 000 mg/kg body weight and IC_{50} > 100 μgmL).

Table 2

Plant parts collected based on ethnopharmacological data, percentage yield of dry ethanolic extract per 100 g of air-dried and antimalarial activity of ethanolic extract plant material used.

Extract	Plant yield (%)	ED_{50} (mg/kg body weight)	IC_{50} (μg/mL)
M. charantia (Momordicacea)	19.61	113.50 ± 10.23	0.0178 ± 0.0100
T. crispa (Menispermaceae)	24.93	271.89 ± 4.32	0.3440 ± 0.2100
O. stamineus (Lamiaceae)	12.60	>1 000.00 ± 21.32	>100
G. mangostana (Clusiaceae)	31.86	548.73 ± 9.87	0.2350 ± 0.1200
P. niruri (Euphorbiacea)	28.68	98.45 ± 6.21	7.5600 ± 0.5400
C. papaya (Caricaceae)	27.41	173.20 ± 3.56	0.1770 ± 0.1500
G. atroviridis (Clusiaceae)	21.01	137.21 ± 5.65	1.0250 ± 0.5400
P. guajava (Myrtaceae)	31.23	274.69 ± 7.65	0.6250 ± 0.2100
A. scholaris (Apocynaceae)	28.35	121.94 ± 7.56	0.1650 ± 0.1100
B. balsamifera (Asteraceae)	20.61	126.56 ± 8.45	8.7500 ± 1.2100
S. sonchifolia (Asteraceae)	19.75	243.27 ± 7.86	27.4500 ± 1.2100
A. muricata (Annonaceae)	25.43	266.32 ± 9.45	0.7150 ± 0.1400
L. cylindrica (Cucurbitaceae)	26.13	>1 000	>100
S. mahagoni (Meliaceae)	17.63	143.39 ± 4.56	45.3500 ± 1.2300
C. siamea (Fabaceae)	8.68	127.05 ± 6.51	4.7500 ± 0.1200

Figure 1. Medicinal plants used in the study.
A: *M. charantia*; B: *T. crispa*; C: *O. stamineus*; D: *G. mangostana*; E: *P. niruri*; F: *C. papaya*; G: *G. atroviridis*; H: *P. guajava*; I: *A. scholaris*; J: *B. balsamifera*; K: *S. sonchifolia*; L: *A. muricata*; M: *L. cylindrica*; N: *S. mahagoni*; O: *C. seamea*.

4. Discussion

The extraction of the plants used as solvent (70% ethanol) and produced high-level yields since the solvent (70% ethanol) had a similar polarity with most of the components contained in the plant. The 70% ethanol can dilute phytochemical compounds in a maximum way, since the solvent contained much water (30%) that could help in the extraction process. In this way, most of the compounds could be attracted in the ethanol and some were attracted in the water.

In vitro antiplasmodial assay on *P. falciparum* culture was conducted on ethanolic extracts which derived from some plants with the aim of finding out antiplasmodial activity of each resulting extract. Furthermore, *in vitro* assay was conducted on strain 3D7 of *P. falciparum* culture to find out the activity of the extract against *Plasmodium* strain. The strain 3D7 is sensitive to chloroquine. Moreover, to find out the antiplasmodial activity, each extract was exposed to *P. falciparum* culture for 48 h. Growth inhibitory activity of *Plasmodium* was calculated based on the decrease of parasitemia from the thin-layer blood smear.

IC_{50} values of the extracts of some plants against strain D10 of *P. falciparum* were < 10 mg/mL except for the extracts of *O. stamineus* and *L. cylindrica* leaves, and IC_{50} < 50 µg/mL was for the extract of *S. mahagoni* leaves. It is said to be active when the IC_{50} value is < 5 mg/mL, and moderate, when the values range from 5 to 50 mg/mL[9]. The criteria proposed by Karou *et al*. was proven that the extracts of *M. charantia, T. crispa, G. mangostana, C. papaya, G. atroviridis, P. guajava, A. scholaris, S. sonchifolia* and *C. siamea* were active against strain 3D7 of *P. falciparum*, while the extracts of *P. niruri*, *B. balsamifera* and *S. mahagoni* were moderately active against strain 3D7 of *P. falciparum*[9]. Muñoz *et al*. proposed a different category from that of Karou *et al*. which was categorized that the plant extracts have excellent antiplasmodial activity if IC_{50} was < 5 µg/mL, good if IC_{50} was 5-10 µg/mL, and inactive if IC_{50} was > 10 µg/mL[9,10].

The plant extracts showed very powerful antiplasmodial activity. Ethanolic extract of *Holarrhena floribunda* stem bark had a strong antiplasmodial activity on the culture of strain W2 of *P. falciparum* with IC_{50} value of 5.2 µg/mL, and on the culture of strain D6 with IC_{50} value of 3 µg/mL[11]. *n*-Butanol extract of *Eurycoma longifolia* Jack root which was empirically used as antimalaria, was found to have antiplasmodial activity on *P. falciparum* culture which derived from the isolate of Gombak A, with IC_{50} value of 0.34 µg/mL[12]. Gessler *et al*. reported that 4 plant extracts out of 43 plants studied and used by the society as antimalaria in Tanzania were found to have a strong antiplasmodial activity with IC_{50} value of < 10 µg/mL[13]. They include *Cissampelos mucronata, Maytenus senegalensis, Salacia madagascariensis* and *Zanthoxylum chalybeum* plants[13].

In vivo antiplasmodial activity was calculated from ED_{50} value. According to Muñoz *et al*., *in vivo* antiplasmodial activity was classified as excellent if ED_{50} was ≤ 100 mg/kg body weight/day, good if ED_{50} was ≤ 101-250 mg/kg body weight/day, moderate if ED_{50} was 251-500 mg/kg body weight/day, and inactive if ED_{50} was > 500 mg/kg body weight/day[10]. In this research, *P. niruri* extract showed ED_{50} value of (98.45 ± 10.41) mg/kg body weight and excellent antiplasmodial activity. Furthermore, the extracts of *M. charantia, C. papaya, G. atroviridis, A. scholaris, B. balsamifera, S. sonchifolius, S. mahagoni* and *C. siamea* showed good antiplasmodial activity with ED_{50} of 101-250 mg/kg body weight. The extracts of *T. crispa*, *P. guajava* and *A. muricata* showed moderate antiplasmodial activity because the three extracts showed ED_{50} of 251-500 mg/kg body weight/day. On the other hand, antiplasmodial activity was found to be weak, if any extracts of *G. mangostana*, *O. stamineus* and *L. cylindrica* showed ED_{50} > 500 mg/kg body weight.

In vitro and *in vivo* antiplasmodial activities of 15 plants are used as antimalarial in Sei Kepayang, North Sumatra. All the plants have *in vitro* and *in vivo* anti-plasmodium activity except *O. stamineus* and *L. cylindrica* (ED_{50} values > 1 000 mg/kg body weight and IC_{50} > 100 µg/mL, respectively).

Conflict of interest statement

We declare that we have no conflict of interest.

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