ORIGINAL ARTICLE

Antiplasmodial Activity and Phytochemical Constituents of Selected Antimalarial Plants Used by Native People in West Timor Indonesia

Batı Timor Endonezya Yerel Halkının Kullandığı Bazı Antimalaryal Bitkilerin Antiplazmodiyal Aktiviteleri ve Fitokimyasal Bileşenleri

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ABSTRACT

Objectives: To document traditional antimalarial plants used by Tetun ethnic people in West Timor Indonesia and evaluate the antiplasmodial activity and phytochemicals of several plants that are widely used as oral medicine.

Materials and Methods: A field study to document antimalarial plants followed by laboratory works to test antiplasmodial activity and identify the phytochemical constituents of some selected plants extract were applied. The inhibitory potency of ethanolic extracts of *Strychnos ligustrina* wood, roots of *Calotropis gigantea, Fatuoa pilosa,* and *Neoalsomitra podagrica*, whole plant of *Cleome rutidosperma* and *Physalis angulata*, stem barks of *Alstonia spectabilis, Alstonia scholaris, Jatropha curcas* and *Plumeria alba*, and leaves of *Melia azedarac*h on the *Plasmodium falciparum* 3D7 strain *in vitro* were tested. Gas chromatography-mass spectrometry instrument was used to analyze the phytochemicals of the extracts.

Results: The Tetun ethnic people use 50 plant species as antimalarials. *P. angulata, J. curcas*, and *A. spectabilis* extracts show strong antiplasmodial activity with IC$_{50}$ values of 0.22, 0.22, and 1.23 µg/mL, respectively; *N. podagrica, A. scholaris, F. pilosa,* and *P. alba* were moderate antiplasmodials with IC$_{50}$ values of 11.60, 15.46, 24.92, and 36.39 µg/mL, respectively; and *C. rutidosperma, M. azedarach, S. ligustrina, and C. gigantea* were weak antiplasmodials with IC$_{50}$ values of 54.25, 63.52, 63.91, and 66.49 µg/mL, respectively. The phytochemicals identification data indicate that these 11 plants contain alkaloids, terpenoids, steroids, coumarins, alcohols, thiols, phenolics, aldehydes, fatty acids, esters, and so forth.

Conclusion: Plants widely used as antimalarials by the Tetun ethnic people is proven to have antiplasmodial activity.

Key words: Ethnomedicine, medicinal plant, malaria, antiplasmodial activity

ÖZ

Amaç: Endonezya Batı Timor'unda Tetun etnik halkı tarafından kullanılan geleneksel antimalaryal bitkileri, yaygın oral ilaçlar olarak kullanılan bazı bitkilerin antiplazmodial aktivitelerini ve fitokimyasallarını belirlemek.

Gereç ve Yöntemler: Antimalaryal bitkileri belirleyen alan çalışmasını takiben, antiplazmodiyal aktiviteyi test etmek ve seçilen bitki ekstraktlarından fitokimyasal bileşenleri belirlemek için laboratuvar çalışması yapılmıştır. *Strychnos ligustrina* bitkisinin odunsu kısımları, *Calotropis gigantea, Fatuoa pilosa* ve *Neoalsomitra podagrica* bitkilerinin kökleri, *Cleome rutidosperma* ve *Physalis angulata* bitkilerinin tamamı, *Alstonia spectabilis, Alstonia scholaris, Jatropha curcas* ve *Plumeria alba* bitkilerinin gövde kabukları ile Melia azedarach yaprakları etanol ekstrelerinin inhibitör potansiyelleri *Plasmodium falciparum* 3D7 suşunda *in vitro* koşullarda belirlendi. Ekstrelerin fitokimyasal içerikleri gaz kromatografisi-kütle spektrometresi ile analiz edildi.

Bulgular: Tetun etnik halkının antimalaryal olarak kullandığı 50 bitki türü olduğu belirlendi. *P. angulata, J. curcas* ve *A. spectabilis* ekstreleri, sırasıyla 0,22, 0,22 ve 1,23 µg/mL IC$_{50}$ değerleriyle güçlü antiplazmodiyal aktivite gösterirken, *N. podagrica, A. scholaris, F. pilosa* ve *P. alba*, sırasıyla 1,60, 15,46, 24,92 ve 36,39 µg/mL IC$_{50}$ değerleriyle, ve *C. rutidosperma, M. azedarach, S. ligustrina* ve *C. gigantea*, sırasıyla 54,25, 63,52, 6391 ve 66,49 µg/mL IC$_{50}$ değerleriyle zayıf antiplazmodiyal aktivite gösterdi. Fitokimyasal tanımlamanın verileri, bu 11 bitkinin alkaloitler, terpenoidler, steroidler, kumarinler, alkoller, tiyoller, fenolikler, aldehitler, yağ asitleri, esterler ve diğer bileşikleri içerdiğini gösterdi.

Sonuç: Tetun etnik halkı tarafından yaygın olarak antimalaryal olarak kullanılan bitkilerin antiplazmodiyal aktivite gösterdiği kanıtlanmıştır.

Anahtar kelimeler: Etnotıp, şifalı bitki, sıtma, antiplazmodiyal aktivite

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INTRODUCTION

Plants are very valuable source in obtaining various pharmacological active substances to deal with various human health problems.[1] It has been the basis and an important part of various traditional medical systems for thousands of years. It is estimated that around 25% or 40,000-70,000 of the total number of plant species known today are used as medicinal plants in various places all over the world.[2,3]

More recently, natural product chemicals isolated from plants have been a good source of lead compounds used to treat various infectious diseases, including malaria. Two examples of phenomenal lead compounds that have greatly contributed in reducing malaria deaths worldwide are quinine isolated from *Cinchona* sp. stem bark and artemisinin from Chinese medicinal plants *Artemisia annua*.[4] Derivatives such as chloroquine, amodiaquine, primaquine, and mefloquine have been synthesized from quinine; and compounds such as artemether, arteether, and sodium artesunate have been produced from artemisinin. However, *Plasmodium* has shown in the last few decades increasing resistance to antimalarial quinine derivatives, especially chloroquine, which made them no longer effective. *Plasmodium falciparum* has also showed an increase in resistance to artemisinin-based antimalarials as reported in several recent publications.[5]

Plasmodium's increasing resistance to currently used antimalarial drugs encouraged researchers to continue searching for new and more effective antimalarials.[6] Plants have become one of the most important sources in searching new potential antimalarials in traditional medicine of various ethnics worldwide.[7] A promising and better approach in finding new antimalarial(s) is selecting plants that have been traditionally used in treating malaria. With this, resources such as high investment and skills are saved, and the time of plant selection and testing of its antimalarial activity is shown to be accelerated compared with the random selection approach. For the development of important active antimalarials, research on medicinal plants of various traditional medicine systems could provide useful leads.[8]

The experience of the Tetun people, a native ethnic in West Timor, in interacting with malaria for a long time led to them to develop their own methods of treatment. They identified malaria as sick of hot body or fever. A variety of medicinal plant formula called ai tahan or kwa were carried out by the Tetun community as a traditional treatment for malaria, which is applied by drinking, bathing, massage, inhaling, and cataplasm.[9] In-depth exploration of the antimalarial plants used in the community's traditional medicine might provide a valuable contribution in discovering new sources of antimalarial substances.

This research is an ethnomedicine study that was first conducted in the Tetun community. In our study, two research steps were performed. For the first step, a field research was conducted to document various species of medicinal plants and formula of traditional medicines for oral treatment of malaria used by the Tetun people. In the second step, some of the high-frequency mentioned plants were selected to test their antiplasmodial activity. *In vitro* antiplasmodial activity testing was carried out against the chloroquine-sensitive *P. falciparum* 3D7 strain.

MATERIALS AND METHODS

Field study

The field research was carried out in several subdistricts of Belu (9°15,0 S, 124°40 E) and Malaka Districts (9°34 S, 124°54 E). These two districts are located along the borderline of East Nusa Tenggara Province (Indonesia) and the Republic Democratic de Timor Leste. In this field study, information was collected through interviews and discussions. This study consists of 94 informants, 42 males and 52 females, who were traditional healers, former malaria patients that have undergone traditional medicine, and others who have knowledge and experience in the traditional treatment of malaria. These informants were between the ages of 40 and 90 years old, and almost all of them have settled in their area since they were born.

Data collected on traditional medicinal plants used for malaria treatment include: the plant's local name, place where the plant was obtained, part of the plant used as a medicine, methods of processing and usage, dosage and duration of use, and the medication's claimed effect. To prepare an herbarium, the plants mentioned by the informants were then collected in parts. First, all plants were identified by matching their local name with the scientific names of the species listed in the Timorese local plants book.[10] Second, experts from the Lembaga Ilmu Pengetahuan Indonesia-Bogor Botanic Garden identified the plants again. Part(s) of some plants mentioned by the informants with high frequency were collected in greater quantities for laboratory evaluation of their antimalarial activity and phytochemicals identification. Those plant part samples were collected from the area where the plant was mentioned.

Preparation of the plant extract

The attached dirt on the plant part samples, that is the whole plant, stem bark, wood, roots, and leaves, were cleaned using tap water. They were then air-dried at room temperature until they were completely dry and then grounded into powder. The extracts were prepared by maceration. Plant powder of 20 g each was macerated with 95% ethanol for 24 h at room temperature and then filtered. Maceration was repeated three times, and the filtrates were collected and then evaporated to dry using a vacuum rotary evaporator at 40°C. The dried extracts were stored in a closed container, and were then used for antiplasmodial activity test against *P. falciparum* and phytochemical analysis.

In vitro antiplasmodial activity test

In vitro antimalarial activity testing was carried out on the chloroquine-sensitive 3D7 strain of *P. falciparum* obtained from the Institute of Tropical Diseases Airlangga University, Surabaya Indonesia. In 96 microwells plate, *Plasmodium* was cultivated according to a method developed by Trager and Jenssen.[11] It was carried out using O type human red blood cells (RBC) with 5% hematocrit suspended in RPMI 1640 medium.

In a CO_2 incubator, the culture was incubated at 37°C, and the medium was replaced every day until parasitemia reaches 1%-2%. Dried plant extracts were dissolved in dimethylsulphoxide and filtered through a 0.22 µm membrane filter. The solution of each extract was then placed in microwells containing *Plasmodium* suspension with 1% parasitemia. To obtain the final concentrations of 100, 10, 1.0, 0.1, and 0.01 µg/mL, the solutions were diluted in a series of tenfold dilution with the RPMI 1640 medium. Two series of controls were set up: as a positive control, in which parasitized blood cells without the addition of plant extract; and as a positive control where parasitized blood cells were added with chloroquine diphosphates. To make final concentrations of 100, 10, 1.0, 0.1, and 0.01 µg/mL, preparation for chloroquine diphosphates were similar to plant extracts. The culture was then incubated at 37°C in a CO_2 incubator for 48 h. Duplicate tests were performed. RBC culture was harvested after 48 h of incubation. On a glass object, a thin blood was smeared, dried and fixed with methanol, and stained with Giemsa. A light microscope with 1000x magnification was then used to count the number of parasitized RBC.

Statistical analysis
A total of 1000s RBC was counted by observing blood smear slides under a microscope, and the number of infected RBC (iRBC) was obtained from the antiplasmodial activity test. The amount of iRBC to the total RBC was expressed as percentage of parasitemia, which was calculated using the numerical formula as follows:

Percentage of parasitemia, $p=[(\Sigma iRBC/\Sigma RBC) \times 100\%]$.

From the percentage of parasitemia data in treated groups and negative control, *Plasmodium*'s growth and inhibition percentages were calculated as follows:

Percentage of growth: $(Pt/Pnc) \times 100\%$,

Percentage of inhibition: 100% - percentage of growth,

where Pt is the percentage of parasitemia in treated groups and Pnc is the percentage of parasitemia in the negative control group. Pt and Pnc were calculated at the beginning of test (0 h, P_0) and when the culture was harvested (48 h, P_{48}). The parasitemia's actual percentage value was $P_{48} - P_0$. The inhibition percentage range is 0%, which means no inhibition, to 100%, which is complete inhibition.

Probit analysis was used to statistically calculate the 50% inhibitory concentration (IC_{50}) by plotting the data on inhibition percentage versus concentration of each extract. To classify the antimalarial potency of each extract, the IC_{50} value was compared with the value given by the literature.

Identification of phytochemicals in extracts
Gas chromatography-mass spectrometry (GC-MS) was used to analyze the phytochemicals contained in each extract. An Agilent 6980N Network GC system with autosampler was linked to a detector of Agilent 5973 inert MSD. A column of J and W Scientific HP-5MS 30 m × 0.25 mm × 0.25 µm was used. To interpret the compounds, the GC-MS instrument used a Wiley version 7.0 database. The GC-MS operational conditions were set up as follows: Inlet temperature of 250°C; and oven temperature programmed at 50°C for 5 min and increased to 10°C/min up to 280°C, maintained constant for 15 min. Temperatures of Aux, MS Quad, and MS Source were 250°C, 150°C, and 230°C, respectively. Used as sample carrier was helium gas with 99.999% purity. Gas flow in the column was set constant at 1.0 mL/min for 50 min running time. Scan mode ranged from 20 amu to 600 amu.

Each extract was first dissolved in 5 mL ethanol, sonicated for 15 min, and filtered through a 0.45-µm nylon membrane filter before running the analysis of the chemical content. The filtrate of 0.2 µL was then injected into the GC-MS system. The results were printed out after the analysis process in the instrument was finished.

Ethics committee approval statement
All field research designs have been approved by the Government of East Nusa Tenggara Province (no: 070/535/DPM-PTSP/2017 and no: 070/535/DPM-PTSP/2017, dated February 28, 2017), the Government of Malaka District (no: 070/150/IV/2017, dated March 3, 2017), and the Government of Belu District (no: BKBP-070/75/III/2017, dated March 29, 2017) to guarantee the ethics and legality of human involvement in this study.

RESULTS AND DISCUSSION

Plants used for malaria treatment
Documented in this study are 50 plant species belonging to 27 families that used by the Tetun ethnic group for malaria treatment in the Malaka and Belu Districts (Table 1). These plants were used as single formula or combination of plants in various recipes for oral application. Some of the plants that are most frequently mentioned by the informants are *Strychnos ligustrina* Blume, *Calotropis gigantea* (L.) R. Br., *Cleome rutidosperma* DC., *Physalis angulata* L., *Carica papaya* L., *Alstonia spectabilis* R.Br., *Alstonia scholaris* (L.) R. Br., *Melia azedarach* L., *Plumeria alba* L., *Swietenia macrophylla* King, and *Momordica balsamina* L.

The highest number of plant species belong to seven families: Apocynaceae and Fabaceae (five species each), Cucurbitaceae (four species), and Meliaceae, Moraceae, Euphorbiaceae, and Rubiaceae (three species each). The most widely used plant parts in its utilization as medicinal materials are the stem bark (21 species), leaves (19 species), and root (11 species). Some plants are used more than one part at a time. Decoction and infusion are the common preparation modes of various formula for oral use of these plants.

Plant selection for antiplasmodial activity test
For antiplasmodial activity testing, 11 plants species with high citation frequency were selected. These are *S. ligustrina* Blume (34.04%), *C. gigantea* (L.) R. Br. (24.47%), *C. rutidosperma* DC. (18.09%), *P. angulata* L. (18.09%), *A. spectabilis* R. Br. (17.02%), *A. scholaris* (L.) R. Br. (13.83%), *M. azedarach* L. (13.83%), *Fatoua pilosa* Gaudich. (7.45%), *Jatropha curcas* (6.38%), *P. alba* L. (6.38%), and *Neoalsomitra podagrica* Steenis (4.26%).

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Table 1. Plants used by the Tetun ethnic people for malaria treatment

Botanic name	Family	Local name	Plants' part used	Frequency of citation n (%)*
Strychnos ligustrina Blume	Loganiaceae	Bakumoru	Wood, stem bark	32 (34.04)
Calotropis gigantea (L.) R. Br.	Asclepediaceae	Fuka	Root	23 (24.47)
Cleome rutidosperma DC.	Capparaceae	Lakaur	Whole plant	17 (18.09)
Physalis angulata L.	Solanaceae	Babotore	Whole plant	17 (18.09)
Carica papaya L.	Caricaceae	Dila	Leaves	16 (17.02)
Alstonia spectabilis R.Br.	Apocynaceae	Kroti metan	Stem bark	16 (17.02)
Alstonia scholaris (L.) R.Br.	Apocynaceae	Kroti mutin	Stem bark	13 (13.83)
Melia azedarach L.	Meliaceae	Samer	Leaves, stem bark	13 (13.83)
Fatoua pilosa Gaudich	Moraceae	Lorowen	Root	7 (7.45)
Jatropha curcas L.	Euphorbiaceae	Badut malaka mutin	Stem bark	6 (6.38)
Swietenia macrophylla King	Meliaceae	Mahoni	Seed	6 (6.38)
Plumeria alba L.	Apocynaceae	Mukrin	Stem bark	6 (6.38)
Momordica balsamina L.	Cucurbitaceae	Bria fuik	Leaves, fruit	5 (5.32)
Neoalsomitra podagrica Steenis	Cucurbitaceae	Masin borat	Root	4 (4.26)
Wrightia pubescens R. Br.	Apocynaceae	Lalitin feto	Leaves, root, stem bark	3 (3.19)
Tabernaemontana pandacaqui Lam.	Apocynaceae	Lalitin mane	Stem bark	3 (3.19)
Aegle marmelos (L.) Correa	Rutaceae	Dilabutak	Stem bark, root, leaves	3 (3.19)
Andrographis paniculata (Burm.f.) Nees.	Acanthaceae	Karlulu	Whole plant	2 (2.13)
Cassia fistula L.	Fabaceae	Liman tohar	Stem bark	2 (2.13)
Cassia siamea Lam.	Fabaceae	Krui	Leaves, stem bark	2 (2.13)
Coccinia grandis (L.) Voigt	Cucurbitaceae	Kabasa	Leaves	2 (2.13)
Ficus callosa Willd.	Moraceae	Salur	Stem bark	2 (2.13)
Ficus hispida L.f.	Moraceae	Baulenuk	Leaves	2 (2.13)
Phyllanthus niruri L.	Phyllanthaceae	Renes	Whole plant	2 (2.13)
Acacia leucophloea (Roxb.) Willd.	Fabaceae	Besak	Stem bark	1 (1.06)
Blumea balsamifera (L.) DC.	Compositae	Fafok	Stem bark	1 (1.06)
Bridelia ovata Decne.	Euphorbiaceae	Knabu	Leaves	1 (1.06)
Brucea javanica (L.) Merr.	Simaroubaceae	Ai lakar	Leaves, stem bark, root	1 (1.06)
Capsicum frutescens L.	Solanaceae	Masimanas	Fruit	1 (1.06)
Ceiba pentandra (L.) Gaertn.	Malvaceae	Kabidawa	Leaves	1 (1.06)
Curcuma domestica Val.	Zingiberaceae	Kinur	Rhizome	1 (1.06)
Dendropthoe pentandra (L) Miq.	Loranthaceae	Tau tiu ten	Leaves	1 (1.06)
Dysoxylum gaudichaudianum (A. Juss.) Miq.	Meliaceae	Meda lasan	Leaves	1 (1.06)
Garuga floribunda Decne.	Burseraceae	Feu	Stem bark	1 (1.06)
Gossypium herbaceum L.	Malvaceae	Kabas fuan mean	Root	1 (1.06)
Grewia koodersiana Burrett	Tilliaceae	Lenok	Root	1 (1.06)
Gymnopetalum chinense (Lour.) Merr.	Cucurbitaceae	Kolokoen	Root	1 (1.06)
Imperata cylindrica (L.) P. Beauv.	Poaceae	Hae manlain	Root	1 (1.06)
Indigofera suffruticosa Mill.	Fabaceae	Taun	Leaves	1 (1.06)
Jatropha gossypifolia L.	Euphorbiaceae	Badut malaka mean	Stem bark	1 (1.06)
Morinda citrifolia L.	Rubiaceae	Nenuk	Leaves, fruit, stem bark	1 (1.06)

Table 1 continue

Nauclea orientalis (L.) L.	Rubiaceae	Kafiru	Stem bark	1 (1.06)
Piper cubeba L.f.	Piperaceae	Kunus aleten	Leaves	1 (1.06)
Sterculia foetida L.	Sterculiaceae	Abano	Stem bark	1 (1.06)
Tamarindus indica L.	Fabaceae	Sukaer	Leaves	1 (1.06)
Uvaria rufa Blume	Annonaceae	Koke	Root	1 (1.06)
Wendlandia burkillii Cowan	Rubiaceae	Katimun	Stem bark	1 (1.06)
Ziziphus timoriensis DC.	Rhamnaceae	Ai sisi	Leaves	1 (1.06)
Not identified	Not identified	Moat tiris	Leaves	1 (1.06)
Not identified	Not identified	Uas laomea	Tuber	1 (1.06)

*: The total percentage is greater than 100% because each informant (N=94) mentioned more than one plant

C. papaya L. (17.02%), *S. macrophylla* King (6.38%), and *M. balsamina* L. (5.32%) were three other plants with high citation frequency but were not included antiplasmodial activity testing because of several reasons. The *C. papaya* was not selected testing for the reason that it is a food plant that is consumed every day as a vegetable. The wild bitter melon *M. balsamina* was also not included because it was difficult to obtain, very rarely cultivated, usually only grow wild, and is seasonal. The *S. macrophylla* was excluded because according to the informants, the use of its seeds as an antimalarial medicine was not sourced from traditional practice of the Tetun people's ancestors.

Antiplasmodial activity

A graphical comparison of the extracts' ability to inhibit *Plasmodium* growth is shown in Figure 1. It can be seen from this graph that with an increase in concentration, three extracts, *P. angulata* L., *J. curcas* L., and *A. spectabilis* R. Br. show a more significant increase in their inhibitory activity compared with the other extracts. The graph presents that on average, a tenfold increase in the concentration of these three extracts increases their antiplasmodial activity twice.

Table 2 lists the antiplasmodial activity of each extract in the form of percentage of inhibition at each concentration level and their IC_{50} values. The concentration that causes 50% reduction in *Plasmodium* growth is represented by IC_{50}. A smaller IC_{50} value indicates the better the extract's antiplasmodial activity; and vice versa, greater IC_{50} indicates low inhibitory activity. In the positive control, with the same experimental condition, chloroquine diphosphate has an IC_{50} value of 0.005 µg/mL.

Ouattara et al.[12] categorized an extract as very active or strong antiplasmodial if IC_{50} <5 µg/mL, active or moderate antiplasmodial if IC_{50}: 5-50 µg/mL, less active or weak antiplasmodial if IC_{50}: 50-100 µg/mL, and inactive if IC_{50} >100 µg/mL. Based on these categories, ethanolic extracts of *P. angulata* L. (IC_{50} 0.22 µg/mL), *J. curcas* L. (IC_{50} 0.22 µg/mL), and *A. spectabilis* R. Br. (IC_{50} 1.23 µg/mL) are strong antiplasmodials; whereas *N. podagrica* Steenis (IC_{50} 11.60 µg/mL), *A. scholaris* (L.) R. Br. (IC_{50} 15.46 µg/mL), *F. pilosa* Gaudich. (IC_{50} 24.92 µg/mL), and *P. alba* L. (IC_{50} 36.39 µg/mL) are moderates. The extracts of *C. rutidosperma* DC. (IC_{50} 54.25 µg/mL), *M. azedarach* L. (IC_{50} 63.52 µg/mL),
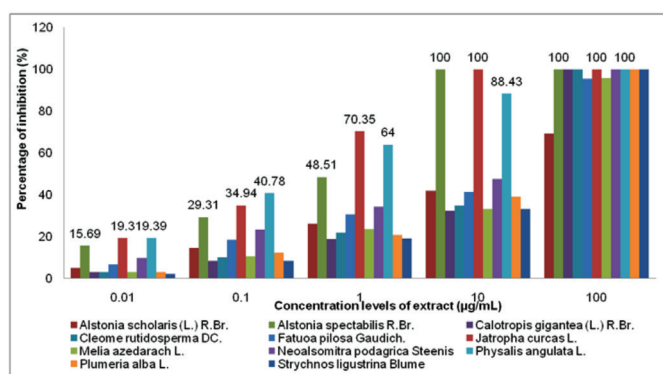

Figure 1. Graphical comparison of the plant extract's inhibitory ability on *Pasmodium falciparum* 3D7 strain *in vitro*

S. ligustrina Blume (IC_{50} 63.91 µg/mL), and *C. gigantea* (L.) R. Br. (IC_{50} 66.49 µg/mL) are weak antiplasmodial. In general, although they are relatively weaker than chloroquine diphosphates, *P. angulata* L., *J. curcas* L., and *A. spectabilis* R. Br. ethanolic extracts showed good antiplasmodial effects on *P. falciparum* 3D7 strain.

Among the 11 plant extracts, *P. angulata* L. showed the strongest antiplasmodial activity. This result is in line with the findings of previous studies using methanol and dichloromethane extracts of *P. angulata* L. leaves, where these extracts presented very high activity with IC_{50} <3 µg/mL against the chloroquine-sensitive 3D7 and chloroquine-resistant W2 strains of *P. falciparum in vitro*. *P. angulata* extracts also showed good *in vivo* parasitemia inhibition on mice infected by *Plasmodium berghei*.[13]

In this study, the ethanolic extract of *J. curcas* L. stem bark showed strong antiplasmodial activity, equivalent to *P. angulata* L. In another study, it was found that mice infected by *P. berghei* treated with 250, 500, and 750 mg/kg body weight doses of aqueous extract of *J. curcas* L. stem bark decreases parasitemia percentage from 9.25% to 7.80%, suggesting that the plant extract possesses antiplasmodial properties.[14]

Results in this study showed that ethanolic extract of *P. alba* L. stem bark is a moderate antiplasmodial. Another study showed that water extract (300 mg/kg body weight) and dichloromethane-methanol extract (300 mg/kg body weight) of this plant's stem bark reduces parasitemia level of mice

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Table 2. Antiplasmodial activity of plant extracts

Plant extract	Inhibition percentage of extract on *Plasmodium* (%) at each level of concentration (µg/mL)					IC_{50} (µg/mL)
	0.01	0.1	1.0	10	100	
Alstonia scholaris (L.) R. Br.	5.06	14.52	26.20	41.89	69.26	15.46
Alstonia spectabilis R. Br.	15.69	29.31	48.51	100.00	100.00	1.23
Calotropis gigantea (L.) R. Br.	2.96	8.43	18.96	32.44	100.00	66.49
Cleome rutidosperma DC.	3.04	10.25	22.09	34.96	100.00	54.25
Fatuoa pilosa Gaudich	6.70	18.69	30.78	41.39	95.56	24.92
Jatropha curcas L.	19.30	34.94	70.35	100.00	100.00	0.22
Melia azedarach L.	2.96	10.78	23.74	33.22	95.91	63.52
Neoalsomitra podagrica Steenis	9.74	23.39	34.26	47.74	100.00	11.60
Physalis angulata L.	19.39	40.78	64.00	88.43	100.00	0.22
Plumeria alba L.	2.98	12.45	20.88	39.04	100.00	36.39
Strychnos ligustrina Blume	2.20	8.56	19.19	33.33	100.00	63.91

infected by *P. berghei* to 16.4% and 20.0%, respectively, in eight days of evaluation.[15]

Although the ethanolic extract of *A. scholaris* (L.) R. Br. stem bark in this study showed a moderate activity, the results in another study showed that methanolic extract of its stem bark showed excellent antiplasmodial activity against *P. falciparum* 3D7 strain, with a mean IC_{50} of 0.1650±0.1100 µg/mL.[16] The stem bark of *A. scholaris* (L.) R. Br. contains villalstonine and macrocarpamine alkaloids, which are antimalarial active, with the IC_{50} values of 0.27 and 0.36 µM, respectively, against chloroquine-resistant *P. falciparum* K1 strain.[17]

Ethanolic extracts of *S. ligustrina* Blume, *C. gigantea* (L.) R. Br., *C. rutidosperma* DC., and *M. azedarach* L. in this study showed weak activity against *P. falciparum* 3D7 strain, with IC_{50} >50 µg/mL. However, it was found in several other studies that these plants showed a good antiplasmodial activity when different solvents were used for extraction. Water extract of *S. ligustrina* Blume wood was classified as a strong antiplasmodial as it inhibited *P. falciparum* growth *in vitro* by 98.1% at a concentration of 1.0 mg/mL.[18] Methanolic extract of *C. gigantea* (L.) R. Br. leaves showed moderate antimalarial activity against *P. falciparum in vitro* with an IC_{50} value of 12.17 µg/mL, and very good activity against *P. berghei in vivo*.[19] Ethanolic extract of *C. rutidosperma* DC. whole plant showed moderate antimalarial activity with IC_{50} of 34.4 µg/mL; however, its water extract was less active with IC_{50} >100 µg/mL against chloroquine-sensitive *P. falciparum* D10 strain *in vitro*.[20]

The results of this antimalarial activity evaluation did not linear to rank of the plants based on their percentage of citation listed in Table 1. In laboratory testing, the informants' most frequently mentioned plants, which are the *S. ligustrina* Blume, *C. gigantea* (L.) R. Br., and *C. rutidosperma* DC., turned out to show weak antiplasmodial activity. However, it does not mean that these plants' effectiveness claims as antimalarials are incorrect. Possible causes of non-synchronous data between frequency of citation of the informants and the laboratory antiplasmodial

evaluation of these plants can be explained as follows. Firstly, the use of ethanol as an extraction solvent can cause differences in type and amount of antiplasmodial active compounds extracted into it, which causes differences in the antiplasmodial activity shown by each plant extract. Secondly, the *in vitro* system is very different from the biochemical system in the human body, therefore, *in vitro* antimalarial activity results cannot directly describe the actual events in human body. Therefore, it is possible for an antimalarial plant to be active in human and be inactive in an *in vitro* testing, and vice versa. Thirdly, certain plants may not be true antimalarial (antiplasmodial) that works to kill or inhibit *Plasmodium* growth. It may more likely be an indirect antimalarial (antipyretic, analgesic or antiinflammatory) that works to heal malaria-related symptoms, and therefore, the test showed no significant activity as antiplasmodial. As is known, traditional treatment of malaria is a symptomatic healing, which mainly aims to reduce heat or fever; therefore, it is possible that a plant used in this treatment is more likely antipyretic than antiplasmodial.[21,22] Plants having antimalarial properties can show a direct effect on *Plasmodium* by inhibiting growth or killing it; or indirect effects on the relationship between parasites and the human body. A plant that inhibits or kills *Plasmodium* is called antiplasmodial or true antimalarial. Other plants may serve as indirect antimalarial, which affects the relationship between the human body as a host and *Plasmodium*, for example as immunostimulant or antipyretic, or causing hemolysis and membrane structure changes, which inhibits *Plasmodium* growth.[23]

This report of antiplasmodial activity of *N. podagrica* Steenis and *F. pilosa* Gaudich. against *P. falciparum* 3D7 strain is the first based on our search of previous studies in any publications. A study on the antimalarial activity of these two plants was not carried out by other researchers before. The *F. pilosa* Gaudich. was only reported to have pharmacological activity as an antimycobacterial,[24] whereas *N. podagrica* Steenis had no known pharmacological activities. Therefore, it is an open

chance to make further evaluation on the antimalarial activity of these two plants, and to identify their antimalarial active compound(s).

Phytochemicals content of the extracts

Table 3 shows the phytochemical analysis results using GC-MS. It can be seen in this table that, overall, these 11 extracts contain various types of natural products, such as alkaloids, terpenoids, steroids, coumarins, alcohols, thiols, phenolics, aldehydes, fatty acids, esters, and so forth. Several studies showed that many secondary plant metabolites, such as alkaloid, flavonoid, xantone, quassinoid, triterpene, and sesquiterpene, have antiplasmodial activity; thus, having the potential to be developed as antimalarial.[4,17]

Although identifying the types and amounts of compounds by GC-MS is limited to volatile compounds, results showed each of the extracts contained quite a number of previously unknown compounds; for example, the alkaloid brucine in *M. azedarach* L leaves. Some of the compounds identified from the 11 plant extracts, such as alstonine, alstomacroline, pleiocarpamine, lupeol, amyrin, and brucine, are known to have antiplasmodial activity.[13,15,17,25-27]

CONCLUSION

The Tetun ethnic people use at least 50 plant species as oral antimalarial medicine. The *A. scholaris* (L.) R. Br., *A. spectabilis* R. Br., *C. gigantea* (L.) R. Br., *C. rutidosperma* DC., *F. pilosa* Gaudich., *J. curcas* L., *M. azedarach* L., *N. podagrica* Steenis, *P. angulata* L., *P. alba* L., and *S. ligustrina* Blume are some of the frequently cited plants. These 11 plants have proven to have antiplasmodial activity, ranged from strong to weak antiplasmodial. The *P. angulata* L., *J. curcas* L., *A. spectabilis* R. Br., *N. podagrica* Steenis, and *F. pilosa* Gaudich. May have a potential to be developed as new sources of antimalarials.

The novelty of this study is the fact that *N. podagrica* Steenis have never been reported used as antimalarial in other traditional medicine systems elsewhere, and this is the first publication of *N. podagrica* Steenis and *F. pilosa* Gaudich.'s antiplasmodial activity against the *P. falciparum* 3D7 strain.

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Table 3. Phytochemical contents of the plants'extracts identified using gas chromatography-mass spectrometry

Plant's extract	Ret-time (min)	Compound	Area (%)
Alstonia scholaris (L.) R. Br.	20.63	*n*-hexadecanoic acid	0.15
	22.25	*Z*-9-oxtadecenoic acid	0.20
	22.41	Linoleic acid ethyl ester	0.08
	26.93	4, 11-dimethoxy-1H-cyclopent[b]anthracene-2,5,10(3H)-trione	0.14
	27.86	2,2-dimethyl-6,11-dioxo-2,3,6,11-tetrahydro anthra[1,2-b]furan-4-carbaldehyde	0.13
	27.94	Pleiocarpamine	0.11
	28.50	*E*-3,3′-bis-ethylmercapto-1,1′-biisoindolylidene	0.18
	29.22	Dihydroxycrinane	0.22
	30.91	Campesterol	0.66
	31.33	2,2-dimethyl-cholest-4-en-3-one	0.83
	32.24	(23*S*)-ethylcholest-5-en-3β-ol	0.97
	32.69	β-amyrin	27.61
	33.17	Aristolone	5.12
	33.77	Isomultiflorenyl acetate	0.54
	34.25	Maragenin I acetate	18.94
	35.17	Lupenyl acetate	32.03
	35.32	Dihydroagnosterol acetate	0.11
	35.85	Friedoursan-3-one	0.11
Alstonia spectabilis R. Br.	18.33	Coniferol	0.71
	20.67	*n*-hexadecanoic acid	0.53
	22.26	*E*-9-octadecanoic acid	0.46
	25.85	Strictamine	0.44
	26.30	Eupomatenoid-17	0.32
	26.43	Pleiocarpamine	1.70
	26.50	10-aza-8-oxyprotoberberine	2.54
	27.07	2,5-bis(3,5-dimethyl-4-methoxyphenyl)-thiophene	0.26
	27.14	Fluorocarpamine	0.43
	28.18	Vincamajine	2.09
	28.70	Alstomacroline	10.27
	30.36	Homoegonol	0.39
	30.91	*E*-5,10-secocholest-1 (10)-en-3,5-dione	0.89
	31.29	2,2-dimethylcoles-4-en-3-one	1.59
	31.99	(23*S*)-ethylcolest-5-en-3β-ol	1.15
	32.56	β-amyrene	0.27
	32.95	α-amyrin	1.26
	33.05	1-amino-8-methyl-3,6-diazahomodamantan-9-ol	0.51
	33.82	Norolean-12-ene	0.71
	34.54	Aristolone	0.40

Table 3 continue

Calotropis gigantea (L.) R. Br.	1.99	1,1-diethoxy-ethane	0.19
	20.56	Ethyl hexadecanoate	0.10
	22.07	Ethyl linoleate	0.30
	32.91	Urs-12-en-24-oic acid, 3-oxo-, methyl ester	0.54
	33.53	α-amyrin acetate	0.98
	35.12	Dammaradienyl acetate	0.49
	38.17	β-amyrene	0.26
Cleome rutidosperma DC.	2.10	1,1-diethoxy-ethane	0.20
Fatuoa pilosa Gaudich	19.40	Angelicin	6.57
	20.67	*n*-hexadecanoic acid	0.18
	21.48	Heraclin	0.48
	21.61	Seselin	0.55
	22.47	Ethyl oleate	0.13
	22.97	Brayelin	0.12
	25.69	Vincanine	1.79
	26.66	Strictamine	0.19
	30.76	Cycloartenol	0.33
	31.75	Friedelin	0.48
	32.08	Aristolone	3.49
	32.46	β-amyrin	2.55
	32.83	Lupene-3-one	14.04
	33.27	Lupeol	15.40
	34.11	Urs-12-en24-oic acid, 3-oxo-, methyl ester	7.76
	34.28	Fern-7-en-3β-ol	1.75
	34.47	Moretenol	0.67
	34.88	α-amyrin acetate	12.38
	35.38	9,19-cyclolanost-7-en-3-ol	1.15
	36.30	3β-Lup-20(29)-en-3-ol acetate	1.15
Jatropha curcas L.	21.71	2,4-bis(1-phenylethyl)phenol	0.47
	24.76	Isocryptotanshinon	1.08
	31.04	5-methoxy-6-[1-(4-ethoxyphenyl)ethyl]-1,3-benzodioxol	1.02
	31.27	7-bromo-cycloisolongifolene	1.07
	31.65	Ferruginol methyl ether	1.95
	31.88	13, 27-cycloursane	2.85
	32.93	4, 14-dimethyl-9, 9-cyclocholestan-3-one	0.55

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Table 3 continue

Melia azedarach L.	2.10	2-allyl-1,3-dioxolan	0.19
	19.05	Ethylpentylacetylene	0.14
	20.51	*n*-hexadecanoic acid	0.81
	22.10	Methyl hexadeca-7,10,13-trienoate	0.56
	22.15	(9*Z*,12*Z*,15*Z*)-octadecatrien-1-ol	0.21
	32.11	(22*E*,24*S*)-stigmasta-4,22-dien-6-one	0.10
	32.92	2,2-dimethylcholest-4-en-3-one	0.35
	35.09	Dibenz[a,h]anthracene	0.23
	36.29	Brucine	0.96
	39.54	4,6-di-m-tolyl-1H-[1,3,5]triazin-2-one	0.28
Neoalsomitra podagrica Steenis	20.63	*n*-Hexadecanoic acid	0.61
	22.26	9, 12, 15-octadecatrien-1-ol	0.99
	22.48	*n*-octadecanoic acid	0.58
	27.62	2, 5, 8-trimethyl-1-naphtol	0.27
	28.25	Plectrinon A	0.43
	29.21	β-tocopherol	0.35
	31.17	3-phenoxyphenol	0.56
	31.96	3β, 5α-stigmasta-7, 25-dien-3-ol	7.94
	32.46	3β, 5α-stigmasta-7, 16-dien-3-ol	2.43
	33.53	Trans, cis-1,2,4-trimethylcyclohexane	1.72
	33.82	Norolean-12-ene	0.77
	34.27	3-methoxy-N-(4-chlorophenyl)sulfonyl benzenecarboximidamide	1.22
	34.51	1-chloro-4-(methylsulfonyl)-benzene	3.91
	34.80	1-(2-thienyl)-1-butanone	2.06
Physalis angulata L.	2.09	1, 1-diethoxy-ethane	0.19
	31.72	Medroxyprogesterone acetate	0.27
	31.88	3β-lupa-1, 20 (29)-dien-3-ol	0.14
	32.40	Ergosta-4, 24 (28)-dien-3-one	0.16
Plumeria alba L.	2.10	1, 1-diethoxy-ethane	0.16
	31.25	3-keto-urs-12-ene	0.37
	31.82	Friedooleanan-3-one	1.07
	32.99	β-amyrin acetate	1.03
	33.86	3β-lup-20 (29)-en-3-ol acetate	9.22
	34.98	Olean-18-en-28-oic acid, 3-oxo-, methyl ester	0.10
Strychnos ligustrina Blume	2.05	1,1-diethoxy-ethane	0.24
	36.10	Brucine	0.73

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