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Research Article

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

Batı Timor Endonezya'da Yerli Halkın Kullandığı Bazı Antimalarial Bitkilerin Antiplazmodial Aktivitesi ve Fitokimyasal Bileşenleri

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ABSTRACT
Objectives: To document traditional antimalarial plants used by the Tetun ethnic people in West Timor Indonesia, and to 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. 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 azedarach* were tested for their inhibitory potency on the *Plasmodium falciparum* 3D7 strain *in vitro.* The phytochemicals of the extracts were analyzed with a GC-MS instrument.
Results: There were 50 species of plants used as antimalarial by the Tetun ethnic people. Extracts of *P. angulata*, *J. curcas* and *A. spectabilis* showed a strong antiplasmodial activity with the IC_{50} values of 0.22, 0.22 and 1.23 µg/mL, respectively. The *N. podagrica*, *A. scholaris, F. pilosa* and *P. alba* were moderate antiplasmodials with the IC_{50} 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 the IC_{50} of 54.25, 63.52, 63.91 and 66.49 µg/mL, respectively. Data of the phytochemicals identification indicate that these 11 plants contain alkaloids, terpenoids, steroids, coumarins, alcohols, thiols, phenolics, aldehydes, fatty acids, esters, etc.
Conclusion: Plants that widely used as antimalarial by the Tetun ethnic people have been proven to have antiplasmodial activity.

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

ÖZ
Amaç: Endonezya Batı Timor'unda Tetun tarafından kullanılan geleneksel antimalarial bitkileri belgelemek ve yaygın olarak oral ilaçlar olarak kullanılan bazı bitkilerin antiplazmodial ve fitokimyasal aktivitelerini değerlendirmek.
Gereç ve Yöntemler: Antimalarial bitkileri belgeleyen bir alan çalışmasını, antiplazmodial aktiviteyi test etmek ve seçilen bitki ekstraktlarından fitokimyasal bileşenleri belirlemek için laboratuvar çalışması izlemiştir. Gelen etanolik ekstreler *Strychnos ligustrina*, *Calotropis*

gigantea, *Fatuoa pilosa* ve *Neoalsomitra podagrica*, *Cleome rutidosperma*, *Physalis angulata*, *Alstonia spectabilis*, *Alstonia scholaris*, *Jatropha curcas*, *Plumeria alba* ve *Melia azedarach* tüm parçaları suşu üzerine inhibitör kuvvete bunları test 3D7 *Plasmodium falciparum in vitro* olarak. Fitokimyasal ekstreler GC-MS cihazları ile analiz edildi.
Bulgular: Tetun etnik halkı tarafından antimalarial olarak kullanılan 50 bitki türü vardı. *P. angulata*, *J. curcas* ve *A. spectabilis*'in özleri, sırasıyla 0.22, 0.22 ve 1.23 μg/mL IC_{50} değerleriyle güçlü bir antiplazmodial aktivite göstermiştir. *N. podagrica*, *A. scholaris*, *F. pilosa* ve *P. alba*, sırasıyla 11.60, 15.46, 24.92 ve 36.39 μg/mL IC_{50}, ve *C. rutidosperma*, *M. azedarach*, *S. ligustrina* ve *C. gigantea*, IC_{50} ile sırasıyla 54.25, 63.52, 63.91 ve 66.49 μg/mL zayıf antiplazmodiyallerdi. Fitokimyasal tanımlamanın verileri, bu 11 bitkinin alkaloitler, terpenoidler, steroidler, kumarinler, alkoller, tiyoller, fenolikler, aldehitler, yağ asitleri, esterler ve diğer bileşikleri içerdiğini gösterir.
Sonuçlar: Tetun etnik halkı tarafından yaygın olarak antimalarial olarak kullanılan bitkilerin antiplazmodial aktivite gösterdiği kanıtlanmıştır.

Anahtar kelimeler: Etnomedicine, şifalı bitki, sıtma, antiplazmodial aktivite

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INTRODUCTION

Plants are a very valuable source for obtaining various pharmacological active substances to deal with various human health problems.[1] For thousands of years, plants have been the basis and important part of various traditional medical systems. It is estimated that of the total number of plant species known today, around 25% or 40,000-70,000 species are used as medicinal plants by people 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 to treat various infectious diseases, including malaria. Quinine isolated from *Cinchona* sp. stem bark and artemisinin from Chinese medicinal plants *Artemisia annua* are two examples of lead compounds that are phenomenal and have contributed greatly to reducing deaths of malaria all over the world.[4] From quinine, various derivatives such as chloroquine, amodiaquine, primaquine, and mefloquine have been synthesized; and from artemisinin, several compounds have been produced, namely artemether, arteether, and sodium artesunate. However, since the last few decades, *Plasmodium* has shown increasing resistance to antimalarial quinine derivatives, especially chloroquine, and made them no longer effective. Several recent publications have even reported that *Plasmodium falciparum* has also shown an increase in resistance to artemisinin-based antimalarials.[5]
The fact that *Plasmodium*'s increasing resistance to antimalarial drugs currently used, has encouraged the researchers to continue searching for new and more effective antimalarials.[6]
Plants in traditional medicine of various ethnics all over the world have become one of the most important sources in searching of new potential antimalarials.[7] Selecting plants that have been traditionally used to treat malaria is a promising and better approach to find new antimalarial(s). This saves more resources such as high investment and skills, and has also

been shown to has accelerated the time of the plant selection and test of its antimalarial activity compared to random selection approach. Research on medicinal plants of various traditional medicine systems could provide useful leads for the development of important active antimalarials.[8]

The experience of the Tetun people –a native ethnic in West Timor interacting with malaria since long time ago led to them having developed their own methods to treat malaria. The Tetun people identify malaria as *sick of hot body* or fever. Traditional treatment of malaria by the Tetun community is carried out using a variety of medicinal plants formula called *ai tahan* or *kwa*, which are applied by drinking, bathing, massage, inhaling, and cataplasm.[9] In-depth exploration of the antimalarial plants used in traditional medicine of the Tetun ethnic community might provide a valuable contribution to the discovery of new sources of antimalarial substances.

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

MATERIALS AND METHODS

Field study

The field research was carried out in several sub-districts of Belu District (9°15'0" S, 124°40' E) and Malaka District (9°34' S, 124°54' E). These two districts are located along the borderline of East Nusa Tenggara Province (Indonesia) and Republic Democratic de Timor Leste. In this field study, we collected information through some interviews and discussions. Ninety-four informants, 42 male and 52 female were involved in this study. They consist of traditional healers, former malaria sufferers who have undergone traditional medicine, and other people who have knowledge and experience in traditional treatment of malaria. These informants were aged between 40-90 years old, and almost all of them have settled in their place since they were born.

Data collected relating to traditional medicinal plants used for the treatment of malaria include: the local name of the plant, the place of obtaining the plant, the part of the plant used as a medicine, the method of processing and use, the dosage and duration of use, and the claimed effect of the medication. Each of the plants informed by the informants was then collected in parts to prepare a herbarium. All of the plants were identified, firstly by matching their local name with scientific name of species listed in book of Timorese local plants.[10] Secondly, those plants were identified again by the expert of Lembaga Ilmu Pengetahuan Indonesia (LIPI)-Bogor Botanic Garden. Part(s) of some plants with high frequency of mention by the informants were collected in greater quantities. Those samples of the plant's part were collected from the area where the plant was mentioned.

Preparation of the plants extract

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

In vitro antiplasmodial activity test

Testing of antimalarial activity was carried out *in vitro* on the chloroquine sensitive 3D7 strain of *Plasmodium falciparum* obtained from the Institute of Tropical Diseases Airlangga University, Surabaya Indonesia. *Plasmodium* was cultivated in 96 microwells plate according to the method developed by Trager-Jensen.[11] Cultivation of *Plasmodium* was carried out using human red blood cells O type with 5% haematocrit suspended in RPMI 1640 medium. The culture was then incubated in a CO_2 incubator at 37°C, and the medium was replaced every day until parasitemia reaches 1-2%. Dried plant extracts were dissolved in dimethylsulphoxide (DMSO) and filtered through a 0.22 µm membrane filter. The solution of each extract was then put into microwells containing *Plasmodium* suspension with 1% parasitemia, then diluted with the RPMI 1640 medium in a series of ten-fold dillution to obtain the final concentrations of 100, 10, 1.0, 0.1 and 0.01 µg/mL. Two series of controls were set up; one with parasitised blood cells without addition of plant extract -as negative control, and the other with parasitised blood cells added with chloroquine diphosphates –as positive control. The chloroquine diphosphates was prepared like plant extract to made final concentrations of 100, 10, 1.0, 0.1 and 0.01 µg/mL. The culture then incubated in a CO_2 incubator at 37°C for 48 h. All tests were performed in duplicate. After 48 h of incubation, the culture of red blood cells was harvested. A thin blood smear was made on the glass object, then dried and fixed with methanol, and stained with Giemsa. The number of parasitised red blood cells were counted using a light microscope with 1000 times magnification.

Data obtained from this antiplasmodial activity was the number of infected red blood cells (iRBC) among a total of 1000s RBC counted, by observing blood smear slides under a microscope. The amount of iRBC to the total RBC was expressed as percentage of parasitemia, which was calculated using the numerical formula:

Percentage of parasitemia, P = [(∑iRBC/∑RBC) x 100%]

By the data of percentage of parasitemia in treated groups and negative control, then the percentage of growth and inhibition of *Plasmodium* were calculated, using the formula:

Percentage of growth = (Pt/Pnc) x 100%

Percentage of inhibition =100% - *percentage of growth*

In those two later equations above, *Pt* stands for the percentage of parasitemia in treated groups, and *Pnc* is the percentage of parasitemia in 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 actual percentage of parasitemia value was P_{48} - P_0. The range of the percentage of inhibition is 0% that means no inhibition, to 100% that is complete inhibition.

By plotting data of the percentage of inhibitions versus concentrations of each extract, the 50% inhibitory concentration (IC_{50}) was then calculated using probit analysis. The IC_{50} value was then compared to the value given by the literature to classify the antimalarial potency of each extract.

Identification of phytochemicals in extracts

Phytochemicals contained in each extract were analyzed using GC-MS. An Agilent 6980N Network GC System with autosampler was linked to a detector of Agilent 5973 inert MSD. A column of J&W Scientific HP-5MS 30 m x 0,25 mm x 0,25 µm was used. This GC-MS instrument used a Wiley version 7.0 database to interpreted the compounds. The operational conditions of the GC-MS was set up as follows: inlet temperature was 250°C; oven tempeature was programmed at 50°C for 5 min and then increased 10°C/min to 280°C, which was maintained constant for 15 min. Temperatures of Aux was 250°C, MS Quad 150°C, and MS Source 230°C. Helium gas with purity of 99.999% was used as sample carrier. Gas flow in the column was set constant at 1.0 mL/min for 50 min running time. Scan mode was ranged from 20 amu to 600 amu.

Before running the analysis of the chemicals content, each extract was first dissolved in 5 mL ethanol, sonicated for 15 min, and then filtered through a 0.45 µm nylon membrane filter. The filtrate of 0.2 µL was then injected in to the GC-MS system. After the analysis process in the instrument was finish, then the results were printed out.

RESULTS AND DISCUSSION

Plants used for the treatment of malaria

In this study, we have documented 50 plant species belong to 27 families that used for the treatment of malaria by the Tetun ethnic people in Malaka and Belu Districts (Table 1). These plants were used in various recipes for oral application, as single formula or combination of plants. The The *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. were some of plants that more frequently mentioned by the informants.

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

Selection of the plants for antiplasmodial activity test

Eleven plants species with high frequency of citation were selected to be tested for their antiplasmodial activity. Those plants were Those plants were *Strychnos ligustrina* Blume (34.04%), *Calotropis gigantea* (L.) R. Br. (24.47%), *Cleome rutidosperma* DC. (18.09%), *Physalis angulata* L. (18.09%), *Alstonia spectabilis* R.Br. (17.02%), *Alstonia scholaris* (L.) R.Br. (13.83%), *Melia azedarach* L. (13.83%), *Fatoua pilosa* Gaudich. (7.45%), *Jatropha curcas* (6.38%), *Plumeria alba* L. (6.38%) and *Neoalsomitra podagrica* Steenis (4.26%). Three other plants of high frequency of citation - *Carica papaya* L. (17.02%), *Swietenia macrophylla* King (6.38%) and *Momordica balsamina* L. (5.32%) were not included to be tested for their antiplasmodial activity for several reasons. The *C. papaya* was not selected for antiplasmodial testing by the reason that it is a food plant that is consumed every day as a vegetable. The wild bitter melon *M. balsamina* was not included too because this plant was difficult to obtain in the field, very rarely cultivated, usually only grow wild and seasonal. The *S. macrophylla* was not included because according to the informants, the use of this plant seeds as an antimalarial medicine was not sourced from traditional practice of the ancestors of Tetun people.

Antiplasmodial activity

Figure 1 shows a graphically comparison of the ability of extracts to inhibit the growth of *Plasmodium*. From this graph, it can be seen that along with an increase of concentration, three extracts, *Physalis angulata* L., *Jatropha curcas* L. and *Alstonia spectabilis* R.Br. show a more significant increase in their inhibitory activity, compared to the other eight extracts. On average, the graph shows that a tenfold increase in concentration of these three extracts increases their antiplasmodial activity twice.

The antiplasmodial activity of each extract in the form of percentage of inhibition at each concentration level, and the IC_{50} values were listed in Table 2. The 50% inhibitory concentration, IC_{50}, represents the concentration that causes 50% reduction in *Plasmodium* growth. The smaller IC_{50} value indicates the better antiplasmodial activity of an extract, and vice versa, the greater the IC_{50} indicates a low inhibitory activity. In the same experimental conditions, the positive control, chloroquine diphosphate, has an IC_{50} value of 0.005 µg/mL.

Ouattara *et al*. categorized an extract with IC_{50} value lower than 5 µg/mL as a very active or strong antiplasmodial, 5–50 µg/mL as active or moderate antiplasmodial, 50–100 µg/mL as less active or weak antiplasmodial, and the IC_{50} value that higher than 100 µg/mL as inactive.[12] Based on these categories, the ethanolic extracts of *Physalis angulata* L. (IC_{50} 0.22 µg/mL), *Jatropha curcas* L. (IC_{50} 0.22 µg/mL) and *Alstonia spectabilis* R.Br. (IC_{50} 1.23 µg/mL) are strong antiplasmodials, while *Neoalsomitra podagrica* Steenis (IC_{50} 11.60 µg/mL), *Alstonia scholaris* (L.) R.Br. (IC_{50} 15.46 µg/mL), *Fatoua pilosa* Gaudich. (IC_{50} 24.92 µg/mL) and *Plumeria alba* L. (IC_{50} 36.39 µg/mL) are moderates. The extracts of *Cleome rutidosperma* DC. (IC_{50} 54.25 µg/mL), *Melia azedarach* L. (IC_{50} 63.52 µg/mL), *Strychnos ligustrina* Blume (IC_{50} 63.91µg/mL) and *Calotropis gigantea* (L.) R. Br. (IC_{50} 66.49 µg/mL) are weak antiplasmodial. In general, the ethanolic extracts of *P. angulata* L., *J. curcas* L. and *A. spectabilis* R.Br. showed good antiplasmodial effects on *Plasmodium falciparum* 3D7 strain, although they were relatively weaker than chloroquine diphosphates. The ethanolic extract of *Physalis angulata* L. showed the strongest antiplasmodial activity among the eleven plant extracts. This result is in line with the findings of other previous study using methanol and dichloromethane extracts of *P. angulata* L. leaves, where, these two extracts presented a very high activity with IC_{50}<3 µg/mL against the chloroquine sensitive 3D7 and chloroquine resistant W2 strains of *Plasmodium falciparum in vitro*. The extracts of *P. angulata* showed also a good inhibition of parasitemia *in vivo* on mice infected by *Plasmodium berghei*.[13]

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

Result of this study showed that ethanolic extract of *Plumeria 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 stem bark of this plant has reduced the level of parasitemia in mice infected by *Plasmodium berghei* to 16.4% and 20.0%, respectively, in eight days of evaluation.[15]

Although the ethanolic extract of *Alstonia scholaris* (L.) R.Br. stem bark in this study showed a moderate activity only, the results of another study showed that methanolic extract of stem bark showed excellent antiplasmodial activity with IC_{50} of 0.1650 ± 0.1100 µg/mL against *Plasmodium falciparum* 3D7 strain.[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]

In this study, ethanolic extracts of *Strychnos ligustrina* Blume, *Calotropis gigantea* (L.) R.Br., *Cleome rutidosperma* DC. and *Melia azedarach* L. showed weak activity against *Plasmodium falciparum* 3D7 strain, with IC_{50}>50 µg/mL. However, in several other studies using different solvents for extraction, it was found that these plants showed a good antiplasmodial activity. Water extract of *S. ligustrina* Blume wood inhibited *P. falciparum* growth *in vitro* by 98.1% at a concentration of 1.0 mg/mL, and was classified as a strong antiplasmodial.[18] Methanolic extract of *C. gigantea* (L.) R.Br. leaves showed a moderate antimalarial activity with an IC_{50} value of 12.17 µg/mL against *P. falciparum in vitro*, and a very good activity against *P. berghei in vivo*.[19] Ethanolic extract of *C. rutidosperma* DC. whole plant showed a moderate antimalarial activity with IC_{50} of 34.4 µg/mL, and 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. The *Strychnos ligustrina* Blume, *Calotropis gigantea* (L.) R.Br., and *Cleome rutidosperma* DC. which were the most frequently mentioned by the informants, were actually turned out to show a weak antiplasmodial activity in the laboratory testing. However, it does not mean that claims about the effectiveness of these plants as antimalarials are incorrect. Possible causes of non-synchronous data between frequency of citation by the informants and the laboratoric antiplasmodial evaluation of these plants can be explained as follows. Firstly, the use of ethanol as a solvent for extraction can cause differences in type and amount of antiplasmodial active compounds extracted into it, and then cause differences in the antiplasmodial activity shown by each plant extract. Secondly, the biochemical system in the human body is very different from the *in vitro* system, therefore, the results of *in vitro* antimalarial activity can not directly describe the actual events in human body. So, it is possible for a plant that is active as an antimalarial in human to be inactive in an *in vitro* testing, and vice versa. Thirdly, certain plant may not be *true antimalarial* (antiplasmodial) that works to kill or inhibit the growth of *Plasmodium*. It may be more likely an *indirect antimalarial* (antipyretic, analgesic or anti-inflammatory) that works to heal the symptoms related to malaria, and therefore, the test showed no significant activity as antiplasmodial. As is known, traditional treatment of malaria is a symptomatic healing with the main goal to reduce heat or fever, so it is possible that a plant used in the traditional treatment of malaria is more likely antipyretic than antiplasmodial.[21,22] The plants that have antimalarial properties can show a direct effect on *Plasmodium* by inhibiting the growth of *Plasmodium* or killing it, or giving indirect effects on the relationship between the human body and parasites. A plant that inhibiting or killing *Plasmodium* is called antiplasmodial or *true antimalarial*. The other plants may serve as *indirect antimalarial*, that affect the relationship between the human body as a host and the *Plasmodium*, for example as immunostimulant or antipyretic, or causing hemolysis and changes in membrane structure which results in the inhibition of the growth of *Plasmodium*.[23]

Searching of previous studies in any publications showed that this report of antiplasmodial activity of *Neoalsomitra podagrica* Steenis and *Fatoua pilosa* Gaudich. against *Plasmodium falciparum* 3D7 strain is the first. There were no studies of the antimalarial activity of these two plants carried out by other researchers before. The *F. pilosa* Gaudich. was only reported to have pharmacological activity as an antimycobacterial,[24] while *N. podagrica* Steenis has no known its pharmacological activities. Therefore, it is an open chance to make a further evaluation of the antimalarial activity of these two plants, and to identify antimalarial active compound(s) of them.

Phytochemicals content of the extracts

The results of phytochemical analysis using GC-MS are as shown in Table 3. From this table, we can see that for overall, these 11 extracts contain various types of natural products, such as alkaloids, terpenoids, steroids, coumarins, alcohols, thiols, phenolics, aldehydes, fatty acids, esters, etc. Several previous studies showed that many secondary plant metabolites such as alkaloid, flavonoid, xantone, quassinoid, triterpene and sesquiterpene have antiplasmodial activity, and thus, have the potential to be developed as antimalarial.[4,17] Although the types and amounts of compounds that can be identified by GC-MS are limited to the volatile compounds, the results showed that there are a quite number of compounds that were previously unknown to be contained in each of the extracts, for example the alkaloid brucine in the leaves of *Melia azedarach* L. Some of the compounds identified from those eleven plants extracts have been known to have antiplasmodial activity, such as alstonine, alstomacroline, pleiocarpamine, lupeol, amyrin and brucine.[13,15,17,25,26,27]

CONCLUSION

There are at least 50 plant species used by the Tetun ethnic people as oral antimalarial medicine. The *Alstonia scholaris* (L.) R.Br., *Alstonia spectabilis* R.Br., *Calotropis gigantea* (L.) R. Br., *Cleome rutidosperma* DC., *Fatoua pilosa* Gaudich., *Jatropha curcas* L., *Melia azedarach* L., *Neoalsomitra podagrica* Steenis, *Physalis angulata* L., *Plumeria alba* L. and *Strychnos ligustrina* Blume are some of the high cited plants. These 11 plants have been 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 potency 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 result is the first publication of the antiplasmodial activity of *N. podagrica* Steenis and *F. pilosa* Gaudich. against *Plasmodium falciparum* 3D7 strain.

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Conflict of interests

We declare that we have no any conflict of interests in publishing this article.

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

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)
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)

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

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

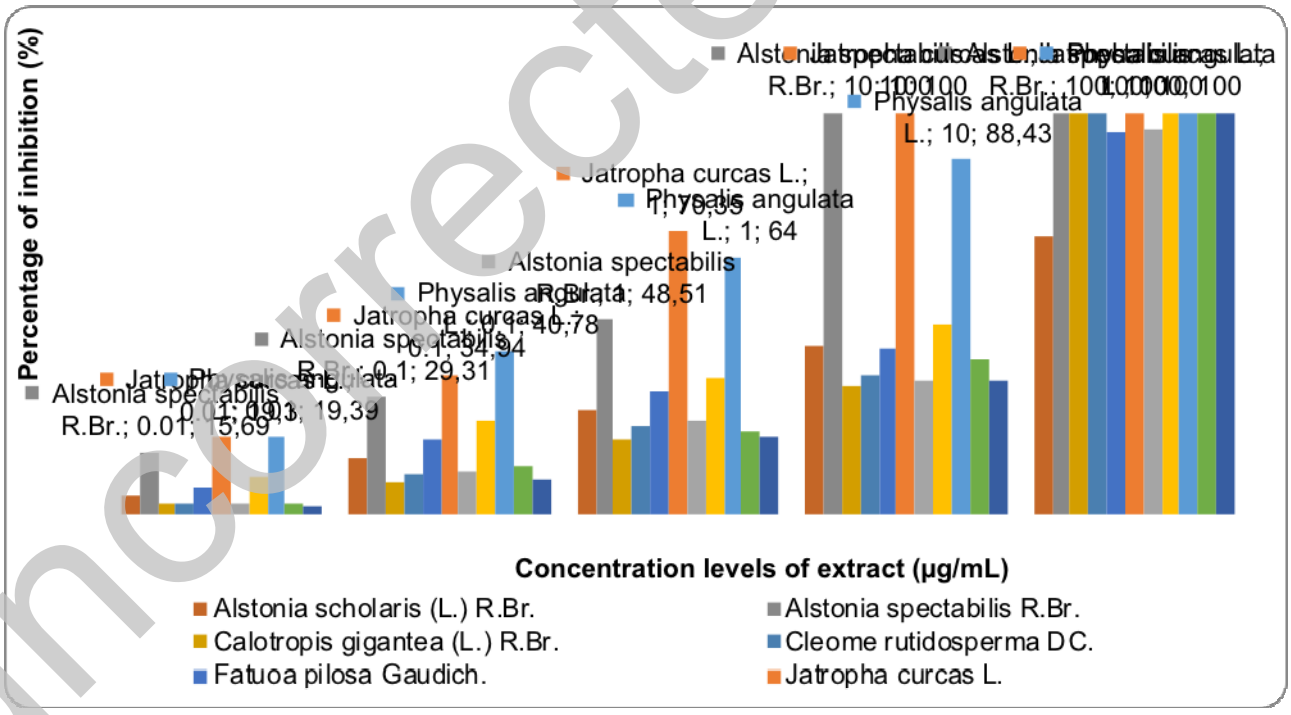

Table 2. Antiplasmodial activity of the plants extract

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

Table 3. Phytochemical contents of the plants'extracts identified using GC-MS

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-tetrahydroanthra[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
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	Bravelin	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
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