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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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ORIGINAL ARTICLE

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Received: 22.10.2019 Accepted: 26.12.2019

Turk J Pharm Sci 2021;18(1):80-90
DOI: 10.4274/tjps.galenos.2019.29000

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INTRODUCTION

Plants are a very valuable source in obtaining various pharmacological active substances to deal with various human health problems. 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.

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 phenominal lead compounds that have greatly contributed in reducing malaria deaths worldwide are quinines isolated from Cinchona sp. stem bark and artemisinin from Chinese medicinal plants Artemisia annua. Derivatives such as chloroquine, amodiaquine, primaquine, and mefloquine have been synthesized from quinines; 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.

Plasmodium’s increasing resistance to currently used antimalarial drugs encouraged researchers to continue searching for new and more effective antimalarials. Plants have become one of the most important sources in searching new potential antimalarials in traditional medicine of various ethnic groups worldwide. 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.

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. 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. 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 phytochemicals 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. It was carried out using O type human red blood cells (RBC) with 5% hematocrit suspended in RPMI 1640 medium.
In a CO₂ 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 CO₂ 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:

\[
\text{Percentage of parasitemia, } \text{P}=\left(\frac{\Sigma \text{iRBC}}{2\text{RBC}}\right)\times 100%.
\]

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:** \(\frac{\text{Pt}}{\text{Pnc}}\times 100%\),

**Percentage of inhibition:** 100% - percentage of growth, where \(\text{Pt}\) is the percentage of parasitemia in treated groups and \(\text{Pnc}\) is the percentage of parasitemia in the negative control group. \(\text{Pt}\) and \(\text{Pnc}\) were calculated at the beginning of test (0 h, \(P_o\)) and when the culture was harvested (48 h, \(P_a\)). The parasitemia’s actual percentage value was \(P_a - P_o\). 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/IIV/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, flowers (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* (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 *Neosolomitra podagrica* Steenis (4.26%).
| 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)                  |
| Siewtenia macrophylla King| Meliaceae          | Mahoni     | Stem bark         | 6 (6.38)                    |
| Plumeria alba L.          | Apocynaceae        | Mukrin     | Stem bark         | 6 (6.38)                    |
| Momordica balsamina L.    | Cucurbitaceae      | Bria fuik  | Leaves, fruit     | 5 (5.32)                    |
| Neolasmota podagrica Steenis | Cucurbitaceae     | Masin borat| Root              | 4 (4.26)                    |
| Wrightia pubescens R. Br. | Apocynaceae        | Lalitin feto| Leaves, root, stem bark | 3 (3.19)                  |
| Tabernaemontana pandacqua L. | Apocynaceae    | Lalitin | Stem bark         | 3 (3.19)                    |
| Adenophyllum correa (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 laker   | 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)                    |
| Dendrothoe 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 ciliaris (L.) P. Beauv. | Poaceae | Hae manlain | Root              | 1 (1.06)                    |
| Indigofera suffrutescens 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**

| Species                        | Family               | Part of Plant | IC₅₀ (μg/mL) |
|--------------------------------|----------------------|---------------|--------------|
| Nauclea orientalis (L.) L.     | Rubiaceae            | Kafiru        | 1 (1.06)     |
| Piper cubeba L.f.              | Piperaceae           | Kunus aleten  | 1 (1.06)     |
| Sterculia foetida L.           | Sterculiaceae        | Abano         | 1 (1.06)     |
| Tamarindus indica L.           | Fabaceae             | Sukaer        | 1 (1.06)     |
| Uvaria rufa Blume              | Annonaceae           | Koke          | 1 (1.06)     |
| Wendlandia burkillii Cowan     | Rhamnaceae           | Ai sisi       | 1 (1.06)     |
| Not identified                 | Not identified       | Moat tiris    | 1 (1.06)     |
| Not identified                 | Not identified       | Uas laomea    | 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 IC₅₀ values. The concentration that causes 50% reduction in *Plasmodium* growth is represented by IC₅₀. A smaller IC₅₀ value indicates the better the extract's antiplasmodial activity; and vice versa, greater IC₅₀ indicates lower inhibitory activity. In the positive control, with the same experimental condition, chloroquine diphosphate has an IC₅₀ value of 0.005 μg/mL.

**Results in this study showed that**

ethanolic extract of *P. alba* stem bark is a moderate antimalarial. 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.
Table 2. Antiplasmodial activity of plant extracts

| Plant extract                          | Inhibition percentage of extract on Plasmodium (%) at each level of concentration (μg/mL) | IC50 (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 |
| Fatua pilosa Gauchic                   |      | 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 | 32.22 | 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 | 21.88 | 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 IC50 of 0.1650±0.1100 μg/mL.16 The bark of *A. scholaris* (L.) R. Br. contains villosamine and macrocarpamine alkaloids, which are antimalarial active, with the IC50 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 IC50 >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 antimalarial 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 IC50 value of 12.17 μg/mL, and very good activity against *P. berghei* in vivo.19 Ethanol extract of *C. rutidosperma* DC. whole plant showed moderate antimalarial activity with IC50 of 34.4 μg/mL; however, its water extract was less active with IC50 >100 μg/mL against chloroquine-sensitive *P. falciparum* D10 strain in vitro.20

The results of this antimalarial activity evaluation did not linear rank to 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 antimalarial 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 antimalplasmodial 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 antimalplasmodial. 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 antimalplasmodial.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 antimalplasmodial activity of *N. podagrica* Steenis and *F. pilosa* Gauchich, 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* Gauchich. was only reported to have pharmacological activity as an antymycobacterial,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, xanthon, quaissinoid, triterpen, and sesquiterpen, 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, alstomacrine, pleiocarpamine, lupeol, amyrin, and brucine, are known to have antiplasmodial activity.13,15, 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. aiba* 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 antimalarial. 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.

**ACKNOWLEDGEMENTS**

This study was supported by The Directorate of Research and Community Service, Ministry of Research, Technology and Higher Education, Republic of Indonesia (Research Contract no: 0668/K8/KM/2018), Father Rector of Widya Mandira Catholic University, and Head of Yayasan Pendidikan Katolik Arnoldus Kupang. Thanks to all the informants for their participation in our field study in Belu and Malaka Districts, and to the analyst of Malaria Laboratory, Institute of Tropical Diseases, Airlangga University, for her assistance in the examination of antiplasmodial activity.

*Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.*
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.** |                | n-hexadecanoic acid                                                     | 0.15     |
| 20.63                          |                | 2-9-octadecenoic acid                                                   | 0.20     |
| 22.25                          |                | 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     |
| 30.91                          |                | Dihydroxyocrinine                                                      | 0.22     |
| **Alstonia spectabilis R. Br.** |                | Coniferol                                                              | 0.71     |
| 18.33                          |                | n-hexadecanoic acid                                                   | 0.53     |
| 20.67                          |                | 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                          |                | Homoeogonol                                                            | 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                          |                | (23S)-ethylcholest-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

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

| Compound Description                          | TAEK et al. Antimalarial Plants of West Timor, Indonesia |
|-----------------------------------------------|--------------------------------------------------------|
| **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 (9Z,12Z,15Z)-octadecatrien-1-ol           | 0.21                                                   |
| 32.11 (22E,24S)-stigmasta-4,22-dien-6-one      | 0.10                                                   |
| 32.92 2,2-dimethylcholest-4-en-3-one            | 0.35                                                   |
| 35.09 Dibenzo[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                                                   |
| 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-thiényl)-1-butanone                  | 2.06                                                   |
| **Nealsomitra podagrica Steenis**              |                                                        |
| 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                                                   |
| **Physalis angulata 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 |
| **Plumeria alba L.**                           |                                                        |
| 2.05 1,1-diethoxy-ethane                       | 0.24                                                   |
| 36.10 Brucine                                  | 0.73                                                   |
| **Strychnos ligustrina Blume**                 |                                                        |
| 2.05 1,1-diethoxy-ethane                       | 0.24                                                   |
| 36.10 Brucine                                  | 0.73                                                   |
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