Effect of selected local medicinal plants on the asexual blood stage of chloroquine resistant *Plasmodium falciparum*

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**Abstract**

**Background:** The development of resistant to current antimalarial drugs is a major challenge in achieving malaria elimination status in many countries. Therefore there is a need for new antimalarial drugs. Medicinal plants have always been the major source for the search of new antimalarial drugs. The aim of this study was to screen selected Malaysian medicinal plants for their antiplasmodial properties.

**Methods:** Each part of the plants were processed, defatted by hexane and sequentially extracted with dichloromethane, methanol and water. The antiplasmodial activities of 54 plant extracts from 14 species were determined by *Plasmodium falciparum* Histidine Rich Protein II ELISA technique. In order to determine the selectivity index (SI), all plant extracts demonstrating a good antiplasmodial activity were tested for their cytotoxicity activity against normal Madin-Darby Bovine Kidney (MDBK) cell lines by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.

**Results:** Twenty three extracts derived from *Curcuma zedoaria* (rhizome), *Curcuma aeruginosa* (rhizome), *Alpinia galanga* (rhizome), *Morinda elliptica* (leaf), *Curcuma mangga* (rhizome), *Elephantopus scaber* (leaf), *Vitex negundo* (leaf), *Brucea javanica* (leaf, root and seed), *Annona muricata* (leaf), *Cinnamomun iners* (leaf) and *Vernonia amygdalina* (leaf) showed promising antiplasmodial activities against the blood stage chloroquine resistant *P. falciparum* (EC₅₀ < 10 μg/ml) with negligible toxicity effect to MDBK cells *in vitro* (SI ≥10).

**Conclusion:** The extracts belonging to eleven plant species were able to perturb the growth of chloroquine resistant *P. falciparum* effectively. The findings justified the bioassay guided fractionation on these plants for the search of potent antimalarial compounds or formulation of standardized extracts which may enhance the antimalarial effect *in vitro* and *in vivo*.

**Background**

Malaria is one of the major public health problems in many tropical regions, including Malaysia. The resistance *Plasmodium falciparum* to common antimalarial drugs such as chloroquine, sulfadoxine-pyrimethamine [1-3] and artemisinin [4] have been reported. In response to this situation, as recommended by World Health Organization (WHO), Malaysian government has changed its first line antimalarial drug regimen to artemisinin-based combination therapy (ACT) such as the use of fixed dose artemether-lumefantrine combination (Riamet®) in the treatment of uncomplicated falciparum malaria (National Antibiotic Guideline 2008, Ministry of Health, Malaysia). However, the ability of the malaria parasite such as *P. falciparum* to develop and become resistant to ACT in the future cannot be denied [5-9]. Therefore, discovering new antimalarial drugs is a priority in the health sector. The challenges in malaria drug discovery are to find safe, cheap and effective antimalarial agents.

Plants have always been the main source for the search of new antimalarial drugs. Until the year of 2003, 1277 plant species from 160 families have been published by 33 tropical countries for their use in treatment of malaria and fevers [10]. In Peninsular Malaysia, about 21 plant species are used by the locals as traditional medicine for malaria treatment [11-13]. So, it is of pivotal to...
know the potential ingredients or candidates which play a major role in killing the malaria parasites. Thus, screening the plant extracts for antimalarial properties prior to bioassay guided fractionation and potent compound isolation is important.

Research on the effectiveness of medicinal plant extracts in inhibiting the growth of malaria parasite has been extensively studied worldwide. One good example is *Artemisia annua* where whole leaves extract of this plant has exhibited better antiplasmodial activity as compared to its isolated compound, artemisinin [14,15]. This shows that there are other unidentified compounds still remain in this plant.

Many of medicinal plants which grow in Malaysian soil have been reported by the local scientists for their antiplasmodial activities in vitro and in vivo (Table 1). In this study, another 14 selected Malaysian medicinal plants (Table 2) with traditional claims were screened for their antimalarial activities.

### Table 1 The list of Malaysian medicinal plants with potential antimalarial properties

| Plant species                      | Local name | Plant part | References |
|------------------------------------|------------|------------|------------|
| *Agathis borneensis*               | Raja Kayu | Leaf       | [17]       |
| *Alpinia galanga*                  | Lengkuas   | Rhizome    | [18]       |
| *Alstonia angustiloba*             | Akar Lumut | Leaf       | [19]       |
| *Alyxia lucida*                    | Mempelas Hari | Leaf    | [20]       |
| *Andrographis paniculata*          | Hempedu Bumi | Leaf    | [20-22]    |
| *Ardisia crenata*                  | Mata Ayarn | Root       | [21]       |
| *Ardisia crispa*                   | Mata Itik  | Leaf       | [17,20]    |
| *Blumea balsamifera*               | Sembong    | Root, Stem | [17]       |
| *Calotropis gigantea*              | Rembega    | Leaf       | [19]       |
| *Canica papaya*                    | Betik      | Leaf       | [21]       |
| *Cinnamomum iners*                 | Teja Lawang | Root     | [21]       |
| *Cocos nucifera*                   | Kelapa     | White flesh | [23]     |
| *Croton argyratum*                 | Semangkok  | Leaf       | [17]       |
| *Cryptocarya nigrig*               | Medang     | Stem       | [24]       |
| *Dyera costulata*                  | Jelutong   | Leaf       | [19]       |
| *Eurycoma longifolia*              | Tongkat Ali | Root     | [25-27]    |
| *Goniolobus macrophyllos*          | Selada     | Stem       | [17]       |
| *Goniolobus sorochealinii*         | Selada Puth | Root, Stem, Leaf | [17,22] |
| *Gynura procumbens*                | Sambung Nyawa | Leaf | [28]       |
| *Jasminum sambac*                  | Melati     | Flower     | [21]       |
| *Kopsia fruticosa*                 | Chabai Hutan | Leaf    | [19]       |
| *Lansium domesticum*               | Langsat    | Leaf, fruit skin | [29] |
| *Leuconotis eugenifolius*          | Cheret Murai | Bark | [30]       |
| *Macaranga triloba*                | Mahang Merah | Inflorescence | [31] |
| *Nigella sativa*                   | Jintan Hitam | Seed | [21]       |
| *Ocimum sanctum*                   | Selasih    | Whole plant | [21]       |
| *Phoebe grandis*                   | Medang     | Leaf       | [32]       |
| *Physalis minima*                  | Letup-ketup | Whole plant | [21]       |
| *Piper betle*                      | Sireh      | Leaf       | [33]       |
| *Piper sarmentosum*                | Kaduk      | Leaf       | [34]       |
| *Rennelia elliptica*               | Segemuk    | Root       | [35]       |
| *Tinospora crispa*                 | Patawali   | Stem       | [21]       |
| *Vallaris glabra*                  | Kerak nasi | Leaf       | [19]       |
| *Xylocarpus granatum*              | Nyireh     | Bark       | [21]       |

Extracts of these plants have been reported for their antiplasmodial (in vitro) and/or antimalarial (in vivo) activities by the Malaysian researchers from year 1995 to 2013.
for their antiplasmodial activity against the malaria parasite, chloroquine (CQ) resistant *P. falciparum* (K1) *in vitro* by using *P. falciparum* Histidine Rich Protein II (HRP2) ELISA technique [16].

### Methods

#### Plant collection and identification

All plant parts except *Brucea javanica* and *Annona muricata* were collected from the herbal garden of Herbal Medicine Research Centre (HMRC), Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. Both *B. javanica* and *A. muricata* were collected from Northern part of Peninsular Malaysia, Tupah Village, Kedah and Malaysian Agricultural Research and Development Institute (MARDI), Kedah, respectively. All plants parts except *B. javanica*, *Curcuma aeruginosa*, *C. mangga*, *C. phaeocaulis* were identified and authenticated by Dr. Richard Chung Cheng Kong and deposited in the Herbarium of Forest Research Institute Malaysia (FRIM), Kepong, Kuala Lumpur. The *B. javanica* was identified and authenticated by Mr. Sani Miran and deposited in the Herbarium of the Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor, Malaysia. The *Curcuma* species were identified and deposited in the Herbarium of Herbal Medicine Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia.

#### Plant extract preparation

The fresh plant materials (rhizome, leaf, root and seed) were cut into small pieces, dried and pulverized into powder before extraction with solvents in increasing polarity (Figure 1). The powdered materials were first

| Family | Plant species | Local name | Traditional claims by the Malays and aborigines | Voucher specimen |
|--------|---------------|------------|------------------------------------------------|-----------------|
| Asteraceae | Vernonia amygdalina | Pokok panjang hayat, Daun bismilah | Leaf: Used as a remedy for the management of diabetes, hypertension and hypercholesterolaemia. | PID231114-18 |
| Simaroubaceae | Brucea javanica | Melada pahit | Leaf: Used as a poultice for scurf, ringworm, boils, centipede bites and over enlarged spleen in fever. Root: Used as a decoction for colic, dysentery, fever, bodily pain and labour pain. Fruit and leaf: Used as an infusion to cure malaria. | UKMB4027 |
| Leeaceae | Lea indica | Mali-mali, memali | Leaf: Used as a poultice in skin complaints caused by poisonous caterpillars and body pains. | PID241114-18 |
| Lauraceae | Cinnamomum iners | Kayu manis hutan, Teja lawang | Root: Used as a decoction after childbirth, fever. Leaf: Used as a poultice for rheumatism. | PID271114-18 |
| Verbenaceae | Vitex negundo | Lenggundi, Lemuni hitam | Leaf: Used as a remedy for cleansing the birth canal and increased production of milk after childbirth. | PID261114-18 |
| Combretaceae | Terminalia catappa | Ketapang | Bark: Act as astringent in dysentery. Leaf: Act as a sudorific and applied to rheumatic joints, used internally for headache and colic. | PID251114-18 |
| Rubiaceae | Morinda elliptica | Mengkudu kecil, Mengkudu hutan | Leaf: Added to rice for loss of appetite, taken for head ache, choler, diarrhoea and fever. Applied in a pounded condition upon the spleen and wounds. A lotion for haemorrhoids and upon the body after childbirth. | MTM193 |
| Annonaceae | Annona muricata | Durian belanda | Leaf: Used as a poultice or an infusion externally for skin complaints in children, and for coughs and rheumatism. | MTA174 |
| Averokaceae | Elephantopus scaber | Tutup bumi, Tapak sulaiman | Used as a decoction (leaf or root) for preventive medicine after childbirth, in tonics, to drive out round worm, for coughs and venereal disease. The leaf decoction used as an antihelmintic, as a diuretic and for abdominal pains. The root decoction also used to arrest vomiting. | MTE174 |
| Zingiberaceae | Curcuma mangga | Temu pauh, Temu manga | Rhizome: Used as stomachic and as a mixture for continuous fever. | RZ14/10 |
| | Curcuma zedoaria | Temu kuning, Temu putih | Rhizome: Used in decoction as a tonic and for indigestion. | MTC0071 |
| | Curcuma aeruginosa | Temu hitam, Temu erang | Rhizome: Used as a tonic, for a cough and asthma. Externally used (pounded in coconut oil) for scurf. | RZ18/10 |
| | Alpinia galanga | Lengkuas | Rhizome: Used as a decoction to cure malaria. Carminative, stomachic and ointment for skin eruptions. | MTA0059 |
| | Curcuma phaeocaulis | Temu merah | Rhizome: Used by the local to treat tumours | RZ19/10 |

As referred in [11,36] and Global Information Hub in Integrated Medicine (www.globinmed.com).

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defatted with hexane and sequentially extracted with dichloromethane (DCM), methanol (MeOH) and sterile deionised water (H₂O) (80°C). Briefly, the resulting solutions from the first extraction using DCM were filtered through filter paper (Whatman No.1, England) to collect the supernatant from the residue. Organic supernatant were evaporated to dryness under reduced pressure with a rotary evaporator (Buchi Rotavapor R-200, Switzerland) at a temperature 40°C. The residue was further extracted by using MeOH similar to the procedure that carried out for the DCM. The resulting residue was air dried and used for further extraction with sterile H₂O at 80°C. The aqueous supernatant were freeze-dried to obtain crude extracts (Figure 1). All crude extracts (DCM, MeOH and H₂O) were stored at 4°C until used.

**The mammalian cells and parasites**
The CQ resistant *P. falciparum* strain, K1 was obtained from American Type Culture Collection (ATCC), The Malaria Research and Reference Reagent Resource Center (MR4). Madin-Darby Bovine Kidney (MDBK) cells were also obtained from ATCC. The cryopreserved parasites and cells were thawed and maintained in culture for further use in this study. Briefly, the cryopreserved parasites were thawed in waterbath at 37°C. The thawed parasites were transferred into 15 ml conical tubes and
equal volume of 3.5% natrium chloride (NaCl) were added drop wise and swirled to mix. The mixtures were centrifuged at 500 × g for 5 minutes and the supernatants were discarded. These steps were repeated twice before adding the complete RPMI 1640 culture medium (Invitrogen, USA) containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.2% sodium bicarbonate (NaHCO₃), 0.02 mg/ml gentamycin supplemented with 10% AB+ human serum (Invitrogen, USA).

For MDBK cells, the cryopreserved cells were thawed immediately in the waterbath at 37°C. The thawed cells were transferred in 15 ml conical tubes containing 1 ml of complete DMEM culture medium (Invitrogen, USA) containing 25 mM HEPES, 0.4% sodium bicarbonate (NaHCO₃), 100U of Penstrep (100U penicillin and 100U streptomycin) supplemented with 10% fetal bovine serum (FBS). The mixtures were centrifuged at 1000 × g, 4°C for 5 minutes. The supernatants were discarded. The cell pellets were transferred into 25 cm² culture flask containing 5 ml of complete DMEM culture medium. The suspension were mixed gently and incubated in 5% carbon dioxide (CO₂) incubator at 37°C.

In vitro culture and synchronization of P. falciparum
The CQ resistant P. falciparum were grown by candle jar technique (3% CO₂ and 17% O₂) [37]. The culture was set up in a 25 cm² culture flask with filtered vent and maintained in complete RPMI 1640 culture medium (Invitrogen, USA). The P. falciparum was grown in ‘O’ type fresh red blood cell (RBC) with the initial culture started with 1% parasitemia at 2.5% hematocrit. The parasite density was monitored daily by making thin blood smears stained with 10% Giemsa solution and observed under the microscope at 1000 times magnification. When the parasitemia of the parasite culture reached approximately 5 to 7%, the parasites were synchronized using 5% sorbitol [38] and cultured for one complete cycle prior to be used in in vitro P. falciparum HRP2 assay.

In vitro P. falciparum HRP2 assay
All crude extracts were evaluated in vitro for their antiplasmodial activities by HRP2 assay [16,39]. The extracts were solubilised in 100% dimethyl sulphoxide (DMSO) or sterile H₂O (H₂O extracts only) to get 5 mg/ml stocks. In preparation of extract or drug stock plates, the extracts (5 mg/ml) were serially diluted (2 fold dilution) to 7 point concentrations (ranging from 5 to 0.08 mg/ml) in DMSO from well A1 to A7 in a 96 well plate. Fifteen microliters of serially diluted stock extracts were transferred correspondingly into watery plates containing 225 µl of sterile H₂O. An aliquote of watery plates will be used in HRP2 assay.

Ring-infected RBCs with 5% parasitemia were adjusted to 0.05% parasitemia and 1.5% hematocrit. A total of 190 µl parasitized RBCs at 1.5% hematocrit were added into each well of the test plates. A total of 10 µl of serially diluted extracts from the watery plates prepared above were transferred into the test plates containing parasitized RBCs and incubated in a candle jar at 37°C for 72 hours. The final tested concentration ranging from 16 to 0.2 µg/ml. The final concentration of DMSO was 0.3%. Chloroquine (CQ) (Sigma, USA), quinine (Q) (Sigma, USA), mefloquine (Mef) (Sigma, USA) and artesiminine (Art) (Sigma, USA) were used as standard control to validate the test. The final tested concentration for standard control ranged from 1772.6 to 27.7 nM for CQ, 3495 to 54.6 nM for Q, 601.3 to 9.4 nM for Mef and 51.2 to 0.8 nM for Art. The negative control was the infected RBC without extracts or with sterile H₂O only. After 72 hours of incubation, the test plates were kept in -80°C overnight. The plates were thawed at room temperature to lyse the infected RBCs. The activity of the parasite-extract exposure (end point) was measured by HRP2 assay. One day prior to the assay, 100 µl of immunoglobulin M (IgM) capture antibody (MPFM-55A, ICL, Inc, Newberg, OR, USA) specific for P. falciparum HRP2 (1 µg/ml in phosphate-buffered saline (PBS)) were added to each well of a 96-well ELISA plates (Microlon 600, Greiner, Germany). The plates were covered and incubated at 4°C overnight. Following incubation, the contents of the wells were removed and the plates were washed three times with 0.05% PBS-Tween 20 (PBST). The non-binding sites of the ELISA plates were blocked with 200 µl/well of 2% bovine serum albumin in PBS for 2 hours at room temperature. Following the blocking step, the ELISA plates were washed 3 times with 200 µl of 0.05% PBST. Hundred microliters of the P. falciparum infected RBC lysates (freeze thawed) were transferred from the test plates into ELISA plates and incubated in humidity chamber for 1 hour at room temperature. The ELISA plates were washed as described above. Hundred microliters of the detector antibody (MPFG-55p, ICL, Inc, Newberg, OR, USA) conjugated with horseradish peroxidase (0.2 µg/ml in PBS) were added to each well, and incubated in humid chamber for 1 hour at room temperature. Following a subsequent washing step similar to the above, 100 µl of 3,3', 5,5'-tetramethylbenzidine (TMB) chromogen (Zymed Lab., Inc., San Francisco, CA, USA) was added to each well and incubated for 10 min in dark, followed by the addition of 50 µl of 1 M sulphuric acid. The absorbance was determined by using ELISA plate reader at a wavelength of 450 nm (FLUOstar Omega, Germany). The collected data were transferred to HN-nonLin software (malaria.farch.net) to get a 50% Effective Concentration (EC₅₀) value directly from the graph.
**In vitro cytotoxicity assay**

The MDBK cells were maintained in complete DMEM culture medium containing 25 mM HEPES, 0.4% sodium bicarbonate (NaHCO₃), 100U of Penstrep (100U penicillin and 100U streptomycin) supplemented with 10% fetal bovine serum (FBS). The cytotoxicity of the extracts were measured by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay [40]. Prior to the day of the test, the stock plates were prepared by serially diluting (2 fold dilution) the stock extracts (5 mg/ml) to 7 point concentration (ranging from 5 to 0.16 mg/ml) with DMSO or sterile H₂O (for H₂O extracts only). Then, 6 μl of serially diluted stocks were transferred into 96 well plates containing 294 μl of complete DMEM media (Medium plates). On the day of the test, MDBK cells were harvested and adjusted to 1 × 10⁴ cell per ml. A hundred microliter of cell suspension was seeded into each well of a 96-well plate and allowed to grow overnight. Then, 100 μl of test extracts taken from medium plate (as prepared above) were added to each well accordingly ranging final concentration of 0.8 to 50 μg/ml. The final concentration of DMSO in all test was less than 1%. All tests were performed in duplicate. The positive control for cell growth is the cell suspension without test substance while the negative control is the cell suspension with 0.05% Triton X 100. The culture was incubated at 37°C in 5% CO₂ incubator for 72 hours. Fifty microliters of MTT solution (5 mg MTT in 1 ml PBS and 2.5 ml DMEM media) were added to each well. The plates were further incubated for 4 hours at 37°C in 5% CO₂ incubator. The medium was removed and replaced with 200 μl of DMSO to solubilise the MTT formazan product. The solution was mixed for 15 min and once for 30 sec before measuring the absorbance at 540 nm with a micro plate reader (FLUOstar Omega, Germany). The percentage of growth inhibition and the EC₅₀ were estimated from a dose response curve.

**Determination of a selectivity index**

A selectivity index (SI), corresponding to the ratio between antiplasmodial and cytotoxic activities was calculated according to the following formula:

\[
SI = \frac{EC_{50\text{ normal cell lines}}}{EC_{50\text{Plasmodium}}}
\]

**Results**

**Antiplasmodial activity against CQ resistant *P. falciparum*, K1 strain**

A total of 54 extracts from different parts of 14 plant species (Table 2) were tested for antiplasmodial activity against chloroquine resistant *P. falciparum* by using HRP2 assay *in vitro*. Each part of the plants was extracted by 3 different solvents with increasing polarity (DCM-MeOH-H₂O). The discrimination of active extracts is based on ranked levels of antiplasmodial activity proposed by Rasoanaivo et al. [41]. The score for the test is classified as extracts with EC₅₀ value less than 0.1 μg/ml is considered to be very good, 0.1 to 1.0 μg/ml is good, 1.1 to 10 μg/ml is good to moderate, 11 to 25 μg/ml is weak, 26 to 50 μg/ml is very weak while more than 100 μg/ml is considered inactive [41]. So, any extracts which exhibit an EC₅₀ value less than 10 μg/ml (EC₅₀ ≤ 10 μg/ml) is considered to have potential or promising antiplasmodial activity. The antiplasmodial HRP2 assay on the plant extracts were performed in parallel with the standard antimalarial drugs such as CQ, Q, Mef and Art which act as a control for the validity of the assay (Table 3). Briefly, the results of each assay were validated by determination of EC₅₀ values produced by the standard antimalarial drugs against CQ resistant *P. falciparum*. In this study, the assay is considered valid when the EC₅₀ value of CQ is more than 100 nM [42].

The DCM, MeOH and H₂O extracts from 14 plants species showed wide range of antiplasmodial activities (Table 4). Overall 41 extracts (76%) from 13 plant species showed promising antiplasmodial activity (EC₅₀ < 10 μg/ml) (Table 5). In detail, there are 11 extracts from 5 plant species fall within a good level of antiplasmodial activity (EC₅₀ < 1 μg/ml) (Table 5). Good to moderate antiplasmodial activities (EC₅₀ = 1.1-10 μg/ml) were detected in 30 extracts from 12 plant species (Table 5). Thirteen extracts from 6 plant species were considered weak or inactive as these extracts exhibited EC₅₀ values of more than 10 μg/ml or less than 50% parasite inhibition at the highest tested concentration (EC₅₀ > 15.7 μg/ml) (Table 5). Overall, DCM and MeOH extracts were more active against CQ resistant *P. falciparum* with good level of antiplasmodial activity (Table 5).

**Cytotoxicity activity to mammalian MDBK cell line**

Forty one extracts from 13 different plant species with promising antiplasmodial activity (EC₅₀ < 10.0 μg/ml), were subjected to MTT cytotoxicity assay and tested for its cytotoxicity activity to mammalian MDBK cell line. Each test was performed in duplicate and validated based on the EC₅₀ threshold for each standard antimalarial drugs against the *P. falciparum* K1 strain in the lab. If the EC₅₀ value of the standard antimalarial drugs is out of range, the test considered invalid.

| Table 3 Representative of *in vitro* antiplasmodial activity of standard drugs against *P. falciparum* K1 strain |
|---------------------------------------------------------------|
| Drugs            | EC₅₀ values (µg/ml) |
| Chloroquine      | 149.43            |
| Quinine          | 152.19            |
| Mefloquine       | 23.49             |
| Artemisinin      | 1.80              |

Data are presented as mean of three independent experiments performed in duplicate assays.
| Plant name          | Parts          | Extracts | EC<sub>50</sub> (μg/ml) | Selectivity index (SI) |
|--------------------|----------------|----------|--------------------------|------------------------|
|                    |                |          | P. falciparum K1 | MDBK cells          |
| Curcuma zedoaria   | Rhizomes       | DCM      | 2.38                     | 37.61                  | 15.92               |
|                    |                | MeOH     | >15.70                   | NT                     | ND                   |
|                    |                | H<sub>2</sub>O | >15.70                   | NT                     | ND                   |
| Vernonia amygdalina| Leaves         | DCM      | 3.36                     | 157.69                 | 47.00               |
|                    |                | MeOH     | 1.09                     | >300                   | >274.39             |
|                    |                | H<sub>2</sub>O | 8.23                     | >600                   | >72.95              |
| Alpinia galanga    | Rhizomes       | DCM      | 1.64                     | 3.92                   | 2.40                |
|                    |                | MeOH     | 8.06                     | 87.95                  | 10.92               |
|                    |                | H<sub>2</sub>O | 1.84                     | >50                    | >27.16              |
| Brucea javanica    | Leaves         | DCM      | 0.55                     | 15.13                  | 27.64               |
|                    |                | MeOH     | 5.27                     | 87.95                  | 16.70               |
|                    |                | H<sub>2</sub>O | 1.29                     | 1.90                   | 1.48                |
|                    | Roots          | DCM      | 0.47                     | 6.54                   | 14.05               |
|                    |                | MeOH     | 0.58                     | 6.95                   | 12.06               |
|                    |                | H<sub>2</sub>O | 4.49                     | 9.59                   | 2.14                |
|                    | Green seeds    | DCM      | >15.70                   | NT                     | ND                   |
|                    |                | MeOH     | 0.25                     | NT                     | ND                   |
|                    |                | H<sub>2</sub>O | 1.47                     | NT                     | ND                   |
|                    | Black seeds    | DCM      | >15.70                   | NT                     | ND                   |
|                    |                | MeOH     | 1.96                     | 7.70                   | 3.92                |
|                    |                | H<sub>2</sub>O | 5.40                     | 24.24                  | 4.49                |
| Leea indica        | Leaves         | DCM      | >15.70                   | NT                     | ND                   |
|                    |                | MeOH     | >15.70                   | NT                     | ND                   |
|                    |                | H<sub>2</sub>O | >15.70                   | NT                     | ND                   |
| Cinnamomun iners   | Leaves         | DCM      | 1.95                     | 199.15                 | 102.58              |
|                    |                | MeOH     | 0.63                     | >400                   | >636.61             |
|                    |                | H<sub>2</sub>O | 0.62                     | >100                   | >161.29             |
| Vitex negundo      | Leaves         | DCM      | 1.10                     | 25.04                  | 22.80               |
|                    |                | MeOH     | 2.17                     | >50                    | >23.10              |
|                    |                | H<sub>2</sub>O | >15.70                   | NT                     | ND                   |
| Terminalia catappa | Leaves        | DCM      | 5.29                     | >50                    | >9.45<sup>a</sup>   |
|                    |                | MeOH     | 5.19                     | >50                    | >9.63<sup>a</sup>   |
|                    |                | H<sub>2</sub>O | 4.28                     | 25.02                  | 5.84                |
| Morinda elliptica  | Leaves         | DCM      | 9.08                     | >50                    | >5.51<sup>a</sup>   |
|                    |                | MeOH     | 7.76                     | >50                    | >6.45<sup>a</sup>   |
|                    |                | H<sub>2</sub>O | 0.50                     | 31.05                  | 62.10               |
| Annona muricata    | Leaves         | DCM      | 0.61                     | 40.48                  | 66.47               |
|                    |                | MeOH     | 0.26                     | >100                   | >387.60             |
|                    |                | H<sub>2</sub>O | 0.27                     | >200                   | >756.14             |
| Curcuma mangga     | Rhizomes       | DCM      | 6.59                     | 36.66                  | 4.09                |
|                    |                | MeOH     | 1.81                     | >50                    | 15.44               |
|                    |                | H<sub>2</sub>O | 2.30                     | 31.85                  | 11.27               |
against the MDBK cell lines (Table 4). In fact, the pharmacological efficacy of the extracts is considered selective and nontoxic when SI is ≥10 [43]. Twenty three of antiplasmodial plant extracts (DCM, MeOH or H₂O) from 11 plant species showed promising antiplasmodial activity (EC₅₀ ≤ 10 μg/ml) with no toxic effect (SI ≥ 10) [43]. Twenty three extracts from 11 plant species showed promising antiplasmodial activity (EC₅₀ ≤ 10 μg/ml) with no toxic effect (SI ≥ 10).

**Discussion**

The present study has identified the antiplasmodial activity in selected plant extracts by HRP2-based assay or HRP2 ELISA technique. There are other reports on antiplasmodial studies using different screening methods such as WHO schizont maturation test [44], isoopic assay [45], pLDH enzymatic assay [46,47], SYBR Green I assay [48-50] or fluorometric assay [51]. Briefly, the HRP2-based assay is a very sensitive and specific measures of *P. falciparum* growth by quantifying parasite specific biomolecule, HRP2. The suitability, reproducibility and sensitivity of HRP2-based assay in antimalarial drug screening is well documented since year 2002 [16,39,52-55]. The HRP2-based assay is comparable with other techniques because the result produced by this assay has been previously shown to be closely parallel those obtained from the isotopic assay, traditional WHO schizont maturation tests [54] and SYBR green I assay [56].

Eleven plants (79%) were identified to possess promising antimalarial properties in at least one of their extracts (DCM, MeOH or H₂O). These findings are based on their potent antimalarial activities (EC₅₀ ≤ 10 μg/ml) and high preferences in killing the malaria parasite rather than mammalian cell line (SI ≥10) (Table 4). Most of the potential antimalarial activity were exhibited by DCM and MeOH extracts which may be related to the presence of alkaloids, terpenoids and flavonoids [57,58]. Theoretically, the purpose of using this extraction technique is to extract specific classes of phytochemical constituents from non-polar compounds to polar compounds [59]. The crude extract of DCM usually contains intermediate polarity of compounds such as alkaloids, steroids and terpenoids [59-61]. These classes of compounds especially alkaloids are well known as active constituents against antimalarial activity. In fact, one of the oldest and most known antimalarial drug, quinine belongs to this class of compounds. In addition, an example of common terpenoids is artemisinin, the most potent antimalarial to date [58]. Extraction with methanol will extract more polar compounds such as flavonoid glycosides, saponin, tannins and anthocyanins [59-61]. The antimalarial activity from these classes of compounds especially flavonoids have been described earlier [57]. Further extraction with water will extract high polarity of compounds such as phenolic acids, sugars and glycosides [59-61].

In another point of view, majority of these plants also possessed at least 1% of CQ antimalarial activity indicating the potential of these plants to be the source of antimalarial candidates. For example, the ethanolic extract of *Artemisia annua* Linn leaves (the source of artemisinin)
inhibited the growth of CQ resistant (K1) and CQ sensitive (3D7) strains of *P. falciparum* with IC$_{50}$ of 10.4 μg/ml and 21.8 μg/ml, respectively [49]. So, even the plant like *A. annua* with weak antiplasmodial activity (only 0.007% and 0.004% of artesunate activity against *P. falciparum* K1 and 3D7, respectively) contains the most potent antimalarial compound to date. This phenomenon may also apply to the plants extracted in this study.

In this study, *A. galanga* (rhizome), *C. iners* (leaf), *C. zedoaria* (rhizome), *E. scaber* (leaf), *C. mangga* (rhizome) and *E. scaber* (leaf) were for the first time reported for their good level of antiplasmodial activities in vitro. Other potent antiplasmodial plants such as *C. aeruginosa* (rhizome) [62], *V. negundo* (leaf) [46], *B. javanica* (leaf and root) [45,51,62], *A. muricata* (leaf) [63] and *V. amygdalina* (leaf) [44,64-68] have been widely studied and were further discussed in this section.

### Table 5 The summary of antiplasmodial level of extracts from different parts of 14 plant species

| Antiplasmodial level | Plant species | Parts | Extracts |
|----------------------|---------------|-------|----------|
| **Good** EC$_{50}$ = 0.1-1.0 μg/ml | *A. muricata* | Leaves | DCM, MeOH, H$_2$O |
|                      | *B. javanica* | Green seeds | MeOH |
|                      | *C. iners* | Roots | DCM, MeOH |
|                      | *E. scaber* | Leaves | DCM |
|                      | *M. elliptica* | Leaves | H$_2$O |
|                      | *C. zedoaria* | Leaves | H$_2$O, MeOH |
| **Good to moderate** EC$_{50}$ = 1.1-10 μg/ml | *V. amygdalina* | Leaves | DCM, MeOH and H$_2$O |
|                      | *A. galanga* | Rhizomes | DCM, MeOH and H$_2$O |
|                      | *T. catappa* | Leaves | DCM, MeOH and H$_2$O |
|                      | *C. mangga* | Rhizomes | DCM, MeOH and H$_2$O |
|                      | *C. aeruginosa* | Rhizomes | DCM, MeOH and H$_2$O |
|                      | *C. zedoaria* | Rhizomes | DCM |
|                      | *E. scaber* | Leaves | DCM |
|                      | *B. javanica* | Roots | H$_2$O |
|                      | *C. iners* | Leaves | DCM |
|                      | *V. negundo* | Leaves | DCM and MeOH |
|                      | *M. elliptica* | Leaves | DCM and MeOH |
|                      | *C. phaeocaulis* | Rhizomes | DCM and MeOH |
| **Weak** EC$_{50}$ = 11-25 μg/ml | *C. phaeocaulis* | Rhizomes | H$_2$O |
|                      | *B. javanica* | Green seeds | DCM |
|                      | *C. zedoaria* | Rhizomes | H$_2$O |
|                      | *V. negundo* | Leaves | H$_2$O |
|                      | *E. scaber* | Leaves | H$_2$O |
|                      | *L. indica* | Black seeds | DCM |
|                      | *C. zedoaria* | Rhizomes | MeOH and H$_2$O |
|                      | *V. negundo* | Leaves | H$_2$O |
|                      | *E. scaber* | Roots | DCM, MeOH and H$_2$O |

The level of efficacy of extracts were ranked according EC$_{50}$ values which based on the threshold for *in vitro* antiplasmodial activity proposed by Rasoanaivo et al. [41]. Forty one extracts from 13 plant species were categorized to have good and good to moderate antiplasmodial level.

The *A. galanga* has been identified as one of the plants traditionally used in some part of Peninsular Malaysia [11]. The *in vitro* antiplasmodial data of *A. galanga* rhizome extracts (EC$_{50}$ < 10 μg/ml) reported by present study is complementing the *in vivo* study conducted by Al-Adhroey et al. [11]. The MeOH extract of the rhizome exhibited a significant suppressive, curative and prophylactic activities on *P. berghei* infected mice. The antimalarial properties of MeOH extract of *A. galanga*
rhizome could be governed by its active constituents such as flavonoids and terpenoids [18]. In addition, both terpenoids and flavonoids related compounds have been previously shown to exhibit antiplasmodial activities against several *P. falciparum* strains [57].

**E. scaber**

In this study, the good level of antiplasmodial activity showed by the MeOH extract of *E. scaber* leaf (EC\(_{50} = 0.27\) µg/ml) was contradictory to the study reported by Kantamreddi and Wright [46]. According to Kantamreddi and Wright [46], the MeOH extract of the leaves of this plant was considered inactive (IC\(_{50} = 133.8\) µg/ml) against CQ resistant *P. falciparum* (K1) [46]. The possible reason for the contradictory results could be due to the differences in the duration of incubation in antimalarial assay. The incubation period for the present study is longer (72 hours) than the Kantamreddi and Wright [46] study (48 hours). The 72 hours incubation period allows the activity of the substance to affect the merozoite reinvasion process whereas the 48 hours incubation period will only affect the intraerythrocytic growth of the malaria parasites. In this case, the MeOH extract of this plant might not so effective against the ring to schizont intraerythrocytic stages of *P. falciparum*. Other species of *Elephantopus* such as *E. mollis* have been shown to possess potential antimalarial activity (IC\(_{50} = 2.2\) µg/ml) against CQ resistant *P. falciparum* (K1) [69].

**M. elliptica**

To our knowledge, this plant had not yet been investigated for the *in vitro* antimalarial activity. However other species of *Morinda*, *M. morindoides* has been shown to exhibit a pronounced antimalarial activity [64].

**C. iners**

The only antimalarial study on *C. iners* was previously reported for its roots extract. However, the MeOH extract of *C. iners* roots exhibited weak antimalarial activity (IC\(_{50} = 12.7\) µg/ml) against CQ resistant *P. falciparum* (FCR-3) [21]. In contrast, the leaves extract (MeOH) of this plant which was prepared by present study exhibited a good antimalarial activity (EC\(_{50} = 0.63\) µg/ml) (Table 4). In addition, other *Cinnamomum* species, *C. griffithii* has been reported to elicit a promising antimalarial activity (IC\(_{50} < 10\) µg/ml) against both CQ sensitive and resistant *P. falciparum* strains [70]. To our knowledge, there is no compound related to this plant reported for antimalarial activity.

**Curcuma sp.**

The member of *Curcuma* plant species such as *C. zedoaria*, *C. mangga*, *C. aeruginosa* and others were well studied for their antiparasiticidal properties [71]. The H\(_2\)O extracts of *C. xanthorrhiza* and *C. aeruginosa* were previously found to be effective in inhibiting *P. falciparum* *in vitro* (40% and 90% inhibition, respectively). However the concentration of the extracted used (1 mg/ml) was too high [62]. Moreover, the major antimalarial compounds like curcumin and its derivatives such as demethoxycurcumin and bis-demethoxycurcumin isolated from *C. longa* exhibited high IC\(_{50}\) value (IC\(_{50} > 5\) µM) [72]. In contrast, by different technique of plant extraction, the present study showed a promising antimalarial activity (EC\(_{50} < 10\) µg/ml) of *C. zedoaria* (DCM extract), *C. aeruginosa* (H\(_2\)O extract) and *C. mangga* (MeOH and H\(_2\)O extracts) with negligible toxic effect on normal cell line (SI >10) (Table 4 and 5). Although the antimalarial activity of *Curcuma sp* isolated compound such as curcumin is considered weak, it was found to be very effective in antimalarial drug combination study [15,73-75].

**V. amygdalina**

The *V. amygdalina* plant is also found in African countries and is widely used traditionally in treating fever, malaria, measles, diabetes, worms, hypertension and others [76,77]. The ethanol, petroleum ether, methylene chloride and MeOH extracts of *V. amygdalina* leaf have been previously reported to elicit IC\(_{50}\) values of less than 10 µg/ml against *P. falciparum* [64,65]. Both ethanolic and H\(_2\)O extracts of *V. amygdalina* leaf has also been shown to inhibit schizont maturation of fresh *P. falciparum* isolates from patients with negligible toxicity in rats [44]. In addition, the *in vivo* antimalarial activity of this plant has also been reported [66-68]. *In vivo* drug combination experiment, the decoction of *V. amygdalina* leaves has the ability to enhance the CQ activity in *P. berghei* infected mice [68]. Furthermore, the infusion of *V. amygdalina* leaves has been clinically tested against the uncomplicated malaria where the parasite clearance has been documented [78]. So, it is not surprising to see a promising antimalarial activity of *V. amygdalina* leaf extracts (DCM, MeOH and H\(_2\)O extracts) as showed by the present study (EC\(_{50} < 10\) µg/ml) (Table 4). The antimalarial property of this plant could be due to the presence of its active constituents, sesquiterpene lactones such as vernolepin, vernolin, vernolide, vernodaline and hydroxyvernodalin [79].

**B. javanica**

The *B. javanica* grows in Asia Pacific region including China, Indonesia, Malaysia and Thailand [45]. Different parts of this plant such as fruit, roots, seeds, stems and bark are traditionally used in treating variety of diseases including babesiosis, malaria and cancer. The H\(_2\)O extract of *B. javanica* leaves, fruits and bark have been shown to possess a strong antimalarial activity against
P. falciparum [62]. In the present study, the DCM and MeOH extracts of B. javanica leaves and roots showed good (EC_{50} < 1 μg/ml) to good to moderate (EC_{50} = 1.1 to 10 μg/ml) level of antiplasmodial activity against CQ resistant P. falciparum, K1 (Table 4). By similar extraction procedure, the DCM, MeOH and H_{2}O extracts B. javanica roots have also been previously reported to possess a good to moderate level antiplasmodial activity against another CQ resistant P. falciparum strain, W2 with IC_{50} ranging from 1.0 to 2.0 μg/ml [51]. The antiplasmodial activity showed by this plant might be governed by its active constituents such as quassinoids, alkaloids (brucecanthinoside) and triterpenoids (Bruceajavanin A and dihydrobruceajavanin A) [80,81]. Other plant from the same family (Simaroubaceae) like E. longifolia also exhibited an antiplasmodial activity ruled by its quassinoids and alkaloids contents [25,82]. In addition, the present study has identified the antiplasmodial activity from seeds of this plant (MeOH and H_{2}O extracts). However, the DCM extract of the seeds seem to have weak or no antiplasmodial activity (EC_{50} > 15.7 μg/ml) (Table 4). In another study with different extraction procedure, the extracts of B. javanica fruits (ethanol, MeOH-ethanol, aqueous-MeOH residue, ethyl acetate and ethyl alcohol extracts) have been shown to have a promising antiplasmodial activity against P. falciparum K1 strain (IC_{50} < 10 μg/ml). In contrast, the H_{2}O extract of the fruits showed weak antiplasmodial activity (IC_{50} > 10 μg/ml) [45].

V. negundo
The V. negundo has been traditionally used in India as antiseptic, anti-inflammatory, anti-pyretic, treating enlargement of spleen and others [83]. The present study has highlighted the potential antiplasmodial activity of DCM (EC_{50} = 1.1 μg/ml) and MeOH (EC_{50} = 2.17 μg/ml) extracts of V. negundo leaves against the CQ resistant P. falciparum. The MeOH extracts of V. negundo leaf has been previously shown to exhibit a promising antiplasmodial activity against CQ sensitive (IC_{50} = 9.5 μg/ml) P. falciparum but not to CQ resistant P. falciparum strain (IC_{50} = 19.8 μg/ml). In similar study, the MeOH extract of the flower of this plant also exhibited a promising antiplasmodial activity with IC_{50} value of against CQ sensitive (IC_{50} = 2.8 μg/ml) but not to CQ resistant P. falciparum (IC_{50} = 17.8 μg/ml) [46].

A. muricata
As reported by Osorio et al. [63], the hexane, ethyl acetate and MeOH extract of A. muricata leaf exhibited good to moderate level of antiplasmodial activities against CQ sensitive P. falciparum strain F32 (IC_{50} ranging from 7.2 to 9.2 μg/ml) but not to CQ resistant strain W2 (IC_{50} ranging from 10.4 to 38.6 μg/ml) [63]. However, the most potent ethyl acetate extracts of this plant is considered toxic to U-937 cells (human monocytes) (SI = 1.1 and 0.2 for F32 and W2 P. falciparum strains, respectively). On the other hand, with different extraction approach, the present study not only showing the promising antiplasmodial activity of A. muricata leaves extracts (DCM, MeOH and H_{2}O extracts) (EC_{50} < 10 μg/ml), but also the non-toxic activity of the extract to MDBK cells (SI = 66-756) (Table 4 and 5).

Conclusions
Twenty three extracts derived from C. zedoaria (rhizome), C. aeruginosa (rhizome), A. galanga (rhizome), V. negundo (leaf), M. elliptica (leaf), C. mangga (rhizome), E. scaber (leaf), B. javanica (leaf and root), A. muricata (leaf), C. iners (leaf) and V. amygdalina (leaf) showed the best antiplasmodial activities against the blood stage chloroquine resistant P. falciparum with no toxic effects on MDBK cells (SI ≥ 10). The present study has also scientifically supported the efficacy of B. javanica and A. galanga which are used traditionally to treat malaria in Peninsular Malaysia. Although these plant extracts were able to kill the P. falciparum in vitro, further in vivo evaluation is needed to demonstrate their efficacy in treating mammalian malaria model. Furthermore, the bioassay guided fractionation is a way forward for determination of bioactive compounds which will lead to the formulation of new anti-malarial drugs or standardized antimalarial extracts.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MRMAR involves manuscript preparation, data interpretation and analysis. AA and RA carried out the plant extraction and cytotoxicity test. NFAJ involves in antiplasmodial screening of plant extracts and data analysis. MIW involves in consultation of plant extraction procedure. SHSZ involves in the preparation of plant specimens for species identification. NRA contributes in overall project design and concept. ZI involves in revising and giving the final approval of the version to be published. All authors read and approved the final manuscript.

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Acknowledgements
The authors would like to thank the Director General of Health Malaysia for the permission to publish this paper. We thank the Director for the Institute for Medical Research (IMR), Kuala Lumpur for her critical review and support in publishing this paper. The authors also wish to thank the staff of Phytochemistry and Information Units, Herbal Medicine Research Centre, IMR, Kuala Lumpur who has contributed in this study. This study, NWIR-13-024-16108 was supported by the National Institute of Health, Ministry of Health Malaysia.

Received: 3 March 2014 Accepted: 11 December 2014
Published: 15 December 2014

References
1. Abdullah NR, Norahmad NA, Jelip J, Sulaiman LH, Mohd Sidek H, Ismail Z, Noedl H. High prevalence of mutation in the Plasmodium falciparum dhfr
and dhps genes in field isolates from Sabah, Northern Borneo. Malar J 2013, 12(1):198.

2. Norahmad NA, Abdullah NR, Yacob N, Jelip J, Dony JF, Ruslan KF, Sulaiman LH, Sidek HM, Noedl H, Ismail Z: High prevalence of pfcrf K76T mutations among Plasmodium falciparum isolates from Sabah, Malaysia. Southeast Asian J Trop Med Public Health 2011, 42(6):1322–1326.

3. Lokman Hakim S, Sharifah Roosi SW, Zukurnia Y, Noor Rain A, Mansor SM, Palmer K, Navaratnam V, Mak JW: Plasmodium falciparum: increased proportion of severe resistance (RfI and RfII) to chloroquine and high rate of resistance to sulfadoxine-pyrimethamine in Peninsular Malaysia after two decades. Trans R Soc Trop Med Hyg 1996, 90(3):294–297.

4. Phyo AP, Nikhoma S, Stepievskas K, Ashley EA, Nair S, McGready R, Ier Moo C, Al-Saai S, Dondorp AM, Lwin KM, Singhasivanon P, Day NP, White NJ, Anderson TJ, Nosten F: Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. Lancet 2012, 379(9803):1960–1966.

5. Baillairre FN, Rosenthal PJ: Prolonged selection of pfmdr1 polymorphisms after treatment of falciparum malaria with artemether-lumefantrine in Uganda. J Infect Dis 2011, 204(7):1120–1124.

6. Famert A, Uising J, Toffenvant T, Rino J, Karlsson L, Sprearel E, Lindergard N: Artemether-lumefantrine treatment failure despite adequate lumefantrine day 7 concentration in a traveller with Plasmodium falciparum malaria after returning from Tanzania. Malar J 2012, 11:76.

7. Wong SK, Lim YY, Abdullah NR, Ismail Z: Antimalarial activity of some Malaysian anti-malarial plants: a community based survey. J Ethnopharmacol 2011, 132(1):364–366.

8. Wongsrichanalai C, Sibley CH: Antimalarial activity of extracts of Malaysian medicinal plants. J Ethnopharmacol 2012, 139(1):198–206.

9. Wongsrichanalai C: Artemisin resistance or artemisinin-based combination therapy resistance? Lancet Infect Dis 2013, 13(2):114–115.

10. Na-Bangchang K, Muhamad P, Ruaengweerayut R, Chaijaroenkul W, Wongsrichanalai C, Al-Saai S, Dondorp AM, Lwin KM, Singhasivanon P, Day NP, White NJ, Anderson TJ, Nosten F: Identification of resistance of Plasmodium falciparum to artesunate-mefloquine combination in an area along the Thai-Myanmar border: integration of clinico-parasitological response, systemic drug exposure, and in vitro parasite sensitivity. Malar J 2013, 12:263.

11. Noor Rain A: Antimalarial activities of selected Malaysian medicinal plants. Phytomedicine 2012, 19(1):29–35.

12. Ashby VA, Kayay K, Chisholm R, Muzzarelli RA, Walker SP, Woodland JG: Histidine-rich protein II: a novel approach to malaria drug sensitivity testing. Antimicrob Agents Chemother 2002, 46(6):1658–1664.

13. Noor Rain A, Khozirah S, Mohd Ridzuan MA, Hong BK, Rohaya C, Rosilawati M, Hamdani I, Badul A, Zakiah I: Antiplasmodial properties of some Malaysian medicinal plants. Trop Bimed 2007, 24(1):29–35.

14. Al-Adroey AH, Nor ZM, Al-Mekhlafi HM, Mahmud R: Ethnobotanical study on some Malaysian anti-malarial plants: a community based survey. J Ethnopharmacol 2010, 132(1):362–364.

15. Ong HC, Chua S, Milow P: Ethnopharmacological studies on plants used for the treatment of malaria. In Traditional Medicinal Plant and Malariology. Edited by Wllcox M, Bodeker G, Raoanoafo P. Florida: CRC Press; 2004:187–197.

16. Deharo E, Arango GJ, Aragon R, Munoz V, Callapa J, Weniger B, Robledo S, Arango G, Deharo E: Ethnobotanical study of antiprotozoal activities of Piper betle L. Molecules 2011, 16(1):107–118.

17. Noedl H, Broennert J, Yingyuen K, Attinayr B, Kollatsch H, Fukuda M: Simple histidine-rich protein 2 double-site sandwich enzyme-linked immunosorbent assay for use in malaria drug sensitivity testing. Antimicrob Agents Chemother 2002, 46(3):857–858.

18. Moorman T: Rapid colorimetric assay for cell growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983, 68(3–4):253–255.

19. Rasoanaivo P, Ramitaritsahambola D, Rafato H, Rakotondramanana D, Robjana B, Rakotozafy A, Ramitaritsahambola S, Labaide M, Greffier P, Allorge L, Mambu L, Frappier F: Screening extracts of Madagascar plants in search of antimalarial compounds. Phytother Res 2004, 18(3):472–477.

20. Ridoux AB, Verderer Pinard D, Fidock DA: Chloroquine resistance in Plasmodium falciparum malaria parasites conferred by pfcrft mutations. Science 2002, 298(5591):210–213.

21. Weniger B, Robledo S, Arango GJ, Deharo E, Arango G, Munoz V, Callapa J, Lobstein A, Anton R: Antiprotozoal activities of Colombian plants. J Ethnopharmacol 2001, 78(3–4):193–200.
activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. J Ethnopharmacol 2004, 93(1):27–32.

56. Zafou D, Tene M, Ngemenya MN, Tane P, Tinjari WP: In vitro antiproliferative activity and cytotoxicity of extracts of selected medicinal plants used by traditional healers of Western cameroon. Malar J Res Treat 2011, 2011:562342.

57. Abosi AO, Razerekha BH: In vivo antimalarial activity of Vernonia amygdalina. Br J Biomed Sci 2003, 60(2):99–91.

58. Njan AA, Adzu B, Agaba AG, Byarugaba D, Diaz-Liera S, Bangsberg DR: The analgesic and antiproliferative activities and toxicology of Vernonia amygdalina. J Med Food 2008, 11(5):74–81.

59. Iwakuma BA: Enhanced antimalarial effects of chloroquine by aqueous Vernonia amygdalina leaf extract in mice infected with chloroquine resistant and sensitive Plasmodium berghei strains. Afr Health Sci 2008, 8(1):25–35.

60. Gachet MS, Lecaro JS, Kaiser M, Bruin R, Navarrete H, Munzra RA, Bauer R, Schuchly W: Assessment of anti-protozoal activity of plants traditionally used in Ecuador in the treatment of leishmaniasis. J Ethnopharmacol 2010, 128(1):184–197.

61. Noedl H, Yingyuen K, Laoboonchai A, Fukuda M, Sirichaisinthop J, Miller RS: Screening of selected indigenous plants of Cambodia for antimalarial activity. J Ethnopharmacol 2006, 107(1):12–18.

62. Noedl H, Yingyuen K, Laoboonchai A, Fukuda M, Sirichaisinthop J, Miller RS: Sensitivity and specificity of an antigen detection ELISA for malaria diagnosis. Am J Trop Med Hyg 2006, 75(5):1205–1208.

63. Noedl H, Attlmayr B, Wernsdorfer WH, Kollaritsch H, Miller RS: A histidine-rich protein 2-based malaria drug sensitivity assay for field use. Am J Trop Med Hyg 2004, 71(6):711–714.

64. Noedl H, Wernsdorfer WH, Kollaritsch H, Looareesuwan S, Miller RS, Wongrichanalai C: Malaria drug-susceptibility testing. HRP2-based assays: current data, future perspectives. Wien Klin Wochenschr 2003, 115(Suppl 3):23–27.

65. Olivier AB, Delabala MF, Braga FC, Jacome RL, Varotti FP, Fovwa MM: Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. Molecules 2009, 14(8):3037–3072.

66. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.

67. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.

68. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.

69. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.

70. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.

71. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.

72. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.

73. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.

74. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.

75. Peters W: Antimalarial drugs. Internationale Pharmaceutica Sciencia 1992, 740.