

In Search of a Potent Antimalarial Agent: Antiplasmodial Assessment of Three Herbs with Folkloric Antimalarial Claims

Modupe Iretiola Builders*

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Bingham University, Karu, Nasarawa, Nigeria

Abstract

Malaria has remained a major cause of morbidity and mortality in the under developed and developing countries of the tropical and sub-tropical regions of the world. In spite of the substantial progress made in the treatment of parasitic diseases, malaria remains a significant therapeutic challenge especially because of the wide spread resistance of malaria parasites to currently available anti-malarial agents, the resistance of the mosquito vectors to currently available insecticides, the limited success in the development of malarial vaccines and the debilitating adverse reactions of conventional anti-malarial drugs. The wide spread resistance of malaria parasites to conventional anti-malarials have stimulated the search for new drug entities with new modes of action. The efficacy of *Agelanthus dodoneifolius* (A.D), *Parkiabiglobosa* (P.B) and *Vernonia ambigua* (V.A) for the treatment of malaria have been widely acclaimed by the Hausa, Ibo and Yoruba communities of the Northern, Eastern and Western Nigeria respectively. The aim of this study is to authenticate this claim as a step in the search for a new anti-malarial. The oral median lethal dose (LD₅₀), phytochemical components and antioxidant activity of the three aqueous extracts were determined. The extracts were also evaluated for *in vivo* and *in vitro* antiplasmodial activities against *Plasmodium berghei* and clinical isolates of *Plasmodium falciparum* respectively. *A. dodoneifolius* showed maximum inhibition (78.7±1.6, 80.1±1.0 and 69.8±1.2%) of parasitaemia and moderate anti-plasmodial activity *in vitro* (21.54µg/ml > IC₅₀ > 50µg/ml) among three plant extracts. The phytochemical analysis revealed the presence of tannins, saponins, steroids, phenols, terpenes and anthraquinones. It also showed a strong free radical scavenging activity on 2,2diphenylpicrylhydrazyl. The aqueous extract of *A. dodoneifolius* contains biologically active principles that are relevant in the treatment of malaria, thus supporting further studies of these components.

Keywords

Potent, Antimalarial Agent, Assessment, Folkloric Claims

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1. Introduction

Malaria remains a major public health problem in the world and Africa is said to be having the highest burden of malaria, this is due to the fact that *Plasmodium falciparum* (which is the most deadliest specie) occurs more in Africa and have led to an increased mortality rate (of about 600,000 deaths yearly) as well as morbidity [1].

Malaria remains a significant therapeutic challenge especially because of the wide spread resistance of malaria parasites to currently available anti-malarial agents, the resistance of the mosquito vectors to currently available insecticides, the limited success in the development of malarial vaccines and the debilitating adverse reactions of conventional anti-

* Corresponding author
E-mail address: modupebuilders@yahoo.com

malarial drugs [2].

Plant-derived compounds played crucial roles in drug discovery and development for the treatment of malaria, the isolation of new bioactive compounds from medicinal plants based on traditional use or ethnomedical data appears to be a very promising approach [1].

The local herbalists in Chaza village in Niger state of Nigeria use the stem bark, leaves of *Parkia biglobosa*, *Agelathus Dodoneifolius* and the whole plant of *Vernonia ambigua* for the treatment of infectious diseases such as malaria. According to them the reputed efficacies of these plants have been experienced and passed on from one generation to the other. *V. ambigua* commonly known as ironweed, in Yoruba it is also known as Orungo. It belongs to the family Asteraceae. *V. ambigua* is an annual herb occurring throughout Nigeria and also widely dispersed in similar parts of tropical Africa. The herb has a bitter juice, Burkill in 1997 reported that the roots are also chewed in Tanaganiyika raw or taken as a decoction for the treatment of cough and colds [3]. *P. biglobosa* is a large tree belonging to the plant family Mimosaceae of the order Leguminosaceae, it is popularly known as African locust bean tree, in Yoruba as Igba or Irugba, in Hausa as Dorowa and Ibo as Orgili. It is common in all parts of Nigeria and is used for seasoning soups. Ajaiyeoba in 2002 reported that a decoction of the bark is also used as a bath for fever and a hot mouth wash to steam and relieve toothache [4, 5]. The leaves of *A. Dodoneifolius* (African mistletoe) called 'Kauchi' in Hausa is a hemi-plant parasite used ethno medicinally by the Hausa and the Fulani tribes of Northern Nigeria to treat different diseases such as malaria, circulatory and respiratory diseases, diabetes, hypertension and sterility [6].

Apparently, lack of scientific proof of efficacies claimed by the traditional healers in Niger state of Nigeria called for this study. The present research was carried out to identify the potent antimalarial agent that can be used as a phytomedicine.

2. Materials and Methods

2.1. Plant Collection and Identification

The plant materials were collected in a busharound Chaza, a village near Suleja, Niger State, Nigeria in the month of February, 2009. It was identified and authenticated by Mallam Ibrahim Muazzam of the Department of Medicinal Plant Research and Development (NIPRD), Abuja, Nigeria, where herbarium and voucher specimens with numbers NIPRD/H/6225, NIPRD/H/6222 and NIPRD/H/6220 were made and deposited. These plants are shown in figure 1.



Figure 1. Agelanthus dodoneifolius Parkia biglobosa Vernonia ambigua.

2.2. Preparation of the Plant Extract

The plant material (stem bark or leaf or whole) was air dried under shade and then ground into coarse powder with a pestle and mortar. A 100 g quantity of the coarse powder was boiled with 1 l of distilled water at 100°C for 30 min. The decoction was decanted, centrifuged at 4500 rpm (Erweka, Germany) for 30 min and freeze-dried. The freeze-dried powder was stored in an airtight container and used for the study [7].

2.3. Phytochemical Screening

The phytochemical screening of the plant extract was carried out to determine the presence of the following compounds; alkaloids, flavonoids, tannins, anthraquinones, saponins, glycosides, sterols, terpenes and phenols using standard procedures [8].

2.4. Antioxidant Potential

The radical scavenging activities of the plant extracts against 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) were determined by UV spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method [9]. The following concentrations of the extracts were prepared, 0.1275, 0.25, 0.5, 1 and 2 mg/ml in methanol. Ascorbic acid (Vitamin C) was used as the antioxidant standard at equal concentrations. 1 ml of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \left\{ \frac{A_b - A_a}{A_b} \right\} \times 100$$

Where A_b is the absorption of the blank sample and A_a is the absorption of the extract

2.5. Determination of LD₅₀

Acute toxicity of each of the aqueous extract was carried out in mice using modified method of Lorke (1983) and Builders *et al* (2012) [10, 11]. The study was carried out in two phases; nine mice randomized into three groups of three mice

per group were used for the first phase. Doses of 10,100 and 1000 (mg/kg b.w; p.o) of each of the extract were used with the aid of stainless metallic feeding cannula (Sigma, USA) for the study. Phase two of the study was carried out as in phase 1 but higher doses of 2000, 3000 and 5000 (mg/kg b.w; p.o) of each of the extract were given to another set of nine fresh mice. Animals were observed for signs of toxicity and mortality during 24 hours post treatment.

2.6. *In vivo* Antimalarial Assay

A series of experiments were carried out to evaluate the *in vivo* anti-malarial activities of the three aqueous extracts at 200, 400 and 600 mg/kg doses as compared to control groups treated with 0.2 ml of normal saline and reference groups treated with standard drugs (Chloroquinediphosphate 25 mg/kg/day). Malaria infection was first established in male mice by the intraperitoneal (i.p.) administration of donor male Swiss albino mouse blood containing about 1×10^7 parasites. The three different methods of treating malaria infections, i.e., 4-Day suppressive test, curative and prophylactic methods were applied. The laboratory tests were started with oral administrations of the compound in the 4-day suppressive tests (early malaria infection) and further screened for their curative (established malaria infection) and prophylactic (residual malaria infection) activities. Thick blood smears were prepared and blood films were fixed with methanol. The blood films were stained with Giemsa, and then microscopically examined with 100-x magnification. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice [12].

2.7. *In vitro* Antimalarial Assay

Three fresh blood specimens were collected from three patients suffering from fever and other malaria symptoms with confirmed infection by *P. falciparum*. Already prepared dried -in-acridine orange -stained thin smears were examined for Plasmodium species identification. The parasite density was determined by counting the number of infected erythrocytes among 20,000 erythrocytes from each patient, 4ml of venous blood was collected in a tube coated with EDTA. Samples with mono-infection due to Plasmodium falciparum and a parasite density between 1 and 2% were used for the *in vitro* antimalarial tests [13].

The assay was performed in duplicate in a 96-well microtiter plate, according to WHO method *in vitro* micro test (Mark III). RPMI 1640 (Sigma Company, USA) was the culture medium used for cultivation of *P. falciparum* [13]. Dilutions were prepared from the three aqueous extracts and the final concentrations prepared by dilution were (5, 1, 0.5, 0.2, 0.1 and 0.05 µg/ml). Negative controls were treated with solvent

and positive controls (artemisinin) were added to each set of experiments. Fifty microliters from blood mixture media was added to each well in plate and incubated in a candle jar (with gas environment of about 3% O₂, 6% CO₂ and 91% N₂) at 37.00C for 24–30 h. After incubation, contents of the wells were harvested and stained for 5 min in an already prepared dried -in-acridine orange reagent. The parasites were counted in five fields of vision (> 200 total cells) using a fluorescence microscope (Partecscope fluorescence microscope, Germany) at a magnification of 40.

2.8. Data Analysis

Data were expressed as the mean ± standard error of mean (SEM). The IC 50 values were determined graphically on a log dose-response curve (log concentration versus percent inhibition curves) by interpolation.

3. Results

3.1. Phytochemical Test

Phytochemical screening showed that three aqueous plant extracts had similar constituents namely anthraquinones, saponins, tannins, steroids, terpenes and phenols. Flavonoids were found in the aqueous stem bark of *P. biglobosa* while the aqueous whole plant extract of *V. ambigua* was found to contain glycosides, flavonoids and alkaloids.

Table 1. Phytochemical analysis of *A. dodoneifolius*, *P. biglobosa* and *V. ambigua*.

Test	<i>A. dodoneifolius</i> leaves	<i>P. biglobosa</i> stem bark	<i>V. ambigua</i> whole plant
Anthraquinones	+	+	+
Glycosides	-	-	+
Flavonoids	-	+	+
Alkaloids	-	-	+
Phenols	++	+	+
Saponins	+	+	+
Steroids	+	+	+
Tannins	++	+	+
Terpenes	++	+	+

Key; - Absence, + Presence, ++ Appreciable amount

3.2. Antioxidant Activity

The comparative antioxidant effect of the three aqueous extracts and the ascorbic acid is illustrated in figure 2. The antioxidant activities were determined by inhibition of the free radical activities of DPPH. The activities of extracts and ascorbic acid were concentration dependent increasing with concentration. The sensitivity of the aqueous extract of *A. dodoneifolius* was higher than two remaining aqueous, *A. dodoneifolius* and ascorbic acid showed maximum DPPH radical-scavenging activity of 94.5 and 97.1%.

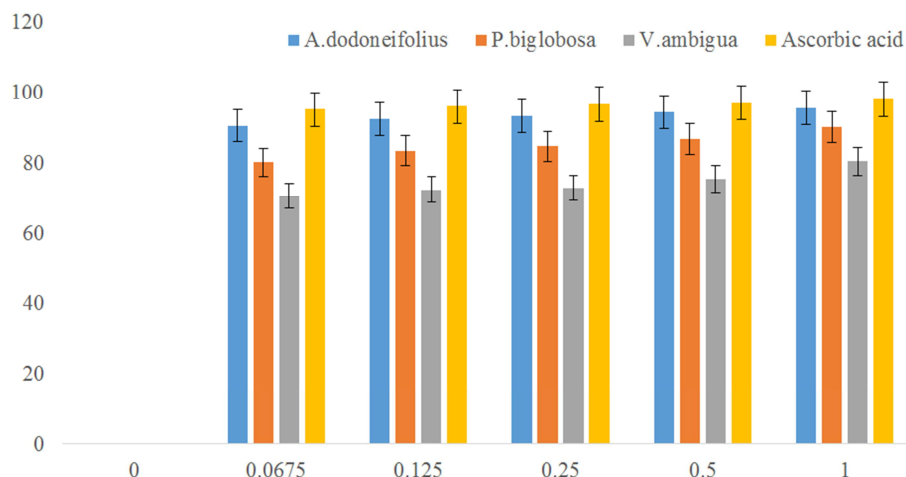


Figure 2. Antioxidant activities of *A. dodoneifolius*, *P. biglobosa* and *Vernoniaambigua* against Ascorbic acid.

3.3. Acute Toxicity Test

The behavioral signs of toxicity exhibited by the animals were vigorous paw licking and dullness. No mortality was observed in the different groups of rats that received the extracts (stem bark of *P. biglobosa*, leaves of *A. dodoneifolius*, and whole plant of *V. ambigua*), even in the ones treated with (5000mg/kg; b.w, P.O).

3.4. In vivo Antimalarial Study

Figure 3 showed the antiplasmodial activities of the three aqueous extracts in relation to chloroquine. The three aqueous plants extracts inhibited parasitemia to a variable extent, the suppressive, curative and prophylactic activities were dose dependent for each of the tested extract. Chloroquine also exhibited a similar reduction in the parasite count. *A. dodoneifolius* showed maximum inhibition (78.7±1.6, 80.1±1.0 and 69.8±1.2%) of parasitaemia.

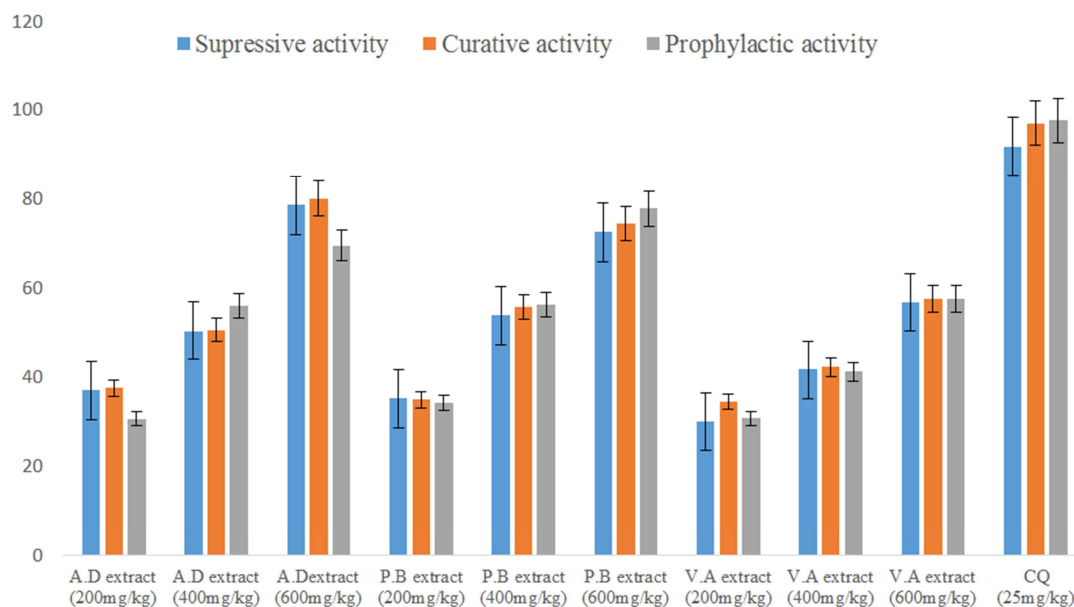


Figure 3. In vivo antiplasmodial activities of *A. dodoneifolius*, *P. biglobosa* and *V. ambigua* in relation to Chloroquine.

3.5. In vitro Antimalarial Assay

The photomicrographs of the aqueous extracts of *A. dodoneifolius*, *P. biglobosa* and *V. ambigua* are presented in figure 4. The three aqueous extracts showed a concentration-dependent inhibition of *P. falciparum*. *A. dodoneifolius*

indicated maximum plasmodia inhibition of 74.9.0±1.0% at a concentration of 100 µg/ml and 100±1.0% for artemisinin at a concentration of 1 µg/ml (figure 5). The IC₅₀ of the aqueous extract of *A. dodoneifolius* was determined as 20.94 µg/ml (figure 6).

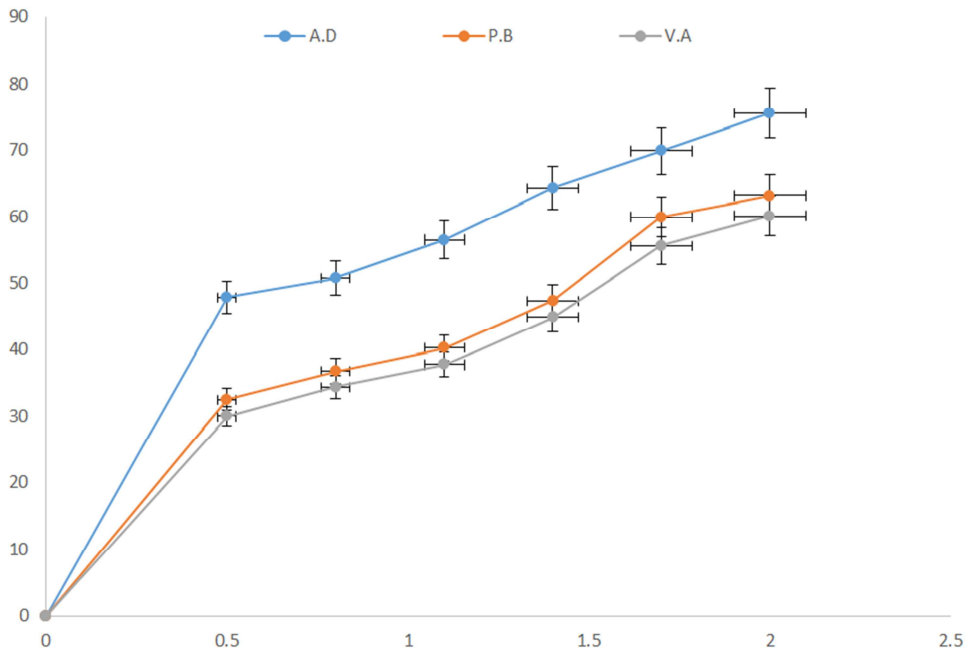


Figure 4. *In vitro* antiplasmodial activities of aqueous extracts of *A. dodoneifolius*, *P. biglobosa* and *V. ambigua*.

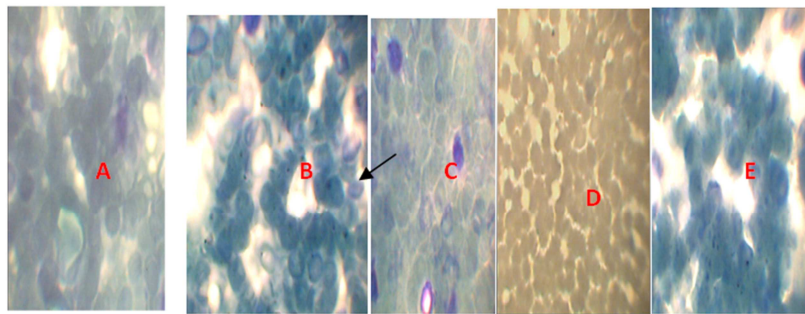


Figure 5. Photomicrographs of *in vivo* antiplasmodial study.

A: Slide showing no *P. berghei* in blood smear of uninfected mouse. B: Slide showing *P. berghei* (black arrow) in blood smear of infected mouse. C: Slide showing no *P. berghei* in blood smear of treated mouse with *A. dodoneifolius* extract.

D: Slide showing scanty *P. berghei* in blood smear of treated mouse with *P. biglobosa* extract. E: Slide showing scanty *P. berghei* in blood smear of treated mouse with *V. ambigua* extract. (X 100 magnifications).

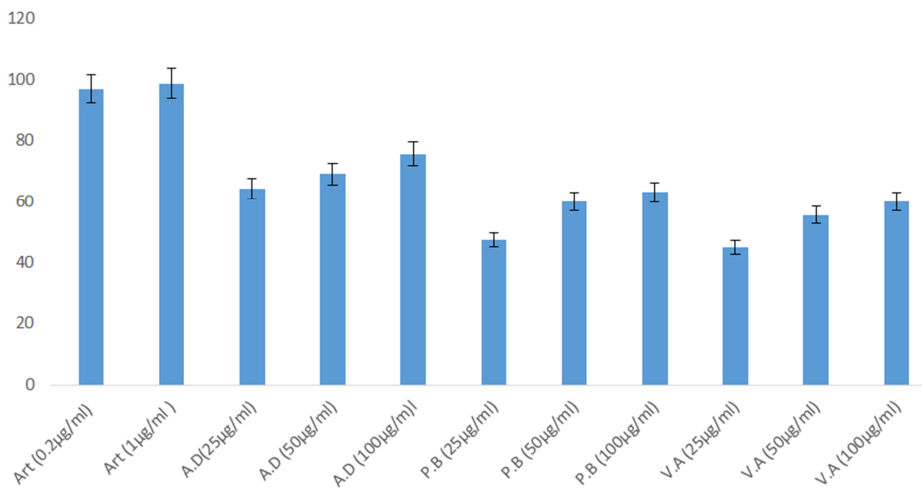


Figure 6. *In vitro* antiplasmodial activities of the *A. dodoneifolius*, *P. biglobosa* and *V. ambigua* against Artemisinin.

4. Discussion

The present study was undertaken to evaluate the antimalarial activity of the most commonly used plants in Nigerian traditional medicine for treatment of malaria [14, 15].

Ethnopharmacological data have been one of the common useful ways of discovery of biologically active compounds from plants [16, 17]. The big advantage of the ethnopharmacological information is that extensive literature may already allow for some rationalization with respect to the biological potential of a reputed use [16]. Ethnopharmacological use of plants can therefore be a basis for phytochemical and phytopharmacological investigation [17].

Phytochemical screening showed that the three aqueous plant extracts had similar constituents namely alkaloids, saponins, tannins, flavonoids, phenols, sterols, anthraquinones, terpenes and glycosides. However there were more appreciable amount of phenols, tannins and terpenes in the aqueous extract of *A. dodoneifolius*.

The aqueous extract of *A. dodoneifolius* possessed the highest antioxidant activity due to appreciable amount of phenols, tannins and terpenes. The presence of phenolic compounds in the plant is likely to be responsible for the free radical scavenging effects observed. Phenolic compounds are tannins and plant phenols are a major group of compounds that act as primary antioxidants or free radicals scavengers [10]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [10].

Substances with LD₅₀ values greater than 5000mg/kg of body weight are considered to show low toxicity. Thus the three aqueous plant extracts can be classified in the category of substances with low toxicity [18]. The high safety profile obtained may have been responsible for their wide spread use in the treatment of malaria and fever.

The three aqueous plants extracts inhibited parasitemia to a variable extent, the suppressive, curative and prophylactic activities were dose dependent for each of the tested extract. However at equal doses aqueous leaves extract of *A. dodoneifolius* gave higher suppressive, curative, and prophylactic activities than the stem bark plant extract of *P. biglobosa* and whole plant of *V. ambigua*.

Many secondary plant metabolites have been assessed either for *in vitro* activity against *P. falciparum* or *in vivo* activity against *P. bergheibergehei* [19]. Various sesquiterpenoids have been reported for their antimalarial activity [20]. Also some triterpenoids have been isolated from different

medicinal plants and were found to exhibit antimalarial property [21, 22]. *Cissusrotundifolia* contains some steroids that indicated antiplasmodial activities [23, 24]. The antiplasmodial activities of many phenolic compounds as well as tannins had been described [12]. The presence of similar phytochemicals in other plants with established antiplasmodial activity corroborates the antimalarial potential of these three aqueous extracts.

Some of these metabolites have been found to exert antiplasmodial effects either by elevating red blood cell oxidation or by inhibiting the parasite's protein synthesis. They may also counteract the oxidative damage induced by the malaria parasite [3]. Thus, the antioxidant activities of these extracts may represent another mechanism that contributes to their antiplasmodial activities.

The aqueous extract of the leaves of *A. dodoneifolius* was more active in *in-vivo* and *in vitro* antiplasmodial screening than the remaining two aqueous extracts as prescribed by the traditional healers. The phytochemical composition result showed that the aqueous extract of *A. dodoneifolius* was richer in phenols, tannins and terpenes than two aqueous extracts. This might explain the higher *in-vivo* and *in vitro* antiplasmodial activity of the aqueous extract of *A. dodoneifolius*.

5. Conclusion

The assessment of phytochemical screening of *A. dodoneifolius* indicated the presence of secondary metabolites with antioxidant and antimalarial activities. The effective *in vivo* and *in vitro* antiplasmodial activities could partly explain the safety, effective and traditional use for the treatment of malaria implicated with the high value of the LD₅₀. Since *A. dodoneifolius* possess the highest antimalarial activity, therefore further studies should be carried out to isolate and characterize the relevant bioactive components in the leaves of the *A. dodoneifolius*.

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