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ORIGINAL PAPER

# In vivo antimalarial evaluation of *MAMA* decoction on *Plasmodium berghei* in mice

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**Abstract** The use of decoctions of different plant materials is common practice in antimalarial ethnomedicine in Africa. Scientific evaluation of such herbal combinations to verify the claims is important. The study has evaluated the antimalarial efficacy of *MAMA* decoction (MD), a multicomponent herbal preparation and its individual plant components, namely leaves of *Morinda lucida* Benth [Rubiaceae] (ML), *Azadirachta indica* A. Juss [Meliaceae] (AI), *Alstonia boonei* De Wild [Apocynaceae] (AB) and *Mangifera indica* L [Anacardiaceae] (MI) in *Plasmodium berghei*-infected mice. Each decoction was prepared by boiling the powdered leaf in water, concentrated in vacuo and freeze-dried. The acute toxicity of MD ($LD_{50}$=3.8 g/kg) was determined using Lorke's method. The antimalarial activities of MD and its plant components were evaluated by oral administration of the freeze-dried extracts (15–240 mg/kg) using the early malaria infection test model. The established malaria infection test was used to evaluate MD (60–240 mg/kg) while amodiaquine [10 mg/kg] (AQ) and distilled water were employed as the positive and negative controls, respectively. From the early malaria infection test, the effective doses at 50 % ($ED_{50}$) and 90 % ($ED_{90}$) for MD, AB, AI, ML, MI and AQ were 43, 79, 140, 134, 208 and 3.9 mg/kg and 202, 276, 291, 408, 480 and 9.2 mg/kg, respectively. For the established infection test, MD (240 mg/kg) and AQ gave parasite clearance of 55 and 95 % on day 5 of treatment. MD possesses antimalarial activity and is relatively safe.

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## Introduction

Malaria remains one of the most prevalent diseases in the developing world, afflicting 216 million people in endemic areas of the world with an estimated mortality of one million, especially in African children (WHO 2010). *Plasmodium falciparum*, the most pathogenic and dominant of the four species of malarial parasites that infect man, is the principal cause of almost every malarial mortality and morbidity in tropical and subtropical countries (Tuteja 2007). The therapeutic use of chloroquine and other affordable mainstay drugs has been discontinued in Nigeria and many other countries in Africa due to *Plasmodium* resistance (FMOH 2005). There is, therefore, the need for sustained research into the development of antimalarial phytomedicines which historically had led to many notable antimalarial drugs especially the two most effective natural antimalarial compounds, namely quinine from *Cinchona succirubra* Pavon and Klutzsch (Rubiaceae) and artemisinin from *Artemisia annua* L (Asteraceae). Although in traditional medicine antimalarial plant remedies are commonly used as multicomponent mixtures, only a few studies have been conducted on such mixtures. *MAMA* decoction consists of the leaves of *Morinda lucida*, *Azadirachta indica* (Neem), *Alstonia boonei* and *Mangifera indica* in 1:1:1:1 ratio. Previous studies have reported the antimalarial activities of the leaves of *M. lucida* and *A. indica* in mice (Isah et al. 2003; Cimanga et al. 2006). The present study reports the in vivo antimalarial evaluation of the freeze-dried extract of *MAMA* decoction as well as those of its plant components—*M. indica*, *A. boonei*, *A. indica* and *M. lucida* leaves.

## Materials and methods

### Collection and preparation of plant materials

The leaves of the plant component of MD were collected in March 2008 on the road leading to the Teaching and Research Farm of Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. They were identified and authenticated by Mr. A.T. Oladele, the Plant Curator of the Department of Pharmacognosy, OAU. Voucher specimens were deposited in the Herbarium of the Botany Department with numbers IFE 16534 (*A. boonei* De Wild [Apocynaceae]), IFE 16536 (*A. indica* A. Juss [Meliaceae]), IFE 16537 (*M. indica* L. [Anacardiaceae]) and IFE 16535 (*M. lucida* Benth [Rubiaceae]). The leaves were oven-dried at 40 °C and powdered separately. The decoction of each plant material was obtained by boiling 100 g each of the powdered material in 1 L of distilled water for 1 h and for MD, 50 g each of the four powdered plant materials were mixed together and boiled in 2 L of distilled water for 1 h (Sofowora 2008). The liquid extracts were filtered and concentrated in vacuo at 70 °C followed by freeze-drying. The freeze-dried powders were stored in air-tight amber-coloured bottles until ready for use. The percentage water-extractive yield was calculated as the percentage of the ratio of the weight of each dried extract ($W_e$) to the weight of the powdered leaf used for extraction ($W_p$) according to the African Pharmacopoeia (AP 1986): $\frac{W_e}{W_p} \times 100\%$

### Animals

Swiss albino mice of both sexes, weighing 18–24 g, were obtained from the Central Animal House, University of Ibadan, Nigeria. The animals were housed under a 12-h light/dark cycle with free access to water and commercial food pellets (Premier Feed Mills Co. Ltd., Ibadan, Nigeria). They were acclimatized for at least 10 days before use and were used in accordance with the "Guide for the care and use of laboratory animals" (National Research Council 1996). An approval for the study was obtained from the Animals Ethics Committee of the Obafemi Awolowo University, Ile-Ife (IPHOAU/12/90).

### Parasite strain

Chloroquine-sensitive *Plasmodium berghei* NK65 strain was obtained from the Institute of Advanced Medical Research and Training, University of Ibadan, Nigeria. The parasites were maintained in continuous blood passage in mice. A standard inoculum of $10^7$ parasitized erythrocytes was prepared by dilution of blood, harvested from a donor mouse (>30 % parasitemia) with normal saline and administered intraperitoneally (200 μl) to each test mouse. A single donor was used to infect the test mice for each experiment.

### Acute toxicity

The acute toxicity of MD was determined in vivo using the procedure described by Lorke (1983). Uninfected mice were orally given MD at doses of 10, 100, 1,000, 1,600, 2,900 and 5,000 mg/kg body weight. The animals were monitored for any toxic symptoms or mortality over a 14-day period after which the median lethal dose ($LD_{50}$) was calculated.

### Antimalarial activity

#### *Early malaria infection (4-day) test*

The evaluation of the freeze-dried extracts of MD and its plant components was carried out based on the 4-day test (Peters et al. 2002). For each extract, five groups of mice (five mice per group) were orally treated at 15, 30, 60, 120 and 240 mg/kg 4 h (day 0) after intraperitoneal inoculation while one group was given 0.2 ml/mouse distilled water (negative control). In addition, AQ (positive control) was orally administered at 1.25, 2.5, 5.0 and 10 mg/kg to four groups of mice. The stock solutions of the extracts and drug were diluted with distilled water such that 0.2 ml of the final concentration of each was administered once daily to each mouse with the aid of a metal feeding cannula for four consecutive days (day 0–day 3). On the fifth day, thin blood smears were made from the tail of the mice, fixed with methanol and stained with Giemsa followed by microscopic assessment (×1,000 magnification) of the parasitized and total red blood cells.

#### *Established malaria infection (curative) test*

The curative effect of MD was assessed by the method described by Ryley and Peters (1970). Twenty-five mice inoculated intraperitoneally with $1\times10^7$ inoculum of CQ-sensitive *P. berghei* NK 65 were randomly distributed into five groups of five mice each. Seventy-two hours after inoculation, three groups were orally administered freeze-dried extracts of MD (60, 120 and 240 mg/kg/day) for five consecutive days (day 3–day 7) while the positive and negative controls groups were treated with amodiaquine (10 mg/kg) and distilled water (0.2 ml/mouse/day), respectively. MD extract and AQ were diluted with distilled water such that 0.2 ml of the final concentration of each was administered with the aid of a metal feeding cannula. Thin blood smears were prepared daily to monitor the level of parasitaemia which was determined by microscopic examination of parasitized and total red blood cells in 10 fields of view.

#### *Rectal temperature and survival time*

The rectal temperature of each mouse was taken daily during the period of treatment. In addition, the mice were observed

for 28 days post-inoculation and any mortality, which occurred during the period, was recorded in order to calculate the survival time of the mice in each group.

*Data and statistical analysis*

The average parasitaemia was determined by obtaining the percentage of the ratio of parasitized to the total number of RBC. Average percentage chemosuppression (or parasite clearance) was calculated as $100\times [(A–B)/A]$, where $A$ is the average parasitemia of the negative control group and $B$ is the average parasitemia of the test group. The effective doses ($ED_{50}$ and $ED_{90}$) were determined using Microsoft Excel (2007) while the one-way analysis of variance between groups and post hoc Dunnett test were used to compare data for the treatment groups. A $P$ value of <0.05 was considered statistically significant.

## Results

Percentage yield of extracts

The extractive yields of the plants obtained were 17.06, 27.26, 11.35, 9.00 and 17.70 % *w*/*w* for AI, ML, AB, MI and MD, respectively.

Acute toxicity

When given as a single dose of 10–5,000 mg/kg, MD produced no signs of acute toxicity in the first 24 h of observation. However, on the 10th day post-treatment, one death was recorded in the 5,000-mg/kg-treated group. There was a gradual decrease in its rectal temperature and weight prior to death. The mortality observed in one mouse may have been caused by some organ (e.g. liver or kidney) damage with the physical signs of reduced temperature and decreased weight representing observed secondary effects. Other groups of mice exhibited stable temperature values throughout the period of observation. The $LD_{50}$ was therefore estimated to be 3,807 mg/kg.

Early malaria infection

The antimalarial activities of MD, the plant components and AQ were assessed and compared in relation to average parasitaemia reduction, effective chemosuppressive doses at 50 % ($ED_{50}$) and at 90 % ($ED_{90}$), mean survival time and the rectal temperature values.

*Average parasitaemia reduction*

Freeze-dried extracts of MD and its plant components as well as AQ exhibited dose-dependent reductions of parasitaemia (Fig. 1). MD showed much higher parasite reduction in the treated animals than any of the individual plants, which was significantly different (F=25.94; $P$=0.0) from the untreated control at all the doses tested. The untreated (negative) control group gave parasitaemia of 30.8±12.8 % which decreased to 3.1±1.0 % with the 240 mg/kg dose. In the groups of mice that received the extracts of *A. boonei*, *A. indica* and *M. lucida*, the percentage parasitaemia reduced significantly from 19.35± 2.2 % (negative control) to 5.5±1.1 % at 240 mg/kg (F= 43.21; $P$=0.0), 15.7±1.6 % to 4.75±1.57 % (F=18.23; $P$= 0.0), 14.0±0.85 % to 5.8±0.9 % (F=9.39; 0.0), respectively. However, in the *M. indica*-treated group, an initial non-significant reduction ($P$>0.05) in parasitaemia was observed in the low-dose groups while a significant reduction (F=4.54; $P$=0.013) from 8.90±1.98 at 60 mg/kg to 7.16±1.23 at 240 mg/kg was observed. The percentage parasitaemia reduction observed in the AQ-treated mice was from 15.66±1.57 to 2.46±0.84 (1.25 mg/kg) which was significantly different (F=59.18; 0.0) when compared with the negative control group. The effective doses for chemosuppression at 50 % ($ED_{50}$) and 90 % ($ED_{90}$) are shown in Table 1.

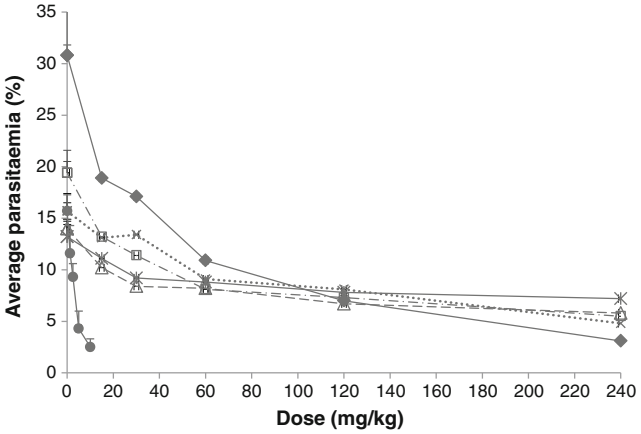


**Fig. 1** Effects of different doses of *MAMA* decoction, its component plants (15–240 mg/kg) and amodiaquine (1.25–10 mg/kg) on parasitaemia in *P. berghei*-infected mice using the early malaria infection test model. *MAMA* decoction (*black-filled diamond*), *M. indica* (*asterisk*), *A. boonei* (*square*), *M. lucida* (*triangle*), *A. indica* (*multiplication sign*) and amodiaquine (*black-filled circle*)

**Table 1** Effective doses ($ED_{50}$ and $ED_{90}$) of *MAMA* decoction, its component plants as well as amodiaquine (10 mg/kg) in the early malaria infection test model

| | $ED_{50}$ (mg/kg) | $ED_{90}$ (mg/kg) |
|---|---|---|
| *MAMA* decoction | 42.52 | 202.04 |
| *A. boonei* | 78.77 | 276.41 |
| *M. lucida* | 134.47 | 407.62 |
| *A. indica* | 139.99 | 290.96 |
| *M. indica* | 208.30 | 480.29 |
| Amodiaquine (positive control) | 3.90 | 9.21 |

*Mean survival time*

The mean survival time (MST) of mice treated with MD and its component plants are presented in Table 2. At 15, 30, 60, 120 and 240 mg/kg, MD-treated mice showed MST of 5.7–9.0 days which were not significantly different ($P>0.05$) from the negative control group (6.7 days). At similar doses, mice treated with extracts of *A. boonei*, *M. lucida*, *A. indica* and *M. indica* gave MST of 9.5–23.0, 7.2–13.8, 8.0–15.7 and 7.3–15.0 days, respectively. Analysis of the survival rates showed that there was significant difference (F=4.31; $P=0.006$) only in the *A. boonei*-treated mice (240 mg/kg) compared to its negative control. The survival time for the AQ-treated mice at 1.25–10 mg/kg ranged between 7.3 and 16.8 days which was not significantly different ($P>0.05$) from the negative control-treated group (10.8 days) [Table 2].

*Effect on temperature of parasitized mice*

The results for the 240-mg/kg-treated groups of the plant components are expressed in °C (day 0 and day 3): *A. boonei* (35.2±0.3, 37.1±0.6; $P=0.022$), *M. lucida* (36.8±0.4, 36.1±0.5; $P=0.306$), *A. indica* (35.6±0.3, 35.6±0.3; $P=1.00$), *M. indica* (36.9±0.8, 36.0±0.9; $P=0.476$) and AQ (35.9±0.5, 35.9±0.1; $P=1.00$).

Established malaria infection model

*Mean percentage parasitaemia*

In this experiment, daily reductions in parasitaemia were observed in the MD-treated groups while the negative control group showed daily increases in parasitaemia (Fig 2). On day 7 (last day of treatment), mice treated with MD at 60, 120 and 240 mg/kg had mean percentage parasitemia values of 11.33, 10.54 and 10.24 % while the positive and negative control-treated groups had values of 0.94 and 22.63 %, respectively.

*Mean survival time*

In the established infection test model, the MST of mice treated with MD ranged from 5.7 to 15 days which were not significantly different ($P>0.05$) from the negative control-treated group. All the mice in the negative control group died between days 3 and 11 giving a mean survival time of 6.4 days. On the other hand, the mice in the AQ-treated group had an MST of 21 days.

*Effect on temperature of parasitized mice*

The rectal temperature of the mice in the negative control declined progressively with the day 7 value being significantly lower (F=4.47; $P=0.014$) than that of day 3 (start of treatment). In the group of mice that received MD at 60 and 120 mg/kg, there were no significant differences while a significant decrease (F=4.22; $P=0.017$) was observed in the 240 mg/kg-treated group on days 6 and 7 of treatment (Table 3).

## Discussion

MD is a traditional herbal antimalarial preparation formulated in a particular ratio based on previous clinical observational experiences which supported the ethnomedical records. It is a decoction prepared from four component plants used ethnomedically for malaria therapy (Elujoba 2009 personal communications) and which had been individually investigated by various workers for their antimalarial properties (Awe et al. 1998; Isah et al. 2003). Furthermore, the antimalarial activities of *A. indica* and *M. lucida* leaves have been

**Table 2** Mean survival time of *P. berghei*-infected mice treated with *MAMA decoction* and its component plants in the early malaria test

| Dose (mg/kg) | | Mean survival time (days ± SD) | | | | |
|---|---|---|---|---|---|---|
| | AQ | *A. boonei* | *M. lucida*[a] | *A. indica*[a] | *M. indica* | *MAMA* decoction |
| 15 (1.25)[b] | 7.3±3.8 | 9.5±1.9 | 9.4±2.1 | 8.0±5.0 | 8.0±2.3 | 7.8±2.3 |
| 30 (2.5) | 12.4±1.2 | 15.5±2.7 | 7.2±1.8 | 15.7±1.2 | 7.3±2.6 | 6.0±0.0 |
| 60 (5.0) | 14.8±4.3 | 20.2±2.4 | 9.2±2.0 | 12.3±0.8 | 8.0±3.0 | 5.7±2.0 |
| 120 (10.0) | 16.8±5.3 | 15.8±2.7 | 9.4±1.7 | 15.3±1.5 | 8.3±0.7 | 8.5±3.5 |
| 240 | | 23.0±2.0[a] | 13.8±1.1 | 12.0±1.6 | 15.0±1.2 | 9.0±1.0 |
| Negative control | 10.8±1.8 | 12.8±2.3 | 10.8±1.8 | 11.8±2.1 | 8.5±2.2 | 6.7±3.5 |

Data are expressed as mean ± standard deviation of five mice per group

*AQ* amodiaquine

[a] Mean value is significantly ($P<0.05$) different from the negative control

[b] The values in parentheses represent the doses of the positive control

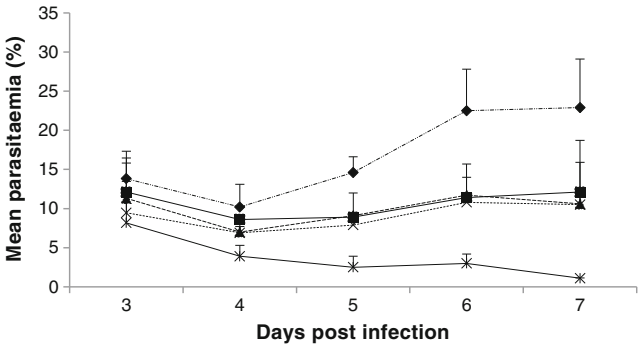


**Fig. 2** Mean percentage parasitaemia of *MAMA* decoction at 60–240 mg/kg using the established infection (curative) model. Negative control (*black-filled diamond*), MD at 60 mg/kg (*black-filled square*), 120 mg/kg (*black-filled triangle*), 240 mg/kg (*multiplication sign*), amodiaquine (*asterisk*)

previously reported with the isolation of the antimalarial constituents (Khalid et al. 1989; Cimanga et al. 2006). However, no pharmacological studies have been reported on the combination of the four plants nor on the respective decoctions of the leaves of *M. indica* and *A. boonei* despite ethnomedicinal claims to their antimalarial activities. The present investigation has shown that MD possesses a significant suppressive activity in addition to about 50 % curative activity. At the maximum dose of 240 mg/kg, parasitological cure was not obtained. It is possible to obtain such level of parasitaemia reduction if the dose of MD had been further increased above 240 mg/kg but with a risk of high toxicity. Furthermore, this study has revealed the comparative biological activities of four of the most commonly investigated antimalarial plants in literature.

*A. boonei* leaf extract gave dose-dependent and statistically significant chemosuppressive antimalarial activity in this study. In addition, it prolonged the survival time of the treated mice and also showed noticeable effect on the temperature alteration in mice on each day of the treatment period. On day 0, the temperature was 35.2 °C, while on day 3, it increased to 37.1 °C. It is noteworthy that in murine malaria, temperature values are known to decrease during malaria infection but increase following effective treatment. Conversely, the body temperatures of human subjects do rise during malaria infection and decrease with successful antimalarial treatment. In both cases, therefore, this phenomenon would mean a favourable 'antipyretic' activity which may assist any antimalarial therapy. Hence, *A. boonei* leaf showed considerable potency in the management of malaria. The result is in agreement with an earlier report on the in vivo antimalarial activity of the leaf of a closely related species, *Alstonia congensis*, which gave chemosuppression of 74.9 % at 200 mg/kg (Awe and Opeke 1990), while in this study, *A. boonei* gave chemosuppression of 72.2 % at 240 mg/kg. Although no previous report was found on the antimalarial activity of *A. boonei* leaf, a number of studies have reported that the stem bark possesses antimalarial activity (Bello et al. 2009; Iyiola et al. 2011). The bioactive compounds in *Alstonia* bark are alkaloids, triterpenes and a lactone (Faparusi and Bassir 1972; Marini Bettolo et al. 1983; Wright et al. 1993; Gosse et al. 1997).

*M. lucida* has been reported consistently to possess antimalarial activity especially in murine malaria which is in agreement with the findings of this work (Makinde and Obih 1985). The leaf has been reported to contain ursolic acid which was isolated as the antimalarial constituent of the plant (Cimanga et al. 2006). It is therefore possible that the triterpene, ursolic acid, also contributed to the antimalarial activity observed in this study. *M. lucida* is an anthraquinone-containing plant (Adewunmi and Adesogan 1984); thus, previous studies reported the in vitro antimalarial activity of some anthraquinones isolated from its root and stembark (Kougmalo et al. 1992; Moody et al. 1994).

*A. indica* leaf, in the present study, demonstrated antimalarial activity despite various previous controversial reports on its efficacy. The present study has therefore provided strong supportive evidence to the ethnomedical claim of efficacy. However, it seemed that the observed activity was due to a direct lethal action on the parasite since it showed minimal effects on the other outcomes of treatment such as the mean

**Table 3** Effects of different doses of *MAMA* decoction (60–240 mg/kg) and amodiaquine (10 mg/kg) on the temperature of *P. berghei*-infected mice using the established malaria infection test model

| Dose (mg/kg) | Temperature (°C ± SEM) | | | | |
|---|---|---|---|---|---|
| | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
| 60 | 36.7±1.0 | 36.1±1.1 | 37.1±1.0 | 35.4±0.6 | 35.2±0.4 |
| 120 | 35.6±1.2 | 34.1±0.6 | 35.0±0.6 | 33.1±0.7 | 34.4±0.8 |
| 240 | 37.5±0.6 | 36.4±0.4 | 36.6±1.0 | 34.0±0.9[a] | 34.9±0.1[a] |
| AQ (10) | 35.5±0.8 | 36.9±0.8 | 38.4±0.8[a] | 37.1±0.3 | 37.6±0.6 |
| Negative control | 36.9±0.8 | 37.3±0.5 | 35.6±1.0 | 35.3±0.4 | 33.9±0.1[a] |

Data are expressed as mean ± standard error of the mean of five mice per group

[a] Value is significantly different ($P<0.05$) compared with day 3 values

survival and reversal of temperature effects. Obaseki and Jegede-Fadunsin (1986) reported a chemosuppression of 95 % for the freeze-dried extract of the fresh leaf of *A. indica* when given orally to mice infected with *Plasmodium yoelii nigeriensis*. Similarly, using the same plasmodium strain, the oral administration of 800 mg/kg of an aqueous suspension of the dried leaf on infected mice gave a suppression of 79.7 % (Isah et al. 2003). The plant is more commonly used in combination with other plants in many antimalarial decoctions and infusions (Willcox et al. 2004). The antimalarial activity of the plant was already attributed to the presence of many compounds including β-sitosterol, nimbolide, gedunin and other limonoids (Willcox et al. 2004; Tan and Luo 2011).

The leaf of *M. indica* has been used extensively for fever and inflammatory disorders in ethnomedicine. However, little or no work has been reported on its evaluation as an antimalarial agent. Mangiferin (2-C-β-D-gluco-pyranosyl-1,3,6,7-tetrahydroxyxanthone; $C_{19}$ $H_{18}$ $O_{11}$), a C-glucoside xanthone was isolated from the fruits, stem bark, heartwood and roots (Wauthoz et al. 2007). The activities of mangiferin include immune-modulatory, antioxidant, anti-nociceptive and anti-inflammatory effects (Sanchez et al. 2000; Garcia et al. 2002; Garrido et al. 2004; Rivera et al. 2006). The immune-stimulating and anti-inflammatory actions of mangiferin could have contributed to the observed antimalarial effects.

Thus, though all the four plants demonstrated antimalarial activity, interestingly, *A. boonei* appears to be the most biologically active and would have played a great role in the antimalarial activity of MD. *Terminalia avicennoides* Gill. and Perr. (Combretaceae), *Xanthium strumarium* L. (Compositae) and *Berghenia ciliata* (Haw) Sternb (Saxifragaceae) are among several medicinal plants that have been reported to demonstrate significant in vivo antimalarial activity (Chandel et al. 2012; Omonkhua et al. 2013; Walter et al. 2013). On the basis of their $ED_{90}$ values, the activities of the four plants can be ranked in order of increasing activity as follows: *M. indica* < *M. lucida* < *A. indica* < *A. boonei*. It is very likely that the antimalarial properties of MD reside in more than one constituent since two of the plant components (*M. lucida* and *A. indica*) have had their antimalarial constituents isolated and characterized. It is also possible that some other chemical constituents, while lacking antimalarial properties, could potentiate the antimalarial property of the isolated compounds. A third possibility is that some constituents may have other beneficial effects such as immune stimulation (*M. indica*) and modulation action on the toxicity potentials of some other components. It is most likely, therefore, that the plants work in synergy to bring about the observed antimalarial chemosuppression in MD.

The use of medicinal plant combinations has existed for ages in various cultures including Africa. *Artemisia annua* (Asteraceae) for example was used in combination with other plants for fevers. *Malarial 5* and *Ayush 64* are poly-herbal antimalarial remedies used in different parts of Africa (Willcox et al. 2004). The findings in this study have therefore demonstrated the scientific justification for poly-herbal formulations for malaria therapy thereby exploiting the advantage of synergistic interactions for enhanced therapeutic efficacy.

## Conclusions

This study represents the first report on the in vivo antimalarial activities of the aqueous extracts of the leaves of *A. boonei* and *M. indica* as single plant preparations. It has also provided the scientific credence to the ethnomedical usage and previous clinical observational records of MD for antimalarial therapy pending its clinical human trials. Similar studies are in progress in our laboratory on MD using chloroquine-resistant *P. berghei* parasites and also to investigate the mechanism of action of the decoction.

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