

# ANTIPLASMODIAL PROPERTIES OF *ALSTONIA BOONEI* STEM-BARK AND *PICRALIMA NITIDA* SEED IN DIFFERENT COMBINATIONS

Ajayi, C. O., Elujoba, A. A.\* and Adepiti, A. O.

Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

\*Corresponding author's e-mail: [tonyelu@yahoo.com](mailto:tonyelu@yahoo.com); Tel. +2348034025633;

**Keyword:** Antimalarial, parasitaemia, pyrimethamine, chemosuppression

## ABSTRACT

The use of mixtures of plants in form of decoctions is common practice in African ethnomedicine. The study evaluated the antiplasmodial activities of the freeze-dried decoctions of different combination ratios of the stem-bark of *Alstonia boonei* De Wild (Apocynaceae) and dry seed of *Picralima nitida* (Stapf) T. & H. Dur. (Apocynaceae) against chloroquine-sensitive *Plasmodium berghei* NK65 strain. Thus, the decoctions of the mixtures of *A. boonei* stem-bark (A) and *P. nitida* seed (P) were prepared in three different ratios 1:1 (AP1), 1:2 (AP2) and 2:1 (AP3), were separately filtered, concentrated *in vacuo* and freeze-dried to obtain the corresponding extractive for each ratio. The acute toxicity test was carried out on each of the ratios using Lorke's method. The antiplasmodial activity of each ratio was assessed using 4-day test at 1.5-100 mg/kg. The ratio with the best chemosuppressive activity was subjected to prophylactic and established malaria infection tests at 6.25-50 mg/kg, with pyrimethamine (1.25 mg/kg) and chloroquine (5 mg/kg) as positive controls, respectively. The LD<sub>50</sub> of each extractive was greater than 5000 mg/kg. The chemosuppressions of AP1, AP2 and AP3 at 12.5 mg/kg were 55.7%, 64.4% and 57.2%, respectively, with AP2 being comparable to chloroquine. The ED<sub>50</sub> for AP1, AP2 and AP3 were 27.8, 26.0 and 29.7 mg/kg, respectively. Furthermore, AP2 exhibited curative activity with parasite clearance between 82.6% and 84% at 12.5 - 50 mg/kg while it showed prophylactic activities between 46.5% and 55.3% at the same doses. The observed antiplasmodial activities of the freeze-dried extractive (AP2) from the decoction of *A. boonei* stem-bark and *P. nitida* seed in ratio 1:2 can therefore be exploited for herbal antimalarial drug development.

## INTRODUCTION

Malaria remains the single most common infectious disease in Africa and other developing countries. It continues to be a global health challenge with about 207 million cases resulting in more than 627,000 deaths per annum while the majority of cases (77%) occur in children under 5 years [1]. The spread of chloroquine-resistant *Plasmodium falciparum* and the alarming emergence of multi-drug resistant strains of the parasite had signaled an urgent need for newer, safer, and stronger anti-resistant antimalarial drugs [2]. The artemisinin group of combination drugs has been the most effective and widely used treatments for malaria but recent literatures [3] have reported the existence of malaria parasites with drug resistance to artemisinin-based combination therapies (ACT). Hence, screening of new herbal therapies used in traditional medicine must continue. Medicinal plant extracts as well as their drug products have been used in the treatment of fever or other symptoms associated with malaria throughout the tropics and subtropics [2]. *Picralima nitida* (Stapf) T. & H. Dur. (Apocynaceae) commonly known as Akuamma seed occurs from Côte d'Ivoire to Uganda, Democratic Republic Congo and Cabinda in Angola [4]. Seeds are extensively used for pain relief, chest and stomach problems, pneumonia and intestinal worms, hypertension, jaundice, gastro-intestinal disorders and malaria [5]. Extracts of *P. nitida* seed, fruit rind, and stem-bark have been investigated *in vitro* for antimalarial activities [6]. *Alstonia boonei* De Wild (Apocynaceae), commonly known as cheesewood, is widely distributed in Africa. The stem-bark is used for treating febrile illnesses, painful urination, rheumatic conditions and jaundice [7, 8], malarial fever [9], intestinal helminthes, rheumatism and reversible antifertility [10]. The ethanolic stem-bark extract possessed potent antimalarial effects [11]. Despite the antimalarial reports on *P. nitida* seed and *A. boonei* stem-bark individually in

the literature, there has been no scientific report on the antimalarial efficacy of their mixtures for possible herbal drug formulation and development. Until now, the rationale for the production and sale of their 1:1 ratio in "MAMA Syrup" remedy by the "Village Chemist", in the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria, for human malaria was based on ethnomedical and arbitrary formulation initiatives.

## RESULTS

The yields (%) of the freeze-dried extractives obtained from the decoctions of AP1, AP2 and AP3 were 6.5% w/w, 6.1% w/w and 6.4 % w/w, respectively. The results of the acute toxicity experiment on each ratio revealed no mortality up to 5000 mg/kg in the treated group after 48 hours and 14 days of observation which showed that the LD<sub>50</sub> was greater than 5000 mg/kg.

### Chemosuppressive (early infection) Test

The results of the chemosuppressive test at 12.5 to 50 mg/kg of the extractive showed a dose-dependent reduction in parasitaemia levels while the highest dose of 100 mg/kg on the other hand, showed a reduction in antiplasmodial activities of all the three plant ratios. Over 50% activity was produced by AP1 and AP2 at 6.25 mg/kg which was significantly different from chloroquine (CQ) at 5 mg/kg (CQ) ( $P < 0.05$ ). AP2 gave the highest activity at 12.5 mg/kg with a chemosuppressive activity of 64.4% which was statistically comparable to the activity of CQ (70.3%) ( $P > 0.05$ ). At 50 mg/kg, AP1 gave the highest activity of 67.7% chemosuppression ( $P > 0.05$ ) which then reduced to 59.1% at 100 mg/kg ( $P < 0.01$ ) as shown in table 1. For the purpose of this study, the chloroquine-equivalent, as a biological index, can be defined

as the potency (in % activity) of each ratio at a specified dose in relation to the potency (in % activity) of chloroquine at 5 mg/kg when tested under the same antimalarial experimental conditions which was calculated and used to rank-order the antimalarial effectiveness of the three ratios. For example in table 1 and at 50 mg/kg, AP1 gave 67.7% chemosuppression while CQ gave 70.3%, hence AP1 has produced 96.3% of CQ potency with a CQ-equivalent (CQE) of 0.96 under the chemosuppressive model (Table 2). Also at 12.5 mg/kg, AP2 gave 64.4% chemosuppression while CQ gave 70.3%, hence AP2 has produced 91.6% of CQ potency with a chloroquine-equivalent (CQE) of 0.92 (Table 2). The effective doses of the extractives for chemosuppression at 50 % ( $ED_{50}$ ) and 90 % ( $ED_{90}$ ) were 27.8 mg/kg and 62.1 mg/kg for AP1, 26 mg/kg and 43.8 mg/kg for AP2, then 29.7 mg/kg and 59 mg/kg for AP3, respectively (Fig. 1), showing that AP2 was the most effective.

### Established Malaria Infection (curative) Test

The results of the curative activity of AP2 at all doses showed dose-dependent activities which were comparable to CQ (except at 6.25 mg/kg) on days 3, 4 and 5 (Table 4). The optimum dose for the curative activity appeared to be 12.5 mg/kg for AP2 with the CQE on day 5 at doses of 6.25, 12.5, 25 and 50 mg/kg being 0.87, 0.96, 0.97 and 0.98, respectively under the established infection

test (Table 2). The survival time of mice treated with CQ (positive control) was the best in prolonging the life of mice (24.8 days), followed by 12.5 mg/kg of AP2 with survival time of 18.3 days and 25 mg/kg of AP2 with the lowest survival time of 13.8 days while the negative control gave a survival time of  $9.25 \pm 1.3$  days (Table 4). The rectal temperature of the negative control of mice reduced progressively on day 5 more than that of day 1 post-administration whereas the temperatures of mice for all the tested doses on day 5 showed no significant difference from negative control (Table 5).

### Prophylactic Test

The results of the prophylactic test for AP2 showed dose-dependent repository activities at 6.25, 12.5, 25 and 50 mg/kg with parasite chemosuppression of 29.6, 46.5, 50.0 and 55.3 %, respectively while pyrimethamine gave 89.2 % (Fig. 3). Although, the result is significantly different from the negative control, at best, the prophylactic activities of the extractive solution of AP2 showed moderate efficacy with 33.2, 52.1, 56.1 and 62.0 % of pyrimethamine potency at 6.25, 12.5, 25 and 50 mg/kg, respectively. These can be expressed similarly as the Pyrimethamine-equivalent (PME) of 0.33, 0.52, 0.56 and 0.62 at the same doses, respectively.

**Table 1:** Effects of AP1, AP2 and AP3 on *Plasmodium berghei-berghei* at different doses in the chemosuppressive test

| Dose (mg/kg)     | AP1                   |                       | AP2                   |                       | AP3               |                       |
|------------------|-----------------------|-----------------------|-----------------------|-----------------------|-------------------|-----------------------|
|                  | % Parasitaemia        | % Chemo-suppression   | % Parasitaemia        | % Chemo-suppression   | % Parasitaemia    | % Chemo-suppression   |
| Negative Control | $7.04 \pm 0.28$       | 0.0                   | $7.04 \pm 0.28$       | 0.0                   | $7.04 \pm 0.28$   | 0.0                   |
| 1.56             | $4.55 \pm 0.57^*$     | $35.4 \pm 8.11^b$     | $4.87 \pm 0.58^*$     | $30.9 \pm 8.29^b$     | $5.04 \pm 0.52$   | $28.4 \pm 7.36^b$     |
| 3.13             | $4.16 \pm 0.23^*$     | $40.9 \pm 3.24^b$     | $3.57 \pm 0.37^*$     | $49.3 \pm 5.30^b$     | $3.99 \pm 0.43^*$ | $43.4 \pm 6.12^b$     |
| 6.25             | $3.44 \pm 0.27^*$     | $51.1 \pm 3.86^b$     | $2.97 \pm 0.16^{*,a}$ | $57.9 \pm 2.32^b$     | $3.61 \pm 0.38^*$ | $48.8 \pm 5.43^b$     |
| 12.5             | $3.12 \pm 0.21^*$     | $55.7 \pm 2.95^b$     | $2.51 \pm 0.38^{*,a}$ | $64.4 \pm 5.38^a$     | $3.01 \pm 0.25^*$ | $57.2 \pm 3.48^b$     |
| 25.0             | $2.65 \pm 0.08^*$     | $62.4 \pm 1.18^a$     | $2.49 \pm 0.60^*$     | $64.6 \pm 8.52^a$     | $3.00 \pm 0.31^*$ | $57.4 \pm 4.33^b$     |
| 50.0             | $2.27 \pm 0.37^{*,a}$ | $67.7 \pm 5.30^a$     | $2.47 \pm 0.38^*$     | $64.9 \pm 5.39^a$     | $2.72 \pm 0.37^*$ | $61.4 \pm 5.27^a$     |
| 100.0            | $2.88 \pm 0.26^*$     | $59.1 \pm 3.73^b$     | $3.69 \pm 0.33^*$     | $47.6 \pm 4.47^b$     | $3.51 \pm 0.61^*$ | $50.2 \pm 8.65^b$     |
| Chloroquine(5)   | $2.09 \pm 0.10^{*,a}$ | $70.3 \pm 1.40^{a,b}$ | $2.09 \pm 0.10^*$     | $70.3 \pm 1.40^{a,b}$ | $2.09 \pm 0.10^*$ | $70.3 \pm 1.40^{a,b}$ |

Data are expressed as Mean  $\pm$  SEM,  $^*P < 0.001$  compared with negative control,  $^aP > 0.05$ ,  $^bP < 0.01$  compared with positive control

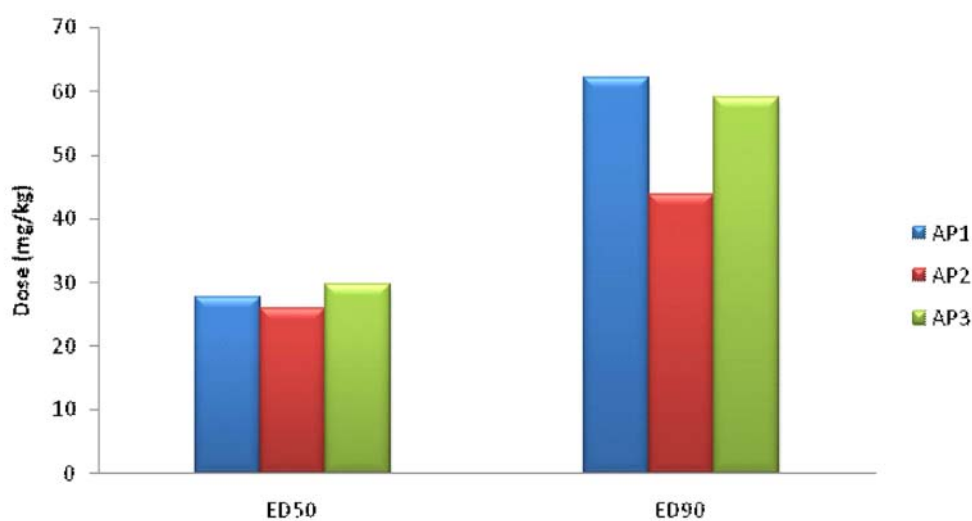
**Table 2** Chloroquine-equivalent (CQE) of AP1, AP2 and AP3 at different doses in the chemosuppressive and curative tests

| Dose (mg/kg) | CQE in Chemosuppressive test |      |      | CQE in Curative test (AP2) |       |       |       |       |
|--------------|------------------------------|------|------|----------------------------|-------|-------|-------|-------|
|              | AP1                          | AP2  | AP3  | Day 1                      | Day 2 | Day 3 | Day 4 | Day 5 |
| 1.56         | 0.50                         | 0.44 | 0.40 |                            |       |       |       |       |
| 3.13         | 0.58                         | 0.70 | 0.62 |                            |       |       |       |       |
| 6.25         | 0.73                         | 0.82 | 0.69 | 0.53                       | 0.54  | 0.82  | 0.90  | 0.87  |
| 12.5         | 0.79                         | 0.92 | 0.81 | 0.66                       | 0.70  | 0.96  | 1.03  | 0.96  |
| 25.0         | 0.89                         | 0.92 | 0.82 | 0.52                       | 0.72  | 1.00  | 1.03  | 0.97  |
| 50.0         | 0.96                         | 0.92 | 0.87 | 1.85                       | 0.80  | 1.03  | 1.05  | 0.98  |
| 100.0        | 0.84                         | 0.68 | 0.71 |                            |       |       |       |       |

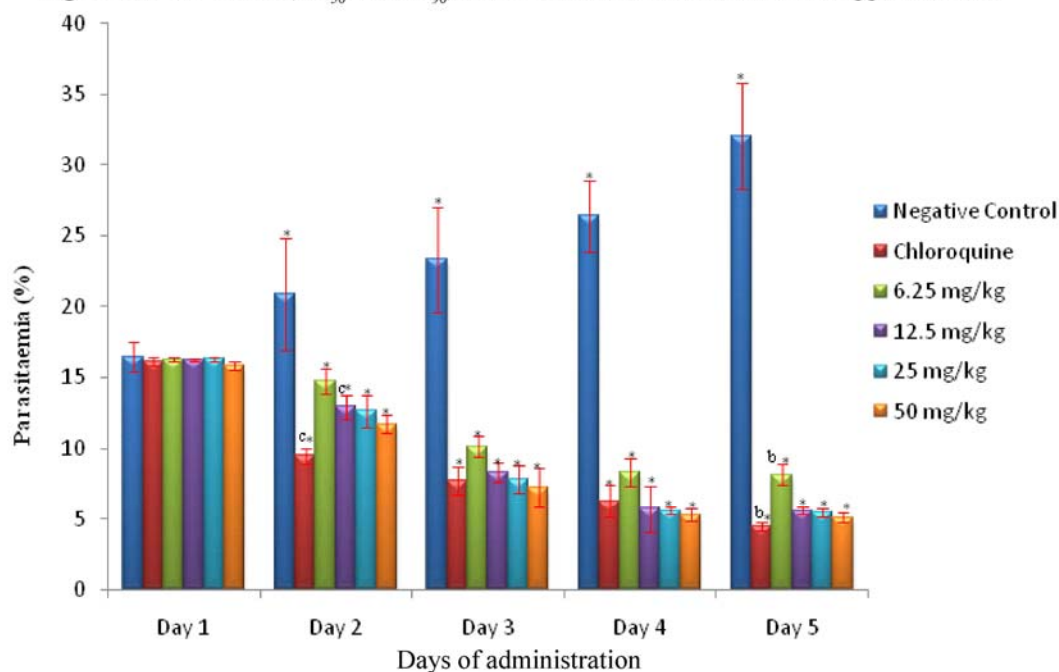
**Table 3:** Mean survival time of *P. berghei-berghei*-infected mice treated with AP1, AP2 and AP3 at different doses in the chemosuppressive test

| Dose (mg/kg)     | Mean survival time (days $\pm$ SEM) |                  |                  |
|------------------|-------------------------------------|------------------|------------------|
|                  | AP1                                 | AP2              | AP3              |
| Negative Control | 10.0 $\pm$ 2.28                     | 10.0 $\pm$ 2.28  | 10.0 $\pm$ 2.28  |
| 1.56             | 14.4 $\pm$ 2.87                     | 13.4 $\pm$ 3.44  | 13.8 $\pm$ 4.92  |
| 3.13             | 15.2 $\pm$ 5.15                     | 17.2 $\pm$ 5.19  | 13.0 $\pm$ 5.39  |
| 6.25             | 14.4 $\pm$ 4.41                     | 16.8 $\pm$ 4.96  | 17.6 $\pm$ 4.59* |
| 12.5             | 22.3 $\pm$ 3.49*                    | 20.2 $\pm$ 1.47* | 20.8 $\pm$ 7.46* |
| 25.0             | 14.6 $\pm$ 4.08                     | 21.0 $\pm$ 4.18* | 22.4 $\pm$ 4.67* |
| 50.0             | 21.5 $\pm$ 4.15*                    | 15.5 $\pm$ 4.50  | 17.0 $\pm$ 2.45* |
| 100.0            | 18.3 $\pm$ 6.06*                    | 19.0 $\pm$ 4.15  | 19.8 $\pm$ 7.00* |
| Chloroquine      | 23.4 $\pm$ 2.33*                    | 23.4 $\pm$ 2.33* | 23.4 $\pm$ 2.33* |

Data are expressed as Mean  $\pm$  SEM, \* $P$  < 0.05 compared to negative control (distilled water)

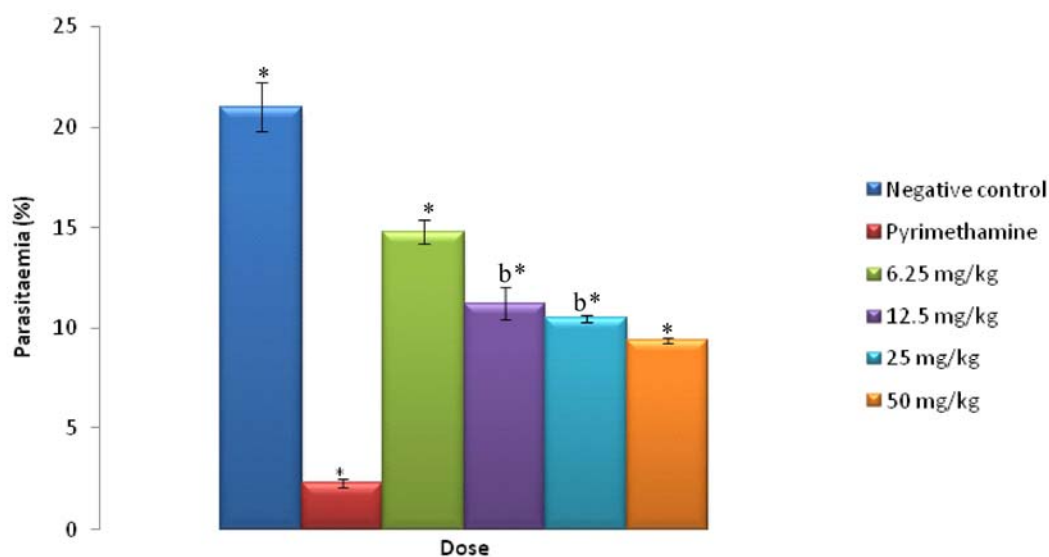


**Fig. 1:** Effective doses (ED<sub>50</sub> and ED<sub>90</sub>) of AP1, AP2 and AP3 in the chemosuppressive test



Data are expressed as Mean  $\pm$  SEM, \* $P$  < 0.001 compared with negative control, <sup>c</sup> $P$  < 0.01, <sup>b</sup> $P$  < 0.05 compared with positive control

**Fig. 2** Mean percentage parasitaemia in mice treated with varying doses of AP2 in the established infection test



Data are expressed as Mean  $\pm$  SEM, \* $P$  < 0.05 and  $^bP$  > 0.05

**Fig. 3** Mean percentage parasitaemia in mice treated with varying doses of AP2 in the prophylactic test

**Table 4** Mean percentage parasite clearance produced by AP2 at different doses and the survival time of mice in the established malaria infection test

| Days                    | Chloroquine<br>(5 mg/kg) | Parasite clearance by AP2 (% $\pm$ SEM) |                   |                   |                   |
|-------------------------|--------------------------|-----------------------------------------|-------------------|-------------------|-------------------|
|                         |                          | 6.25 mg/kg                              | 12.5 mg/kg        | 25 mg/kg          | 50 mg/kg          |
| 1                       | 2.01 $\pm$ 1.83          | 1.07 $\pm$ 0.67                         | 1.34 $\pm$ 0.70   | 1.05 $\pm$ 0.82   | 3.71 $\pm$ 2.03   |
| 2                       | 54.59 $\pm$ 2.63         | 29.20 $\pm$ 4.26*                       | 37.98 $\pm$ 4.06* | 39.42 $\pm$ 5.57* | 43.81 $\pm$ 3.25* |
| 3                       | 66.82 $\pm$ 4.31         | 54.49 $\pm$ 3.21*                       | 64.43 $\pm$ 2.92  | 66.51 $\pm$ 4.18  | 68.91 $\pm$ 5.77  |
| 4                       | 76.23 $\pm$ 4.28         | 68.48 $\pm$ 3.67*                       | 78.21 $\pm$ 6.17  | 78.80 $\pm$ 0.97  | 79.90 $\pm$ 1.64  |
| 5                       | 85.98 $\pm$ 0.85         | 74.52 $\pm$ 2.29*                       | 82.58 $\pm$ 0.85  | 82.98 $\pm$ 1.06  | 84.07 $\pm$ 1.21  |
| Survival time<br>(days) | 24.80 $\pm$ 1.30         | 17.80 $\pm$ 5.89                        | 18.30 $\pm$ 5.80  | 13.80 $\pm$ 6.61  | 16.30 $\pm$ 4.32  |

Data are expressed as Mean  $\pm$  SEM, \* $P$  < 0.01 compared with positive control

**Table 5** Effects of different doses of AP2 on the temperatures of *P. berghei-berghei*-infected mice during the established infection test

| Dose (mg/kg)     | Temperature ( $^{\circ}$ C $\pm$ SEM) |                 |                 |                 |                 |
|------------------|---------------------------------------|-----------------|-----------------|-----------------|-----------------|
|                  | Day 1                                 | Day 2           | Day 3           | Day 4           | Day 5           |
| Negative Control | 35.4 $\pm$ 1.7                        | 35.8 $\pm$ 1.4  | 35.3 $\pm$ 1.3  | 34.9 $\pm$ 0.77 | 34.6 $\pm$ 0.80 |
| 6.25             | 35.6 $\pm$ 0.79                       | 35.9 $\pm$ 0.91 | 35.7 $\pm$ 0.88 | 35.7 $\pm$ 0.92 | 36.1 $\pm$ 0.40 |
| 12.5             | 35.5 $\pm$ 0.21                       | 35.6 $\pm$ 0.77 | 36.1 $\pm$ 0.61 | 36.1 $\pm$ 0.33 | 36.1 $\pm$ 0.56 |
| 25.0             | 35.1 $\pm$ 0.99                       | 35.6 $\pm$ 0.78 | 35.7 $\pm$ 0.41 | 36.0 $\pm$ 0.49 | 36.1 $\pm$ 0.20 |
| 50.0             | 35.7 $\pm$ 0.29                       | 35.6 $\pm$ 0.28 | 35.8 $\pm$ 0.15 | 35.7 $\pm$ 0.22 | 36.0 $\pm$ 0.54 |
| Chloroquine (5)  | 35.7 $\pm$ 0.33                       | 35.9 $\pm$ 0.40 | 35.7 $\pm$ 0.33 | 35.7 $\pm$ 0.07 | 36.2 $\pm$ 0.15 |

Data are expressed as Mean  $\pm$  SEM

## DISCUSSION

The use of different plant materials in form of a decoction for malaria therapy is a common practice in African ethnomedicine. Pharmacological investigations on the antimalarial activities of *A. boonei* stem-bark and *P. nitida* seed individually as well as their active chemical constituents especially the indole alkaloids have been reported by different authors [12]. Until now, the ethnomedical claims on the safety and antimalarial activities of their mixtures, as often found in African traditional medicine practice, have not been verified despite the safety report on the individual plants [13, 14]. The present investigation on three different mixtures of the two plants has reaffirmed their safety (> 5000 mg/kg), using the freeze-dried extractives of the three ratios. The results showed that all the ratios possessed chemosuppressive activities in the order: AP2 > AP1 > AP3. Thus, AP2 produced the best activity with the lowest ED<sub>50</sub> and ED<sub>90</sub> values as well as showed the greatest potentials of being a possible candidate for antimalarial drug development and it was consequently studied for the prophylactic and curative antimalarial activities. The report has thus supported the antimalarial work of Iyiola *et al* [11] who used 400 mg/kg ethanolic extract of *A. boonei* stem-bark with a significant activity and the report of Okokon *et al* [15] on *P. nitida* seed with a significant antimalarial activity at 35 mg/kg of ethanolic extract while the present work showed that the mixtures of the two plants (AP1, AP2 and AP3) have produced higher antimalarial activities at lower doses of the freeze-dried extractives obtained from their decoctions. For example, AP2 gave a curative activity of 82.6 % at 12.5 mg/kg which was statistically comparable to CQ with 86.0 % at 5 mg/kg. Moreover, it was observed that AP2 had highest activity on day 4 with chloroquine-equivalent (CQE) of 1.03, 1.03 and 1.05 at doses of 12.5, 25 and 50 mg/kg, respectively, although it did not exhibit any antipyretic effect (Table 2). We have introduced the use of chloroquine-equivalent (CQE) or pyrimethamine-equivalent (PME) as a valuable antiparasmodial index of comparison which can be employed to rank-order the potencies of any group of antimalarial medicinal plants. Senna-equivalent was previously used for comparing the laxative activities of *Cassia* species [16, 17] while vanillic acid- and *p*-hydroxy benzoic acid-equivalents were used to rank-order the antisickling activities of *Zanthoxylum* species in Nigeria [18]. Having being more dose-efficient with higher potency than when used individually [11, 15], the mixture of the two plants as in AP2 can be developed as an antimalarial alternative drug product. From this study, it is also noted that the plant decoctions required lower doses (e.g. 12.5 mg/kg) for higher activities than when compared with the studies of Iyiola *et al* [11] and Okokon *et al* [15] who used ethanolic extracts at much higher doses (400 mg/kg and 35 mg/kg, respectively), thereby justifying the preference of decoctions in African ethnomedicine. At prophylactic level, the AP2 was not as potent as in the chemosuppressive and curative experiments. Hence, the current marketed herbal product in Nigeria (*MAMA Syrup* of the "Village Chemist") which was based on the AP1 formula (ratio 1:1), will be replaced by AP2 formulation (ratio 1:2) as a result of the present findings on the toxicity and curative experiments.

## CONCLUSION

The study has obtained the best *in vivo* antiplasmodial activities in ratio 1:2 mixture of *A. boonei* stem-bark and *P. nitida* seed (AP2) among the three different mixtures investigated. Further herbal drug development work on AP2 has progressed in our laboratories and will be reported soon.

## MATERIALS AND METHODS

### Plant Materials

The stem-bark of *Alstonia boonei* De Wild (Apocynaceae) was collected from the campus of Obafemi Awolowo University (OAU), Ile-Ife, Nigeria and chopped into small pieces while the whole seed of *Picralima nitida* (Stapf) T. & H. Dur. (Apocynaceae) was purchased from Oja-Oba market, Osogbo, Nigeria in January, 2014. They were identified and authenticated by comparison with the herbarium specimens of previously collected and preserved samples in the IFE Herbarium of Botany Department, OAU, Ile Ife, Nigeria with the Voucher numbers IFE 16536 (*A. boonei* De Wild [Apocynaceae]) and IFE 17430 (*P. nitida* (Stapf) T.&H.Dur.[Apocynaceae]). Both plant materials were separately oven-dried at 50 °C.

### Preparation of Decoctions

Mixtures, consisting of *A. boonei* stem-bark (in small chips) and the dried whole seed of *P. nitida*, in ratios 1:1 (AP1), 1:2 (AP2) and 2:1 (AP3), respectively to make 300 g of each mixture, were separately boiled in 1.5 L of distilled water for 1 h [19]. Each decoction was cooled, filtered and concentrated *in vacuo* at 70°C followed by freeze-drying to obtain the freeze-dried extractive which was kept in a sealed amber bottle until ready for use.

### Animals

Swiss albino mice of both sexes, weighing 18-22 g, were purchased from the animal house of the Faculty of Basic Medical Sciences, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were housed under a 12 h light /dark cycle with free access to water and were fed with commercial food pellets purchased from Capsfeed Limited, Osogbo, Nigeria. All the procedures performed in this study involving the use of animals, were in accordance with the ethical standards of Obafemi Awolowo University Ile-Ife, Nigeria where the study was conducted. The mice were acclimatized for two weeks before the assay and handled in accordance with NIH Guide for the use of laboratory animals [20].

### Parasite Strain

Chloroquine-sensitive *Plasmodium berghei-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 1×10<sup>7</sup> parasitized erythrocytes was prepared by dilution of blood harvested from a donor mouse (> 30% parasitaemia) with normal saline and administered intraperitoneally (200 µl) to each test mouse

### Acute Toxicity

The median lethal dose ( $LD_{50}$ ) of each of the freeze-dried extractives of the decoctions was determined *in vivo* using the procedure described by Lorke [21]. For each ratio and in the first phase, nine mice were randomly divided into three groups of three mice each and each group received the freeze-dried extractive solution at 10, 100 and 1000 mg/kg body weight orally (*via* a feeding cannula). The mice were then observed for signs of adverse effects and mortality for 48 h and then for 14 days. In the second phase and for each ratio, four mice were divided into 4 groups of 1 mouse each and were each similarly treated at doses of 1000, 1600, 2900 and 5000 mg/kg orally. The animals were then monitored for any sign of toxicity or mortality over a period of 14 days.

### Chemosuppressive (early Infection) Test

One hundred and fifteen (115) animals were intraperitoneally inoculated, 2 h prior to dosing [22]. The animals were grouped into 23 groups of 5 mice per group. Groups 1–7, 8–14 and 15–21 for AP1, AP2 and AP3 (7 animal groups per ratio) and were respectively dosed orally with the freeze-dried extractive solution at 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/kg/day for 4 consecutive days ( $D_0$ – $D_3$ ). Mice in Groups 22 and 23 were similarly given CQ at 5 mg/kg/day (positive control) and 0.2 ml distilled water/mouse/day (negative control), respectively. Doses were given once daily, using a metal feeding cannula. On the fifth day ( $D_4$ ), the parasitaemia level was assessed by examining and counting the cells in the Giemsa-stained blood smears. The percentage of parasitaemia suppression was determined by recording the number of parasitized erythrocytes out of 500 red blood cells counted in random fields under light microscope and calculating the average percentage parasitaemia suppression using the formula  $100\{(A - B)/A\}$ , where *A* is the average percentage parasitaemia in the negative control group and *B* is the average parasitaemia in the test group.

### Prophylactic (Repository) Test

Evaluation of the prophylactic potential was carried out according to the method of Peters [23]. Thirty mice were grouped into six (6) groups of 5 mice each; Group 1–4 were orally administered with AP2 at 6.25–50 mg/kg/day while the control animals (Group 5) were given 1.25 mg/kg/day pyrimethamine (positive control) and Group 6 received 0.2 ml/mouse/day distilled water (negative control) for three consecutive days ( $D_0$ – $D_2$ ), respectively. On day 4 ( $D_4$ ), the mice were inoculated with *P. berghei-berghei*-infected red blood cells. Blood smears were then made from each mouse after seventy-two hours (post-inoculation) to evaluate the parasitaemia levels to calculate the percentage chemosuppression.

### Established Infection (curative) Test

Thirty (30) mice were inoculated with *P. berghei-berghei*, 72 h prior to drug administration [24]. The mice were randomly divided into 6 groups of 5 mice each and Groups 1–4 were orally treated with AP2 at 6.25, 12.5, 25 and 50 mg/kg/day. Chloroquine at 5 mg/kg/day and distilled water (0.2 ml) were administered to Groups 5 and 6 as positive and negative controls, respectively. The dosing, preparation of blood smears and microscopic examination of

parasitized cells to assess the parasitaemia levels were repeated daily for 5 days ( $D_1$ – $D_5$ ).

### Calculation of Chloroquine-equivalent (CQE)

The chloroquine-equivalent (CQE) of any antimalarial plant material is the relative antiplasmodial activity of that plant at a specific dose in relation to the antiplasmodial activity of chloroquine at 5 mg/kg when tested under the same antimalarial experimental conditions. It measures how much CQ is more or less potent than the plant material under investigation using the following formula:  $AMP_{activity}/CQ_{activity}$ , where  $AMP_{activity}$  = % antiplasmodial activity at a given dose (mg/kg) of any antimalarial plant being investigated;  $CQ_{activity}$  = % antiplasmodial activity of chloroquine at 5mg/kg.

### Rectal Temperatures and Survival Times

The rectal temperature of each mouse was taken daily throughout the period of experiment. The mice were then observed for 28 days post-inoculation and any mortality, occurring during the period, was recorded in order to calculate the survival times of the mice in each group.

### Data Analysis

The percentage parasitaemia reduction was expressed as mean  $\pm$  SEM while the effective doses were determined using Microsoft Excel (2010) and the variation in a set of data was analyzed through the one-way analysis of variance. The difference among the means was considered at 95 % confidence level using the post-hoc method of Newman-Keuls using GraphPad Prism software (2011).

### Acknowledgements

The authors wish to acknowledge Professor O. G. Ademowo of the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan, Nigeria, for supplying the rodent parasite, *Plasmodium berghei-berghei* NK65.

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