

## Full Length Research Paper

# ***In vivo* anti-plasmodial activity and histopathological analysis of water and ethanol extracts of a polyherbal antimalarial recipe**

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Received 21 February, 2017; Accepted 12 April, 2017

*Anthocleista djalonensis* A. Chev. (stem bark), *Azadirachta indica* A. Juss (stem bark and leaf), *Cajanus cajan* (L.) Huth. (leaf), *Crescentia cujete* L. (stem bark), *Lawsonia inermis* L. (leaf), *Lophira alata* Banks ex C.F. Gaertn. (stem bark), *Myrianthus pruessii* Engl. (leaf), *Nauclea latifolia* Sm. (stem bark), *Olax subscorpioidea* Oliv. (root), and *Terminalia glaucescens* Planch ex Benth. (stem bark and root) are combined for use in the treatment of malaria in Akure, Southwestern Nigeria. The powdered plant samples were screened for phytochemical constituents, proximate composition and mineral elements according to standard protocols. *Plasmodium berghei* infected mice were screened for parasitemia and administered with water and ethanol extracts of the combined plant sample. Toxicity and histopathological studies were carried out on the liver and kidney sections of the mice. Data were statistically analyzed. The powdered herbal recipe contained appreciable phytochemicals and important minerals. The concentrations administered for LD<sub>50</sub> did not elicit adverse reactions in the experimental animals, and no mortality was recorded. Histological studies revealed some pathology caused by the malaria parasite, as well as side effects of the extracts administered. This is discussed in relation to safety considerations.

**Key words:** Malaria, herbs, phytochemical, histopathology, Nigeria.

## INTRODUCTION

Malaria, an infectious disease caused by *Plasmodium* species, has been a menace to the health conditions of both rural and urban populations in Nigeria (NGA, 2005). Although, it is a global epidemic the incidence and severity are higher in the tropics especially in the sub-Saharan Africa, where pregnant women and children are the most susceptible (Nmorsi et al., 2007; WHO, 2008; Nguta et al., 2010; Akanbi et al., 2012). It is prevalent in

the tropical and subtropical regions because environmental factors such as rainfall, warm temperatures, and stagnant water provide the ideal habitats for the development of the mosquito that serve as the vector. Approximately 40% of the world's population is susceptible to malaria. Records have shown that 3.3 billion people all over the world live in areas at risk of malaria with episodes in 106 countries and

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territories (African Medical and Research Foundation - AMRF, 2012). In the year 2010, an estimated 655,000 people died of malaria - most of whom were young children in sub-Saharan Africa where one in every five childhood deaths is due to malaria (Ibrahim et al., 2004). Moreover, 91% of deaths in 2010 were in the African region, followed by the South-East Asian region (6%), and the Eastern Mediterranean region (3%), and about 86% of death in children globally (WHO, 2011). However, more than three million deaths and 300 to 500 million cases are still reported annually in the world (Sachs and Malaney, 2002).

In recent years, malaria has become more difficult to control and treat with conventional drugs of western medicine because malaria parasites have become resistant to drugs, and mosquitoes that transmit the disease have become resistant to insecticides. Plants, particularly herbs, are the basis for the development of modern drugs, and medicinal plants have been used for many years in daily life to treat diseases all over the world (Ates and Erdogru, 2003). At present, traditional medicine is still the predominant means of health care in developing countries where about 80% of their total population depend on it for their wellbeing (WHO, 1978).

Ethnomedicinal investigation revealed that *Anthocleista djalensis* A. Chev. (stem bark), *Azadirachta indica* A. Juss (stem bark and leaf), *Cajanus cajan* (L.) Huth. (leaf), *Crescentia cujete* L. (stem bark), *Lawsonia inermis* L. (leaf), *Lophira alata* Banks ex C.F. Gaertn. (stem bark), *Myrianthus pruessii* Engl. (leaf), *Nauclea latifolia* Sm. (stem bark), *Olex subscorpioidea* Oliv. (root), and *Terminalia glaucescens* Planch ex Benth. (stem bark and root) are combined for use as a polyherbal recipe in the treatment of malaria in Akure, Southwestern Nigeria.

Akure is a popular metropolis in Ondo State and supports a population of over 400,000 people. The mean annual rainfall is about 1350 mm with bimodal distribution spanning between March and November; the relative humidity averaged 80% with temperature range between 23 and 30°C which is suitable for agricultural production. Civil servants are the major inhabitants of the city which is the centre of administration of the Ondo State Government. However, farming and trading are other occupations of the residents who majored in food crops and livestock production.

This study aimed to screen the plant parts (root, stem bark, leaf) of some herbs used in the treatment of malaria and also to evaluate the toxicity and histopathological effects of the plant extracts on tissues of the kidney and liver using albino mice infected with *Plasmodium berghei* as a model.

## MATERIALS AND METHODS

### Procurement and identification of plant materials

Fresh samples of *A. djalensis* (stem bark), *A. indica* (stem bark and leaf), *C. cajan* (leaf), *C. cujete* (stem bark), *L. inermis* (leaf), *L.*

*alata* (stem bark), *M. pruessii* (leaf), *N. latifolia* (stem bark), *O. subscorpioidea* (root), and *T. glaucescens* (stem bark and root) were bought from herb sellers in Akure and identified at the University of Ibadan herbarium by comparing them with representative specimens. Voucher specimens were deposited in the herbarium.

### Preparation of plant samples

The fresh samples were air-dried for 2 to 3 weeks depending on the plant part. The dried plant samples were then pulverized to coarse powder using a laboratory mill (Model 4 Arthur Thomas, USA). The coarse powder was screened for phytochemical, proximate, and mineral compositions.

### Phytochemical screening

The powdered plant samples were screened for alkaloids, anthraquinones, flavonoids, glycosides, polyphenols, saponins, and tannins according to the methods described by Harbone (1973), Evans (2002) and Sofowora (2008).

### Mineral element analysis

The method of the Association of Analytical Chemists (AOAC, 1990) was used. Calcium (Ca), Sodium (Na), Potassium (K), Phosphorus (P), Copper (Cu), Iron (Fe), Zinc (Zn), Magnesium (Mg), Manganese (Mn), and Lead (Pb) were quantified. Sodium and potassium were estimated with Gallenkamp Flame Analyzer. Phosphorus was determined using phosphor-vanado-molybdate colorimetric techniques, whereas calcium, iron, magnesium, manganese, zinc, lead, and copper were determined using Spectrumlab 23A Spectrophotometer.

### Preparation and concentration of extracts

combination (recipe) comprising 50 g each of the 10 powdered plant samples was dissolved in distilled water (500 g in 3 L of distilled water for 24 h) and ethanol (500 g in 3 L of 96% ethanol for 72 h) separately. The individual preparation was stirred every 2 h, decanted and filtered using Whatman No 1 filter paper. The solvent containing the extract was collected, filtered again and concentrated using a rotary evaporator at 40°C. The crude ethanolic extract was further concentrated in a vacuum oven set at 40°C with a pressure of 600 mmHg so as to further remove any traces of solvent. The crude extract of water solvent was further concentrated in a thermo-regulated water bath at 40°C. The concentrate was retrieved and weighed. The extract was refrigerated at 4°C prior to use.

### Experimental animals (Swiss albino mice)

The Swiss albino mice weighing between eighteen and twenty-two grammes (18 to 22 g) were purchased from the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan. The animals were housed in iron cages in the animal house of IAMRAT. The animals were acclimatized for two weeks at room temperature with 12 h dark/light periodicity and fed with commercial chow (purchased from Cap Feeds Ibadan, Nigeria) and water *ad libitum*.

### Experimental design

The experiment was in two phases (Phases 1 and 2). In Phase 1,

the median lethal dose (LD<sub>50</sub>) was determined and in Phase 2, the antiparasitodal activity of the recipe was examined.

### Phase 1: Determination of median lethal dose

Twelve-four (24) mice were used. Four (4) mice received 1000 mg/kg body weight of water extract of the combined plant samples; another four (4) mice received 2000 mg/kg body weight; and still another four (4) mice received 3000 mg/kg body weight. The set-up was the same for the ethanol extract. All the animals were monitored for loss of appetite, pains, distress, change in respiration, behavioural manifestations, and most importantly death for a period of 24 h. Oral administration of extract was carried out using gastric feeding tube for 28 days, for long-term possible lethal outcomes (Lorke, 1983).

### Phase 2: Antimalarial activity of plant extract

Forty (40) mice were used in all. Five (5) mice received 200 mg/kg body weight of water extract of the combined plant samples; another five (5) mice received 300 mg/kg body weight; and still another five (5) mice received 500 mg/kg body weight. The set-up was the same for the ethanol extract. The control groups were administered with distilled water (5 mice) and chloroquine (5 mice).

### Malaria parasite specimen

*P. berghei* (NK65) was obtained from the Department of Parasitology, Institute of Advanced Medical Research and Training (IAMRAT), University College Hospital (UCH) Ibadan, Nigeria.

### Ethical consideration

Ethical guidelines for the use of animal models in research were followed; clearance was sought and obtained from the University of Ibadan/University College Hospital Ethical Committee through the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria.

### Inoculation of mice

A Swiss albino mouse (which served as the donor mouse) was intraperitoneally administered with a standard inoculum of *P. berghei* on day 0. On the 5<sup>th</sup> day (when the parasite had stabilized in the host mouse), blood was withdrawn from the heart of the donor mouse by cardiac puncture and diluted with isotonic saline. Normal saline and 0.1 ml of acid citrate dextrose (ACD) were drawn into the syringe to make inoculum for infecting experimental mice. Thereafter, the experimental mice were inoculated with 0.2 ml of parasite specimen (containing about  $1 \times 10^7$  parasitized cells).

### Determination of parasitemia

Blood was obtained from each of the experimental mice via a tail cut from which thin blood smears were prepared. Smears were fixed with methanol for 5 min and stained with 10% Geimsa. The slides were observed with compound microscope under x100 to determine the number of parasitized cell per magnification field. For each blood smear specific for a given mouse, four magnification fields were observed and the number of parasitized cells and the total number of cells in the magnification field were recorded. The data obtained was used to determine percentage parasitemia using

the method described by Hilou et al. (2006).

$$\% \text{ Parasitemia} = \frac{\text{Total number of parasitized red blood cells}}{\text{Total number of red blood cells}} \times 100\%$$

### In vivo antimalarial study

By the 5<sup>th</sup> day from inoculation, parasites were fully established in the blood of infected mice. 200, 300 and 500 mg/kg body weight of water and ethanol extracts were administered to the experimental mice. Distilled water and 0.2 ml of Chloroquine were administered as negative and positive controls, respectively.

### Evaluation of toxicity

On the 29<sup>th</sup> day, the experiment was discontinued. The mice were weighed, anaesthetized with chloroform and blood samples collected by cardiac puncture for serum biochemical and haematological analyses. Blood samples obtained by cardiac puncture were analyzed for white blood cells (WBC), red blood cells (RBC), platelet (Plt), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Serum biochemical parameters evaluated were total protein, albumin, globulin, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), bilirubin, creatinine, sodium, potassium, calcium, magnesium, and chloride ion (Dacie and Lewis, 1991; Bergmeyer et al., 1986; Roy, 1970).

### Histopathological studies

The experimental mice were sacrificed and their liver and kidney were excised, weighed, trimmed and fixed in Bouin's solution. Fixed tissues were dehydrated, in ascending series of alcohol, cleared in xylene, and embedded in paraffin wax melted at 60°C. Serial sections (5 µm thick) were mounted in 3-aminopropyl triethsilane-coated slides and allowed to dry for 24 h at 37°C. The sections on the slides were de-paraffined, hydrated and stained with Mayer's haematoxylin and eosin dyes, dried and mounted, and thereafter examined under a light microscope (Drury et al., 1967).

### Data analysis

Data were statistically analyzed and where necessary expressed as mean ± SD. Differences in means were assessed for significance with Duncan multiple range test (DMRT) at p>0.05 using IBM SPSS Statistics version 20.

## RESULTS

### The medicinal plants profile

The plant profile of the ten (10) medicinal plants used in the treatment of malaria in Akure, Southwestern Nigeria is presented in Table 1.

### Quantitative phytochemical composition

Table 2 shows the phytochemical constituents of the

**Table 1.** Plant profile of ten medicinal plants used in the management of malaria in Akure, Southwestern Nigeria.

S/N	Botanical name	Family	Local name (Yoruba)	Common name	Habit	Part used
1	<i>Anthocleista djalonensis</i> A. Chev	Loganiaceae	Sapo	Cabbage tree	Tree	Stem bark
2	<i>Azadirachta indica</i> A. Juss	Meliaceae	Dongoyaro	Neem	Tree	Stem bark, leaf
3	<i>Cajanus cajan</i> (L.) Huth.	Fabaceae	Otili	Pigeon pea	Shrub	Leaf
4	<i>Crecentia cujete</i> L.	Bignoniaceae	Igi-sogba	Calabash tree	Tree	Stem bark
5	<i>Lawsonia inermis</i> L.	Lythraceae	Laali	Henna	Shrub	Leaf
6	<i>Lophira alata</i> Banks ex C.F. Gaertn.	Ochnaceae	Ponhan	Red ironwood	Tree	Stem bark
7	<i>Myrianthus pruessii</i> Engl.	Urticaceae	Ogunsere	-	Tree	Leaf
8	<i>Nauclea latifolia</i> Sm.	Rubiaceae	Egbesi	African peach	Tree	Stem bark
9	<i>Olax subscorpioidea</i> Oliv.	Olacaceae	Ifon	-	Shrub	Root
10	<i>Terminalia glaucescens</i> Planch ex Benth.	Combretaceae	Idi	-	Tree	Stem bark, root

**Table 2.** Phytochemical constituents of combined medicinal plants used in the treatment of malaria in Akure, Southwestern Nigeria.

Parameter	Concentration (mg/100 g)
Alkaloids	0.78±0.01
Flavonoids	0.00±0.00
Saponins	0.31±0.01
Tannins	0.05±0.00
Polyphenols	0.18±0.00
Anthraquinones	0.00±0.00
Cardiac glycosides	0.35±0.00

Values are mean ± SD; n=3.

**Table 3.** Proximate composition of combined medicinal plants used in the treatment of malaria in Akure, Southwestern Nigeria.

Parameter	Composition (%)
Protein	16.52±0.30
Fat	12.28±0.20
Fibre	15.50±0.30
Ash	8.50±0.50
Carbohydrate	40.60±0.70
Moisture	6.60±0.50

Values are mean ± SD; n=3.

combined plant sample. The recipe contained appreciable quantity of alkaloids, saponins, tannins, polyphenols, and cardiac glycosides. Flavonoids and anthraquinones were altogether absent.

#### Proximate and mineral elements contents

The powdered recipe also contained important nutritive and mineral elements and are presented in Tables 3 and

4, respectively. Carbohydrate, protein, fat, and fibre were relatively high while ash and moisture were low. Phosphorus, calcium, magnesium, iron, and zinc were found to be high while sodium, potassium, and copper were found to be relatively low. Lead was absent.

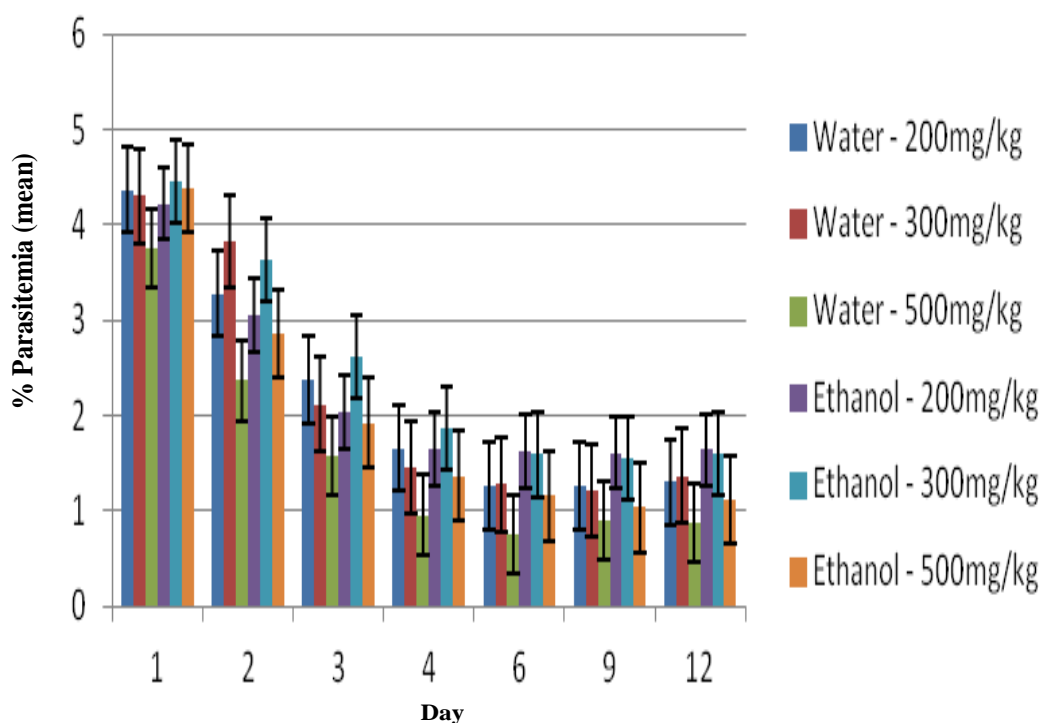
#### Antiplasmodial activity of the combined plant sample

Figures 1 and 2 show the comparative antiplasmodial

**Table 4.** Quantitative mineral contents of combined medicinal plants used in the treatment of malaria in Akure, Southwestern Nigeria.

Parameter	Concentration (mg/kg)
Phosphorus	1800.50±15.00
Calcium	25280.00±30.50
Magnesium	4870.30±23.30
Sodium	0.90±0.03
Potassium	1.50±0.01
Manganese	95.60±2.80
Iron	770.37±16.77
Copper	21.64±0.60
Zinc	250.34±10.58
Lead	

Values are mean ± SD; n=3.



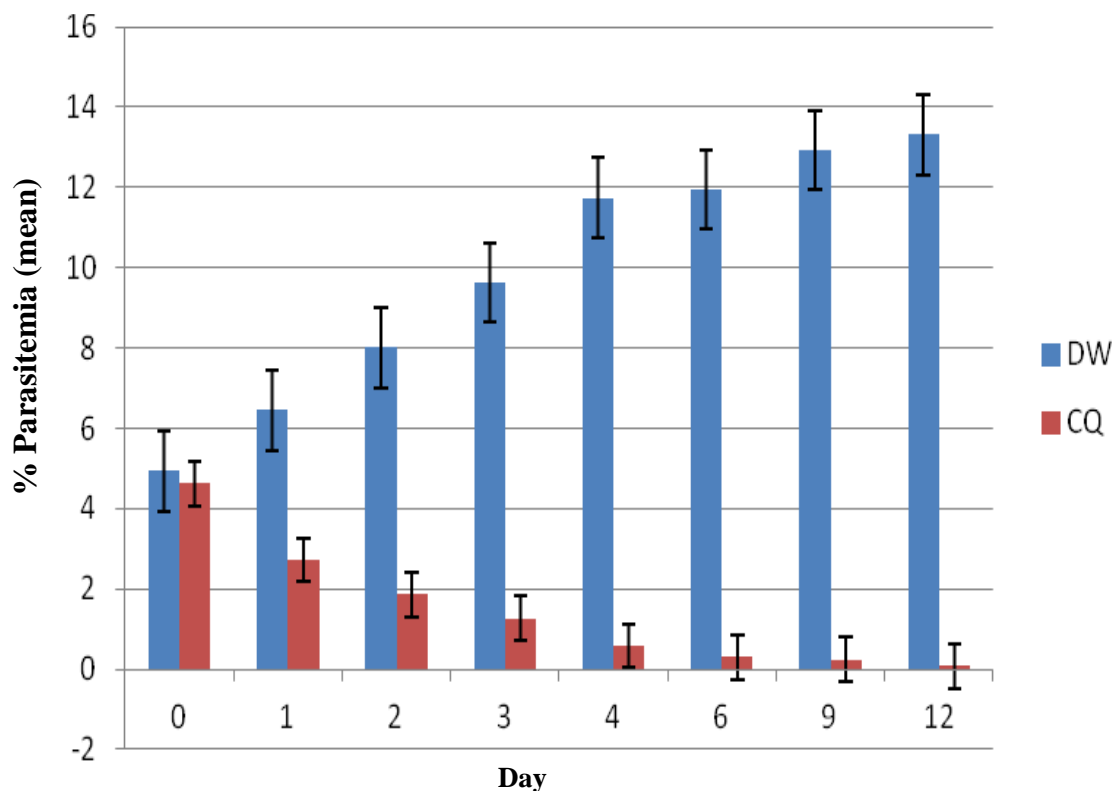
**Figure 1.** Comparative percentage parasitemia (mean) of *Plasmodium berghei* infected mice treated with water and ethanol extracts of the combined extract till 12<sup>th</sup> day post establishment.

activity of the recipe at graded concentrations of 200, 300, and 500 mg/kg body weight and the control group (negative and positive). Optimum activity was recorded on Day 4. Activity was highest with water extract of recipe at 500 mg/kg.

#### Serum biochemical, haematological, and histopathological studies

Serum biochemical and haematological values are

presented in Tables 5 and 6, respectively. Histological studies revealed some pathology caused by the malaria parasite and presentations of conditions after administration of extracts. These conditions (indicated on Plates 1 to 16) include interstitial nephritis, widespread severe flattening of the epithelium of renal tubules, congestion of interstitial renal blood vessels, hepatocellular necrosis, severe necrosis of epithelial renal tubules, nephrosis, congestion of hepatic sinusoids, and the presence of *Plasmodium* gametocytes (extra-erythrocytes). The damages were severe in the negative



**Figure 2.** Comparative percentage parasitemia (mean) of *P. berghei* infected mice treated with distilled water and chloroquine till 12<sup>th</sup> day post establishment.

control (the group administered with distilled water after infection and establishment of malaria parasite).

## DISCUSSION

This study reports the anti-plasmodial activity of a polyherbal recipe and serves as a ground breaking report of such combination. The traditional uses and the scientific justification of individual plants that formed the recipe used in this study have been reported. For example, the leaf and stem bark of *Nauclea latifolia* in the traditional treatment of yellow fever, toothache, dental caries, dysentery, septic, mouth, high blood pressure and diarrhoea have been reported by Benoit-Vicala et al. (1998) while Kayode (2006) reported anti-plasmodial activity of leaves, stem bark, root and seed of *Lophira alata*. Also, Priyanka et al. (2013) reported the antiplasmodial potentials demonstrated by *A. indica* in *P. berghei* infected mice model. Udobre et al. (2013) reported that the methanol leaf extract of *N. latifolia* reduced parasitemia in a dose dependent manner in albino mice infected with *P. berghei*. Akpanabiatu et al. (2005) also confirmed the vasodilatory property of the ethanol extract of *N. latifolia* and its action on aorta as well as the lipid profiles of rat upon administration with an increase in potassium concentration. All these reports are

in line with the activity obtained for the combination of the plants.

A polyherbal comprising *A. indica*, *C. papaya*, and *M. indica* showed antiplasmodial property (Ofori-Attah et al., 2012). Similar investigation on the prophylactic effect of a multi-herbal extract (*C. cajan* leaf, *E. laterifolia* leaf, *M. indica* leaf and stem, *Cymbopogon giganteas* leaf, and *Uvaria chamae* bark) by Nwabuisi (2002) gave noteworthy antimalarial activity with no apparent significant side effects. Binary combination of *Artocarpus altilis*, *Enantia chlorantha*, or *Murraya koenigii* with *N. latifolia* significantly increased the prophylactic and suppressive activities of the individual plants (Adebajo et al., 2014). In contrast, Arise et al. (2012) reported hepatotoxic and nephrotoxic potentials of the aqueous extract of *N. latifolia* stem. The toxic potentials of *N. latifolia* on the kidney and liver of the animals presents a great concern in the consumption of this plant since this study also reports the pathological changes observed upon administration of the plant extract.

Alkaloids, saponins, and flavonoids have been implicated to be responsible for antimalarial activity (Etebong et al., 2015) as these secondary metabolites elicit bioactivity wholly or in combination with other plants (Shigemori et al., 2003). Malaria parasites in wreaking havoc synthesize protein and produce free radicals in the human body. These vices are corrected in the presence

**Table 5.** Serum biochemical of *Plasmodium berghei* infected mice after 12 days post infection treatment with water and ethanol extracts of antimalarial recipe.

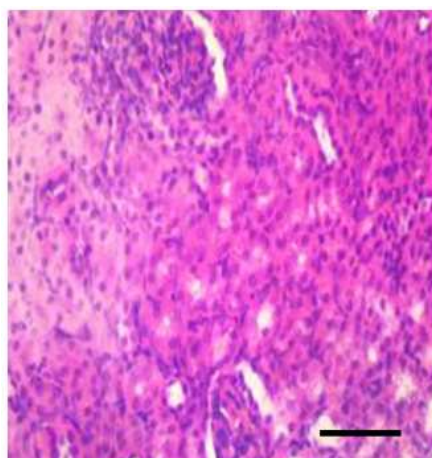
Group	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb-Glob. ratio	AST ( $\mu$ l)	ALT ( $\mu$ l)	ALP ( $\mu$ l)	BUN (mg/dl)	Creatinine (mg/kg)	Sodium (mg/l)
Combination <sub>Water</sub>	7.35 $\pm$ 0.47 <sup>e</sup>	3.28 $\pm$ 0.38 <sup>g</sup>	4.07 $\pm$ 0.32 <sup>c</sup>	0.83 $\pm$ 0.12 <sup>abcd</sup>	88.67 $\pm$ 5.45 <sup>def</sup>	47.83 $\pm$ 5.31 <sup>a</sup>	88.83 $\pm$ 12.70 <sup>a</sup>	18.83 $\pm$ 4.20 <sup>abc</sup>	0.40 $\pm$ 0.12 <sup>a</sup>	133.33 $\pm$ 11.45 <sup>a</sup>
Combination <sub>Ethanol</sub>	8.32 $\pm$ 0.61 <sup>f</sup>	3.42 $\pm$ 0.29 <sup>g</sup>	4.90 $\pm$ 0.35 <sup>d</sup>	0.63 $\pm$ 0.05 <sup>a</sup>	89.17 $\pm$ 10.26 <sup>def</sup>	54.17 $\pm$ 7.73 <sup>a</sup>	142.00 $\pm$ 24.75 <sup>b</sup>	22.67 $\pm$ 0.99 <sup>d</sup>	0.98 $\pm$ 0.22 <sup>e</sup>	155.50 $\pm$ 9.72 <sup>d</sup>
Control <sub>DW</sub>	4.33 $\pm$ 0.26 <sup>a</sup>	1.88 $\pm$ 0.07 <sup>a</sup>	2.45 $\pm$ 0.25 <sup>a</sup>	0.80 $\pm$ 0.06 <sup>abc</sup>	52.83 $\pm$ 13.41 <sup>a</sup>	49.67 $\pm$ 6.79 <sup>a</sup>	89.83 $\pm$ 13.81 <sup>a</sup>	19.67 $\pm$ 4.21 <sup>abcd</sup>	0.47 $\pm$ 0.12 <sup>abc</sup>	148.67 $\pm$ 14.01 <sup>cd</sup>
Control <sub>CQ</sub>	7.40 $\pm$ 0.76 <sup>e</sup>	3.15 $\pm$ 0.40 <sup>g</sup>	4.25 $\pm$ 0.06 <sup>c</sup>	0.75 $\pm$ 0.06 <sup>abc</sup>	96.50 $\pm$ 4.04 <sup>f</sup>	57.00 $\pm$ 4.62 <sup>a</sup>	152.00 $\pm$ 47.34 <sup>b</sup>	21.00 $\pm$ 1.16 <sup>abcd</sup>	0.60 $\pm$ 0.12 <sup>d</sup>	168.00 $\pm$ 17.32 <sup>e</sup>

Values are mean  $\pm$  SD; n = 15 except control where n = 5. Values with the same letter in the same column are not significantly different with Duncan's multiple range Test (DMRT), p>0.05.

**Table 6.** Haematological effects of water and ethanol extract of antimalarial recipe on *P. berghei* infected mice.

Group/Plant	PCV (%)	Hb (mg/dl)	WBC ( $10^3/\text{mm}^3$ )	MCV ( $\text{m}^3$ )	MCH ( $\times 10^{-5}$ )	MCHC (g %)	RBC ( $10^6/\text{mm}^3$ )
Combination (water)	32.00	9.07	12.47	53.87	18.50	34.47	4.89
Combination (ethanol)	32.00	10.20	11.50	55.87	19.37	34.63	5.32
Control (Distilled water)	37.00	9.50	4.80	55.60	19.20	34.50	4.96
Control (CQ)	44.00	13.70	5.30	56.80	18.70	33.00	7.33

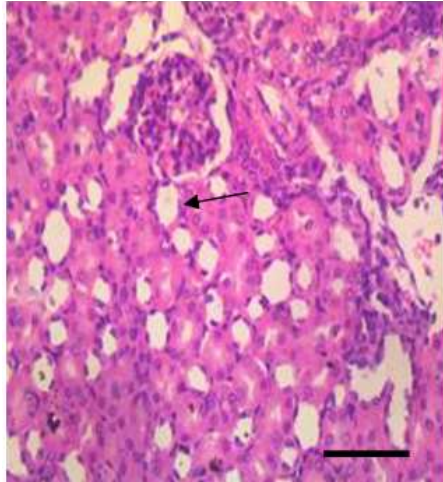
Values are mean of 6 determinations. PCV = Packed cell volume; Hb = Haemoglobin; WBC= White blood cell; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; RBC = Red blood cell.



NVL except for a mild congestion of renal interstitial blood vessels

(H&E stain, X400). Scale bar: 20  $\mu$ m

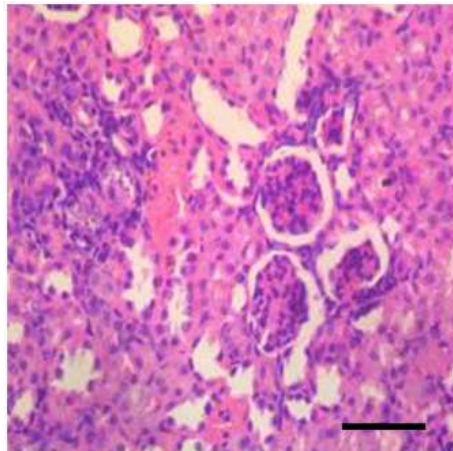
**Plate 1.** Effect of 200 mg/kg water extract of combination of plants on kidney.



← = Mild sloughing off of epithelium of tubules in the renal cortex.

(H&E stain, X400). Scale bar: 20  $\mu$ m

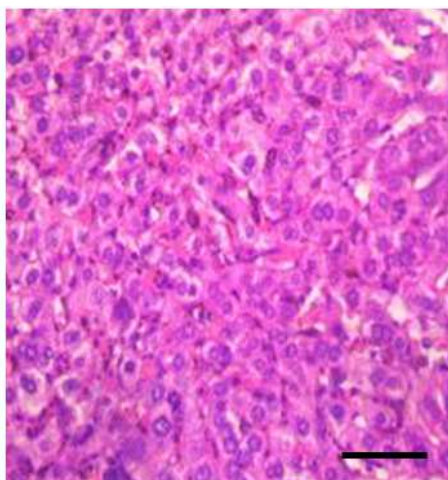
**Plate 3.** Effect of 500 mg/kg water extract of combination of plants on kidney.



☆ = Moderate congestion of renal interstitial blood vessels

(H&E stain, X400). Scale bar: 20  $\mu$ m

**Plate 3.** Effect of 500 mg/kg water extract of combination of plants on kidney.



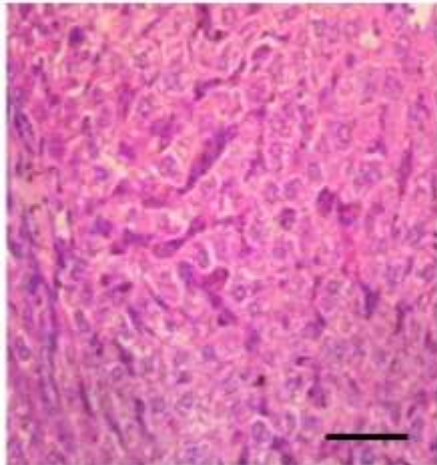
= Bile stasis within the canaliculi

= Widespread congestion of hepatic sinusoids

(H&E stain, X400). Scale bar: 20  $\mu$ m

**Plate 4.** Effect of 200 mg/kg water extract of combination of plants on liver.

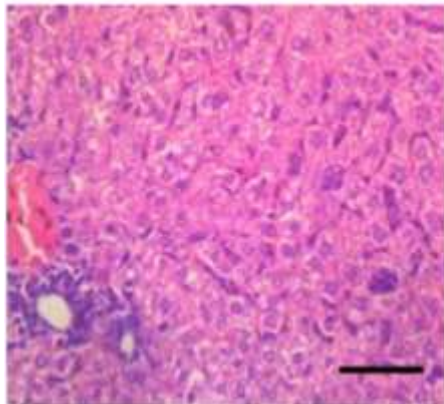




= Moderate KCH with some of the cells containing dark brown pigments.

(H&E stain, X400). Scale bar: 20  $\mu$ m

**Plate 5.** Effect of 300 mg/kg water extract of combination of plants on liver.

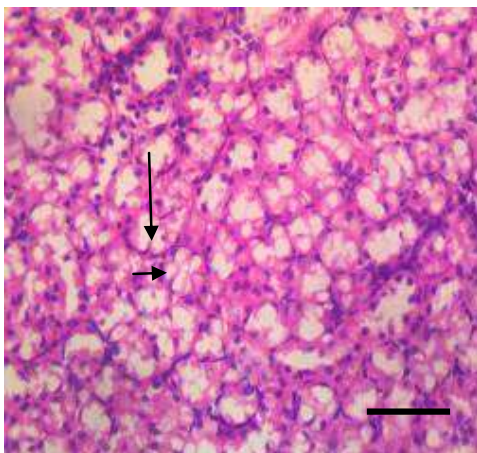


= Moderate KCH with some of the cells containing dark brown pigments and formation of multinucleated giant cells

= Widespread congestion of hepatic sinusoids and portal vessels.

(H&E stain, X400). Scale bar: 20  $\mu$ m

**Plate 6.** Effect of 500 mg/kg water extract of combination of plants on liver.



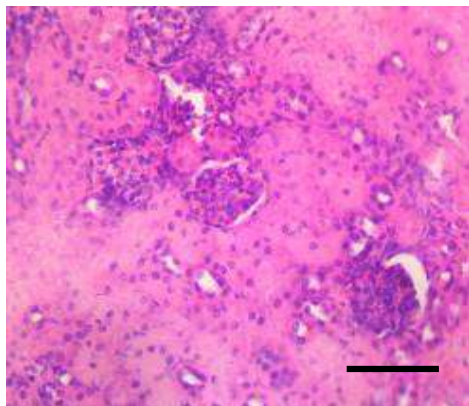
↓ = Moderate vacuolar change of the epithelial cells of tubules in the renal medulla

(H&E stain, X400). Scale bar: 20 $\mu$ m

**Plate 7.** Effect of 200 mg/kg ethanol extract of combination of plants on kidney.

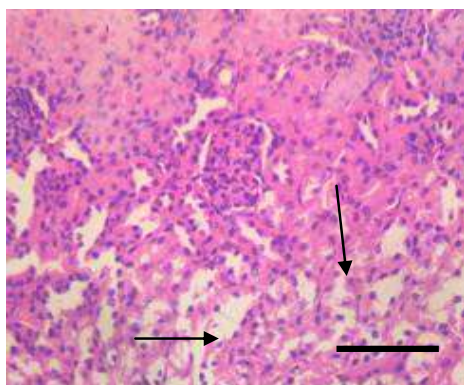
of alkaloids which block protein-synthesis of *Plasmodium* species, and flavonoid, saponin, and tannin which are

involved in primary anti-oxidation of free radicals and other reactive oxygen species (David et al., 2004). In



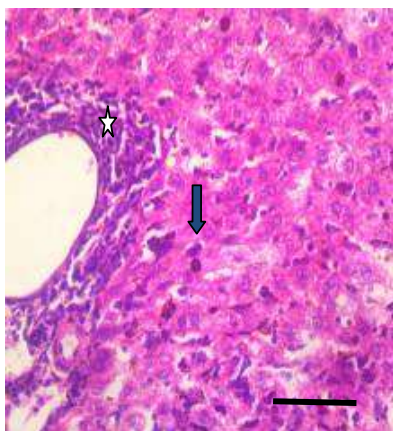
No visible lesion  
(H&E stain, X400). Scale bar: 20  $\mu$ m

**Plate 8.** Effect of 300 mg/kg ethanol extract of combination of plants on kidney.



= A few foci of mild sloughing off of epithelial cells of tubules in renal cortex  
(H&E stain, X400). Scale bar: 20  $\mu$ m

**Plate 9.** Effect of 500 mg/kg ethanol extract of combination of plants on kidney.



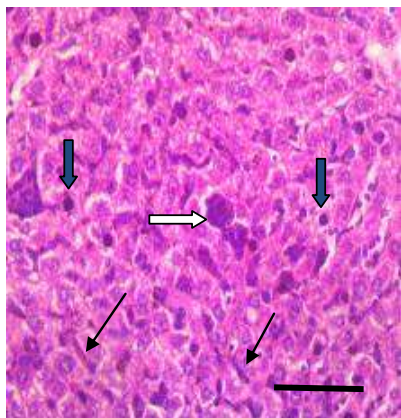
↓ = Numerous round/oval dark structures likely to be *Plasmodium* gametocytes/extra-erythrocytic stages  
☆ = Moderate aggregates of MNCs in portal tracts

**(H&E stain, X400). Scale bar: 20 $\mu$ m**

**Plate 10.** Effect of 200 mg/kg ethanol extract of combination of plants on liver.

many plants, antiplasmodial activity is associated with the presence of total polyphenols, flavonoids and alkaloids (Kaur et al., 2009). For instance, alkaloids occur in plants in association with characteristic acids (Evans, 2002) and

are known to have anticancer, anti-aging and antiviral properties with marked physiological actions on man and animals. Tannins in the root of the plant could be an essential astringent. They act as astringent by

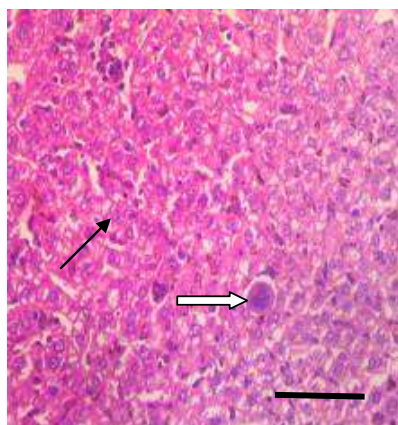


↓ = Numerous round/oval dark structures likely to be *Plasmodium* gametocytes/extra-erythrocytic stages

⇨ = Moderate aggregates of MNCs in portal tracts

↙ = Moderate KCH with some of the cells containing dark brown pigments. **(H&E stain, X400). Scale bar: 20µm**

**Plate 11.** Effect of 300 mg/kg ethanol extract of combination of plants on liver.

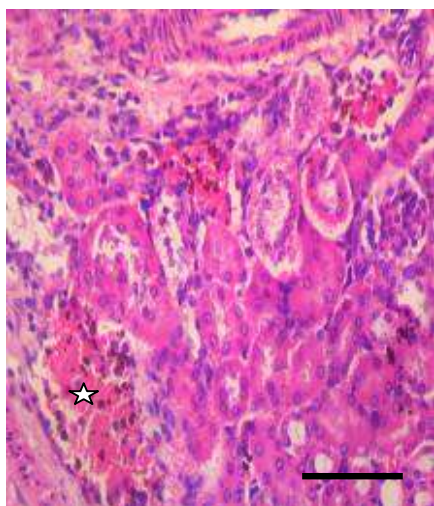


⇨ = Megakaryocyte suggestive of extra-medullary haematopoiesis

↗ = Mild widespread bile stasis in the bile canaliculi

**(H&E stain, X400). Scale bar: 20µm**

**Plate 12.** Effect of 500 mg/kg ethanol extract of combination of plants on liver.



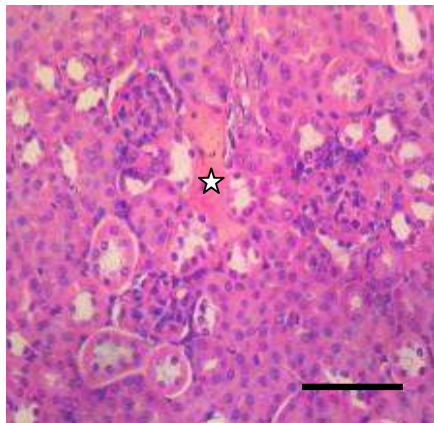
☆ = A few foci of haemorrhages in the renal medulla.

**(H&E stain, X400). Scale bar: 20µm**

**Plate 13.** Kidney section of control mouse administered with distilled water.

precipitating proteins in living tissues, on gastrointestinal tract and on skin abrasions (Sofowora, 2008).

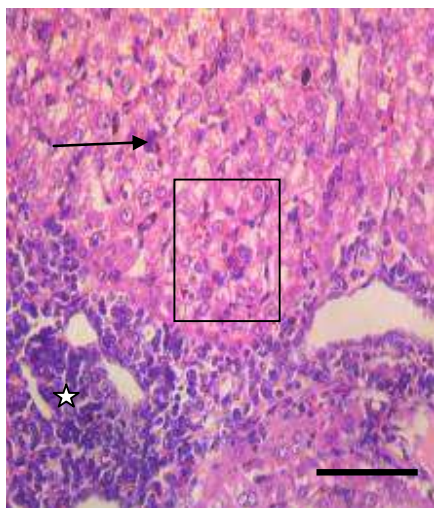
Polyphenols may aid in the prevention of age-associated diseases such as cardiovascular diseases, cancers, and



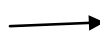
No visible lesion.

(H&E stain, X400). Scale bar: 20µm

Plate 14. Kidney section of control mouse administered with chloroquine



= Multiple foci of moderate vacuolar change of hepatocytes



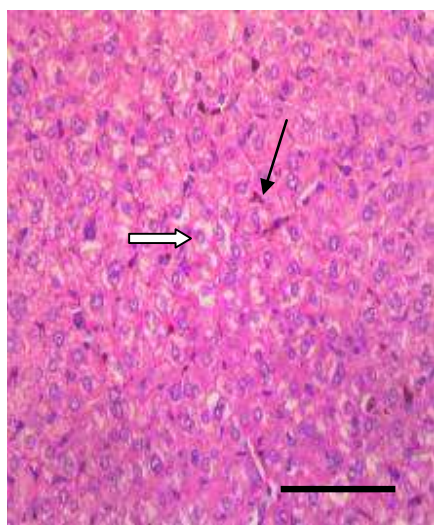
= Multinucleated giant cells



= marked aggregates of MNCs in portal tracts.

(H&E stain, X400). Scale bar: 20µm

Plate 15. Liver section of control mouse administered with distilled water.



= Moderate KCH with some of the cells containing dark brown pigments.



= A few foci of mild vacuolar change of hepatocytes

(H&E stain, X400). Scale bar: 20µm

Plate 16. Liver section of control mouse administered with chloroquine.

osteoporosis. The moisture content of the plant sample screened in this study was very low; this is an indication that the recipe could withstand long storage.

In food and drug industries, the evaluation of toxicity is important because it presents the likely physiological and pathological conditions associated with administration. The toxic effects noticed in animal models (mice, rats, rabbits etc.) could serve as baseline for comparison in mammalian anatomy and physiology. The concentrations administered for LD<sub>50</sub> did not elicit adverse reactions in the animals, and no mortality was recorded. According to Hodge and Sterner (2005), these concentrations are practically safe. Ofori-Attah et al. (2012) evaluated the acute toxicity of aqueous leaf extract of *A. indica* by administering 12 mice with 1250, 2500 and 5000 mg/kg and found the concentrations to be safe. LD<sub>50</sub> between 500 and 5000 mg/kg is considered as moderately toxic (Agaie et al., 2000), or may be classified as practically non-toxic, and fall within the safety margin considered acceptable (Hodge and Sterner Scale, 2005).

At the tested dosages, the recipe showed no significant lysis and could be said to boost the immune system of the mice. The haemoglobin concentration was fairly constant; this suggests that the oxygen-carrying capacity of the blood of the animals was not affected. The control (chloroquine) showed significantly high PCV and reduced WBC compared with the recipe. Histology of kidney and liver sections indicated that caution must be exercised in the use of the plants that make up the recipe especially in high doses. The combination of the plants (recipe) showed significant activity, although it compared less with the positive control (chloroquine).

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

## ACKNOWLEDGEMENT

Special appreciation go to the research team at the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. Thanks to Prof. O. G. Ademowo for granting laboratory space, facility, and the malaria parasite used for the study. The financial support from Tertiary Education Trust Fund (TETFund) through Adeyemi College of Education, Ondo, Ondo State is gratefully acknowledged.

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