

# Nutritional quality assessment and antiplasmodial activity of *Cajanus cajan* (L.) Huth., *Crescentia cujete* L. and *Myrianthus preussii* Engl. from Akure, Southwestern Nigeria

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## Abstract

Many plants are now considered to have dual purpose usefulness in terms of their therapeutic effects and nutritional benefits. *Cajanus cajan* (L.) Huth., *Crescentia cujete* L. and *Myrianthus preussii* Engl. 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 administered with water and ethanol extracts of plant samples and blood samples screened for parasitemia. Data were statistically analyzed.

Alkaloids, glycosides, saponins, tannins, and polyphenols were present in all the three samples. Anthraquinones and flavonoids were altogether absent. *C. cajan* had the highest ash (11.69%), crude protein (17.76%) and fat (17.34%) whereas *C. cujete* was richest in carbohydrate (58.52%). Calcium was found to be highest in *C. cujete* (22672.43mg/kg) and least in *C. cajan* (13288.33mg/kg). *C. cujete* was richest (898.37mg/kg) and *C. cajan* (304.22mg/kg) least in iron. However, magnesium was found to be highest in *M. preussii* (5837.03mg/kg) and least in *C. cujete* (2166.48mg/kg). The ethanol extract of the recipe was most active at 200mg/kg.

Dietary or mineral elements serve structural, functional and biochemical roles. The three plants contained appreciable major and minor elements. The leaf of *C. cajan* could serve as a complement for animal protein. The activity observed in the ethanol extract could be as a result of the complete dissolution of the phytochemicals in ethanol. Toxicity studies on the plants will confirm their safe application, although lead tested negative in the plant samples.

**Keywords:** Nutrition, Malaria, *Cajanus cajan*, *Crescentia cujete*, *Myrianthus preussii*, Nigeria

## Introduction

Many plants are now considered to have dual purpose usefulness in terms of their therapeutic effects and nutritional uses [1] [2]. Although, the fundamental needs of human beings provided by the plant world include food, clothing, and shelter, the place of plants in the management of diseases (mild or chronic) cannot be overstated. The nutritional compositions of some of these medicinal plants have encouraged their incorporation in diets as food supplements in various forms such as powder, pill, and tablet or in liquid form. These botanicals, when administered, not only correct, modify or improve physiological functions in humans but also serve as foodstuffs in supplementing normal diets presenting nutritional and metabolic effects. Well known of these are *Vernonia amygdalina*, *Ocimum gratissimum*, *Telfaria occidentalis*, *Mentha* spp., *Thymus* spp., *Persia americana* and a host of others [2] [3] [4].

Malaria, an infectious disease caused by *Plasmodium* spp., has been a menace to the health conditions of both rural and urban

populations in Nigeria. 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 [5] [6]. Worldwide, malaria afflicts about 40% amounting to over 300 million people annually [7] affecting more than 100 countries in virtually all the continents of the world [8]. In Nigeria, reports indicated that pregnant women are most vulnerable and hence malaria causes 10% of all deaths in pregnant women [9]. The cost of management or eradication of malaria with conventional approach and its attendant effect on the standard of living as well as the economy of a nation calls for an urgent review of natural products to combat the ancient scourge. *Cajanus cajan* (L.) Huth., *Crescentia cujete* L. and *Myrianthus preussii* Engl. are combined for use in the treatment of malaria in Akure, Southwestern Nigeria.

*Cajanus cajan* (L.) Huth. – Fabaceae is a cultivated shrub, 2.5m high, trifoliolate leaves with bright yellow flowers and large pods. Traditionally, the leaf is used in the management of jaundice, oral infections, malaria, and as antibacterial [10]. *C. cajan* (seeds or peas) is an ideal source of protein and vitamins in human diet, especially to vegetarians [11]. The pea is also used as feed and



fuel [12] and contains amino acids comparable to that of soybean [13].

*Crescentia cujete* L. – Bignoniaceae is a spreading tree, with cauliflorous flowers, green calyx and corolla with pink brown stripes; fruits are large and globose. In Nigeria, the tree is widely grown in the Northern states, but few stands occur in other parts of the country [14]. The fruit of *C. cujete* is part of herbal mixtures reported in various traditional medicine for respiratory ailments, bronchitis, cough, colds, toothaches, headaches, as laxative, anti-inflammatory and febrifuge [15]. In Nigeria, the fruit is reported to have medicinal values [16] [17].

*Myrianthus preussii* Engl. – Cecropiaceae is a small tree or single-stemmed shrub, 3-4m tall; leaves digitate, with pale leaflets; infructescence green, fruits beaked. There is no published information on the traditional/indigenous uses of *M. preussii*. However, herbsellers in Akure claimed the leaf of *M. preussii* forms part of a recipe used in the management of malaria. In Nigeria and Congo, the leaves of a sister species (*M. arboreus*) is used as analgesic to treat children with fever, as an enema to treat back pain, for pregnancy complications, bronchitis, sore throat, toothache, dysmenorrhoeal, and incipient hernia [18] [19]. We can expect similar medicinal properties in *M. preussii*.

This study therefore reports the nutritional composition and antiparasmodial activity of three antimalarial plants used in Akure, Southwestern Nigeria.

## Materials and Methods

### Study Area

Akure is a popular metropolis in Ondo State. Akure South Local Government supports a population of over 400,000 people [20]. The mean annual rainfall is about 1350mm 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 [21]. 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 [21].

### Collection and Identification of Plant Materials

Leaves of *Cajanus cajan*, stem barks of *Crescentia cujete* and leaves of *Myrianthus preussii* - all fresh - were collected, and identified by comparison with specimens deposited by various workers in the field at the University of Ibadan Herbarium (UIH) [*C. cajan* - UIH 21708; *C. cujete* - UIH 14814; *M. preussii* - UIH 17895] and thereafter air-dried at room temperature (25±2°C).

### Preparation of Plant Samples

The dried plant samples were pulverized to coarse powder using a laboratory mill (Model 4 Arthur Thomas, USA).

### Preparation and Concentration of Extracts

The powdered plant materials were each divided into two parts. 500g of each plant sample was soaked in 3liters of 96% ethanol for 72 hours, and another 500g in 3litres of distilled water for 24 hours. A combination (comprising 150g each of the powdered samples) was also dissolved in distilled water and ethanol respectively. The preparations were stirred every 2hours, thereafter 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 600mmHg 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 percentage yield was calculated. The extract was refrigerated at 4 C prior to use.

### Plant Analysis

The powdered plant samples (*Cajanus cajan* leaf, *Crescentia cujete* stem bark, *Myrianthus preussii* leaf) were screened for phytochemical constituents, proximate composition and mineral elements according to standard protocols reported by [22] [23] [24] [25] [26].

### Phytochemical Constituents

#### Alkaloids

The powdered plant sample (500mg) was weighed and extracted with 10ml of hydrochloric acid (HCl). The HCl extract was then filtered with Whatman filter paper (No. 1). The filtrate of about 2.5ml was treated with few drops of Dragendoff's reagent. A precipitate indicated the presence of alkaloids.

#### Anthraquinones

The powdered plant sample (500mg) was shaken with 10ml of benzene. The solution was filtered and 5ml of 10% ammonium hydroxide (NH<sub>4</sub>OH) solution was added to the filtrate. A violet colour was observed in the lower phase. It indicated presence of anthraquinones.

#### Cardiac glycosides

One gram (1g) of sample was extracted with 40ml of distilled water; the extract was placed in the oven at 100°C for 15min. 1ml of the preparation was added to 5ml distilled water and 2ml Glacial Acetic Acid, and a drop of FeCl<sub>3</sub>. Thereafter, 1ml of concentrated H<sub>2</sub>SO<sub>4</sub> was introduced from the side of the test tube. A brown ring (with violet or green ring) signifies the presence of cardiac glycosides.

#### Flavonoids



A few drops of concentrated hydrochloric acid (HCl) were added to a small amount of an extract (0.5g) of the plant material; development of red colour was taken as an indication of the presence of flavonoids.

### Saponins

The sample (200mg) was shaken with 5ml of distilled water and then heated to boil. Persistent frothing showed the presence of saponins.

### Tannins

The sample (500mg) was mixed with 10ml of distilled water and heated on a water bath. The mixture was filtered and ferric chloride (FeCl<sub>3</sub>) was added to the filtrate. Appearance of blue black colouration showed the presence of tannins.

### Polyphenols

One gram (1g) of sample was added to 25ml of water. The preparation was put in oven at 100 C for 15mins. The presence or absence of polyphenols was determined by adding a few drops of 1% (w/v) solution of ferric chloride followed by 1% (w/v) gelatin in sodium chloride of the same concentration. The formation of a precipitate indicated the presence of polyphenols.

### Proximate Composition

#### Carbohydrate

Carbohydrate content was estimated by difference using the formula: % Available carbohydrate = 100 - (% protein + % moisture + % ash + % fibre + % fat)

#### Crude fibre

Two grams (2g) of each sample was digested with 20% H<sub>2</sub>SO<sub>4</sub> and NaOH solutions.

#### Crude protein

Half a gram (0.5g) of each sample was weighed into a filter paper and put into a Kjeldahl flask; 10cm<sup>3</sup> of concentrated H<sub>2</sub>SO<sub>4</sub> was added and then digested in a fume cupboard until the solution became colourless. Distillation was carried out with 10cm<sup>3</sup> of 40% NaOH. The distillate was received with 5cm<sup>3</sup> of 4% boric acid in a mixed indicator till the boric acid solution turned green. Titration was done in the receiver flask with 0.01M HCl until the solution turned red.

#### Fat (Ether extract)

Two grams (2g) of each sample was extracted with petroleum ether for 5hours in a Soxhlet extractor.

### Moisture content

Two grams (2g) of each sample was put into the crucible and dried in an oven at 105 C overnight. The dried samples were cooled in a dessicator for 30 minutes and weighed to a constant weight. The percentage loss in weight was taken as the moisture content.

### Total Ash

Two grams (2g) of each sample was placed in a crucible and ashed at 600 C for 3hours. The hot crucibles were cooled in a dessicator and weighed. The percentage residual weight was taken for ash content.

### Mineral Analysis

After wet digestion, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), and lead (Pb) were analyzed using Atomic Absorption Spectrophotometer (FC 210/211 VGP Bausch Scientific AAS); phosphorus was determined using Vanadomolybdate (Yellow method). Percentage transmittance was determined at 400nm using Spectronic 20 (Bausch and Lomb) Colorimeter.

### Animals

The Swiss albino mice (18-22g) 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.

### Treatment of Animals

The animals were acclimatized for two weeks at room temperature with 12h dark/light periodicity and fed with commercial chow purchased from Cap Feeds (Ibadan, Nigeria) and water *ad libitum*.

### Experimental Design

In Phase 1, the median lethal dose (LD50) was determined. Eight (8) plant extracts (4 water extracts and 4 ethanol extracts) were prepared from the three plants and a combination of the plant samples. The extracts were graded into 1000mg/kg, 2000mg/kg, and 3000mg/kg. 4 mice per concentration per extract were used. In this phase, 96 mice were used. The antiplasmodial activity of the plant extracts was determined in Phase 2. The set-up had nine (9) groups; 6 groups for the 3 individual plant parts (water and ethanol extracts); 7<sup>th</sup> and 8<sup>th</sup> group for the combination of plant parts (water and ethanol extracts) while the 9<sup>th</sup> group represented the control (administered with distilled water and chloroquine). Each group has randomly attached animals to remove bias. The concentration of the drugs was graded into 200mg/kg, 300mg/kg and 500mg/kg. 15 mice per group/extract (5 per concentration) for were used for groups 1 - 8, whereas the control group (administered with distilled



water, and chloroquine) had 10 mice. In this phase, 130 mice were used.

### Determination of Median Lethal Dose (LD<sub>50</sub>)

Animals were labelled with picric acid for identification, and were administered with 1000mg/kg, 2000mg/kg, and 3000mg/kg of the extracts. 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 hours. Oral administration of extracts was carried out using gastric feeding tube for 7 days, for long-term possible lethal outcomes [27].

### Malaria Parasite

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

### Inoculation of Animals

A Swiss albino mouse (which served as the donor mouse) was intraperitoneally administered with a standard inoculum of *P. berghei*. Blood was taken from the heart of the donor mouse through cardiac puncture and diluted with isotonic saline. 0.1ml of acid citrate dextrose (ACD) was drawn into the syringe and normal saline was added. Thereafter, the experimental groups of healthy animals were injected with the preparation.

### Determination of Parasitemia

Parasitemia was monitored in blood obtained from the tail of Swiss mice infected with *P. berghei*. The number of parasites was determined microscopically at X100 magnification by counting the average number of parasites in 4 magnification fields of at least 1000 red blood cells using tally counter as described by [28].

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

### *In vivo* Antiplasmodial Study - Extract and Drug Administration

After the 5<sup>th</sup> day of inoculation at which the parasites were fully established, the infected mice in groups 1-8 were treated with 200mg/kg, 300mg/kg and 500mg/kg of water and ethanol extracts of plants, whereas the control group (9) was administered with distilled water, and 0.2ml of Chloroquine (0.3ml of 4mg of chloroquine made up to 8ml of distilled water).

### Data Analysis

Data were statistically analysed and expressed as mean  $\pm$  SD. Differences in means were assessed for significance by Duncan's Multiple Range Test (DMRT) at  $p < 0.05$ .

## Results and Discussion

Table 1 shows the phytochemical profile of *C. cajan*, *C. cujete* and *M. preussii*. Alkaloids, glycosides, saponins, tannins, and polyphenols were present in all the three samples. Alkaloid was found to be highest in *C. cujete* (0.64%), followed by *C. cajan* (0.52%) and least in *M. preussi* (0.47%). The same trend was observed for cardiac glycosides, saponins, and polyphenols in the samples. *C. cajan* had the highest (0.20%) tannin content, followed by *C. cujete* (0.06%) and least in *M. preussii* (0.01%). Anthraquinones and flavonoids were altogether absent. Oyeyemi *et al.* (2014) reported the presence of flavonoids and the absence of saponins in a sister species of *M. preussii* (*M. arboreus*). This difference may be as a result of species identity due to genetic make-up as well as ecological and/or soil factors of species location. This chemical identity has also served useful purpose in the taxonomy of plant groups [29]. According to [25], different parts of a plant may contain different phytochemicals in varying concentrations. Secondary metabolites have played key roles as antimalarial, antimicrobial, anti-hypertensive agents such as the prophylactic effect of a multi-herbal extract containing *C. cajan* leaf [30], anti-hypertensive activity of *Nauclea latifolia* [31], and antiplasmodial property of *Lophira alata* [32].

The proximate composition of the plant samples is presented in Table 2. *C. cajan* had the highest ash (11.69%), crude protein (17.76%) and fat (17.34%) whereas *C. cujete* was richest in carbohydrate (58.52%). The moisture content was 8.44% in *C. cujete* and 7.37% in *C. cajan*. Fibre was highest in *M. preussii* (17.40%) followed by *C. cujete* (15.61%) and least in *C. cajan* (14.44%). Ash represents the inorganic mineral matter of food samples and is also nutritionally important [33]. The high protein value in *C. cajan* is an indication that the plant could be of help in the maintenance of body tissues. Crude fibre, made up of cellulose with little quantity of lignin, is indicative of the level of non-digestible carbohydrate [34] [35]. Carbohydrate is known for the supply of energy; it may also serve as a necessary material for the production of aromatic amino acids and phenolics [36]. The moisture content of the plant samples was very low; this is an indication that the plants could withstand long storage. Compared with proximate compositions reported for *M. arboreus* [19], leaf of *M. preussii* had higher protein, fibre, ash and carbohydrate contents and lower moisture content. *C. cajan* leaf contained higher crude fat, crude fibre, ash, and moisture and lesser crude protein and dry matter when compared with the proximate composition of raw and roasted pigeon pea seeds [12] [37]. [37] also reported that pigeon pea seeds can replace up to 75% of maize in broiler finisher diets without negatively affecting performance and carcass yield. However, pigeon pea seeds have low value for humans for reasons of cooking time and low



palatability [38]; the leaf could serve as substitute for animal protein in human diet.

The plant samples were screened for mineral constituents (Table 3).

**Table 1: Phytochemical constituents of three antimalarial plants used in Akure, Southwestern Nigeria**

Parameter	Composition (%)		
	<i>C. cajan</i>	<i>C. cujete</i>	<i>M. preussii</i>
Alkaloids	0.52 <sup>b</sup> ± 0.00	0.64 <sup>a</sup> ± 0.01	0.47 <sup>c</sup> ± 0.00
Anthraquinones	0.00 <sup>a</sup> ± 0.00	0.00 <sup>a</sup> ± 0.00	0.00 <sup>a</sup> ± 0.00
Cardiac glycosides	0.19 <sup>a</sup> ± 0.00	0.21 <sup>a</sup> ± 0.00	0.17 <sup>a</sup> ± 0.00
Flavonoids	0.00 <sup>a</sup> ± 0.00	0.00 <sup>a</sup> ± 0.00	0.00 <sup>a</sup> ± 0.00
Saponins	0.25 <sup>b</sup> ± 0.01	0.34 <sup>a</sup> ± 0.01	0.23 <sup>c</sup> ± 0.01
Tannins	0.20 <sup>a</sup> ± 0.00	0.06 <sup>b</sup> ± 0.00	0.01 <sup>b</sup> ± 0.00
Polyphenols	0.12 <sup>a</sup> ± 0.00	0.14 <sup>a</sup> ± 0.00	0.11 <sup>a</sup> ± 0.00

Values are mean ± SD of triplicate determinations. Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test (DMRT) at p<0.05.

**Table 2: Proximate composition of three antimalarial plants used in Akure, Southwestern Nigeria**

Parameter	Composition (%)		
	<i>C. cajan</i>	<i>C. cujete</i>	<i>M. preussii</i>
Ash	11.69 <sup>a</sup> ± 0.34	3.72 <sup>b</sup> ± 0.05	11.51 <sup>a</sup> ± 0.42
Carbohydrate	33.75 <sup>c</sup> ± 4.08	58.52 <sup>a</sup> ± 0.83	44.64 <sup>b</sup> ± 0.45
Crude Fibre	14.44 <sup>c</sup> ± 0.44	15.61 <sup>b</sup> ± 0.50	17.40 <sup>a</sup> ± 0.43
Crude Protein	17.76 <sup>a</sup> ± 0.41	11.59 <sup>c</sup> ± 0.16	16.35 <sup>b</sup> ± 0.11
Fat	17.34 <sup>a</sup> ± 0.19	2.28 <sup>b</sup> ± 0.23	2.67 <sup>b</sup> ± 0.11
Moisture	7.37 <sup>b</sup> ± 0.16	8.44 <sup>a</sup> ± 0.29	7.42 <sup>b</sup> ± 0.41

Values are mean ± SD of triplicate determinations. Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test (DMRT) at p<0.05.

**Table 3: Mineral element composition of three antimalarial plants used in Akure, Southwestern Nigeria**

Minerals	Composition (mg/kg)		
	<i>C. cajan</i>	<i>C. cujete</i>	<i>M. preussii</i>
Calcium	13288.33 <sup>c</sup> ± 36.68	22672.43 <sup>a</sup> ± 26.74	22287.63 <sup>b</sup> ± 31.98
Copper	18.00 <sup>b</sup> ± 2.01	18.81 <sup>b</sup> ± 0.91	22.31 <sup>a</sup> ± 3.14
Iron	304.22 <sup>c</sup> ± 5.93	898.37 <sup>a</sup> ± 16.77	366.73 <sup>b</sup> ± 4.01
Lead	0.00 <sup>a</sup> ± 0.00	0.00 <sup>a</sup> ± 0.00	0.00 <sup>a</sup> ± 0.00
Magnesium	3224.33 <sup>b</sup> ± 4.04	2166.48 <sup>c</sup> ± 30.23	5837.03 <sup>a</sup> ± 31.22
Manganese	92.33 <sup>b</sup> ± 3.05	43.64 <sup>c</sup> ± 2.46	101.82 <sup>a</sup> ± 1.76
Phosphorus	2279.51 <sup>a</sup> ± 3.24	566.35 <sup>c</sup> ± 2.90	848.70 <sup>b</sup> ± 3.33
Potassium	1.11 <sup>a</sup> ± 0.01	0.63 <sup>a</sup> ± 0.01	0.87 <sup>a</sup> ± 0.01
Sodium	1.26 <sup>a</sup> ± 0.06	0.68 <sup>b</sup> ± 0.01	0.96 <sup>b</sup> ± 0.03
Zinc	77.33 <sup>a</sup> ± 1.53	76.34 <sup>a</sup> ± 0.58	62.41 <sup>b</sup> ± 4.26

Values are mean ± SD of triplicate determinations. Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test (DMRT) at p<0.05.

Calcium was found to be 22672.43mg/kg in *C. cujete*, 22287.63mg/kg in *M. preussii* and 13288.33mg/kg in *C. cajan*. The same trend was observed for iron in which *C. cujete* was richest (898.37mg/kg) followed by *M. preussii* (366.73mg/kg) and least in *C. cajan* (304.22mg/kg). However, magnesium was found to be highest in *M. preussii* (5837.03mg/kg), 3224.03mg/kg in *C. cajan* and least in *C. cujete* (2166.48mg/kg). Potassium and sodium were

fairly present in all the three samples. Copper was found to be highest in *M. preussii* (22.31mg/kg), 18.81mg/kg in *C. cujete* and 18.00mg/kg in *C. cajan*. Manganese was 101.82mg/kg in *M. preussii*, 92.33mg/kg in *C. cajan* and 43.64mg/kg in *C. cujete*. *C. cajan* had the highest zinc (77.33mg/kg), closely followed by *C. cujete* (76.34mg/kg) and least in *M. preussii* (62.41mg/kg). Phosphorus was richest in *C. cajan* (2279.51mg/kg), 848.70mg/kg



in *M. preussii* and lowest in *C. cujete* (566.35mg/kg). Lead tested negative in the three samples. Calcium, iron, and magnesium were found to be higher in *M. preussii* compared to those reported for *M. arboreus* by [19]. The recommended dietary allowance (RDA) of these elements has been reported by [7] [39] and also published at [www.mii.org/periodic/LifeElement.html](http://www.mii.org/periodic/LifeElement.html). Dietary or mineral elements are required for the physiological and biochemical processes in humans. Hence, the human body requires major dietary elements (calcium, magnesium, potassium, and phosphorus) as well as trace or minor elements (copper, zinc, and manganese). Some of these elements serve structural and functional roles. For example, potassium and sodium work together in the co-regulation of ATP; calcium is needed for bone, muscle, and heart and important in the function of blood. Phosphorus is involved in the regulatory processes of ATP and DNA whereas magnesium is required for bone formation and phosphate regulation. Iron is necessary for the synthesis of many proteins and enzymes, and features in haemoglobin production. Manganese presents as a co-factor in enzyme functions whereas copper is a necessary component of many redox reactions [34] [40] [41] [42]. These elements when taken in diets or as supplements help to maintain optimal health; a deficiency or an excess may lead to irregular biochemical reactions of metabolism [41].

For the LD<sub>50</sub>, no mortality was recorded in all the groups in 24h, 72h, and up to a week (for possible long-term lethal action). Therefore, the experimental mice were adjudged to be healthy and normal with no record of weight loss, hair loss, and allergy; however, a little discomfort leading to aggressiveness, restiveness, ruffled hair, weakness, withdrawal from food and water, and

temporary immobility was noticed. By the 5<sup>th</sup> day after inoculation, parasitemia had established in all the experimental groups. In the establishment, the percentage parasitemia was not significant in all the groups. Figure. 1 shows the antiplasmodial activity of water and ethanol extracts of *C. cajan* leaf. Activity was negligible between days 1 and 2 of treatment, considerable at days 3 and 4, and constant for days 6 and 9. 500mg/kg each of the water and ethanol extracts were noted to have fair activities. The water and ethanol stem bark extract of *C. cujete* showed minimal activity (Figure. 2). The ethanolic stem bark of *C. cujete* was more active at 300mg/kg whereas the water extract was more active at 500mg/kg. Figure. 3 shows a slow reduction in the level of parasitemia in mice treated with water and ethanol extract of *M. preussii* leaf. The malaria parasite was resistant to 300mg/kg of water extract between days 6 and 12 of treatment. Figure. 4 shows the antiplasmodial activity of the water and ethanol extracts of the recipe (combination of the plant samples). The percentage parasitemia significantly reduced up to day 6; the parasitemia level was fairly constant for days 9 and 12. The ethanol extract of the recipe was most active at 200mg/kg. The positive control (chloroquine) showed significant activity compared to the other test drugs (Figure. 5). Investigation on the prophylactic effect of a multi-herbal extract (consisting *Cajanus cajan* leaf, *Euphorbia laterifolia* leaf, *Mangifera indica* leaf and stem, *Cymbopogon giganteas* leaf, and *Uvaria chamae* bark) by [30] gave noteworthy antimalarial activity with no apparent significant side effects. No published antimalarial activity has been reported for *C. cujete* stem bark and *M. preussii* leaf. This study therefore presents a groundbreaking recipe and premier antimalarial potentials of the plants.

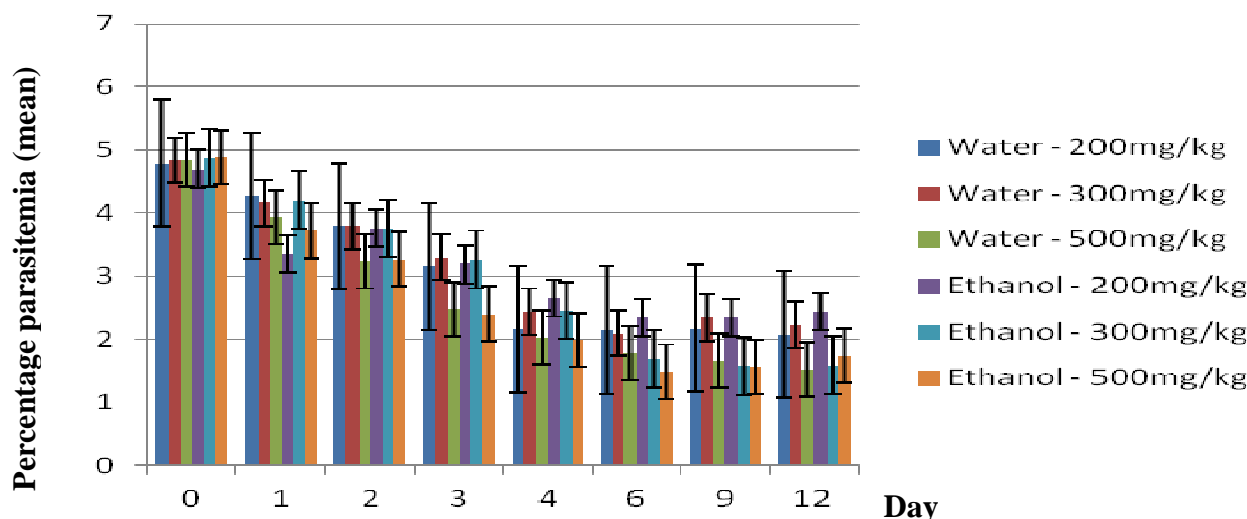


Figure. 1: Comparative percentage parasitemia (mean) of *Plasmodium berghei* infected mice treated with water and ethanol extracts of *Cajanus cajan* leaf till 12<sup>th</sup> day post establishment



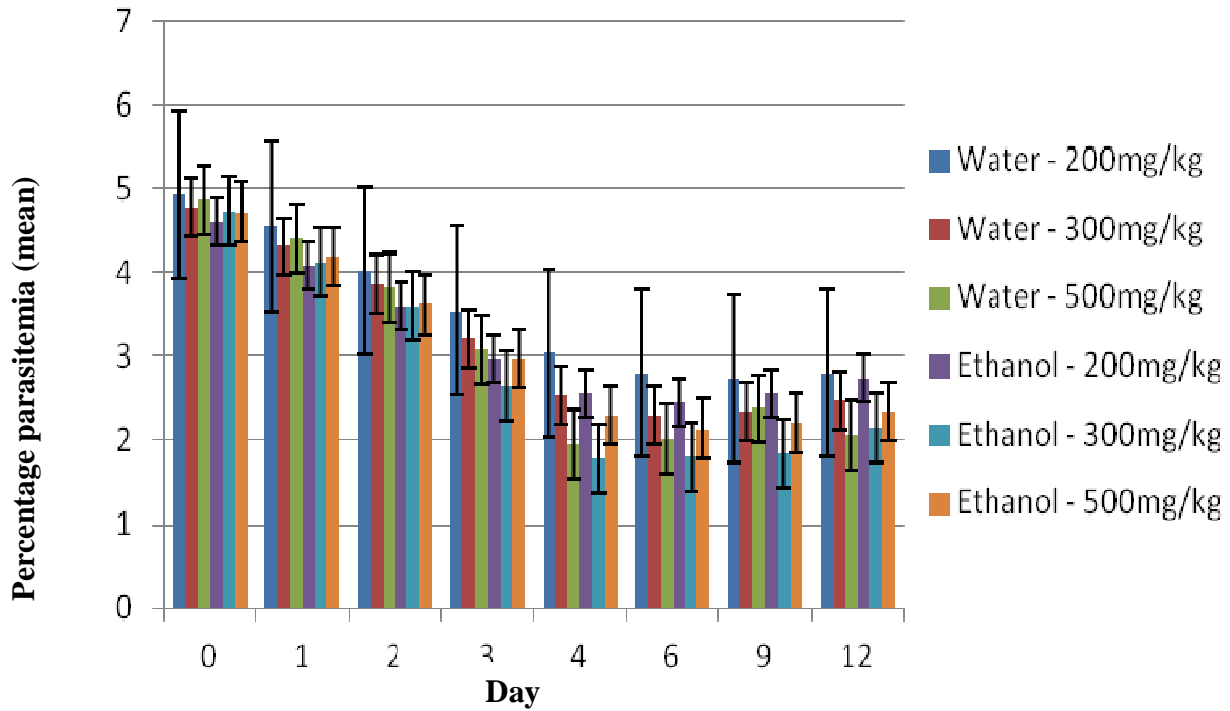


Figure. 2: Comparative percentage parasitemia (mean) of *Plasmodium berghei* infected mice treated with water and ethanol extracts of *Crescentia cujete* leaf till 12<sup>th</sup> day post establishment

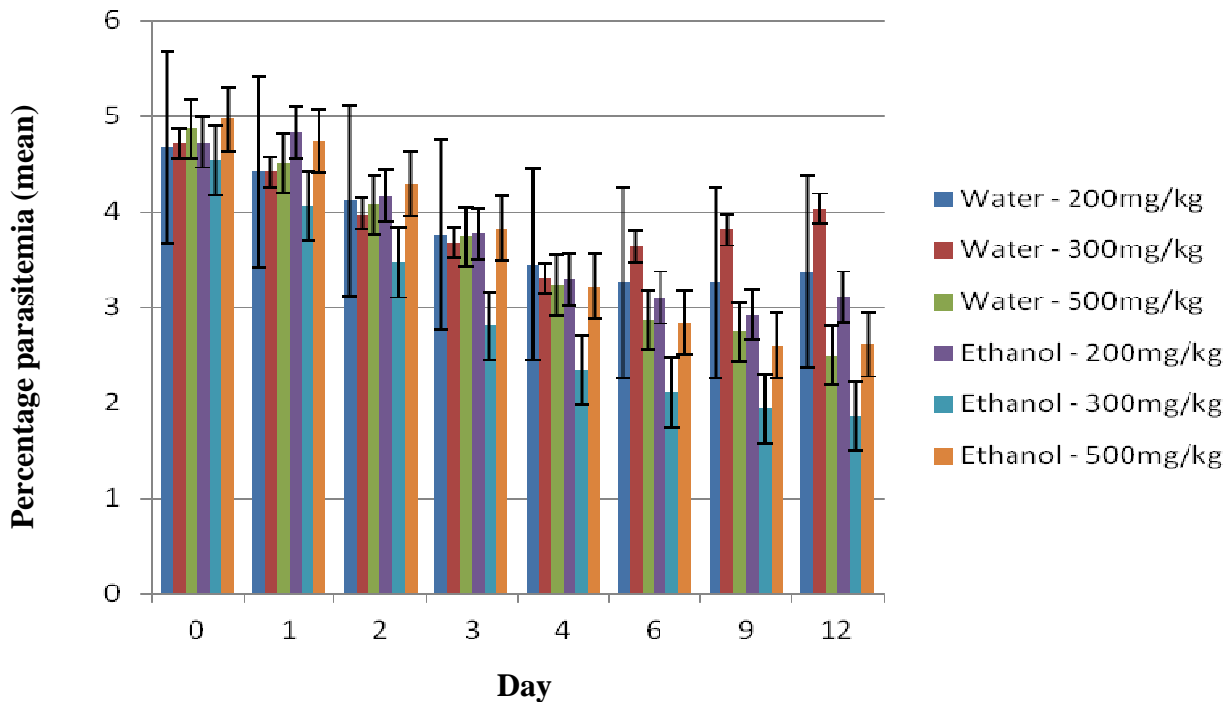


Figure. 3: Comparative percentage parasitemia (mean) of *Plasmodium berghei* infected mice treated with water and ethanol extracts of *Myrianthus preussii* leaf till 12<sup>th</sup> day post establishment



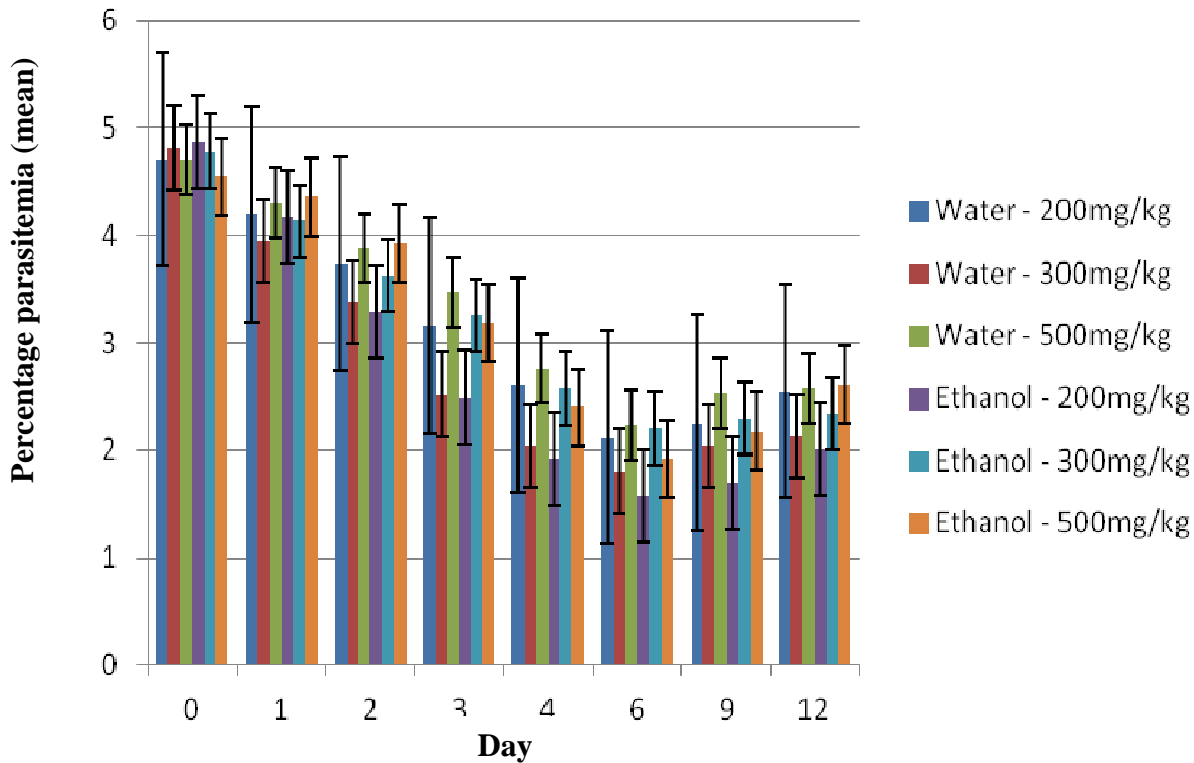


Figure. 4: Comparative percentage parasitemia (mean) of *Plasmodium berghei* infected mice treated with water and ethanol extracts of the combination of plants till 12<sup>th</sup> day post establishment

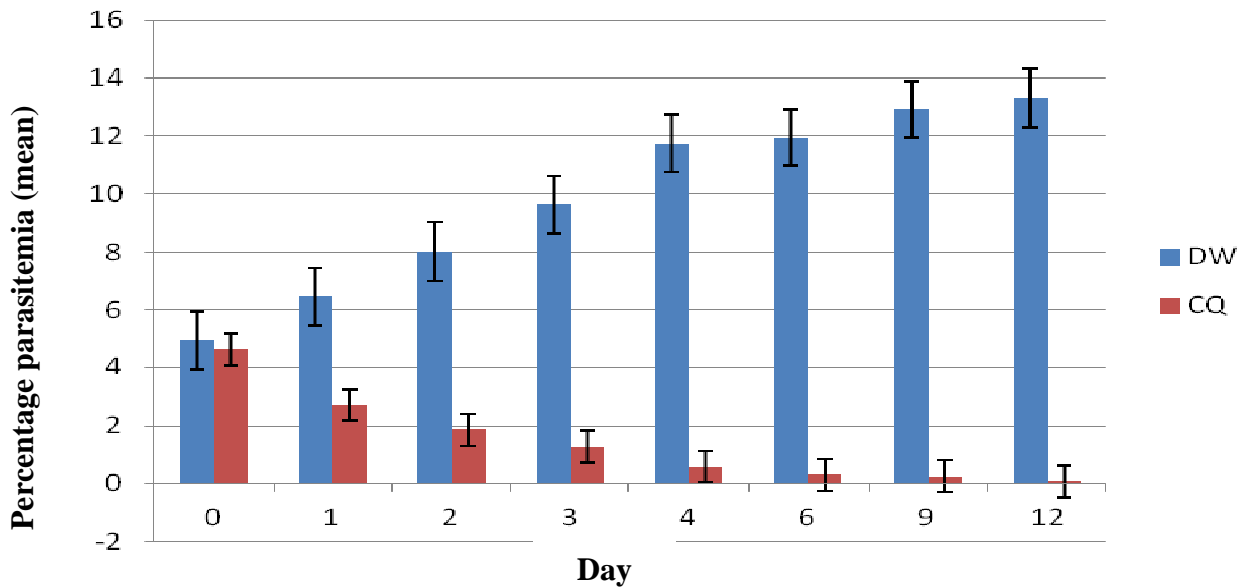


Figure. 5: Comparative percentage parasitemia (mean) of *Plasmodium berghei* infected mice treated with distilled water and chloroquine till 12<sup>th</sup> day post establishment



## Conclusion

This study has provided an assessment of the nutritional qualities and antiparasitocidal activities of three antimalarial plants. *C. cajan*, *C. cujete* and *M. preussii* contained appreciable major and minor elements sufficiently adequate to supply human necessity of minerals. The leaf of *C. cajan* could serve as a complement for animal protein. The medicinal properties could be attributed to the presence of phytochemicals present in the parts studied. The ethanol extract of the recipe had the highest activity. This activity could be as a result of the complete dissolution of the phytochemicals in ethanol. Although lead tested negative in the plant samples, toxicity studies on the plants will confirm their safe application.

## Conflict of Interest Statement

The authors declare no conflict of interest.

## Authors' Contributions

TAO: conceived and designed the experiment; he also revised the manuscript draft. MRI: did the literature search, conducted the experiment, acquired data, and drafted the manuscript. EOD: analyzed and interpreted the data. All authors read and approved the final manuscript.

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