

## Evaluation of the Prophylactic Activity of Ethanolic Extract of *Ricinus communis* L. against *Plasmodium berghei* in Mice

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### Abstract

The continuous quest to annihilate the scourge of malaria parasites and the need for exploration of new drugs for the ever endemic killer disease opens the way for continued research. In the present study, ethanolic extract of *Ricinus communis* leaves was evaluated for possible antimalarial activity against *Plasmodium berghei* (NK65) infections in mice. Prophylactic antimalarial activity of the extract was investigated to determine the efficacy of the extract. Phytochemical screening of the extract was conducted with the view to elucidating the feasible mechanism of the antimalarial potentials. It was established from our findings that 141.42mg/kg was the acute oral toxicity (LD<sub>50</sub>) of the ethanolic extract of *Ricinus communis* in mice. Consequently, three doses of the extract (10mg/kg, 20mg/kg and 40mg/kg) were prepared. A remarkable antimalarial activity was portrayed by the three doses of the extract with 10mg/kg dose having the highest percentage suppression of 58.7%. Result of the phytochemical screening revealed the presence of chemical compounds noted for their antiplasmodial activities such as alkaloids and flavonoids. The leaves extract of *Ricinus communis* thus possess antimalarial activity. It is therefore recommended for consideration in further researches on new anti-malarial drug development.

**Keywords:** *Ricinus communis*, ethanolic extract, antimalarial activity, median lethal dose.

### INTRODUCTION

One of the most debilitating diseases from ancient to recent times is malaria which is associated with considerable morbidity and mortality having significant social and economic impact around the globe (Balogun *et al.*, 2009). It is the most common protozoan parasitic disease in the tropical and subtropical regions of the world with more than 40% of the world population at risk (Snow *et al.*, 2005). The disease has been escalating at an alarming rate, especially in Africa where it accounts for an estimated 300 to 500 million cases each year and cause 1.5 to 2.7 million deaths with more than 90% in children below 5 years of age (Good, 2001).

In 2015, 214 million cases of malaria were recorded worldwide resulting in an estimated 438,000 deaths with 90% cases of the death in Africa (WHO, 2016). In Nigeria, malaria pose a major public health problem where it accounts for one-third of the global prevalence (WHO, 2012) as transmission of the disease occur all year round in the southern part of the country while in the northern part, the disease is more seasonal mostly occurring during the rainy season when conditions are more favourable for the breeding of mosquitoes (Greenwood *et al.*, 2005).

Malaria is caused by *Plasmodium* species that are transmitted by the bite of female

Anopheles mosquito, *Plasmodium falciparum*, *P. malariae*, *P. vivax*, *P. ovale* and *P. knowlesi* cause malaria in humans (Collins, 2012). It is traditionally believed that *P. falciparum* accounts for the majority of death (Sarkar *et al.*, 2009) though recent evidence suggests that *P. vivax* is associated with potentially life-threatening conditions (Baird, 2013). In rodents, malaria is caused by a number of *Plasmodium* species of which *P. berghei* stands out as a model organism in the evaluation of human malaria due to its similarities to species that cause human malaria and the ease by which it can be genetically manipulated (Craig *et al.*, 2012).

The use of medicinal plants in the treatment of ailments has been in existence since time immemorial (Hoareau *et al.*, 1999; Ahn, 2017) and about 80% of the population of third world countries are dependent on medicinal plants for their primary health care needs due to poverty and lack of access to modern medicine (WHO, 1997). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Rabe and Vanstoden, 2000).

In recent years, prevalence of malaria has increased due to the spread of drug-resistant malaria parasites (Sinha *et al.*, 2014). About 75% of the African population does not have

direct access to conventional drugs for the Due to rise in drug-resistance strains of the parasites, researches are continuously seeking alternatives in many of the local herbs used in the treatment of malaria in rural areas. Albeit, these herbs are easily available and affordable, there is little or no clinical information as to their safety and efficacy (Etkins, 2003).

*Ricinus communis*, commonly known as Castor oil plant is a tropical flowering plant belonging to the family Euphorbiaceae and has been known to be ubiquitous (Eudmar *et al.*, 2011). Inhabitants of rural communities often exploit different parts of this plant for treatment of various ailments (Jena and Gupta, 2012). Studies have revealed that various parts of the plant such as flowers, fruits, leaves, roots and stems have medicinal potentials (Kensa and Syhed, 2011; Naz and Bano, 2012). Extracts obtained from the plant exhibited arrays of valuable effects ranging from analgesic, diuretic, antidiarrhea, anthelmintic and a host of other medicinal benefits (Taur and Patil, 2011; Khurshed *et al.*, 2012; Naz and Bano, 2012).

*Ricinus communis* has antioxidant, antiasthmatic and antitumor properties (Taur and Patil, 2011; Khurshed *et al.*, 2012), but its potential benefits are yet to be fully utilized due to the limited knowledge about the plant (Amir and Manisha, 2016). These properties are believed to be due to its possession of certain compounds such as ricin, ricinolein and triglycerides (Taur and Patil, 2011). Despite the fact that extracts from the leaves and barks of this plant are used traditionally to cure some ailments mostly in the northern part of Nigeria, there is little or no information regarding its antimalarial potentials. Furthermore, since most of the conventional antimalarial prophylaxis are becoming less effective as a result of resistance developed by the parasites coupled with their costly nature, it thus calls for seeking alternatives in local plants with prophylactic potentials. Hence, the current study intends to investigate the prophylactic antimalarial efficacy of *Ricinus communis*.

## MATERIALS AND METHODS

### Collection of Plant materials and preparation of extracts

The leaves of the *R. communis* was collected and identified by Professor Khalimullah Sagir of the Department of Biology, Umaru Musa Yar'adua University, Katsina State. Voucher specimen's number 20180927-58 has been deposited at the departmental herbarium. Fresh leaves of *Ricinus communis* were obtained from Dutsin-Ma Local Government Area of the State. The leaves were washed and

treatment of malaria (Azas *et al.*, 2002). air dried at room temperature, pulverized into powder and stored in a clean air tight plastic container to avert moisture absorption and contamination. The extraction method used was cold extraction as described by Barisi and Omodele (2014) viz; Two hundred (200 g) of the powdered leaves were dissolved in 600ml of absolute ethanol in a closed container. The mixture was placed on a Stuart Orbital Shaker SSL1 (Bibby Scientific Ltd, UK) at 150 rpm and was shaken vigorously for 72 hours at room temperature. The content was allowed to settle and the supernatant was filtered using Whatman No. 1 filter paper, Buckner funnel. The resulting filtrates was centrifuged using a C5 bench top centrifuge (LW Scientific Inc. GA, USA) for 10 minutes at 1,000 rpm to remove insoluble particles and any fatty layer. Afterwards, the filtrate was decanted and concentrated to about 10% initial volume using a Stuart RE-52A water bath-rotary evaporator (Bibby Scientific Ltd, UK). The extract was dispensed into evaporating dishes and placed on Clifton water bath (Clifton Lab equipment, UK) and evaporated to dryness. The dried extract was kept in an air tight container and stored in a refrigerator at 4°C till it was needed (Barisi and Omodele, 2014).

### Phytochemical Test

#### Qualitative phytochemical test

Phytochemical screening was conducted to deduce the active chemicals present in the plant extract. These tests were carried out using standard procedures to identify the constituents as previously described by (Trease and Evans, 1989; Sofowara, 1993). The tests include; Tannins, flavonoids, Terpenoids, Cardiac Glycosides, Anthraquinones, Alkaloids and saponins.

#### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas Chromatography-Mass Spectrometry analysis of the ethanolic extract of *R. Communis* was carried out using a GC-MS - QP2010 PLUS at National Research Institute For Chemical Technology (NARICT) Zaria. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70eV (Imad *et al.*, 2014; Muhanned *et al.*, 2015). The instrument was set to an initial temperature of 110°C and was maintained for 2 mins. The oven temperature was raised to 280°C at the rate of an increase of 5°Cmin<sup>-1</sup> and maintained for 9mins. Helium was used as carrier gas at a constant flow rate of 1ml/min with an injection volume of 2ml. The injector temperature was maintained at 220°C and the ion source temperature was 200°C. Mass spectra were taken at 70eV, a scan

interval of 0.5s and fragments from 45-450 Da.  
**Experimental Animals and the Determination of Median lethal dose**

Fifty adult Swiss albino mice (both male and female) with 20-25g weights were obtained from the animal breeding unit of the Department of Pharmacognosy, Ahmadu Bello University Zaria, Kaduna State. The mice were housed in plastic cages and maintained under standard laboratory conditions and fed with mice pellets and tap water ad-libitum. The research strictly adhered to the principle of Laboratory Animal Care (NIH publication #85-23, revisited in 1985). Permission and approval was obtained from the Departmental Postgraduate research committee as well as the Research ethics review committee of the Katsina State Ministry of Health.

Acute oral toxicity of the ethanolic extract of *Ricinus communis* was studied using Lorke's method (Lorke, 1983). This involved intraperitoneal administration of *R. communis* extract (10 - 1000 mg/kg) to three groups each containing three mice. The mice were kept on fasting overnight prior to administration and returned to feeding 3 hours later (Abdulelah *et al.*, 2011). After the administration of the plant extract, all the mice were observed for mortality and signs of toxicity for 24 hours. The number of deaths in each group within 24 h was recorded. The LD<sub>50</sub> was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b) as shown in the formulae below:  $LD_{50} = \sqrt{ab}$

#### Parasite Inoculation

A chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) in donor mice were obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Oyo State. Inoculum preparation was made from blood of donor mouse infected with *Plasmodium berghei*. The desired blood volume was drawn from the donor mouse and diluted in normal saline solution. The final suspension contains about  $1 \times 10^6$  infected red blood cells in every 0.2 ml suspension (Abdulelah *et al.*, 2011). Each mouse was intraperitoneally inoculated on day 0 (D<sub>0</sub>) with 0.2ml of infected blood containing about  $1 \times 10^6$  *P. berghei* parasitized red blood cells (Ishih *et al.*, 2004).

#### Antimalarial Assay

In vivo antimalarial assay was carried according to the method of Abdulelah *et al.*, (2011) out in order to evaluate the prophylactic antimalarial activities of ethanolic extract of *Ricinus communis* at 10, 20 and 40 mg/kg doses as compared to control groups treated with 0.5ml of distilled water and reference drug groups

The total GC-MS running time was 28 mins. treated with standard drugs (Pyrimethamine 1.2 mg/kg). The percentage parasitaemia was determined by counting the number of parasitized red blood cells in random fields of microscope. The average percentage (%) suppression of parasitaemia was calculated in comparison to control as shown below (Abdulelah *et al.*, 2011).

Average % suppression is given by;

$$\frac{\text{Average parasitaemia in control groups} - \text{Average parasitaemia in treated groups}}{\text{Average parasitaemia in control groups}} \times 100$$

The prophylactic activity of the extract was evaluated using the method described by Abdulelah *et al.* (2011). The experimental mice were randomly divided into five (5) groups of six (6) mice each. The mice were orally administered 10, 20 and 40 mg/kg/day of the extract, 1.2 mg/kg/day of Pyrimethamine was administered to the reference drug group and 0.5ml distilled water to the control group. The treatment lasted for three (3) consecutive days (D<sub>0</sub>-D<sub>2</sub>). On the fourth day (D<sub>3</sub>), all the mice were injected with 0.2 ml of suspension containing  $1 \times 10^6$  *P. berghei* and kept for the next three (3) days. On D<sub>7</sub>, thin blood film was prepared from the tail of each mouse and the percentage of suppression of parasitaemia was calculated.

#### Statistical analysis

All data were expressed as mean  $\pm$  S.E.M. Statistical analysis was performed using Graphpad instat version 6.0 via analysis of variance (ANOVA). Differences at 5% level ( $P \leq 0.05$ ) were considered significant. Tukey-Kramer multiple comparison test was used to make comparisons between groups treated with different doses of the extracts, pyrimethamine and the control.

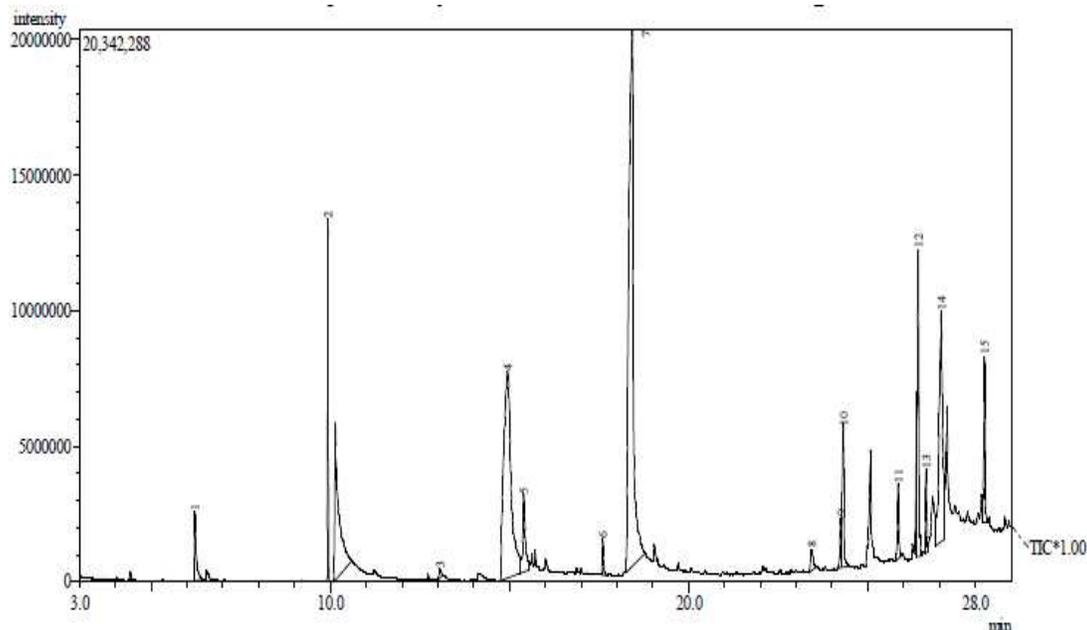
## RESULTS AND DISCUSSION

### Phytochemical test

Qualitative phytochemical investigation of ethanolic extract of *Ricinus communis* revealed that the leaves contain secondary metabolites such as alkaloids, anthraquinones, flavonoids, saponins and tannins. These metabolites could be the active materials responsible for the activities. The GC-MS analysis of the ethanolic extract of *Ricinus communis* leaves revealed fifteen peaks with each peak corresponding to a particular phytochemical. Below is the GC-MS profile for ethanolic extract of *Ricinus communis* (Fig 1), the result indicated the presence of phenolic compounds, long chain fatty acids (such as oleic acid, methylricinoleate, octadecanoic acid, octadecenoic acid, hexanoic acid) and benzene-like compounds (such as azulene, benzamine,

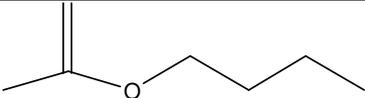
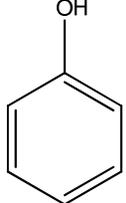
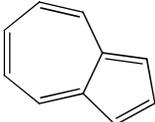
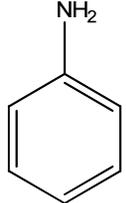
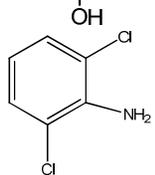
naphthalenamine, isoquinoline and Alkaloids, anthraquinones, flavonoids and tannins are usually implicated in the possession of antimalarial activity. Similar results to the potentials of these phytochemicals were obtained by (Miliken, 1997; Abdulalah *et al.*, 2010). Flavonoids are forms of phenolic

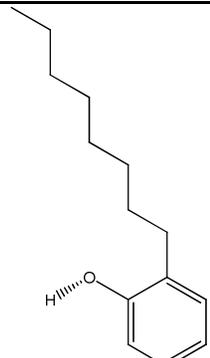
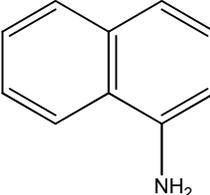
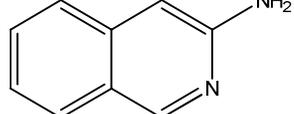
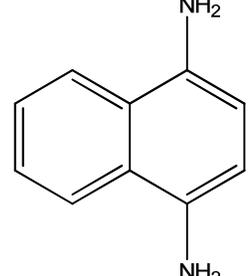
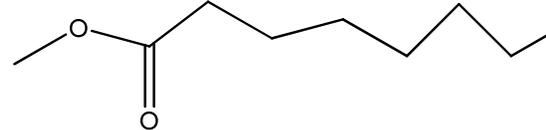
naphthalenediamine). compounds and exhibit significant antiparasitic activities against different strains of *Plasmodium*, *Trypanosoma* and *Leishmania* species (Kim *et al.*, 2004; Monbrinson *et al.*, 2006; Tasdemir *et al.*, 2006).

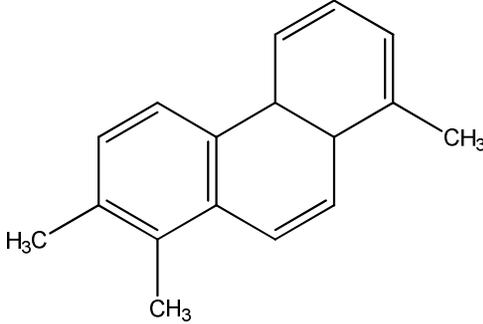
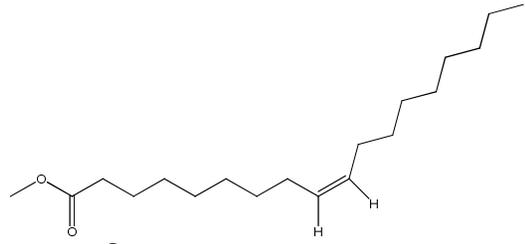
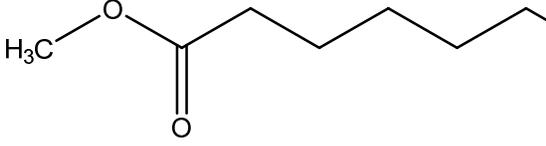
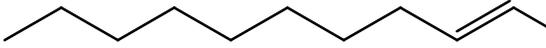
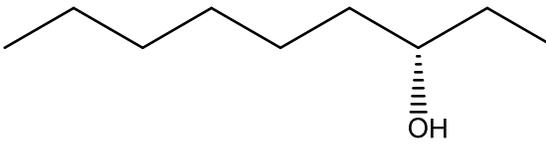


**Figure 1:** GC-MS profile of ethanolic extract of *Ricinus communis* leaves (The description of the compounds present is given in Table 1).

**Table 1** The chemical constituents of the ethanolic extracts of the leaves of *Ricinus communis* as revealed by GC-MS analysis

S/N	Phytochemical compounds	RT(min)	Area (%)	Formula	Molecular weight	Chemical structure
1.	Butyl acetate	6.228	1.66	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	116	
2.	Phenol	10.141	9.05	C <sub>6</sub> H <sub>6</sub> O	94	
3.	Azulene	13.054	0.27	C <sub>10</sub> H <sub>8</sub>	128	
4.	4-Aminophenol	14.947	22.22	C <sub>6</sub> H <sub>7</sub> NO	109	
5.	Benzanamine	15.396	3.19	C <sub>6</sub> H <sub>5</sub> Cl <sub>2</sub> N	161	

6.	3,5-di-tert-butylphenol	17.607	0.68	C <sub>14</sub> H <sub>22</sub> O	206	
7.	1-Naphthalenamine	18.430	35.38	C <sub>10</sub> H <sub>9</sub> N	143	
8.	3-Isoquinolinamime	23.421	0.80	C <sub>10</sub> H <sub>10</sub> N <sub>2</sub>	158	
9.	1,4-Naphthalenediamine	24.249	1.28	C <sub>10</sub> H <sub>10</sub> N <sub>2</sub>	158	
10.	Hexadecanoic acid	24.312	2.81	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	

11.	Phenanthrene	25.847	1.57	$C_{17}H_{16}$	220	
12.	9-Octadecenoic acid	26.403	5.03	$C_{19}H_{36}O_2$	296	
13.	Octadecanoic acid	26.626	1.23	$C_{19}H_{38}O_2$	298	
14.	Oleic acid	27.043	11.91	$C_{18}H_{34}O_2$	282	
15.	Methylricinoleate	28.253	2.92	$C_{19}H_{36}O_3$	312	

**Median lethal dose (LD<sub>50</sub>)**

After the test for the median lethal dose, a value of 141.42 mg/kg was obtained as the dose that kills half the test subjects (mice). Going by this value, doses of 10, 20 and 40 mg/kg of the extract were chosen for the bioassay. Asthenia, lethargy and ataxia were observed as the symptoms of toxicity of the extract. At the highest dose of 1000 mg/kg, these symptoms continued till death of the mice.

**Prophylactic antimalarial activity**

The antimalarial activities of ethanolic extract of *Ricinus communis* in the prophylactic test is

shown in Table 2 below. The results of the antimalarial evaluation assays of the test extract, standard drug (pyrimethamine) and control groups at different doses in mice parasitized with *P. berghei* are expressed as percentage (%) for suppression of parasitemia. P values  $\leq 0.05$  were considered to be statistically significant. Parasitaemia are expressed as mean  $\pm$  S.E.M. (standard error of mean) (n=6). Significance was obtained by comparing extract tested groups with control. The antimalarial activities of the extract proved to be of high statistical significance (P < 0.001) when compared with the control.

**Table 2** The prophylactic antimalarial activity of ethanolic extract of *Ricinus communis* in mice

Drug or extract	Dosage (mg/kg/day)	Average parasitaemia	Average % suppression	Significance
<i>Ricinus communis</i>	10	9.0 $\pm$ 1.8	58.7	P < 0.001
	20	11.3 $\pm$ 1.8	48.2	
	40	11.7 $\pm$ 0.9	46.3	
Pyrimethamine (standard)	1.2	5.6 $\pm$ 0.4	74.3	
Distilled water (control)	0.5ml	21.8 $\pm$ 0.5	-	

The Analysis of variance (ANOVA) revealed statistically significant difference in the antimalarial activity of ethanolic extract of *R. communis* among the control, treated and standard drug groups (P < 0.0001). The post test using (Tukey-Kramer multiple comparisons test) revealed that there was no significant differences among the groups treated with 10, 20 and 40 mg/kg doses of the extract (P > 0.05). Moreover, there was also no statistically significant difference between the reference drug group (Pyrimethamine) and the group tested with 10 mg/kg dose of the extract (P > 0.05). The suppression of parasitaemia by pyrimethamine at 1.2 mg/kg/day appeared to be in tandem with previous studies (Abdulelah *et al.*, 2010).

The decline in activity at higher doses of the extract could be as a result of reduced or no effect of the components present in the extract at higher doses (Rao *et al.*, 2001). Plants that exhibit antimalarial activities are known to do this either by initiating elevation of red blood cells oxidation or by preventing protein synthesis depending on the phytochemical constituents within them (Etkin, 1997). Phytochemicals such as saponins and phenols (flavonoid is also a phenolic compound) are reported to be good antioxidants in a study by (Barisi and Omodele, 2014). These compounds could be responsible for

the activity displayed by *Ricinus communis* extract since antioxidant property is another mechanism by which antimalarial effect can be exerted.

This implies that 10 mg/kg of the extract was as effective as pyrimethamine in the prevention of *P. berghei* infection. However, there was statistically significant difference in prophylactic antimalarial activity between the groups of mice treated with different doses (10, 20 and 40 mg/kg) of the extract and the control group (P < 0.001).

Previous works by (Abdulelah *et al.*, 2011) agrees that anti-plasmodial activities could be related to antioxidant effects of some phytochemicals. Saponins are known to aid in the fight against infections caused by parasite by boosting the immune system while other phytochemicals having good antioxidant properties exhibit capabilities of protecting or elevating resistance of red blood cells to oxidative damage (Barisi and Omodele, 2014). Similarly, fatty acids such as hexadecanoic acid, 9-octadecenoic acid, octadecanoic acid and oleic acid as revealed by the GC-MS analysis may also be responsible for the antimalarial activity exhibited by the extract. This corroborates the findings of Melariri *et al.*, (2012).

Fatty acids cause the degeneration of intra-erythrocytic stage of the parasites and antiplasmodial activity increases with increase in unsaturation of the fatty acids (Melariri *et al.*, 2012). The antimalarial activity of this extract could be due to single or synergistic actions of these chemical compounds.

### CONCLUSION

Ethanol extract of *Ricinus communis* exhibited remarkable antimalarial properties by virtue of its suppression of parasitemia in the prophylactic

test in mice. Ten (10 mg/kg) dose of the extract exhibited the highest percentage suppression of 58.7%. The antimalarial properties possessed by *Ricinus communis* in mice can be related to the presence of different phytochemicals such as alkaloids, flavonoids, anthraquinone and saponins which are often implicated to have antiplasmodial activities. This thus validates the rationale for the usage of the plant by the local populace in the study area for the prevention of malaria.

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