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Towards accomplishing the roll back malaria initiative: Phytochemical screening and antimalarial activity of ethanolic leaf extract of *Ricinus communis* L. (Euphorbiaceae)

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Malaria is a major debilitating disease caused by *Plasmodium* species and spread by female *Anopheles* mosquitoes. This research was conducted to determine the efficacy of ethanolic leaf extracts of *Ricinus communis* L. against *Plasmodium berghei* (NK65) infection in mice. Phytochemical components of the extract were analyzed and elucidated in order to reveal the constituents with antimalarial potentials. The safety of the extract in the experimental mice was ascertained by determining the median lethal dose (LD_{50}). Result of the phytochemical screening revealed the presence of compounds notable for antimalarial effects such as alkaloids, flavonoids and anthraquinones. From the findings, it was established that a dosage of 141.42 mg/kg of the extract represents the acute lethal dose (LD_{50}) in mice. Hence, three separate doses of the extract (10, 20 and 40 mg/kg) were prepared for the curative test. All the three doses portrayed a remarkable antimalarial activity as compared to the standard reference drug (chloroquine, 5 mg/kg). The extract dosage of 20 mg/kg showed the highest average suppression of 81.6% among the treatments. No significant differences were however observed among the treated groups ($P>0.05$). On the other hand, a highly significant difference was observed between the treated and control groups ($P\leq0.001$). The leaf extracts of *R. communis* thus possess antimalarial properties and is therefore recommended as a new candidate for antimalarial drug development.

Key words: *Ricinus communis,* chloroquine, phytochemical compound, antimalaria, dose, *Plasmodium berghei*.

INTRODUCTION

Malaria is an infectious debilitating disease which is continuously associated with considerable morbidity, mortality as well as significant social and economic impact around the globe (Balogun et al., 2009). It is the most common protozoan parasite disease in the tropical and subtropical regions of the world with more than 40%

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of the world's population at risk (Snow et al., 2005). It was estimated that by the year 2015, there were 214 million cases of malaria worldwide resulting in about 438,000 deaths, with about 90% of all cases of mortality due to malaria occurring in Africa (WHO, 2016).

Nigeria has the highest prevalence of malarial cases in Africa as transmission of the disease occurs all year round in the southern part of the country while in the northern part, the disease is more seasonal occurring mostly during the rainy season (WHO, 2008). Pregnant women, their unborn foetus and children below the age of 5 years are more vulnerable to malaria which serves as the major cause of maternal and infant anemia (Hartman et al., 2010).

Malaria in human is transmitted by the bite of a female *Anopheles* mosquito infected with *Plasmodium* species. Species that generally cause malaria in humans are *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, Plasmodium ovale* and *Plasmodium knowlesi* (Collins, 2012). It is traditionally believed that *P. falciparum* accounts for the majority of deaths due to malaria (Sarkar et al., 2009). Recent evidence also suggests that *P. vivax* is associated with potentially life-threatening conditions (Baird, 2013).

It was earlier estimated that 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). Despite recent developments in modern health care development, traditional medicine is still a norm in many parts of the world. Medicinal plants have been used since time immemorial in all cultures to treat ailments (Hoareau et al., 1999). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. Medicinal plants are plants that have one or more of their parts containing important phytochemicals that can be used for therapeutic purposes or as additives in pharmaceutical products (Bentley and Trimen, 2007). These phytochemicals appear to be of great benefits to humans and their consumption has less side effects as compared to pharmaceutical synthetic drugs (Barisi and Omodele, 2014). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Rabe and Vanstoden, 2000). A major challenge in the efforts to curtail malaria is the drug resistance (Randrianarivelojosia et al., 2003). Drug resistance is responsible for the spread of malaria to new areas and resurgence in areas where it had been eradicated (Ridley, 1997). As a result of this increasing problem, there is every need for the quest to develop novel antimalarial drugs to combat the spreading drug of resistant parasites (Abdulelah et al., 2010).

Ricinus communis, commonly known as Castor oil plant is a tropical flowering plant belonging to the family Euphorbiaceae. It is ubiquitous in nature (de Assis Junior et al., 2011). Inhabitants of rural communities often exploit different parts of this plant for the treatment of various ailments (Jena and Gupta, 2012). In the northern part of Nigeria, rural dwellers occasionally utilize the bark and leaves of this plant for local treatment of malaria. To the best of our knowledge there is no any published research that investigates the potentials of this plant in malaria therapy. This study therefore is designed to investigate the *in vivo* antimalarial potentials of *R. communis* leaves extract against chloroquine sensitive strain of *Plasmodium berghei.*

MATERIALS AND METHODS

Plant collection

The leaves of the plant were collected within the months of June and July, 2017 from Dutsin-ma area, Katsina State (Latitude 12°27'18" N and Longitude 7°29'29" E) by the researchers and identified by a taxonomist at the Department of Biology, Umaru Musa Yar'adua University, Katsina State, Nigeria. Further confirmation was done by the departmental herbarium officer and voucher specimen was deposited for future reference, before embarking on the research.

Preparation of the plant extract

Fresh leaves of *R. communis* obtained were washed and air dried at room temperature. The dried leaves were later blended into powder and stored in a clean air tight plastic container to avert moisture absorption and any possible contamination. The plant extracts were prepared via cold extraction method as described by Barisi and Omodele (2014).

Phytochemical screening

A preliminary phytochemical screening of the leaf extract of *R. communis* was carried out to detect the presence or absence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, tannins and terpenoids using Dragendoff's reagent, sulphuric acid-chloroform test, Keller-Killani's test, ammonium test, frothing test, ferric chloride test, and Salkowski's test, respectively. This was done with adherence to standard procedures as described by Trease and Evans (1989) and Sofowara (1993).

Experimental animals

Swiss albino mice (both male and female) with considerable weights were obtained from the animal breeding unit of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria, Kaduna State. The mice were housed in metal cages and maintained under standard laboratory conditions with free access to standard pelleted feed and water *ad-libitum*.

Ethical consideration

The research protocol was conduct by strictly adhering to the principle of Laboratory Animal Care (NIH publication #85-23, revised in 1985). The research protocols were also approved by the Postgraduate Research Committee of the Department of Biology and the Umaru Musa Yar'adua University Board of Research. Further permission and approval was obtained from the Research Ethics Review Committee of Katsina State Ministry of Health via

clearance certificate No. MOH/ADM/SUB/1152/1/149 dated 13th July, 2017. This was done to ensure conformity with the ethical provisions expected of this kind of research.

Determination of median lethal dose (LD_{50})

The LD_{50} of the extract (Table 2) was estimated using Swiss albino mice by oral administration route as previously described (Lorke, 1983).

Parasite inoculation

A chloroquine-sensitive strain of *P. berghei* (NK-65) was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Oyo State, Nigeria. Inoculum preparation was made from blood of donor mouse infected with *P. berghei*. The desired blood volume was drawn from the donor mouse by slaughtering and dilution in normal saline solution. The final suspension produced contains about 1×10^6 infected red blood cells in every 0.2 ml of the suspension (Abdulelah et al., 2011). Each mouse was intraperitoneally inoculated on the first day or day 0 (D_0) with 0.2 ml of infected blood containing about 1×10^6 *P. berghei* parasitized red blood cells (Ishih et al., 2004).

In vivo antimalarial assay

In vivo curative antimalarial assay was carried out in order to evaluate the possible antimalarial activity of the ethanolic leaf extract of the plant (*R. communis*) at 10, 20 and 40 mg/kg doses as compared to control groups treated with 0.5 ml of distilled water and reference drug group treated with standard drugs (chloroquine 5 mg/kg). The percentage parasitaemia was determined by counting the number of parasitized red blood cells in random fields of microscope. This experiment was conducted in duplicate. The average percentage suppression of parasitaemia was calculated in comparison with the control as described previously (Abdulelah et al., 2011).

Average percentage suppression is given by:

$$\frac{Average\ \%\ parasitemia in control groups\ \ -Average\ \%\ parasitemia in treated groups}{Average\ \%\ parasitemia in control groups}\times 100$$

Curative antimalarial activity

Thirty Swiss albino mice were inoculated intraperitoneally with 0.2 ml suspension containing 1×10^6 *P. berghei* each on day zero (D_0). The mice were then divided into five groups with each group containing six mice. After 72 h, different doses (10, 20 and 40 mg/kg/day) of the extract were orally administered to the experimental groups. The reference drug group was treated with chloroquine (5 mg/kg) and the control group received 0.5 ml of distilled water. The treatment was continued once daily for five days (Etetim et al., 2008). On the sixth day, thin blood film was prepared from the blood obtained from the tail of each mouse, stained with Giemsa stain and examined under microscope to determine the percentage of parasitaemia and average percentage of suppression following treatment.

Statistical analysis

All the data obtained were carefully cleaned, filed and entered into GraphPad InStat 3 statistical software for further analysis. The results were expressed as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) was used to determine the mean differences between the different treatments. Differences at 95% level of confidence ($P \leq 0.05$) were considered significant. Tukey-Kramer multiple comparisons test was also used to compare the variations between the treated groups to ascertain the level of significance of the various treatments on the mice.

RESULTS

Phytochemical screening

Qualitative phytochemical investigation of ethanolic extract of *R. communis* revealed that the leaves contain secondary metabolites such as alkaloids, anthraquinones, flavonoids, saponins and tannins shown in Table 1.

Median lethal dose

After the two-phase test for the median lethal dose (Table 2), a value of 141.42 mg/kg was obtained as the dose that will kill half the test subjects (mice). Going by this value, doses of 10, 20 and 40 mg/kg of the extract was 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.

Curative antimalarial activity of *R. communis* ethanolic leaf extract

The results as presented in Table 3, indicates that the ethanolic extract of *R. communis* leaves exhibited a remarkable reduction of parasitaemia in mice infected with *P. berghei*. Analysis of variance (ANOVA) indicated an extremely significant difference between the control, treated and standard drug groups ($P < 0.001$). The post-test (Tukey-Kramer multiple comparisons test) showed that there were no significant differences among the treated groups administered with the doses of 10, 20 and 40 mg/kg ($P > 0.05$) of the extract. These findings however showed high significant differences between the treated groups (administered with doses 10, 20 and 40 mg/kg) and the control group ($P < 0.001$). Furthermore, there were no significant differences between the groups

Table 1. The tests/reagents and the presence or absence of secondary metabolites.

Chemical constituent	Test/reagent	Result
Alkaloids	Dragendoff's reagent	+
Anthraquinones	Sulphuric acid-chloroform layer test	+
Cardiac glycosides	Keller-Killani's test	−
Flavonoids	Ammonium test	+
Saponins	Frothing	+
Tannins	Ferric chloride test	+
Terpenoids	Salkowski's test	−

+: Present; −: Absent.

Table 2. The acute oral toxicity of the ethanolic extract of *Ricinus communis* leaves administered orally to mice.

Dose (mg/kg)	Mortality	Toxic symptoms
10	0/3	None
*50	0/1	None
100	1/3	Lethargy, asthenia
*100	0/1	Lethargy
*200	1/1	Lethargy, asthenia
*400	1/1	Lethargy, asthenia, ataxia
1000	3/3	Lethargy, asthenia, ataxia

*Refers to the second phase of LD_{50}.

Table 3. The curative antimalarial activity of ethanolic extract of *Ricinus communis* in mice.

Drug or extract	Dosage (mg/kg/day)	Average parasitemia	Average % suppression	P-value
Ricinus communis	10	9.4 ± 1.8	74.2	P < 0.001
	20	6.7 ± 1.5	81.6	P < 0.001
	40	6.8 ± 0.9	81.3	P < 0.001
Chloroquine (standard)	5	3.9 ± 0.3	89.3	-
Distilled water (control)	0.5 ml	36.4 ± 3.5	-	-

Data are expressed as Mean ± SEM for six mice per group.

treated with different doses of the extract and those treated with the standard or reference drug group (P >0.05). This implies that the doses are comparatively effective in the reduction of parasitaemia.

DISCUSSION

The phytochemical screening of the ethanolic extract of *R. communis* revealed that the leaves contain alkaloids, anthraquinones, flavonoids, saponins and tannins. Alkaloids and flavonoids are usually implicated in the possession of antimalarial activity. Similar results to the potentials of these phytochemicals were obtained in previous studies (Miliken, 1997; Abdulelah et al., 2010). Flavonoids are forms of phenolic compounds which are known to exhibit significant anti-parasitic activities against different strains of *Plasmodium, Trypanosoma* and *Leishmania* species (Kim et al., 2004; Monbrison et al., 2006; Tasdemir et al., 2006). The antimalarial activity of this extract could be due to single or synergistic action of these chemical compounds. Nonetheless, it is important to identify and ascertain the active principle which could be isolated and purified to improve the potency of theextract.

In the curative test, the plant extract showed a dose-dependent antimalarial activity which was found to be statistically significant when compared with the control.

The lack of significant differences among the doses when compared with the standard drug (chloroquine) confirms their competitive curative antimalarial efficacy against *P. berghei* infection. The result of this study agrees with the findings of a previous similar research conducted in Malaysia (Abdulelah et al., 2010). Thus, the doses are fairly equally effective in the reduction of parasitaemia with regards to their high percentage suppression.

The decline in activity at higher doses of the extract could be as a result of reduced or no effect of thecomponents present in the extract at higher doses. This agrees with the findings from the research conducted by 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 *R. communis* extract since antioxidant property is another mechanism by which antimalarial effect can be exerted. Previous works by Abdulelah et al. (2011) suggests that anti-plasmodial activities could be related to antioxidant effects of some phytochemicals. Saponins are known to aid in the fight against parasitic infections by boosting the immune system while other phytochemicals having good antioxidant properties that exhibit capabilities of protecting or elevating resistance of red blood cells to oxidative damage (Barisi and Omodele, 2014). On another note, it has been suggested by Bapna et al. (2014), that the smell of some medicinal plants may repel mosquitos thereby reducing the incidence of malaria infection. Based on the findings from this research, the ethanolic extract of *R. communis* proved to be very effective for malaria therapy.

Conclusion

The results of the present study revealed the potent antimalarial properties possessed by the ethanolic leaf extract of *R. communis* due to its abilities to significantly suppress *P. berghei* infection in the evaluation test conducted. The antimalarial potentials exhibited by the leaf extracts of *R. communis* could be attributed to the active anti-plasmodial components within the extracts which are mainly alkaloids, flavonoids, anthraquinones and saponins. The use of the *R. communis* plant extract as a potential candidate for future anti-malaria drug development is therefore recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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