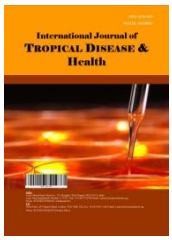

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Evaluation of the Antiplasmodial Potential of the Aqueous Leaf Extract of *Ricinus communis* L (Euphorbiaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: As the problem of resistance to antiplasmodial agents persists, the need to develop new and more effective drugs continue to arise. The antiplasmodial potential of the aqueous leaf extract of *Ricinus communis* using Albino mice injected with Plasmodium berghei NK65 was studied using the suppressive, prophylactic and curative models.
Methods: Animals were divided into five groups consisting of six mice each. Artesunate (5mg/kg/day) and pyrimethamine (1.2mg/kg/day) served as positive controls while distilled water (10ml/kg/day) served as negative control. The extract was administered at doses of 73, 145 and 217mg/kg/day through the intraperitoneal route.
Results: Results obtained showed that the extract achieved a Chemosuppression of 81.50% 89.90% and 92.90% which are greater than that achieved by the standard drug pyrimethamine in the prophylactic model experiment.
Conclusion: The leaf of R. *communis* has good potentials for the development of a new antiplasmodial.

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Keywords: Antiplasmodial; plasmodium berghei; extract; chemosuppression; Ricinus communis.

1. INTRODUCTION

Malaria is a haematological disease caused by a protozoan of the genus Plasmodium and transmitted through the bite of an infected female anopheles mosquito. Malaria is one of the common killer diseases of tropical Africa with pregnant women and children under the age of five being the most vulnerable [1]. The World Health Organisation (WHO) malaria report in 2019 confirmed that an estimated 228 million cases of malaria occurred worldwide in 2018 with 405,000deaths within the same period with the African region accounting for 94% of deaths. It is one of the major public health problems in Nigeria contributing to a quarter of the malaria burden in Africa. The burden of malaria has remained high in most African countries [2] and the emergence of malaria resistance to artemisin derivatives has become one of the greatest challenges to its control and elimination [3]. This resistance has been reported to be caused by a nucleotide polymorphism in parasites K13 gene which leads to deregulated protein response. This has manifested in slow parasite clearance in patients and is observed as increased survival of early ring stage parasites *In-vitro*. [4].

Malaria is usually associated with poverty [5] and has a major effect on economic development [6]. These facts along with the high cost of orthodox medicine, have led most African countries to encourage the use of herbs in the treatment of malaria.

R. communis belongs to the Euphorbiaceae family and is widely distributed across the world [7]. It invades grassland and farmlands [8]. Although it is indigenous to Eastern Africa and the Mediterranean basin, today, it is widespread in tropical regions. The leaves of *R. communis* are alternate, curved and cylindrical ovate. They have found so much application in traditional medicine in so many countries where they are found. The leaves, for example, are used to relieve flatulence in children [9], while they are recommended in the form of a decoction for women as a lactogogue [10]. Different parts of the plant and seed have also been used for so many other purposes due to a wide range of activities which they exhibit including antiashmatic, anti-fertility, anti-inflammatory, wound healing, antiulcer and anti- diabetic activities [11]. Remarkably, three unique peptides referred to as RCB 1-3 which have both antibacterial and antifungal properties have been reported [12].

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh leaves of *R. communis* were collected from a farm at Itak Ikot Atap village in Ikono local Government Area of Akwa Ibom State, Nigeria. The plant was identified with the aid of taxanomic keys provided by the Department of Pharmacognosy and Natural Medicine, the University of Uyo where a sample with herbarium number UUPH31P was deposited for future reference.

2.2 Plant Extraction

Leaves of R. *communis* were washed, air dried for 18 days and then pulverized using a mortar and pestle. Three hundred gramme (300g) of the powdered leaves were soaked in 1L of water for 72 hours. The liquid extract was then obtained by filtration using a clean muslin cloth. This was further filtered using a grade1 Whatman filter paper and the filtrate was then evaporated to dryness in vacuo at 4^{o}C. The extract obtained was stored in the refrigerator at 4^{o}C until used.

2.3 Animals

Male and female albino mice weighing 20-25g were used. They were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. The animals were kept in standard cages with proper ventilation, fed with feed from Grand cereal limited, Jos, Nigeria and given clean drinking water *ad libitum*.

2.4 Phytochemical Studies

The leaf extract of *R. communis* was screened for the presence of certain phytochemical constituents using the methods described by [13].

2.5 Acute Toxicity Studies

This was done using a modified Lorke method to determine the safety of the leaf extract using Swiss albino mice. The experimental animals were divided into three groups with three mice in

each group. Each group was administered with different doses of the extract from 500, 1000, 1500 up to 5000mg/kg body weight. The animals were then observed within a 24 hour period for visible signs of toxicity and death. The median lethal dose (LD_{50}) was calculated using the method of Lorke [14] thus:

$LD_{50} = \sqrt{AB}$

Where A=Maxmum dose producing 0% mortality
B=Minimum dose producing 100% mortality

2.6 Parasite

Plasmodium berghei (NK65) obtained from the National Institute for Medical Research (NIMR) Lagos was used for the study. They were preserved by the serial passage of 0.2mL parasitized blood from an infected mouse to an uninfected mouse

2.7 Parasite Inoculation

The mice used for the experiments were first confirmed to be free from malaria parasites by the absence of these parasites in the smears made with blood obtained from the tails of the animals. Those confirmed to be free were then used for the experiments by the administration of 0.2mL ($1.0x\ 10^7$) parasitized blood for the infection of each mouse (Ettebong et al, 2015).

2.8 Antiplasmodial Activity Studies

2.8.1 Suppressive activity

The 4- day suppressive test described by Peters et al., [15] with modifications was used to evaluate the suppressive activity of the leaf extract. Thirty (30) mice were randomly allocated to five groups of six mice each. On day1 (D_0), 0.2mL standard inoculum containing approximately $1x10^7$ parasitized erythrocyte was administered through the intraperitoneal route to all the mice. Groups 1-3 were given 73, 145 and 217mg of the of the leaf extract per kg/day respectively. Group 4 received 5mg/kg/day of Artesunate (Standard drug) while group 5 which served as the control received 10ml/kg bodyweight of distilled water. This continued at the same time for the next 4 days. On the fifth day (D_4) thin blood smears using blood from the tail tip of each mouse in the model were made on microscopic slides. The slides were Giemsa stained and examined under the microscope after which the percentage suppression was determined at each dose.

Average percentage suppression was calculated below

Average % parasitemia in negative control- Average % parasitemia in positive control

Average % parasitemia in negative control

2.8.2 Prophylactic or repository activity

The method described by Okokon and Nwafor [16] with modifications was employed for this experiment. Thirty (30) albino mice were randomly divided into five groups of six mice each. Groups 1-3 were administered 73, 145 and 217mg/kg of the extract while group 4 and 5 received 1.2mg/kg/day of pyrimethamine (Positive control) and10mL/kg of distilled water (Negative control). The drug and extract administrations continued at the same time of the day for three days. On the fourth day, the mice were injected with 0.2mL of infected blood containing approximately 1×10^7 parasitized erythrocytes through the intraperitoneal route. After 72 hours, thin blood films were prepared from blood obtained from the tail of each mouse on a slide. The slides were Giemsa stained to show parasitized erythrocytes in fields observed under the microscope and the percentage suppression due to its prophylactic activity was determined at each dose.

2.8.3 Curative activity

This test was employed to determine the schizontocidal activity of the extract after a confirmed infection and the method described by Okokon and Nwafor [16] with modifications was employed. 0.2mL blood containing approximately 1×10^7 parasitized erythrocytes was administered to the mice through the intra peritoneal route and left for 72 hours. Before the administration of the extract, blood was taken from the tail of each mouse and the level of parasitaemia determined. Mice in groups 1-3 were given 73, 145 and 217mg/kg/ of the leaf extract respectively. Those in group 4 were given 5mg/kg of Artesunate while those in group 5 which served as the negative control group were given10ml/kg of body weight of distilled water. The administration of the extract, distilled water and standard drug was done once daily for five

days after which blood samples were collected from the tail of each mouse on each day of the treatment. The blood was Giemsa stained and the effect of the extract treatment determined by monitoring parasitaemia level. The mean survival time (MST) which is the period of survival (in days) of each group of mice after the administration of extracts and standard drugs over a period of 30 days was calculated thus

MST = No of days survived x 100

Total No of days (30)

2.9 Evaluation of Parasitaemia

The parasitaemia levels were determined using the method described by [15]. The number of parasitized erythrocytes out of 200 in random fields of the microscope was noted and percentage parasitaemia was calculated thus

Average % Parasitaemia in negative control─ Average % Parasitaemia in positive control
Average % parasitaemia in negative control

2.10 Statistical Analysis

The one- way analysis of variance (ANOVA) was used for the analysis of data which was presented as the mean of six dimensions ± SEM while the Turkey Kramer post hoc was employed to check for multiple comparisons. Differences were considered to be statistically significant at $p<0.05$.

3. RESULTS

3.1 Phytochemical Constituents

Results obtained showed the presence of saponins, alkaloids, flavonoids and glycosides in the aqueous leaf extract of *Ricinus communis.*

3.2 Acute Toxicity (LD_{50}) Test

On the administration of varying doses of the extract, different signs of toxicity including convulsion and then death were observed. The animals which received 750-5000mg/kg body weight of the extract showed these visible signs of toxicity and died while those which received 700mg/kg all survived. The (LD_{50}) median lethal dose which is the geometric mean of the maximum dosage that produced zero percent lethality and the minimum dosage that produced 100% mortality was thus calculated to be 724.57mg/kg.

3.3 Pre-screening

Animals that were pre-screened for the prophylactic test had parasite count within the range of 59-69.5% while those pre-screened for the curative test had parasite count of 74-83.5%

3.4 Evaluation of Suppressive Effect

Results obtained in this model showed a percentage chemo suppression of 47.80, 53.20 and 68.90% at doses of 73, 145 and 217mg/kg respectively, while Artesunate (standard drug) produced suppression of 88.0%. (Table 1).

3.5 Evaluation of Proplylactic Effect

The extracts on administration to the P. *berghei* produced chemosuppression of 81.50, 89.90 and 92.90% at doses of 73, 145 and 217mg/kg respectively, while Pyrimethamine (standard drug) caused a suppression of 63.9%. (Table 2).

3.6 Evaluation of Curative Effect

Results obtained showed that after 6 days post administration of extract a significant decrease in parasitaemia ($p< 0.05$) was observed (Table 3.).

Table 1. Suppressive activity of aqueous leaf extract of *R. Communis* in mice infected with *Plasmodium berghei*

Treatment	Dose(mg/kg)	Parasitaemia	Percentage suppression
Leaf Extract	73	$77.7\pm 2.43^{a,b}$	47.80
Leaf Extract	145	$67.9\pm2.56^{a,b}$	53.20
Leaf Extract	217	$46.3\pm 1.80^{a,b}$	68.90
Artesunate	5	17.3 ± 0.92^{a}	88.0
Distilled water	10mL/kg	149.0 ± 2.28	-

Values are expressed as mean± SEM, [a]=p<0.05 (Significant relative to control (distilled water)), [b]=p< 0.05 significant relative to control (artesunate), n=6 Turkey Kramer (Post hoc), ANOVA

Table 2. Prophylactic activity of aqueous leaf extract of *R. communis* in mice infected with *Plasmodium berghei*

Treatment	Dose(mg/kg)	Parasitaemia	Percentage suppression
Leaf Extract	73	27.0± 3.52[a,c]	81.50
Leaf Extract	145	14.7±0.92[a,c]	89.90
Leaf Extract	217	10.3± 0.76[a,c]	92.90
Pyrimethamine	5	52.7± 2.70[a]	63.90
Distilled water	10mL/kg	146.0± 3.600	-

Values are expressed as mean± SEM, [a]=p<0.05 (Significant relative to control (distilled water)), [b]=p< 0.05 significant relative to control (pyrimethamine), n=6, Turkey Kramer (Post hoc), ANOVA

Table 3. Curative activity of aqueous leaf extract of *R. Communis* in mice infected with *Plasmodium berghei*

Treatment	Dose(mg/kg)	Parasitaemia Levels		
		D2	D4	D6
Leaf Extract	73	125.0± 0.56[c]	122.0± 1.10[a,b]	102.0± 1.38[a,b]
Leaf Extract	145	123.0±0.15[a]	117.0± 0.76[a,b]	79.7± 0.69[a,b]
Leaf Extract	217	121.0± 0.73[a]	113.0± 0.81[a,b]	53.0± 1.12[a,b]
Artesunate	5	122.0± 0.76[a]	103.0± 0.52[a]	26.7± 0.39[a,b]
Distilled water	10mL/kg	135.0± 0.86	147.0± 1.18	158± 0.88

Values are expressed as mean± SEM, [a]=p<0.05 (Significant relative to control (distilled water)), [b]=p< 0.05 significant relative to control (artesunate), n=6 Turkey Kramer (Post hoc), ANOVA

Table 4. Mean Survival time of mice infected with *Plasmodium berghei* after treatment with different doses of the aqueous leaf extract of *R. communis*

Treatment	Dose(mg/kg)	Survival Time(Days)
Leaf Extract	73	10.7±0.33
Leaf Extract	145	13.0±0.58
Leaf Extract	217	14.7± 0.67
Artesunate	5	21.7± 0.88
Distilled water	10mL/kg	8.67± 0.33

Values are expressed as mean± SEM, [a]=p<0.05 (Significant relative to control (distilled water)), [b]=p< 0.05 significant relative to control (artesunate), n=6 Turkey Kramer (Post hoc), ANOVA

4. DISCUSSION

While malaria has continued to be a significant health problem especially in the tropics, plant products which are known to be a rich source of bioactive chemicals have continued to give scientists hope of the development of novel antiplasmodial drugs for its control [17,18].

Phytochemical analysis of the extract showed the presence of alkaloids, saponins, flavonoids and glycosides. Several of these phytochemicals are known to be responsible for antimalarial activity. Flavonoids for instance have been reported to have antiplasmodial activity which they exhibit by inhibiting the fatty acid biosynthesis of the parasite [19]. They have also been reported to chelate with the nucleic acid base pairing of malaria parasites [20]. Alkaloids have been reported to show antimalarial properties by blocking protein synthesis in plasmodium [21]. Saponins and tannins act as primary antioxidants or free radical scavengers which can counteract oxidative damage induced by malaria parasite [22]. This antioxidative property is believed to present yet another mechanism that contributes to antiplasmodial activity [23]. These secondary metabolites may be responsible for the observed antiplasmodial activity.

The greatest problem with the use of plant parts extract in the management of most infections has been that of toxicity. Results obtained however showed that the plant extract was only slightly toxic. This is confirmed by the fact that the experimental animals which received doses of the extract above the LD_{50} (724.57mg/kg) first showed visible signs of toxicity and then died.

A decrease in parasitaemia with an increasing dose levels of the extract was observed through the experiment with the highest dose (217.37mg/kg) showing highest

chemosuppression confirming a dose dependent activity of the extract.

Although the standard drug artesunate produced a higher percentage of suppression (Table 1) the effect due to the extract is significant ($p<0.05$) relative to the control. It is important to note that the extract used for the experiments are still in the crude form. When the active compounds responsible for this activity are obtained and purified, their effect will no doubt be better.

The result of the prophylactic test showed a better effect of the extract than the standard drug pyrimethamine (Table 2). This highlights the good potentials of the extract as a prophylactic agent.

Upon established infection there was also an observed significant ($p<0.05$) reduction in parasitaemia level at various doses of the leaf extract when compared with the control (Artesunate) (Table 3). Altogether, these significant reduction in parasitaemia levels by the extract in all the models of experiments, translated into a longer mean mean survival time when compared with water as a control (Table 4). Generally, a chemical compound is considered to be active when it is able to achieve a percentage parasitaemia suppression of at least 30% [24]. The chemosuppression effect of the aqueous leaf extract used in the study against *P. berghei* was found to be above 30% (Tables 1-3) and is therefore considered effective when used either for prophylactic or curative purposes [25].

5. CONCLUSION

Results obtained from this study showed that the leaf extract of R. *communis* has a significant suppressive, prophylactic and curative antiplasmodial activity when compared to the standards used [26-29]. This explains why the people of Itak Ikot Akap village in Ikono Lcal Government Area of Akwa Ibom State, Nigeria use this leaf extract as an antimalarial. The dose dependent activity is believed to be due to the presence of certain secondary metabolites found in the leaves which could serve as a lead for the development of new and effective antiplasmodial.

ETHICAL APPROVAL

Approval for the use of animals in the experiment was granted by the animal ethics committee of the Faculty of Pharmacy, University of Uyo, Nigeria and all the animal experiments followed the ethical standards for the care and use of laboratory animals. (Guide for the Care and Use of Laboratory Animals, 2011).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Olashinde GI, Ajayi AA, Taiwo SO, Adekeye BT, Adeyeba OA. Prevalence and Management of Falciparium malaria among infants and children in Ota, Ogun State. Afr J clin Exper Microbiol. 2010;11:159-163.
2. Griffin JT, Hollings TD, Worth LC, Okell TS, White M. Reducing Plasmodium falciparum Malaria Transmission in Africa. A model based Evaluation of Intervention strategies. Plos Med. 2010;7(10):1371.
3. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, Kim S, Duru V, Bouchier C. A Moloecular Marker of Artemisinin-Resistant Plasmodium falciparum Malaria. Nature. 2014;505: 7481:50-55.
4. Fairhust RM, Dondorp AM. Artemisin-resistant plasmodium malaria Emerging Infections , Wiley Online Library; 2016.
5. Francesco Ricci. Social implications of Malaria and their relationship with poverty. Mediterr J Haematol Infect Dis. 2012;4(1). DOI: 10.4084/MJHID.2012.048
6. Oriol Mitja, Raymond Paru, Inoni Betuela, Quique Bassat. Malaria epidemiology in Lihir Island, Papua New Guinea. Mal. Journ. 2013;12(1):98.
7. Eudinar MJ, Ismael MS, Ferrandes SS, Luciene XM, Rogerio AP, Patricio MB, Benito, SB. Toxicity of castor bean (Ricinus Communis). Pollen to Honey bees. Agri. Ecosyst Env. 2001;141:221-223.
8. Weber E. The Most Complete Global overview of Invasive species in Natural areas. Diver. Distrib. 2003;10:505.
9. Haider Mashkoor Hussein, Rafid Hadi Hameed, Imad Hadi Hameed. Screening of Bioactive compounds of Ricinus communis

using GC-MS and FTIR and evaluation of its antibacterial and antifungal properties. Ind. J Pub. Healt. Res and Dev. 2018;9(5) 463-469.
10. Bentley R. Medicinal plants. Forgotten Books, New York; 2008.
11. Sarfaraz khan Marwat, Fazal-UrRrehma, Ejaz Ahmad khan, Mohammad Safdar Baloch, Muhammad Sadiq, Imdad ulla, Sadaf Javaria and Salma Shaheen. Ricinus communis: Ethnomedicinal uses and Pharmacological activities. Pak. J. Pharm. Sci. 2017;30(5):1815-182.
12. Delgarbat Bolbaater, Sunithi Gunasekera, Hesham R. El-seedi and Ulf Goranssor. Synthesis, Structural Characterization and Bioactivity of thestable peptide RCB-1 from Ricinus communis. J. Nat. Prod. 78;2545-2551.
13. Harborne JB. Phytochemical Methods. London: Chapman and Hall. 1984;166-226.
14. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983;54:275-287.
15. Knight DJ, Peters W. The antimalarial action of nbenzyloxy dihydrotriazines: The action of cycolguanil CBRL 50216 against rodent malaria and studies on its mode of action. Ann. Trop. Med. Parasitol. 1980;74:393-404.
16. Okokon JE, Nwafor PA. Antiplasmodial activity of ethanolic root extract and fractions of Croton zambesicus. J Ethnopharmacol. 2009;121:74–78.
17. Homburger F. *In vivo* testing in the study of toxicity and safety evaluation In: A Guide to General Toxicology 2nd ed. New York; 1989.
18. Das N, Goseami D, Rahba B. Preliminary Evaluation of Efficacy of Plant Extract. Jour. Vect Born Dis. 2007;44:145-148.
19. Freundlich JS, Anderson JW, Saratakis D, Shich HM, Valderramos JC, Lucumi E, Kuo M. Synthesis, Biological action and X-ray of crystal structural analysis of Diaryl ether inhibitors of Malarial Enoyl Acyl carrier protein reductase. Bioorg. Med Chem. 2005;15(23):5247-5252.
20. Okokon JE, Ettebong E, John UA, Obot, JA. Antipasmodial and antiulcer activities of Melanthera Scandens. As Pac J Trop Biomed. 2012;2:16-20.
21. Dawodu AO, Moses UD, Apena A. Adetoro A, JO Dairo JO. The Proximate Evaluation and Phytochemistry of Enantia chlorantha Stem Bark in Aqueous and Ethanolic Extract. Mid-East Jour Sci Res. 2014; 21(11):2145-2148.
22. David AF, Philip JR, Simon LC, Reto B, and Solomon N. Antimalarial drug discovery: efficacy models for compound screening. Nat. Rev. Drug Disc. 2004;3:509-520.
23. Abdulelah H, Zurainee MN, Hesham MA and Rohela M. Median Lethal Dose, Antimalarial Activity, Phytochemical Screening and Radical Scavenging of Methanolic Languas galangal Rhizome Extract. Molecul. 2010;15:8366-8376.
24. Adugna MF, Taddese TW, Admasu P. *In vitro* Antimalarial Activity of Crude extract of Aerial part of Artemisa abyssinica against plasmodium berghei in Mice. Glob J Pharm. 2014;8:460-468.
25. Ezekiel Tambari V. Dornu. In vivo antiplasmodial activities of Nauclea latifolia. Asian Journ. Med Sci. 2015;6(3): 6-11.
26. Evans WC. Trease and Evans Pharmacognosy. 15th ed W. B. Saunders company Ltd. 2002; 135-150.
27. Guide for the care and use of laboratory animals. Eight Ed. Washington D.C. The National Academies; 2011.
Available:http://doi.org//10.17226/12910
28. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Ibadan: Spectrum Book Ltd; 2006.
29. Ubulom PM, Ettebong EO, Udofa EJ, Inyang Etuk RS. *In vivo* antiplasmodial potential of aqueous seed extract of Ricinus communis J.Herbmed Pharmacol. 2019;8(2):133-138.

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