

The Efficacy of *Andrographis paniculata* and *Chromolaena odorata* Plant Extract against Malaria Parasite

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Abstract—Malaria constitutes one of the major health problems in Nigeria. One of the reasons attributed for the upsurge was the development of resistance of *Plasmodium falciparum* and the emergence of multi-resistant strains of the parasite to anti-malaria drugs. A continued search for other effective, safe and cheap plant-based anti-malaria agents thus becomes imperative in the face of these difficulties. The objective of this study is therefore to evaluate the *in vivo* anti-malarial efficacy of ethanolic extracts of *Chromolaena odorata* and *Andrographis paniculata* leaves. The two plants were evaluated for their anti-malaria efficacy *in vivo* in a 4-day curative test assay against *Plasmodium berghei* strain in mice. The group treated with 500mg/ml dose of ethanolic extract of *A. paniculata* plant showed parasite suppression with increase in Packed Cell Volume (PCV) value except day 3 which showed a slight decrease in PCV value. During the 4-day curative test, an increase in the PCV values, weight measurement and zero count of *Plasmodium berghei* parasite values was recorded after day 3 of drug administration. These results obtained in group treated with *A. paniculata* extract showed anti-malarial efficacy with higher mortality rate in parasitaemia count when compared with *Chromolaena odorata* group. These results justify the use of ethanolic extracts of *A. paniculata* plant as medicinal herb used in folklore medicine in the treatment of malaria.

Keywords—Anti-malaria, Curative, Plant-based anti-malaria agents.

I. INTRODUCTION

MALARIA is one of the most common vector-borne diseases prevalent in tropical and subtropical areas of the world, including regions in Africa, Asia, and America [20]. It is an endemic infectious disease that is wide spread in tropical and subtropical regions of the world and one of the six most important parasitic diseases of man [19]. Malaria remains an incredible health burden in tropical areas. Malaria is caused by the protozoan parasites, belonging to the genus *Plasmodium*, residing in female Anopheles mosquitoes. Of all Plasmodia, only *P. malariae*, *P. ovale*, *P. falciparum*, *P. vivax* [11] and *P. knowlesi* [4] infect humans thereby cause diseases. *Plasmodium falciparum* is responsible for the most severe form of human malaria and causes a tremendous economic burden [17], leading to at least one million deaths per year,

particularly in developing countries where failure to eradicate the anopheline mosquito vector leads to occasional epidemics [10], [2]. Approximately 250 million people are infected with malaria worldwide every year, mainly consisting of pregnant women and children under the age of five years [2].

Malaria remains one of the world's most debilitating infectious diseases [16]. In Nigeria, Malaria is endemic throughout the country. World Health Organization (WHO) estimated malaria mortality rate for children under five in Nigeria at 729 per 100,000. Medicinal plants have been used in the treatment and prevention of malaria in various part of the word. Plants have been a great source of medicine useful in the treatment of various diseases [3]. Disease remains a formidable medical challenge due to resistance by *Plasmodium* to commonly available drugs e.g. chloroquine. The search for antimalarial drug from plant origin cannot be neglected since that the antimalarial drugs in use today (quinine and artemisinin) were isolated from plants [8].

In Nigeria, various plants are used for the management of malaria and these vary from one locality to another [1], [12]. The usefulness of these medicinal plants may hold the key to another new and effective antimalarial drug in the future. Indigenous medicinal plants in Nigeria used in combating malaria are yet to be projected in conferences as the foreign plants in spite of our rich flora diversity. Therefore, this present study aimed at confirming the medicinal property of the plant used for malaria therapy in Olode area, Adegboyi, Ibadan Oyo State and south-west of Nigeria.

II. MATERIALS AND METHODS

A. Plant Collection and Preparation

The leaf part *Andrographis paniculata* plant and *Chromolaena odorata* leaves were collected from Akinleye Local Govt. Area, Ibadan, Oyo state and south-west Nigeria in October 2011. Voucher number was received for the two plants which are 109503 for *A. paniculata* specimens and 109494 for *C. odorata* were deposited at the Herbarium Department of the Institute of Forestry Research of Nigeria, Ibadan.

B. Plant Extract Preparation

The leaves samples of the plants were air-dried under shade at room temperature for four days, with adulterant picked out of the leaves. The leaves were thereafter pulverized; 112.0g and 16.2 of *Chromolaena odorata* and *Andrographis paniculata* were weighed with digital weighing machine.

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112.0g of *Chromolaena odorata* and 16.2g of *Andrographis paniculata* were respectively soaked in 95% ethanol for 3days. The crude plants were extracted after concentrated over water bath and dried at 40°C. The crude extracts were weighed and stored in a refrigerator for further use.

C. *In vivo* Antiplasmodial Testing Parasites Strain

In vivo antimalarial testing in mice was done using chloroquine sensitive strain of *Plasmodium berghei* (donated by the animal unit the Institute for advanced Medical Research and training, College of Medicine, UCH, Ibadan). The parasites stock was maintained by continuous re-infection in the mice.

D. Animal Bioassay

Male and female BALB/mice (20-25g), used in the study were kept in a ventilated room and feed *ad libitum* with food and water during the whole period of the study. The animals were obtained from the unit of the institute of Advance Medical Research and Training, College of Medicine, UCH, Ibadan. The animals were housed in cages in group of five (five in each group)

E. Inoculum

A donor mouse infected with rodent malaria parasites *P. berghei*, a chloroquine sensitive strain of *P. berghei* and blood collected through cardiac puncture with a sterile and a pyrogenic disposable needle and syringe. The blood was diluted with normal saline making up 14ml, each mice was injected intraperitoneally, i.e. through the peritoneum (A transparent membrane that lines the abdominal cavity in mammals and covers most of the viscera) with 0.2ml of blood containing approximately 1×10^6 infected red cells.

F. Four Day Curative Test

The experiment procedure for the curative test is similar to that of the suppressive test [14]. The differences as in the administration of compound test, which required treatment after 72 hrs of infection with inoculum. After 72 hrs of infection with parasites, the mice were randomly assigned into treatment groups of four as follows

- i. Infected untreated group (vehicle)
- ii. Infected treated with chloroquine as positive control
- iii. Infected treated with *Chromolaena odorata*
- iv. Infected treated with *Andrographis paniculata*

The infected group was given 500mg/kg of tween 80, the infected treated with chloroquine group was given 10mg/kg body weight of chloroquine and the remaining groups was given 500mg/kg body wt of 95% ethanolic extracts of the plants. The extracts were administrated orally once daily for four consecutive days (Day 0 (D0) till day 3, during which parasitaemia level was monitored daily and mean survival times (days) were recorded. Thin smear of blood were obtained from the peripheral blood on the tail from each mouse on day four after infection. The smears were placed on microscopic slides, fixed with methanol and stained with giemsa stain at pH 7.2 and examined under microscope at X 100 (oil immersion).

Determination of Parasitaemia

$$\% \text{ parasitaemia} = \frac{\text{total number of PRBC}}{\text{Total number of RBC}} \times 100$$

where; PRBC: Parasitized red blood cells; RBC: red blood cells.

The body weights of the mice were measured to observe whether the test extracts prevented the weight loss that is commonly reduced with increasing parasitaemia in infected mice. The weights were taken on D0 to D4.

G. Packed Cell Volume

The packed cell volume (PCV) was measured to predict the effectiveness of the test extracts using the modified wintrobe's method. Blood from the tail of the animals was then drawn into capillary tube and determinations were done by measuring the relative volume of the blood occupied by erythrocytes, using the relation:

$$\text{Packed Cell Volume} = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}}$$

PCV is a measure of the proportion of red blood cells to plasma. This test was done for each mouse just before infection and on the D0 to D3 of extracts administration and D4 (7days after treatment) and D5 (14 days after treatment) for follow up.

H. Data Analysis

The mean (MN) and standard deviation (SD) data of the parasitaemia count, Body weight and PCV readings of the animals used for the experiments are analyzed using SPSS.

III. RESULT

Chromolaena odorata and *Andrographis paniculata* plants are both in abundance at Olode, Adegbayi, Egbeda local Govt. Area of Ibadan respectively.

The result of the study indicated that *in vivo* test of ethanolic extract of *A. paniculata* displayed a very good activity against the *P. berghei* malaria parasite in Table I. The comparison analysis indicated in Table II that 500mg/kg ethanolic extract of *A. paniculata* and 500mg/kg of *C. odorata* showed statistically significant difference on day4 parasitaemia level, compared to the positive control.

The results of the packed cell volume (PCV) showed that the infected mice treated with *A. paniculata* had a more improved result than the ones treated with *C. odorata*. This is shown in Tables III & IV.

TABLE I
PARASITAEMIA COUNT AGAINST CURATIVE TEST OF ETHANOLIC EXTRACT OF *C. ODORATA*

GRP	D0	D1	D2	D3	D10	D17
Co	9.69±11.38	12.36±18.39	13.88±12.81	9.83±7.14	6.38±9.55	
CQ	9.62±9.87	10.04±15.50	3.56±7.68			
Veh	6.45±4.84	15.2±9.04	13.12±15.25	13.10±10.25		

Results are expressed in (Mn±S.D), GRP=Group, Vehicle (Veh) = Diluents (Tween 20), Cq=Chloroquine and *C. odorata* all in 500mg/ml dose.

TABLE II
PARASITAEMIA COUNT AGAINST CURATIVE TEST OF ETHANOLIC EXTRACT OF *A. PANICULATA*

GRP	D0	D1	D2	D3	D10	D17
Ap	6.47±2.02	7.09±8.15	3.53±3.50	4.79±3.45	1.31±2.92	
Q	9.62±9.87	10.04±15.50	3.56±7.68			
Veh	6.45±4.84	15.20±9.04	13.12±15.25	13.10±10.25		

Results are expressed in (Mn±S.D), GRP=group, Vehicle (veh) = Diluents (Tween 20), Cq = Chloroquine and *A.p=Andrographispaniculata* all in 500mg/ml dose.

TABLE III
THE PCV READING OF INFECTED MICE TREATED WITH *C. ODORATA*

GRP	D0	D1	D2	D3	D10	D17
Co	39.16±5.50	30.46±3.47	29.06±3.62	27.00±2.91	23.36±8.67	
CQ	40.52±13.26	35.92±5.02	40.40±1.14	37.06±7.24	41.96±1.90	44.80±1.90
Veh	40.92±9.70	32.82±5.41	27.00±5.83	25.70±5.84	38.50±5.00	45.50±0.00

Results are expressed in (Mn±S.D), Vehicle= Diluents (Tween 20), Cq=Chloroquine and Co= *C. odorata* all in 500mg/ml dose.

TABLE IV
THE PCV READING OF INFECTED MICE TREATED WITH *A. PANICULATA*

GRP	D0	D1	D2	D3	D10	D17
Ap	30.08±6.13	29.67±5.39	30.92±4.38	28.23±4.60	32.10±7.63	38.50±0.00
CQ	40.52±13.26	35.92±5.02	40.40±1.14	37.06±7.24	41.96±1.90	44.80±1.90
Veh	40.92±9.70	32.82±5.41	27.00±5.83	25.70±5.84	38.50±5.00	45.50±0.00

Results are expressed in (Mn±S.D), GRP=group, Vehicle (veh)= Diluents (Tween 20), Cq=Chloroquine and *A.p=Andrographispaniculata* all in 500mg/ml dose.

TABLE V
THE BODY WEIGHT (G) OF INFECTED MICE TREATED WITH *C. ODORATA*

GROUPS	D0	D1	D2	D3	D10	D17
Vehicle	20.14±2.23	18.10±1.80	18.24±1.60	17.73±1.96	15.70±0.00	20.80±0.00
CQ treated	19.20±1.80	19.36±2.51	20.08±2.53	18.88±2.63	20.40±2.60	18.80±3.90
Co treated	19.16±1.46	19.80±1.64	19.73±2.44	19.33±3.15	20.10±1.83	20.60±0.00

TABLE VI
THE BODY WEIGHT (G) OF INFECTED MICE TREATED WITH *A. PANICULATA*

GROUPS	D0	D1	D2	D3	D10	D17
Vehicle	20.14±2.23	18.10±1.80	18.24±1.60	17.73±1.96	15.70±0.00	20.80±0.00
CQ treated	19.20±1.80	19.36±2.51	20.08±2.53	18.88±2.63	20.40±2.60	18.80±3.90
Ap treated	18.82±2.01	19.12±1.01	18.18±1.10	17.74±1.02	10.20±3.90	

The results of the body weight showed that the infected mice treated with *A. paniculata* plant extract had increase in body weight when compared with those treated with *C. Odorata* extract the latter had reduction in body weight as shown in Tables V and VI respectively.

IV. DISCUSSION

Plants have remained the ultimate source for the treatment of various ailments and their extracts have been used for centuries by human to prevent and/or treat many diseases. *P. berghei* has been used in studying the activity of potential antimalarials in mice [18] and in rats [13]. Rodent models of antimalarial study have been validated through the identification of several conventional antimalarials especially with the success of quinine and more recently artemisinin

derivatives [7]. The results obtained in this work indicated that ethanolic extracts of *C. odorata* and *A. paniculata* are effective in terms of anti-plasmodial action against *P. berghei*, although *A. paniculata* showed more activity than *C. odorata*. This implies that the extracts possess suppressive and curative effects against early and established Plasmodium infection.

The antimalarial activities showed by the ethanolic extracts could be due to the presence of some phyto-constituents. These effects may be attributed to the presence of alkaloids, terpenes and flavonoids that have been implicated in antiplasmodial activity [15], [6]. However, the actual compound(s) responsible for the known activity should be identified.

The reduction in the parasitaemia level of rats treated with ethanolic extract of *A. paniculata* and *C. odorata* justify their

use as anti-malaria in folklore medicine. This result agree with the work of Chandel [5] who got similar results when infected mice were treated with *Azadirachta indica*, well known anti-malaria plant in Nigeria. The fact that ethanolic extract of *A. paniculata* displayed a very good activity against the *P. berghei* malaria parasite showed that it could be used in human to treat malaria caused by *P. falciparum*.

The body weights of the mice are observed to be reducing in measurement (g), when they were infected with the malaria parasite. Also, some other symptoms were also observed in the mice such as sluggishness and loss of fur. All these symptoms could be attributed to loss of appetite and stimulation of the immune response that is associated with malaria illness in humans [5]. However, the body weights showed slight increase after treatment with the extracts began.

The increase in PCV of those infected and treated with *C. odorata* also indicates the effectiveness of the plant as blood supplement. According to Hopkins [9] extracts with this ability are rich blood supplements as such should be analyzed for the possibility of using it both for therapeutic purpose and immune-stimulatory potentials.

These results justify the use of ethanolic extract *Andrographis paniculata* plant as medicinal herb used in folklore medicine in the treatment of malaria. The result suggests that the leaf extracts of *A. paniculata* possess potential properties in the search for novel antimalarial drugs. Meanwhile, it is clear that this plant can make a major contribution in the control and treatment of malaria.

REFERENCES

- [1] Aiyelaja, A. A. and Bello, O. A. (2006). Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu state. *Educ. Res. Rev.* 1(1):16-22.
- [2] Anopheles. Available online: <http://en.wikipedia.org/wiki/Anopheles> (accessed on 10 May 2012).
- [3] Bako SP, Bakfur MJ, John I, Bala EI (2005). Ethnomedicinal and phytochemical profile of some savanna plant species in Nigeria. *Int. J. Bot.* 1(2):147-150.
- [4] Bronner, U.; Divis, P.C.; Farnert, A.; Singh, B. Swedish Traveller with Plasmodium Knowlesi Malaria After Visiting Malaysian Borneo. *Malar. J.* 2009, 8, 15.
- [5] Chandel, S. Bagai, U. (2010). Anti-plasmodial activity of *Ajugabraceosa* against *P. bergi* infected BALB/C mice. *Indian J. Med Res.* 131: 440-444.
- [6] Christensen SB, Kharazmi A (2001). Antimalarial natural products. Isolation, characterisation and biological properties. In: Bioactive compounds from natural sources: Isolation, characterisation and biological properties. Tringali C. Ed. Taylor & Francis: London, pp. 379-432.
- [7] David AF, Philip JR, Simon IC, Reto B, Solomon N (2004). Antimalarial drug discovery: Efficacy models for compound screening. *Nassture Rev.*, 3: 509-520.
- [8] Gessler MC, Nkunya MHH, Mwasumbi LB, Heinrich M, Tanner M (1994). Screening Tanzanian medicinal plants for antimalaria activity. *Acta Trop.* 56:65-77.
- [9] Hopkins, H.; Talisuna, A.; Whitney, CJM, Marsh, K. (2007). Impact of home based management of malaria on health outcomes in Africa: A systemic Review of evidence. *Malaria Journal* 6:134-139.
- [10] Murray, C.J.L.; Rosenfeld, L.C.; Lim, S.S.; Andrews, K.G.; Foreman, K.J.; Haring, D.; Fullman, N.; Mohsen, N.; Rafael, L.; Lopez, A.D. Global malaria mortality between 1980 and 2010: A systematic analysis. *Lancet* 2012, 379, 413-431.
- [11] Oaks, S.C.; Mitchell, V.S.; Pearson, G.W. Malaria: Obstacles and Opportunities; Carpenter, C.C.J., Ed.; National Academy: Washington, WA, USA, 1991.
- [12] Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO (2007). Medicinal plants useful for malarial therapy in Okeigbo, Ondo State, Southwest Nigeria. *Afr. J. Trad. CAM* 4(2):191-198.
- [13] Pedroni HC, Betton CC, Spalding SM, Coaster TD (2006). Plasmodium: Development of an irreversible experimental malaria model in Wister rats. *Exp. Parasitol.*, 113: 193-196.
- [14] Peters W (1965). Drug resistance in *Plasmodium berghei*. *Vinka and Lips. Exp. Parasitol.*, 17: 80-89.
- [15] Phlipson JD and Wright CW (1990). Antiprotozoal compound from plants sources. *Planta Medica.* 98(7): 733-739.
- [16] Prudencio M, Rodriguez A, Mota MM (2006). The silent path to thousands of merozoites: the plasmodium liver stage. *Nat. Rev. Microbiol.* 4:84-856.
- [17] Sachs, J.; Malaney, P. The economic and social burden of malaria. *Nature* 2002, 415, 680-685.
- [18] Thomas AM, Van Der Wel AM, Thomas AW, Janse CJ, Waters AP (1998). Transfection systems for animal models of malaria. *Parasitol. Today*, 14: 248-249.
- [19] World Health Organization (1998) Malaria: Know the facts. *World Health Organization Newsletter* 13(1): 6-7
- [20] World Health Organization Regional Office for South-East Asia. Anopheline Species Complexes in South and South-East; World Health Organization Regional Office for South-East Asia: New Delhi, India, 2007; pp. 102.