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ANTIPLASMODIAL ACTIVITIES OF COMBINED EXTRACTS OF ZANTHOXYLUM ZANTHOXYLOIDES, ANOGEISSUS LEIOCARPUS AND ANTHOCLEISTA VOGELII IN PLASMODIUM BERGGHEI INFECTED MICE

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ABSTRACT

The search for new compounds for the treatment of malaria is growing mainly due to the acquired resistance of parasites to commonly used malaria drugs. An in vivo study to evaluate the anti-plasmodial activity of combined leaf extracts of Zanthoxylum zanthoxylum, Anogeissus leiocarpus and Anthocleista vogelii which are medicinal plants commonly used in the treatment of malaria in Afuze, South-South Nigeria was conducted using Plasmodium berghei infected mice. The leaves of the plants were washed, air-dried and grinded into powder, and extracted sequentially, using methanol solvent. Using five mice each, three different concentrations of the combined leaf extracts 200 mg/ml, 400 mg/ml and 800 mg/ml were used to determine their anti-plasmodial activity in Plasmodium berghei infected mice. Each mouse was inoculated with 1 x 10^4 infected erythrocytes and leaf extract administration was based on the average body weight (AV.BW) of the mouse. 0.1 mg/ml body weight of the combined leaf extract was administered to each infected mouse and monitored for four days. At the end of the fourth day, the three different concentrations of the combined leaf extracts produced 41.50%, 47.20% and 49.22% parasite suppression respectively. The mean parasitaemia were 11.30 ± 6.81, 10.20 ± 5.25 and 9.80 ± 9.22 respectively. The results indicated that, there was some level of parasite suppression, with 800 mg/ml having the highest level of parasite suppression and 200 mg/ml having the lowest level of parasite suppression. The study reveals the anti-plasmodial activity of the three plants and concludes that the higher the concentration of the combined leaf extract the higher the level of parasite suppression and the lower the parasitaemia.

Keywords*: Anti-plasmodial activity, Combined leaf extracts, Parasite suppression, Parasitaemia, Plasmodium berghei*

1.0 INTRODUCTION

Recent surveys conducted in different parts of Nigeria revealed many plants that have been identified by users to be potent against malaria (Nwachukwu *et al*., 2010; Idowu *et al*., 2010). *Vernonia amygdalina* commonly found in West and Central Nigeria and *Morinda lucida* commonly found in Northern and Southern Nigeria, have been proven to have anti-malarial activities against drug sensitive *Plasmodium berghei* (Abosi and Raseroke, 2003; Bello *et al*., 2009; Ebiloma *et al*., 2011; Paula *et al*., 2011; Lawal *et al*., 2012). Elujoba *et al*. (2005) reported the roots of Aristotle plant being used in Nigeria against malaria. Several other medicinal plants have been used locally to treat malaria. Such plants include *Enantia chloranta, Nauclea nautifolia, Salacia nitida* (Ogbonna *et al*., 2008), *Acalypha fruticosa*, *Azadirachta indica*, *Cissus rotundifolia*, *Echium rauwalfii*, and *Boswellia elongate* (Merlin, 2004), *Cymbopogon giganteus and Morinda lucida* (Awe and Makinde, 1997; Azas *et al*., 2002). Studies have documented over 1,200 plant species from 160 plant families used for the treatment of malaria (Wilcox and Boderker, 2001). Traditional healers claim that some of these medicinal plants are more efficient to treat malaria than the synthetic anti-malaria drugs.

There is an increasing resistance of malaria parasites to chloroquine, one of the affordable and commonly used drugs for malaria in Nigeria.

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Anti-malaria drug resistance has emerged as one of the greatest challenges facing malaria control today and has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated (Bloland, 2001). The increasing prevalence of strains of *Plasmodium falciparum* resistant to anti-malaria drugs poses a serious problem for the control of malaria. The search for new natural compounds is growing mainly due to the acquired resistance of the malaria parasite to commonly used drugs.

The use of plants with anti-malaria properties is now very common in the country. Today, not much has been done to project the anti-malaria properties of indigenous medicinal plants which can be a possible source for new potent drugs to which malaria parasites are not resistant (Elujoba *et al.,* 2005;Ogbonna *et al*., 2008).Thus, the development of new drugs that are capable of overcoming parasite resistance is critical.

In Nigeria, malaria has become a household name and the use of herbs to treat it is not new. Idowu *et al*. (2010) opined that about 80% of Nigerian homes maintain some sort of private family traditional medicine practitioner especially for the treatment of malaria.

The present study was designed to evaluate the anti-plasmodial activity of combined leaf extracts of three plants, *Zanthoxylum zanthoxylum, Anogeissus leiocarpus* and *Anthocleista vogelii* which are medicinal plants commonly used in the treatment of malarial in Afuze, Owan-East Local Government Area, Edo State, South-South Nigeria, using *Plasmodium berghei* infected mice. The research adds to the efforts of the World Health Organization (WHO) in the search for natural anti-malarias and provides a basis for future research on these plants.

2.0 MATERIALS AND METHODS

2.1 Collection and identification of plant materials

The plants *Zanthoxylum zanthoxloides* (Ota), *Anogeissus leiocarpus* (Uto) and *Anthocleista vogelii* (Umiegugu) were collected from Afuze, Edo State, South-South Nigeria. The plants were identified and confirmed at the herbarium of the Department of Biological Sciences, Federal University Lokoja.

2.2 Preparation of plant extract

The leaves of the collected plants were thoroughly washed off dirt in running tap water and air dried at room temperature for three weeks. The leaves were then pounded and grounded into powder using pestle and mortar and a milling machine respectively. The powdered leaves were packed in plastic containers and stored in a cool dried place, until the extraction process.

50 g of the powdered leaves were weighed using the analytical weighing balance and 300 ml of 70% methanol was used for solvent extraction, using the soxhlet extractor for three hours. The extract was further subjected to heat for one week at 40^0C, in order to obtain the pure extract. The extract was then stored in tightly closed bottles and kept in a refrigerator until when they were to be used for the experiment.

2.3 Animals

Twenty-five pure strains and adult Swiss albino mice with an average weight of 24 g obtained from the Department of Biological Sciences, Federal University, Lokoja animal house were used in the study. The mice were kept in cages at room temperature (29 ^{0}C) in the Department of Biological Sciences and were fed with mice pellet diet standard ration, obtained from Vital Feeds Limited, Ibadan and with clean water. The mice were randomized into five experimental groups of five animals per group, based on their body weights. The groups were designated as group A (200 mg), B (400 mg), C (800 mg), D (Negative control) and E (Positive control).

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2.4 Parasites

The NK-65 strain of *Plasmodium berghei* obtained from Dr. Aina's Laboratory, Biochemistry Department, Nigerian Institute of Medical Research, Yaba, Lagos (NIMR) was used for the experiment.

2.5 Inoculation of experimental mice

An infected donor mouse with the *Plasmodium berghei* strain was used for parasite inoculum preparation. The required blood was obtained from the infected mouse by ocular puncture from the ocular plexus using a heparinized sterile capillary tube. 0.1 ml of blood containing 3×10^8 parasitized erythrocytes was collected and diluted with phosphate buffer saline (PBS). Each of the 25 mice used in the experiment was infected with parasites by inoculating them intraperitonially with 0.1 ml of the prepared blood solution. Blood smears were made and observed under the microscope on daily basis, to confirm the establishment of the parasitic infection in the experimental mice. Treatment began when a parasitaemia of 30-40% was established in the infected mice.

2.6 Suppressive treatment of infected mice

A four days suppressive test according to Idowu *et al.* (2010) was adopted and used for this experiment. The mice were divided into five groups, with each group having five mice in a cage. Groups A, B and C were administered with the combined leaf extracts of 200 mg/ml, 400 mg/ml and 800 mg/ml respectively while group D served as a negative control and were given distilled water and the last group E which served as the positive control, were given Chloroquine. Mice in groups A, B and C were treated with the combined leaf extracts using oral administration with a single dose per day. A dosage of 0.01 mg/ml was administered to each of the mice for treatment with the combined leaf extract. The untreated group D served as the negative control while the group E treated with Chloroquine at a dosage of 0.1 mg/ml, served as positive control for the experiment. The treatment continued daily for four consecutive days.

2.7 Estimation of parasitaemia in experimental mice

Each day, a thin blood film smear was made from blood collected from the caudal vein (tail) of each mouse. The smear was prepared by spreading the blood on a clean glass slide over an area of 1.5 cm × 2.5 cm. The smear was allowed to dry and fixed with methanol, stained with 3% Giemsa stain for 45 minutes and examined with the light microscope (Olympus CX, Japan) under the X100 oil immersion objective lens. This was necessary as to monitor the level of parasitaemia. The suppression of parasitaemia in relation to the control was assessed using the recommended formula (Mohammed *et al*., 2007).

The number of parasitized red blood cells was divided by the total number of red blood cells and then multiplied by 100 to express it as a percentage (Mohammed *et al*., 2007).

$$\%\ \text{Parasitaemia} = \frac{\text{Number of Parasitized red blood cells}}{\text{Total Number of red blood cells}} \times 100$$

2.8 Estimation of percentage suppression

The percentage suppression was estimated by subtracting the average percentage parasitaemia for the untreated group (control) from the average percentage parasitaemia for the treated group and then divided by the average percentage parasitaemia for the untreated group (control) and then multiplied by 100 (Mohammed *et al*., 2007).

$$\text{Average(Av)\%Suppression} = \frac{\text{Av\% Parasitaemia in control - Av \% Parasitaemia in Test}}{\text{Av \% Parasitaemia in control}} \times 100$$

2.9 Statistical analysis

Statistical significance was determined using one way Analysis of Variance (ANOVA) to compare % suppression between groups. The probability level of $P<0.05$ was considered as significant. The results were analyzed using Statistical Package for Social Sciences 20.0 Version.

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3.0 RESULT AND DISCUSSION

There was a systematic increase in level of parasite suppression by all the combined leaf extracts from day 1 to day 4. The level of parasite suppression varied with the different concentrations of the combined leaf extracts. The combined leaf extracts showed lowest percentage of parasite suppression (41.50%) at concentration of 200 mg/ml and highest (49.22%) at 800 mg/ml (Table 1). The combined leaf extract at concentration of 800 mg/ml body weight produced the highest reduction in parasitaemia when compared with the other concentrations of 200 mg/ml and 400 mg/ml (Table 1; Figure 1).

The mean parasitaemia of the test groups A, B, and C ranged from 11.30 ± 6.81 to 9.80 ± 9.22 while the negative control group D had a mean parasitaemia of 19.30 ± 8.58. The mice in the positive control group E were completely free from any parasitaemia on day 4 (Table 1). No parasite suppression was observed in the negative control group D since no drug nor extract were administered to them.

Table 1: Mean parasitaemia and chemo-suppression combined leaf extracts of *Zanthoxylum zanthoxyliodes, Anogeissus leiocarpus* and *Anthocleista vogelii* in *Plasmodium berghei* infected mice

Treatment Groups	Treatments	Mean Parasitaemia (% ± S.D)	Percentage Parasite suppression (%)
A	200mg/ml	11.30±6.81	41.50
B	400mg/ml	10.20±5.25	47.20
C	800mg/ml	9.80±9.22	49.20
D	Untreated	19.30±8.58	-
E	Chloroquine	-	100

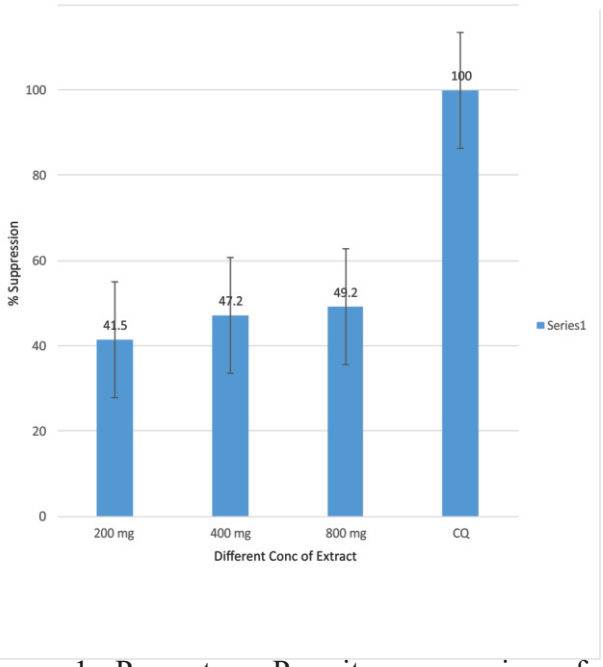

Figure 1: Percentage Parasite suppression of different concentrations of combined leaf extracts and chloroquin in *Plasmodium berghei* infected mice

et al. (2012) reported a total of 39 plant species belonging to 31 families as plants been used for the treatment of malaria in Sokoto State, North Western Nigeria. 28 (71.8%) of the plants have been scientifically validated to have anti-plasmodial and anti-malaria properties. Eleven plant species were identified by Ogbuehi and Ebong (2015) used for the treatment of malaria in Onitsha, South Eastern Nigeria. 60% of the concoction studied contained plant combinations than single plants. The scientific validation of the traditional claims of anti-malaria properties of the plants is imperative as this will contribute positively to the search for newer and more effective anti-malarial drugs.

In this study, the anti-plasmodial effects of the combined leaf extracts of the three plants at different concentrations was observed in a four days treatment of *P. berghei* infected mice.

The combined leaf extracts at the three different concentrations produced lower percentage parasite suppression compared to the 100% produced by chloroquin the positive control. This might be due to the fact that the combined leaf

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extracts were still in their crude forms, with the active ingredients not been isolated and compressed in active drugs like chloroquin which is a standard drug (Adzu and Haruna, 2007; Ebiloma *et al*., 2011). Chloroquine therefore, seems to produce a higher parasite suppressive effect than any of the concentrations of the combined leaf extracts and thus, could still be a better drug for the treatment of malaria in Afuze than any of the studied plants. The combined leaf extract at 800 mg/ml concentration produced a percentage parasite suppression of 49.22 %, higher than that of the 400 mg/ml and 200 mg/ml concentrations. This could be an indication that the higher the concentration of the combined leaf extract, the higher the percentage parasite suppression. The combined leaf extract is likely to respond quickly as an anti-plasmodial agent if the concentration is increased and could be more effective or efficacious as chloroquin if the concentration is increased to 1600 mg/kg.

All the concentrations of the combined leaf extracts showed anti-plasmodial properties which were significantly different when compared to the infected untreated group (Negative control). Although the phyto-chemical screenings of the aqueous leaf extract of these plants have not been carried out, their anti-plasmodial activity could be attributed to the presence of some phyto-chemicals like alkaloids, tannins, saponins, flavonoids, terpenes, etc which have been implicated in anti-plasmodial activities (Idowu *et al*., 2010; Akuodor *et al.*, 2010). Thus, the anti-malarial activity of these plants could be related to the presence of these compounds. Idowu *et al*. (2010) reported that *Morinda lucida* possesses anti-malaria properties attributed to anthraquinones. Obih *et al*. (1985) and Ebiloma *et al*. (2011) reported the stem bark and aqueous leaf extracts of *Morinda lucida* to have chemo-suppression properties with 96.4% and 85.05% respectively in *P. berghei* infected mice. The anti-malaria activity of the combined leaf extracts supports their traditional use for the local treatment of malaria. In traditional medicine, the use of herbal plants for the treatment of ailments is either singly or a combination of different plants or plant parts (Rasoanaivo *et al*., 2011). Asase *et al*. (2005) reported that the use of *Morinda lucida* to treat malaria is usually done in combination with other plants. The anti-plasmodial activities observed in this study for the combined leaf extracts, could probably not be due to a single plant but a combined effect of the three plants as the plants work in synergy. This confirms the reason why most herbal medicinal practitioners advocate for the use of different plants (multitherapy) as against the use of a single plant (monotherapy) (Azas *et al*., 2002). Olliaro and Taylor (2002) opined that to protect drugs from resistance, there is now clear evidence that combining them can improve their efficacy without increasing their toxicity. Medicinal plants combination proves to cure malaria within a short period which shows high efficacy and potent active ingredient found against the parasite.

The worsening anti-malarial resistance, fake or ineffective anti-malarial drugs and the difficulties of families to access and afford genuine and effective anti-malarial drugs, has probably lead to the promotion and utilization of herbal medicines and has become the most probable sustainable solution to malaria treatment.

The standard drug, CQ gave a maximum efficacy when compared with the activities of the plant extracts. This could be attributed to the fact that the combined leaf extracts were in their crude form, with the active ingredient not having been isolated and compressed into active drugs (Adzu and Haruna, 2009; Ebiloma *et al*., 2011). *Plasmodium berghei* infection in mice in a dose dependent manner, may partly justify the claim of traditional medical practitioners in Afuze in the use of these plants for the treatment of malaria.

Studies revealed that some plants are highly toxic despite their high chemo-suppression of

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parasitaemia. For example, *Morinda lucida* which is on the list of frequently used plants has shown *in vitro* cytotoxicity, with the stem bark reported to be extremely toxic (Shariff, 2001). Idowu *et al*. (2010) reported 65% of 38 plants studied to have toxic effects on the liver and kidney of experimental mice. The fact that no mortality was recorded in the combined leaf extract groups/treatments, is an indication that the leaf extracts of the plants are not toxic or have low toxicity. The highest chemo-suppression of parasiteamia was observed at the dose of 800 mg / ml indicating that dose has a significant effect on the chemo-suppression of parasiteamia.

4.0 CONCLUSION

This study concludes that the combined leaf extracts of *Zanthoxylum zanthoxyliodes, Anogeissus leiocarpus* and *Anthocleista vogelii* have anti-plasmodial activity.

This study therefore recommends that further evaluation of the three plants used in the study be carried out in order to isolate, identify and characterized the active ingredients responsible for the observed anti-malarial activities of the plants. This study also recommends that locally used medicinal plants for the treatment of malaria could be fully investigated, with the view of establishing their efficacy and also determine their potential as sources for the production of new anti-malarial drugs. It is also recommended that a standard concentration level / dosage higher than the 800 mg/ml of combined leaf extracts used in this study should be comprehensively worked out for the total clearance of parasitaemia.

The major challenge to the use and development of herbs in the treatment of malaria is lack of evidence of the required dosage of the remedies, established evidence of efficacy, safety and quality assurance mechanism. Guidelines should therefore be developed on the method of evaluating the dosage, safety and efficacy of these herbs to enable them overcome the resistance developed by the malaria parasite.

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