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INVESTIGATION OF ANTI-PLASMODIUM ACTIVITY OF A COMBINED EXTRACT OF *Bryophyllum pinnatum* and *Aloe barbadensis* LEAVES

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ABSTRACT
Anti-malaria drug resistance is a major public health challenge causing a serious setback to the roll back malaria programme in Africa. This study therefore attempted to explore the combinatorial effect of *Aloe barbadensis* and *Bryophyllum pinnatum* on malaria parasitemia with the aim of producing an effective anti-malaria preparation. Extracts of both plants were prepared at varying concentrations of 10^{-1} to 10^{-9} mg/ml and were administered orally to *Plasmodium berghei* infected albino mice. The mode of extract administration was both single, using individual extracts and combined, using mixture of both extracts. The infected animals in test group were given the extract at a dose of 0.5ml per day for 3 consecutive days, the negative control animals were administered with placebo while the positive control animals were treated with Lumefantrine/arthemisinin combination. After the third day, thin and thick blood films were made from the blood collected from the tail of the animal and examined for malaria parasites. Pre and post test malaria parasitemia and test and control malaria parasitemia were determined and compared. At all the concentrations of *Aloe barbadensis* extract administered, no significant variation in malaria parasitemia was observed before and after application of the extract. Similarly, with *Bryophyllum pinnatum* extract, data obtained also showed no significant reduction in the density of malaria parasite when post-test density was compared with pre-test. A combination of both extracts however revealed a synergistic effect. Post-application results of the extract indicated that at 10^{-1} and 10^{-3} mg/ml concentrations, significant reduction in malaria density was recorded when post –treatment malaria density was compared with pre-treatment.($P < 0.05$). However none of the extract concentrations was able to clear malaria parasitemia completely in the experimental animal. In conclusion although the combinatorial effect of both plants produced remarkable reduction in malaria parasitemia, no case of complete clearance was recorded

KEYWORDS: Combinatorial effect, Natural products, Malaria parasitemia.

INTRODUCTION

Malaria is the commonest parasitic infection in Africa (Anderson, *et al*., 1996 and Brewer *et al.*,1994) and most important cause of death especially among children under five years and pregnant women (Kilama, 2005 and Malaney *et al*., 2004). Anti – malaria drug resistance is a major public health challenge causing a serious setback to the roll back malaria programme in Africa. Resistance of *Plasmodium falciparium* to chloroquine had been reported by many researchers. Khan *et al.* (1978) reported on the rate of chloroquine resistant malaria while Kean (1979) documented *P. falciparium* resistance to drug in African countries. Sulfadoxine – Pyrimethamine resistance of *P. falciparium* was reported in Kenya (Wenigar, 1986). Similarly, *P. falciparium* drug resistant strain was documented in Gabon by the Centre for Disease Control (CDC) in 1983 (Salako *et al.*, 1984). Malaria resurgence was reported in Kenya (Wenigar, 1986), similarly, *P. falciparium* drug resistant strain was documented in Garbon by centre for Disease control in 1983 (Salako *et al.*, 1984)). In 1982, American Health Organization documented resistance of *P falciparium* to combination drug (Markwelder *et al.*, 1983). While *P. falciparium* resistant to drug combinations such has fansider and other unbranded sulfadoxine/pyrimethamin combinations was also documented in many countries of the world such as Zambia, New Guinea, Colombia Malaysia, Indonesia and Brazil (WHO, 1998). *Bryophyllum pinnatum* is a common natural product and a perennial herb widely used by traditional healers in many regions of the world. The extract from this plant is effective for use as an immuno – modulator, analgestic, antibiotics, antiulcer, antifungal, sedative, anti viral and anti – inflammatory (Kamboj and Saluja, 2009). *Aloe barbadensis* is another important medicinal herb that belongs to the family Aloeceae. Also this divine herb composes of gel, concentrate or extract, juice and latex that are all medicinal. It is a bitter herb with anti – inflammatory, astringent, emolient, antifungal, antibacterial antiviral and anti – parasitic properties. (Redbook, 2004 and Krimsky *et al.*, 2003). The application of natural products is gaining popularity daily because of the herbs are readily available and generally affordable (Leonardo *et al.*, 2000). Many countries of the world such as Mali, Vietnam, China, Sri Lanka and India had integrated natural products with orthodox medicine in their health care systems for more effective delivery. (Kazambe and Munyarari, 2006 and Larsen, 1999) Similarly, combination therapy had been reported by some researchers as a way of checking the increasing rate of multiple anti-malaria drug resistance ravaging not only the

African continent but the World at large (Kazambe *et al.*, 2012 and Kazambe, 2007). Alternative, effective and affordable substitutes are essential if malaria is indeed to be rolled back. It is therefore imperative to research into some of the natural herbs to discover such effective replacements. Several reports had confirmed efficacies of *B. pinnatum* and *Aloe barbadensis*, on different types of ailments as documented by Rocha *et al.* (2005), Arunkumer and Muthuselvam (2009) and Akinpelu (2000), however, no combinatorial effects of both plants on diseased conditions had been documented. Combination of two or more extracts for malarial treatment could be antagonistic, synergistic or none of both (Kazambe *et al.*, 2012). The combinatorial effect of both plants on malaria parasitemia was therefore investigated in this study

MATERIALS & METHODS

Collection of herbs and preparation of crude extract

Fresh *Bryophyllum pinnatum* leaves were collected from Isale Aluko garden near Baboko market in Ilorin while the fresh *Aloe barbadensis* pant was collected from potted plant at Adewole Estate in Ilorin. All the plants and leaves were identified by Professor Oladele of the Plant Biology Unit in Kwara State University. Manual aqueous extraction was employed on each of the plants. The *Bryophyllum pinnatum* leaves were washed and grinded in sterilized electric blender, the paste obtained was squeezed out through a sterilized mesh of about 0.20mm in size and the residue (extract) was kept in an electronic oven, maintained at 60^0 C for drying. The granule (powdery) obtained was weighed, redried and weighed again until constant weight was obtained. The same procedure was repeated for the *Aloe barbadensis* leave to obtain coarse resultant granules which was later grinded with sterilized ceramic mortar and pestle .Ten-fold dilution was performed on the combined extract to obtain various concentrations such as:10^{-1},10^{-3},10^{-5},$10^{-7}$and 10^{-9} . 0.5ml of each concentration of each extract was orally administered to the infected mice daily for three days.

Experimental animals

A total of sixty (60) albino mice infected with *Plasmodium bergei* parasite, weighing between 25g and 30g were procured from Biochemistry Unit of the Nigerian Institute of Medical Research, Yaba-Lagos The animals were fed with commercial feeds (Ewu growers from the Bendel Feeds and Flour Mill Ewu, Nigeria) and allowed to drink water freely. They were allowed to acclimatize for two (2) weeks. The infected animals were grouped into three, each group containing twenty male mice. The animals in first group were treated with extract of *Bryophyllum pinnatum*, those in second group treated with *Aloe barbadensis* extract while animals in the third group were administered with the mixture of both extracts. Infected animals in each group were further sub-divided into test group and control group each containing ten infected mice. In test group, two infected mice each were administered orally with extract concentration of 10^{-1}, 10^{-3}, 10^{-5}, $10^{-7}$and 10^{-9} respectively at 0.5ml of extract per day for three days. Five of the ten infected animals in the control group were administered with Placebo (Negative control) while the remaining five were given Lumefantrine/arthemisinin combination.

Preparation and examination of blood films

After the third day of extract administration, blood samples were collected aseptically in duplicates from the animals by cutting the tip of the tail and squeezing blood onto slides to make thin and thick films from each animal. All the thick films were stained by Giemsa method while the thin smears were stained by Leishman method as describe by Monica (2002). All films were examined under the oil immersion lens of the microscope. The thick film was used to ascertain if the animal was actually infected with malaria parasites or not while the thin film was used to determine the rate of parasitemia. The rate of malaria parasitemia was assessed by counting the total number of parasitized red blood cells out of the two hundred red blood cells differentiated in the blood film of the experimental animal

Statistical analysis

Results obtained were analyzed using the SPSS version 17 software (SPSS Inc, USA). The data were expressed as mean $\pm$ Standard Deviation. The test of Significance was performed using student t – test for analysis of data from the test and control groups of the experimental animal's. The level of significance was based on P value less than 0.05.

RESULTS & DISCUSSION

Malaria remains one of the five major life-threatening childhood diseases in Africa according to World Health Organization, (1997) with the increasing rate of anti-malarial drug resistance in the continent (Salako *et al.*, and Wenigar *et al*., 1986). In view of this challenge, phyto-medicine is fast gaining recognition all over Africa for treatment of malaria, in contrast to the use of synthetic products. This study therefore investigated combinatorial activity of the extracts of *Aloe barbadensis* and *Bryophyllum pinnatum* on Plasmodiasis. Table 1 shows the impact of *Aloe barbadensis* extract on malaria parasitemia in experimental animal when administered and compared with the negative control group which was administered with no extract but placebo. The P-values obtained at different extract concentrations were also indicated in the Table. At all the concentrations of the extract administered (10^{-1} to 10^{-9} mg/ml), there was no significant difference in malaria parasitemia before and after intervention. Also, no significant difference in the parasite density in the test and control animals was observed. Assessment of post intervention malaria parasite densities after administering with extract of *Bryophyllum pinnatum* is as depicted in Table 2. The Table also compared post-test malaria parasitemia with that of negative control animals The p-values obtained at different concentrations of the extract were as indicated Analysis of the data obtained after treatment with extract showed no significant reduction in density of malaria parasites when the post-test density was compared with pre-test malaria densities at all the concentrations of the extract administered. The post-test malaria parasite density was also not significantly different from the density in negative control animals. Table 3 and Figure 1 show the effect of synergistic combination of *Bryophyllum pinnatum* and *Aloe barbadensis* extract on malaria parasitemia. Post application results of the combined extract indicated that

at 10^{-1}mg/ml and 10^{-3} mg/ml concentrations of the combined extract, significant reduction in malaria parasite density was recorded when post-treatment malaria density was compared with pre-treatment and control malaria densities. The study however revealed that none of these extract concentrations was able to clear completely the malaria parasitemia in the experimental animal. Acute toxicity of the combined extract on the animal explained the reason for low concentrations of the extract administered in this study. Several attempts to increase the concentration above 0.10mg/ml led to the death of some animals. Meanwhile with the extract concentrations of 10^{-5}, 10^{-7} and 10^{-9} mg/ml, there was no significant reduction in malaria density when compared with malaria parasite densities in pre-treatment and negative control groups. The p-values obtained at different concentrations of combined extract are as indicated in the Table-3

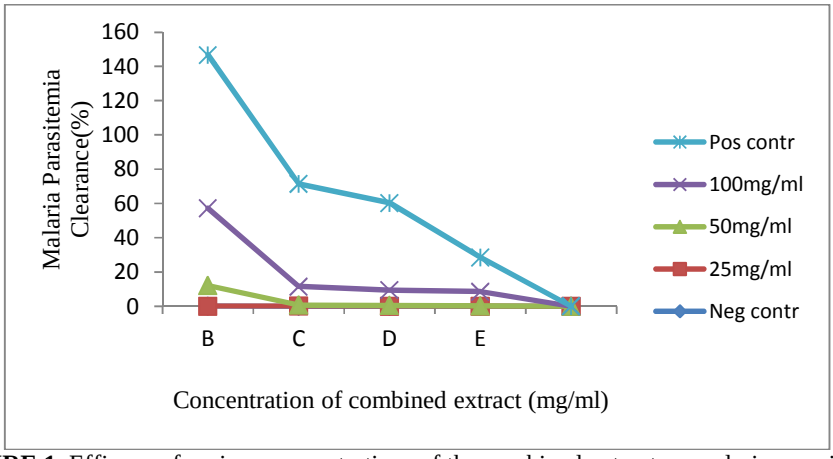

FIGURE 1: Efficacy of various concentrations of the combined extract on malaria parasitemia
B= 10^{-1} mg/ml, C= 10^{-3} mg/ml, D= 10^{-5} mg/ml, E= 10^{-7} mg/ml, Negative control= Placebo
Positive control = Arthemiter/artemisin combination

TABLE 1: Impact of *Aloe barbadensis* extract on malaria parasitemia in experimental animal

Extract Conc	Mean Parasite Count ±SD			P-value	Significance Level
	Pre-test	Post-test	Neg. Control	(t- test)	
10^{-1}	80.4±1.21	80.0±2.11	80.5±1.94	0.9742	P>0.05
10^{-3}	79.5±1.09	79.5. ±1.09	80.0±2.11	0.9411	p>0.05
10^{-5}	90.7±1.93	90.5±2.01	90.5±2.01	0.6432	P>0.05
10^{-7}	75.6±1.77	75.5±1.84	75.5±1.84	0.6473	P>0.05
10^{-9}	90.2±2.61	90.0±2.34	90. ±2.34	0.8314	P>0.05

TABLE 2: Effect of *Bryophyllum pinnatum* extract on malaria density in mice infected with *Plasmodium berghei*

Extract	Mean Parasite Count ±SD			P-value	Significance
Concent.	Pre-test	Post-test	Neg. Control	(t- test)	Level
10^{-1}	75.3±1.05	75.0± 1.8	75.5±1.8	0.6474	P>0.05
10^{-3}	80.1± 2.11	80.0±2.11	80.0± 2.11	0.9411	p>0.05
10^{-5}	80.0± 2.11	79.5±1.09	89.0± 2.11	0.9723	P>0.05
10^{-7}	90.4±2.21	90.0± 2.34	90.0± 2.34	0.8314	P>0.05
10^{-9}	90.7± 2.01	90.7± 2.01	90.7±2.01	0.6432	P>0.05

TABLE 3: Synergistic effect of *Bryophyllum pinnatum* and *Aloe barbadensis* extracts on malaria parasitemia.

Extract Conc	Mean Parastite Count ±SD			P-value	Significance
	Pre-test	Post-test	Neg. Control	(t- test)	Level
10^{-1}	75.4±1.05	12.0±0.80	75.5±1.8	7.146	P<0.05
10^{-3}	80.1±2.11	35.0±1.23	80.0±2.11	5.431	P<0.05
10^{-5}	80.3±2.03	79.5±1.61	80.0±2.11	1.093	P>0.05
10^{-7}	90.5±2.01	73.5±1.05	90.0±2.34	0.863	P>0.05
10^{-9}	90.7±2.01	82.5±1.74	90.7±2.01	0.047	P>0.05

In view of some limitations of this study, further study on the combined extract which could lead to complete clearance of malaria parasitemia is advocated. The study also suggests more research works on the in-vivo and in-vitro combinatorial effect of *Bryophyllum pinnatum* and *Aloe barbadensis* extract on other agents of communicable diseases such as viruses, bacteria and fungi. Similarly, the impact of the combined extract against non-communicable diseases such as diabetic, hypertension and peptic ulcer is suggested. In conclusion, although the combinational effect of *Bryophyllum pinnatum* and *Aloe barbadensis* produced a remarkable reduction in malaria parasitemia in

mice infected with *Plasmodium berghei*, no case of complete malaria parasite clearance in the animal was recorded throughout the study.

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