

ANTIPLASMODIAL ACTIVITY OF CRUDE EXTRACTS AND ESSENTIAL OIL OF *LIPPIA MULTIFLORA* IN MICE

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ABSTRACT

The search for common and cheaper antimalarial drug in Nigeria cannot be over-emphasis considering the endemicity of the disease. Traditional medicines (whole plants or Parts of plants) have proven to have good antimalaria efficacy. The evaluation of the anti-malarial properties of the crude extract and essential oil of *Lippia multiflora* was carried out using standard procedure (curative) tests in laboratory mice. The result of the curative test showed 5% essential oil to have good antimalaria efficacy (75.00%) against *Plasmodium berghei*. Other experimental essential oils (20%, 10%) and crude extracts (100%, 50%, 25%) employed in this study all showed significant difference in parasitemia percentage reduction when compared to the negative control group. All essential oils and crude extracts only reduced the parasitemia in the mice but could not clear it all after termination of treatment. The constituents of the essential oils can therefore be isolated to test for the efficacy of each constitute.

Keywords: Antiplasmodial, Crude extract, Essential oil, *Lippia multiflora*

1. INTRODUCTION

Malaria remains one of the major killers of humans worldwide, threatening the lives of more than one third of the world's population. It thrives in the tropical areas of Asia, Africa, and Central and South America, where it strikes millions of people. Each year 350 to 500 million cases of malaria infection are reported worldwide (WHO, 2014). Malaria is caused by a single-celled parasite from the genus *Plasmodium*. More than 100 different species of *Plasmodium* exist. They produce malaria in many types of animals and birds, as well as in humans (WHO, 2014). Malaria has been known since antiquity is still the world's most prevalent disease. Two to three million people die each year from malaria and at least one million of these deaths are young children and today malaria is largely confined to tropical and sub-tropical countries in Asia, Africa, central and South America (WHO, 2014).

L. multiflora tea is commonly consumed in Northern Nigeria as remedy for malaria fever. Literature reports also indicate the plant as having antimalaria properties (Abena *et al.*, 1998; Aquaye *et al.*, 2001). Antiplasmodial test was thus carried out with crude and oil leaf extracts of the plant in an effort to validate these claims.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of *Lippia multiflora* were collected in the early hours of the day between April and July in Chaza village located in the Suleja Local Government Area of Niger State, Northern Nigeria and authenticated by a botanist Mr. Segun Olayanju of the Department of Biological Sciences Herbarium, University of Abuja. The leaves were allowed to air dry at room temperature in the Herbarium for duration of 10 days.

2.2 Preparation of Crude Extracts

6 kilograms of the fresh leaves were collected and 1.5 kg of the dried plant leaves were pounded for the crude extracts preparation. Methanol was used for the crude extraction carried out at the Toxicology Department, NIPRD (National Institute for Pharmaceutical Research Development) Idu, Abuja.

2.3 Preparation of Essential Oils

4.5kg of the dried and powdered leaves was used for the extraction of the essential oils through hydro distillation carried out at the Toxicology Department NIPRD Idu, Abuja.

2.4 Experimental Animals

50 healthy Swiss albino mice of both sexes of about 6 weeks old weighing between 15 – 20 g each was obtained from the animal house of the Department of Pharmacology, University of Jos, Nigeria. The mice were fed using standard rodent feed with free access to water.

2.4.1 Parasites

The malaria parasite *P. berghei* NK65 chloroquine sensitive strain was obtained from the Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria. A donor mouse with parasitaemia level of (++) = 11 – 50 parasites was anaesthetized with chloroform. 1ml of blood was extracted through cardiac puncture using 1ml needle and syringe, 19ml of normal saline was added making up to 20ml (Adzu *et al.*, 2007).

2.4.2 Antiplasmodial activity (Curative test)

The crude extract and essential oil of the plant were used for curative test against the malaria parasite following the procedure described by Adzu *et al.* (2007) and Saidu *et al.* (2000). The curative treatment started on day three (D3) after infection was established and the treatment continued for four (4) days (D3 – D7). The mice were treated with different dosages of the plant according to their body weight once a day. (5kg, 10kg and 20kg) of the oil dissolved in

Dimethylsulfoxide and 25 mg/ml, 50mg/ml and 100mg/ml of the extract dissolved in normal saline.

2.4.3 Identification and estimation of parasites

After infection was established in the mice, on the D3 a pretreatment thick blood smear was collected from each mouse to estimate the number of parasites in each sample.

Also on the D7 of post treatment thick blood smear was also collected from each mice and the parasites were counted. The thick blood smear was gotten by a small cut from the tail of each mouse with a pair of scissors, the blood was gently squeezed unto a microscope slide which was allowed to air dry and stained with Geimsa stain before viewing under the microscope (Cheesbrough, 1998).

2.5 Statistical analysis

Parasite counts obtained before treatment (pretreatment) and after treatment (post treatment) were subjected to the student t-test. The student t-test was used to compare means of treated groups and control for any significant difference in parasitemia of treated mice and the control groups. Results were expressed as mean \pm standard error of mean (SEM). The formula used for the calculations is:

$$t = \frac{X - \mu}{S/\sqrt{n}}$$

All data were analyzed at a 95% confidence interval ($\alpha = 0.05$).

$$\% \text{ reduction} = \frac{\text{Pre-treatment} - \text{Post-treatment}}{\text{Pre-treatment}} \times 100$$

3. RESULTS

Table 1 shows that 5% of essential oil greatly reduced the parasitaemia level by 18.4 ± 1.14 on day three (D3) of parasite establishment to 4.6 ± 0.71 observed after the termination of treatment (D7) compared to the control where there was an increase in the parasitaemia level 18.4 ± 1.14 observed in day three to 18.8 ± 0.84 on day seven (D7) after treatment.

Table 1: Antimalaria activities of essential oil and crude extract of *Lippia multiflora* against *P. berghei* in mice (curative test)

Test substance	Dose (mg/kg)	Parasitaemia count		% Reduction
		D-3	D-7	
Essential oil	20	18.8 ± 0.84	8.2 ± 0.84	56.38
	10	18.4 ± 1.34	10.8 ± 1.30	41.30
	5	18.4 ± 1.14	4.6 ± 0.71	75.00
Crude Extract	100	18.6 ± 1.14	9.4 ± 1.14	49.46
	50	18.4 ± 1.52	11.2 ± 0.84	39.13
	25	18.6 ± 1.14	12.0 ± 0.71	35.48
Chloroquine	10	18.6 ± 1.14	5.8 ± 0.84	68.82
Normal Saline	2 ml	18.4 ± 1.14	18.8 ± 0.84	-

D - 3 = day 3, D - 7 = day 7 after infection was initiated.
Each result is with a mean of 5 mice.

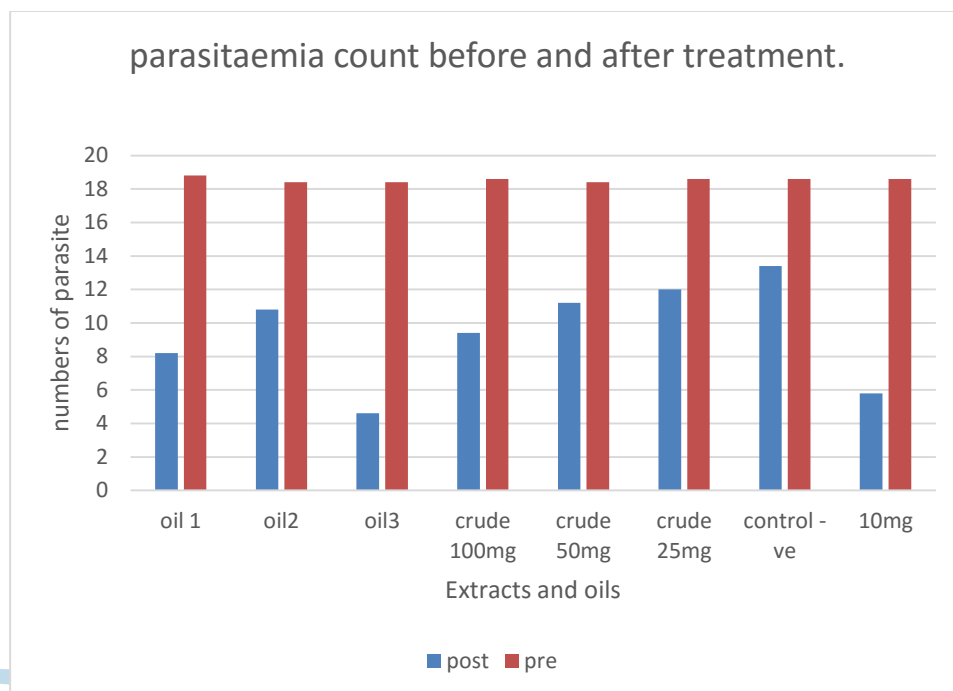


Figure 2: Bar chart showing differences in mean between the different groups treated.

Keys: Oil 1 = 20% essential oil

Oil 2 = 10% essential oil

Oil 3 = 5% essential oil

Crude = crude extract at different doses

Figure 2 showed a mean difference of all test substance to parasitaemia reduction used in this study with oil3 having a more suppressing effect on the parasite. From the observation, all test substance showed a percentage reduction in parasitaemia with a significant difference between the treated and the control ($P < 0.05$).

4. DISCUSSION

From reported works, *L. multiflora* plant parts has proven to be of great effect in pharmacology as antifungal, antihypertensive, antiviral and its traditional use in these cases (Pham *et al.*, 1988a, 1988b; Pousset, 1989; Abena *et al.*, 1998; Valentin *et al.*, 1995).

The 7-day curative test is a standard which is commonly used in screening of antimalarial activities of plants (Peters and Ryley, 1970). The essential oil and crude extracts showed a

significant difference ($P < 0.05$) when compared to the negative control in the percentage reduction in the parasitaemia with all the essential oils showing more activity than the crude extracts. The 5% essential oil gave a high percentage reduction of parasitaemia of 75.00% even more than the chloroquine (Positive control) which have been the most active antimalaria. All essential oils and crude extracts employed in this study only recorded suppression in the parasitaemia count but could not clear it with the chloroquine as it has been previously reported (Malann *et al.*, 2015; Adzu *et al.*, 2007). The crude extracts of 50% and 25% recorded only a slight parasitaemia suppression of 39.13% and 35.48% respectively. This study have therefore improve on the quest for plants for antimalaria with the efficacy shown by the oils and extracts to anti-malaria. A more investigation can be conducted into isolating the constituents to know the exact isolate with the much potential for the malaria problem to be combated.

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