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Antiplasmodial activity of aqueous leaf extract of *Cymbopogon citratus* against *Plasmodium falciparum* infected rats

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Abstract: In Nigeria, malaria is a major public health problem and there are high cost of the effective antimalarial drugs, poor quality drugs and increased emergence of Plasmodial resistance. Thus, there is a need for alternative source of medicine in malaria treatment and prevention. The antimalarial potential of aqueous leaf extracts of *Cymbopogon citratus* was investigated in this present study. Twenty five Swiss albino rats with average weight of 30.80g were distributed into five groups (A, B, C, D and E) with five mice per group. Group B was infected with 0.2 ml O^+ human parasitized blood of *Plasmodium falciparum* and 0.1ml Chloroquine (Bini Laboratories Pvt Ltd). Group C, D, and E were infected with 0.2 ml O^+ human parasitized blood of *Plasmodium falciparum* treated with 40 mg/kg, 80 mg/kg and 120 mg/kg of *Cymbopogon citratus* extracts respectively for three days. The mice infected with 0.2 ml O^+ human parasitized blood of *Plasmodium falciparum* were observed for 72 hours for general symptoms of malaria. The mice tail was punctured, blood was examined under light microscope (x10) resolution) and several malaria parasites were found. Significant decrease of parasitemia levels was observed in 120 mg/kg body weight treated group compared to 0.1 ml Chloroquine the positive control. The result showed that *Cymbopogon citratus* possessed a good antimalarial property and can be use for prophylactic and chemotherapeutic purposes.

Keywords: *Cymbopogon citratus*, Chloroquine, Blood, *Plasmodium falciparum*

1. Introduction

In Sub-Saharan Africa, medicinal herbs have been used in the treatment of malaria in endemic regions of the world. Malaria is a major cause of morbidity and mortality in the world and it account for about 216 million cases and about 655 000 deaths in 2010 according to WHO. Malaria is caused by different species of Plasmodia including *Plasmodium vivax*, *Plasmodium ovale, Plasmodium malariae* and *Plasmodium falciparum*. Thus, *Plasmodium falciparum* is the most deadly specie and it gives the highest malaria cases in the world [1,2]. In Nigeria, malaria is a major public health problem and there are high cost of the effective antimalarial drugs, poor quality drugs and increased emergence of Plasmodial resistance. Hence, there is a need for alternative source of medicine in malaria treatment and prevention. *Cymbopogon citratus* also known as Lemon grass is a perennial grass of about 55 species found mostly in warm region, especially in tropical and subtropical countries [3]. *Cymbopogon citratus* belongs to the Poaceae family which is monocotyledonous aromatic perennial having slender sharp edged leaves and pointed apex [4]. *Cymbopogon citratus* consists of citral as the biologically active constituent with more than 75% (w/w) of its essential oil [5]. *Cymbopogon citratus* is widely used in Asia as cuisines because of its sharp lemon flavour. In India it is used as sedatives, febrifuge and immunostimulant [6,7] In Nigeria, it is used for stomach problem and typhoid [8,9]. Since the beginning of human

history, all natural plants are rich source of medicinal agents and have been in practice as folk medicine, especially in traditional medicine [10]. Medicinal plants have one or more parts of substances that can be useful for the therapeutic purpose [11]. It has been reported that *Cymbopogon citratus* are being used as the therapeutic agent for the treatment of gastrointestinal disturbances, nervous and hypertension. The essential oils of *Cymbopogon citratus* were found to produced 86.6% suppression in growth of *Plasmodium berghei* when compare to a standard drug chloroquine [12] The extract of *Cymbopogon citratus* consist majorly citral approximately 65-85% and small quantity of geranyl acetate, monoterpene olefins and geraniol [13]. The extracts of *Cymbopogon citratus* are also used in elephantiasis, coughs, flu, headache, gingivitis, leprosy, ophthalmia, vascular disorders and pneumonia. [14,15,16,17,18]. Moreover, studies of *Cymbopogon citratus* extracts have been reported to have antifungal, antibacterial and antiviral activities antiviral [19]. Gore *et al* reported the anti helminthes activities of *Cymbopogon citratus* [20]. World Health Organization estimated 2 billion people with malaria infection (WHO, 2012). The purpose of present study is to investigate the antiplasmodial effect of *Cymbopogon citratus* on *Plasmodium falciparum* infected mice.

2. Material and Methods

2.1. Drug

Chloroquine was manufactured by Bini Laboratories Pvt Nashik India and obtained from Pharmacy section of Bingham University Clinic, Karu, Nasarawa State Nigeria.

2.2. Plant Material

Fresh leaves of lemon grass (C*ymbopogon citratus*) was bought from Masaka market a suburb of Abuja and was identified by Prof Asenge, Department of Biological sciences, Bingham University, Nigeria. The leaves were dried at room temperature and blended into powder, then stored in a plastic container to prevent moisture absorption and contamination. The powdered sample was extracted in 200ml of 95% methanol at a temperature of 95^0C for an hour and 30 minutes using the soxhlet apparatus as described by Harbone (1993). The crude methanolic extracts obtained was concentrated by evaporation on a water bath at 100^0C for 1 hour then stored in a sample bottle. The percentage yield determined as follows:

$$\text{Percentage yield (\%)} = \frac{\text{weight of the crude extract}}{\text{Weight of the dried plant sample used}} \times 100$$

The extract was reconstitute by dilution (in distilled water) to various concentrations of 250, 200, 150, 100 and 50mg/ml as described by Akujobi et al. (2004) before using for treatment.

2.3. Experimental Design

A total number of 25 albino mice of average weight 30.80g were used for the experiment. They were distributed into five groups (A, B, C, D and E) with five mice per group. Group B (Experimental control) was administered with 0.2 ml O^+ human malaria parasite and 0.1ml Chloroquine (Bini Laboratories Pvt Ltd). Group C, D, and E represents the Treatment groups and was administered with 0.2 ml O^+ human malaria parasite treated with 40 mg/kg, 80 mg/kg and 120 mg/kg of extracts respectively as can be seen in the table below:

Table 1. *Experimental Design*

Groups	Descriptions	Treatment
A	Normal control	Administered with distilled water, no infection, no treatment.
B	Experimental control	Administered with 0.2ml O^+ human malaria parasite and treated with 0.1ml chloroquine.
C	Treatment group 1	Administered with 0.2ml O^+ human malaria parasite and treated with 40mg/kg of *Cymbopogon citratus* extracts.
D	Treatment group 2	Administered with 0.2ml O^+ human malaria parasite and treated with 80mg/kg of *Cymbopogon citratus* extracts.
E	Treatment group 3	Administered with 0.2ml O^+ human malaria parasite and treated with 120mg/kg of *Cymbopogon citratus* extracts

2.4. Infection of Malaria in Mice

0.2ml of parasitized human blood of blood group O was obtained from the haematology Department of the Asokoro General Hospital, Abuja. This was injected into a mouse weighing 30.85g intra-peritoneally and was observed for about 72 hours for general symptoms of malaria. The tail was punctured and a representative quantity of blood was obtained which was smeared on a glass slide to prepare a thick blood film. On examination under the low power of a light microscope (x10 resolution), several malaria parasites were found, showing that the mouse was positive for malaria parasite. The parasitized mouse was sacrificed and from it, blood was obtained and diluted, using normal saline solution in the proportion 9:1 (9ml of blood: 1ml of normal saline), with which the remaining experiment mice were infected with malaria parasite.

2.5. Phytochemical Screening of Plant Extract

Chemical tests were carried out on the methanolic

extracts using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

2.5.1. Test for Tannis

About 0.5ml of the methanolic extracts was put in a test tube and a few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration.

2.5.2. Test for Flavonoids

1ml of the methanolic extract was heated with 10ml of ethyl acetate over a steam bath for 3 min. the mixture was filtered and 4ml of the filtrate was shaken with 1ml if dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

2.5.3. Test for Terpenoids (Salkowski Test)

1ml of the methanolic extract was mixed in 2ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

2.5.4. Test for Cardiac Glycosides (Keller-Killani Test)

0.5ml of the methanolic extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.5.5. Test for Anthraquinones

1ml of the extract was boiled with 5ml diluted H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of diluted ammonia was added. The resulting solution was observed for color change.

2.5.6. Test for Alkaloids

0.5ml of the methanolic extracts was stirred in 5ml of 1%HCL_{aq} on a steam bath for 5mins. The mixture was then filtered using Whatman's no 1 filter. To filtrate, 2-4 drops of Dragendoff's reagent was added to 1ml of the filtrate. An orange color was observed indicating the presence of alkaloids.

2.6. Determination of Parasitemia Level in Experimental Animals

The level of parasitemia in the experimental animals was determined haematologically, using microscopic technique. (Herbert and Lumsdem 1976) The blood samples were obtained from the experimental animals, thick and thin film was prepared on a glass stand. The film was stained (Giemsa stain), and then viewed under low powered microscope (x 10 resolution) to determine the parasitemia level. The level of parasitemia was determined once daily and was closely monitored for 3 days.

3. Results

Table 2. *Phytochemical Screening of Cymbopogon citrates leaf extract*

Phytochemicals	*Cymbopogon citrates* leaf extract
Alkaloids	+++
Flavonoids	+++
Tannins	+++
Saponins	+++
Terpenoids	++
Anthraquinones	++
Cardiac glycosides	+++

Legend: +++ = High, ++ = Moderate; + = Low; ± = Inconclusive.

Table 3. *Number of parasites per field in experimental animals.*

		Post Infection Days		
Group	Treatment	Day 1	Day 2	Day 3
A	Normal control	-	-	-
B	Experimental control	81+2.10	16+2.22	10+1.65
C	Treatment group	80+2.05	16+1.55	8+1.85
D	Treatment group	70+2.32	15+2.10	4+1.42
E	Treatment group	67+2.22	12+1.05	0+0.50

Values are mean for five determination +SD.

Table 4. *Amount of daily consumption by mice.*

		Post Infection Days		
Group	Treatment	Day 1 (G)	Day 2 (G)	Day 3 (G)
A	Normal control	50.00	50.00	50.00
B	Experimental control	4.50	5.70	30.50
C	Treatment group	4.00	10.00	28.00
D	Treatment group	3.00	12.00	35.50
E	Treatment group	2.50	8.00	41.00

4. Discussion

Cymbopogon citratus are very well known to provide a rich and diverse source therapeutic agent for the treatment of gastrointestinal disturbances, nervous and hypertension. [21]. A number of previously conducted report indicated that that *Cymbopogon citratus* has been used against gastrointestinal disturbances and complications [22]. It has been reported that Chromatographic fraction of *Cymbopogon citratus* essential oil in agar plate was active on *Bacillus subtilis, Escherichia coli, Staphylococus aureus* [23,24] and *Salmonella paratyphi, Shigella flexneri*. [25,26] In this study the aqueous extract of *Cymbopogon citratus* showed antiplasmodial activity. Administration of 0.1ml of chloroquine, 40 mg/kg, 80 mg/kg and 120 mg/kg body weight of aqueous extracts of *Cymbopogon citratus* reduced the level of parasitemia significantly after three days of treatment in the different treatment groups. The group treated with 120 mg/kg body weight of extracts of *Cymbopogon citratus* produced the most significant

reduction in the level of parasitemia. It was observed that the level of parasitemia was highest on the first day of treatment while the amount of food consumed was lowest. The animals showed sign of weakness and sluggishness prior to commencement of treatment. Level of feed consumption was also observed to be low in the experimental animals when compared to the normal control group. Appetite level was found to be the highest on the last day of treatment, probably due to the facts that parasitemia has almost be completely eradicated (Table 4). The experimental groups treated with 40 mg/kg and 80 mg/kg recorded minimal troughs of parasitemia on day 3 the group treated with 120 mg/kg body weight extract recorded a total eradication of the third day of treatment.

5. Conclusion

From the results of this preliminary work, it is concluded that aqueous extract of *Cymbopogon citratus* showed antiplasmodial activity and could be apply as an effective agent in future after further exploration. Studies should be needed in next steps of the undertaken work for understanding the mechanism of action by using in vitro models to figure out the effectiveness and pharmacological rationale of using *Cymbopogon citratus* as an antimalaria drug with chloroqiune as positive control.

6. Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH Publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of the Bingham University, Karu, Nigeria. All authors hereby declare that all experiments have been examined and approved by appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Conflict of Interest

There are no conflicts of interests on this research work.

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