EVALUATION OF THE CURATIVE POTENTIALS OF METHANOL EXTRACT OF *Citrus aurantifolia* LEAVES IN *Plasmodium berghei* - INFECTED MICE

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Abstract

Malaria remains a public health problem, with most front line drugs not able to clear parasite infection completely due to parasite resistance. With the increased tempo in the search for new chemotherapeutic agents against malaria, the use of medicinal plants by extracting active principles still remains the most appealing option. Phytochemical screening and *in vivo* antiplasmodial potential of methanol extract of *Citrus aurantifolia* leaves in *Plasmodium berghei* infected mice were carried out using standard methods. Infected mice were grouped into five groups of five mice each. Treatment commenced 72 hours post infection and lasted for five consecutive days, with daily parasitaemia levels monitored. The packed cell volume (PCV) of the experimental animals was also monitored before infection, before the commencement of treatment and after treatment. Three groups A, B and C were administered 200, 400 and 600 mg/kg body weight (b.w) of methanol extract of *Citrus aurantifolia* leaves respectively. Group D was administered 5 mg/kg b.w Chloroquine (standard drug). Group E was left untreated, while a sixth group of five mice was not infected and not treated. The animals were also monitored for any mortality for a period of 28 days and the mean survival time calculated in days. The methanol extract of *Citrus aurantifolia* leaves was found to contain tannins, steroids, terpenoids, glycosides, anthraquinones and flavonoids. The percentage parasite inhibition as well as the mean survival time were calculated to be (50.51 %, 12.67±1.45 days), (57.78 %, 17.33±0.88 days), (63.59 %, 22.67±0.67 days) and (70.67 %, 28.33±0.88) for groups treated with 200, 400, 600 mg/kg b.w of the extract and 5 mg/kg b.w of chloroquine respectively. There was no significant difference in percentage packed cell volume after treatment. The results obtained from this study indicate that *Citrus aurantifolia* leaves have appreciable antiplasmodial activity against *Plasmodium berghei* infected mice in a dose dependent manner. However, bioactive-guided purification of the crude extract could bring about specific bioactive compound(s) for antiplasmodial activity which could result in a much higher percentage parasite inhibition.

Keywords: Antiplasmodial, *Citrus aurantifolia*, *Plasmodium berghei*, chloroquine, percentage inhibition

1. Introduction

Malaria is a disease caused by protozoan micro parasite of the genus Plasmodium. It is a leading cause of mortality especially in children below the age of five across many tropical and subtropical countries (Monroe *et al.*, 2022). The discovery of ACT could be considered as the most noteworthy achievement of ethnopharmacological research in the 20th century. However, clinically relevant Artemisinin resistance is likely to occur since it has been obtained in laboratory models (WHO, 2016). Although global priority is to reduce the high malaria burden while retaining the long-term vision of eradication, the world malaria report 2022 estimates 247 million cases of malaria and 619,000 deaths in 2021 (WHO, 2022). This might be attributed to resistance to frontline antimalarial drugs which result to treatment failure in significant number of cases and the emergence of COVID-19 infections, owing to limited access to malaria health care services in WHO African region. Moreover, the recently licensed malaria vaccine exhibits limited efficacy (Atanu *et al.*, 2021). Hence, the need for cost effective and more efficacious alternative. Folklore use of medicinal plants is a common global practice due to accessibility, cultural acceptability, and relative affordability (Qayum *et al.*, 2016). In fact, many reviews of plant-derived drugs and their contribution to the global disease pandemic agree that the future of drug discovery lies in isolation, identification and characterization of secondary metabolites from medicinal plants (Calixto, 2005). *Citrus aurantifolia* commonly known as "Lime", is widely used traditional medicine in West Africa, particularly in Nigeria (Odediran *et al.*, 2020) for a plethora of diseases including fever, headache, jaundice, sore throat, oral thrush, arthritis, colds, coughs, as an antiseptic, antiviral, antifungal, anthelmintic, mosquito repellent, etc. (Enejoh *et al*., 2015). This research was carried out to investigate the curative potentials of methanol extract of *Citrus aurantifolia* leaf in *Plasmodium berghei* infected mice.

2. Materials and Methods

Parasites

The chloroquine sensitive *Plasmodium berghei berghei* (NK-65) was obtained from National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria. The parasites were kept alive by continuous re-infestation (I.P) in mice (Carvalho *et al*., 1991) every 10 days.

Animals

Thirty Albino mice of both genders, weighing between 20 g to 31 g were obtained from Nigerian Institute of Veterinary Research (NIVR), Jos, Nigeria. The animals were fed ad *libitum* with standard feed and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12 hrs light/darkness cycle. The animals were acclimatized for two weeks before the commencement of the study. A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulations (ENV/MC/CHEM (98) 17, 1998). The principle of laboratory animal care (NIH Publication No. 85-23, 1985) was also followed in this study.

Sample Collection

Leaves of *Citrus aurantifolia* (key lime) were collected with permission from a garden in Maikunkele, Bosso Local Government Area of Minna, Niger State and identified in the Department

of Plant Biology, Federal University of Technology, Minna. They were washed in clean tap water, shade dried at room temperature and pulverized.

Sample Extraction

The pulverized *C. aurantifolia* leaves (90 g) was extracted with 100 % methanol using a reflux method as described Cho *et al.* (2003). The filtrate obtained was concentrated by subjecting it to solvent recovery using a rotary evaporator set at 60 ^{o}C. The extract was placed in a water bath set at 60 ^{o}C to remove all traces of methanol and to yield the extract concentrate.

Screening of the presence of Secondary Metabolites in the Plant Extract

Qualitative phytochemical screening of the plant extract was carried out according to methods described by Trease and Evans (1989), Siddiqui and Ali (1997) and Sofowora (2006).

Inoculums

Parasitized erythrocytes were obtained from a donor- infected mouse by cardiac puncture in heparin and made up to 20 mL with normal saline. Animals were inoculated intraperitoneally with infected blood suspension (0.2 mL) containing about $1x10^7$ parasitized erythrocytes.

Induction of Malaria and Monitoring of Parasitaemia

A total of twenty mice were used for this study. On the first day (D_0), standard inoculums of $1x10^7$ *P. berghei berghei* infected red blood cells were injected into the mice intraperitoneally. Seventy-two hours later (after confirmation of infection), the mice were divided into five groups of four mice each. Different doses of the extract (200, 400 and 600 mg/kg/day) were administered orally to three groups. Chloroquine phosphate (5 mg/kg/day) was administered to the positive control group and 0.2 mL/day of distilled water was administered to the negative control group (infected not treated). The treatment lasted for five days at a single dose per day and daily blood smears were collected and examined microscopically to monitor the parasitemia level. The percentage parasite inhibition was calculated using the formula:

$$\frac{\text{Mean parasitemia in negative control group} - \text{Mean parasitemia in treated group}}{\text{Mean parasitemia in negative control group}} \times 100$$

The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculation) in each group over a period of 28 days (D_0-D_{27}) (Ryley and Peters (1970); Chandel and Bagai (2010)).

Determination of Packed Cell Volume

Packed cell volume (PCV) was determined using the capillary method for each mouse after inoculation and after treatment. Tail blood was collected into a heparinized hematocrit tubes, filled to 2/3rd of their volume and sealed at one end with plasticing, arranged on the micro hematocrit centrifuge and thereafter centrifuged for about 10,000 rpm for 5 minutes. The volume of cells was calculated as shown below:

$$PCV = \frac{\text{Erythrocyte Volume}}{\text{Total Blood Volume}} \times 100$$

Data Analysis

Data were calculated as mean ± SEM and were analysed statistically using One-way ANOVA followed by Duncan multiple comparison test and values of $p<0.05$ were considered significant. Statistical Package for Social Sciences (SPSS), 20th version was used.

3. Results

Table 1: **Composition of Secondary Metabolites in Methanol Extract of *Citrus aurantifolia* Leaves**

SECONDARY METABOLITES	INFERENCE
Alkaloids	-
Glycosides	+
Steroids and Terpenoids	+
Tannins	+
Reducing Sugars	+
Anthraquinones	+
Phlobatannins	-
Saponins	-
Flavonoids	+
Phenols	-

Key: + = Present, - = Not detected.

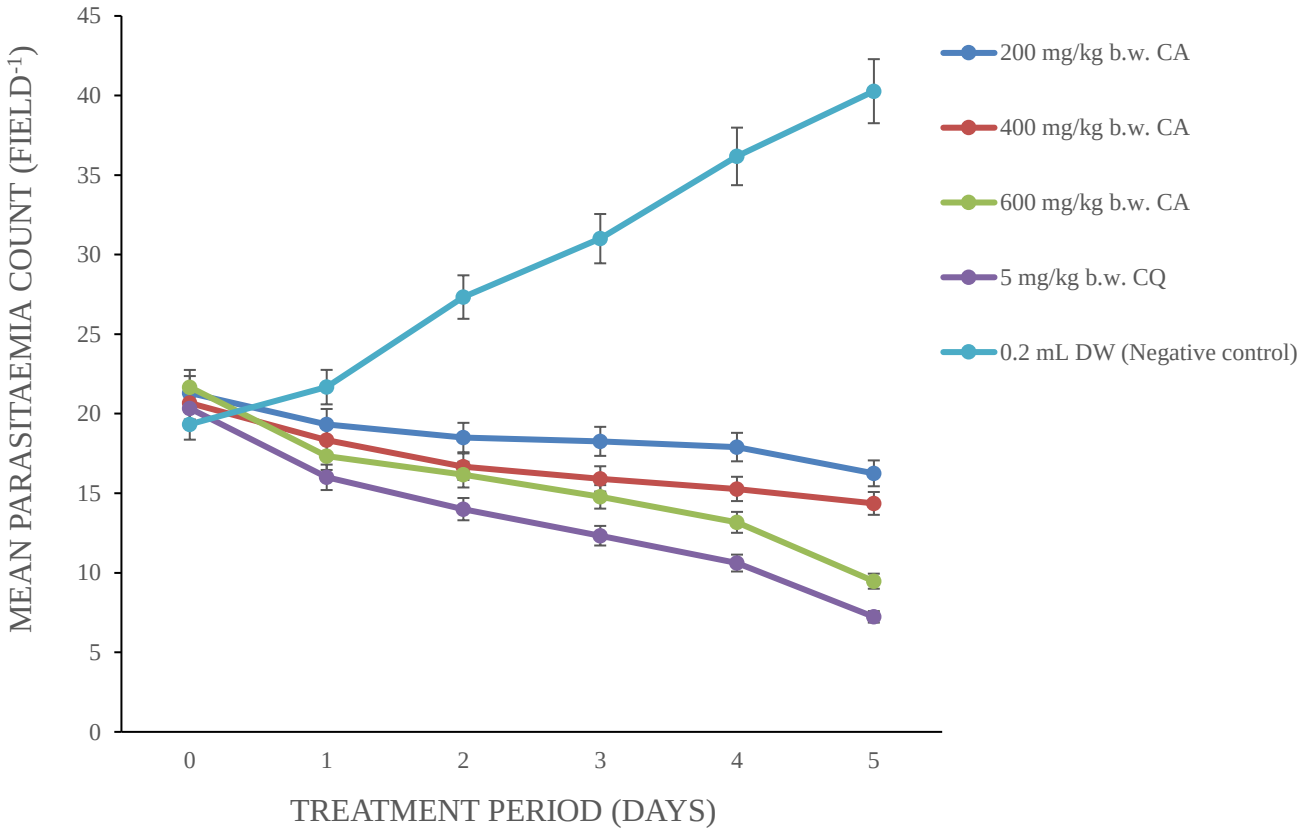

Figure 1: Antiplasmodial Effect of Methanol Extract of *Citrus aurantifolia* Leaves in Plasmodium berghei-infected Mice

Keys:
CA: Methanol extract of *Citrus aurantifolia*
CQ: Chloroquine phosphate
DW: Distilled water
mg/kg b.w.: milligram per kilogram body weight

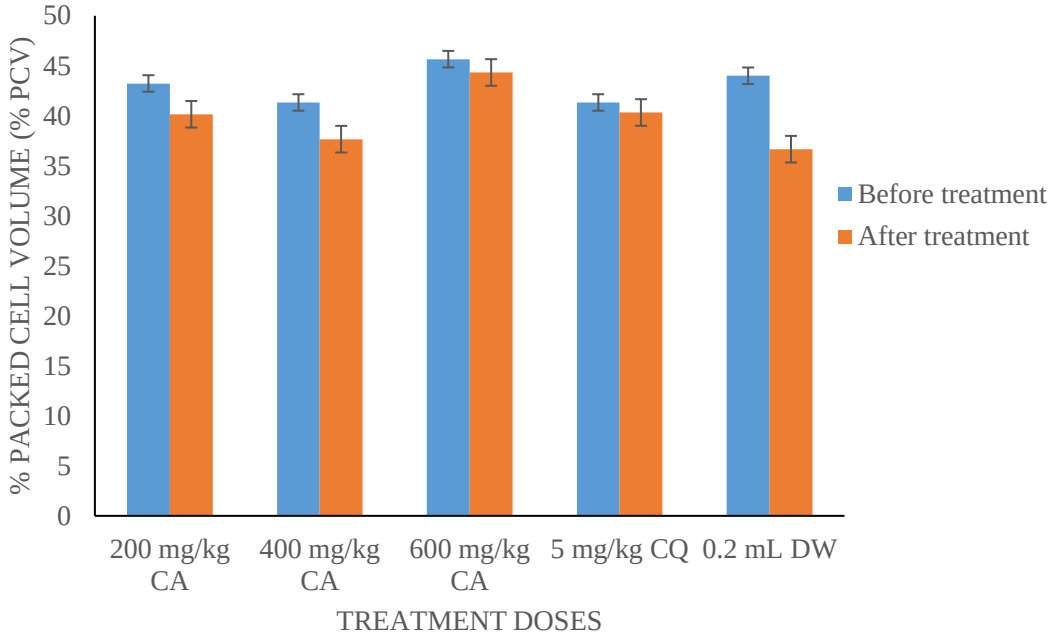

Figure 2: Percentage Packed Cell Volume (PCV) of Plasmodium berghei-infected Mice treated with Methanol Extract of *Citrus aurantifolia* Leaves

Table 2: Mean Survival Time (Days) of Plasmodium berghei-infected Mice treated with Methanol Extract of *Citrus aurantifolia* Leaves

Group	Dose (mg/kg b.w.)	Percentage parasite inhibition (%)	Mean survival time (Days)
Methanol extract of *Citrus aurantifolia*	200	50.51	12.67±1.45
Methanol extract of *Citrus aurantifolia*	400	57.78	17.33±0.88
Methanol extract of *Citrus aurantifolia*	600	63.59	22.67±0.67
Chloroquine phosphate	5	70.67	28.33±0.88
Distilled water	0.2 mL	-	3.33±0.33

4. Discussion

The secondary metabolites present in the methanol extract of *Citrus aurantifolia* leaves such as alkaloids, flavonoids, tannin and anthraquinones, have been implicated in antimalarial activity of many medicinal plants (Christenzen and Kharazmi, 2001). When a standard antimalarial drug is used in mice infected with *P. berghei*, it suppresses parasitaemia to non-detectable level (Kamei *et al*., 2000), which is in agreement with the effect of chloroquine and *Citrus aurantifolia* (at a dose of 600 mg/kg) in this study. The observed antimalarial activity in this study was dose dependent, with the group treated with 600 mg/ kg body weight having the highest percentage reduction in parasitaemia amongst the extract treated groups and the effect of the extract compared favourably with chloroquine. This is similar to the work of Ettebong *et al*. (2019) who reported the antimalarial activity of methanol extract of *Citrus aurantifolia* leaves at dose of 960 mg/kg body weight. A similar work by Laksemi *et al*. (2023) reported the percentage parasite suppression of Plasmodium berghei when *Citrus aurantifolia* was combined with stingless bee honey to be 78.8 % (Laksemi *et al*., 2023). There was no significant ($p \geq 0.05$) difference in the packed cell volume (PCV) of the chloroquine and methanol extract of *Citrus aurantifolia* leaves (600 mg/kg b.w.) treated groups before and after treatment. However, there was significant drop in the PCV of the infected and not treated group. The drop in the PCV that is responsible for malarial anaemia occurs both through an increase in the rate at which old Red blood cells are broken and a decrease in the rate at which new ones are produced. Plasmodium not only causes the rupture of parasitized red blood cells, but stimulates the activity of macrophages in the spleen, which then destroys both parasitized and unparasitized red blood cells. The observed high survival time of the group treated with 600 mg/kg body weight compared to the other extract treated groups might not be unconnected with the dose dependent parasite inhibition.

Conclusions

The present study revealed that oral administration of methanol extract of *Citrus aurantifolia* leaves exhibited antimalarial activity against *P. berghei*–infected mice. This suggests the beneficial effects of the plant. The findings in this study have also lent scientific support to the folkloric use of *Citrus aurantifolia* in the treatment of several ailments including fever and malaria.

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PETROGRAPHIC INTERPRETATION OF CHARNOCKITIC ROCKS FROM OSUNTEDO AND WASIMI, SOUTHWESTERN NIGERIA

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Abstract
Samples of charnockitic rocks from Osuntedo and Wasimi were subjected to petrographical analysis to determine the mineral present and the textural relationship of the mineral assemblage. It was observed that the charnockitic rocks contain pyroxene, biotite, amphibole, plagioclase feldspar, orthoclase and quartz with apatite and titanite present as accessory mineral. The textural relationship shows pyroxene included in biotite, and biotite mantling pyroxene. Also, amphibole reaction rims were noted around pyroxene, biotite, plagioclase and quartz. In addition, there were inclusions of euhedral apatite in biotite, antiperthite is surrounded by orthoclase feldspars. Plagioclase feldspar show zoning and bent twin lamellae. The sequence of crystallization of minerals follows the order; pyroxene, biotite and plagioclase, with amphibole showing a late appearance. The textural relationship of the minerals in the charnockitic rocks of Wasimi and Osuntedo demonstrate that the rocks are of igneous origin. Charnockitic rock of Wasimi contain more quartz compared to that of Osuntedo.

Key words: Hypersthene, antiperthite, reaction rim, zoning, apatite

1. Introduction

Charnockites are orthopyroxene (hypersthene) bearing rocks. Charnockite are used to describe orthopyroxene-bearing igneous rocks as well as orthopyroxene-bearing granitic orthogneisses associated with granulite terranes (Frost and Frost, 2008). Charnockite can be of igneous or metamorphic origin (Bohlender et al., 1992; Touret and Huizenga, 2012). Orthopyroxene formed from prograde form of metamorphism occur in very low water activity environment ((Newton et al., 1980; Waters, 1988; Ballèvre et al., 1997). Charnockitic rocks has been recognized as one of the major group of the rocks of the Basement complex of southwestern Nigeria (Hubbard, 1975; Rahaman, 1976). Charnockite remnants have been recognized in the north and center of the large elongate Ikire-Iwo complex (Figure 1). Field observation complemented with geochemical evidence show that the charnockite-granite association in Ado-Ekiti of southwestrn Nigeria forms a cogenetic sequence similar to rapakivi granite-anorthosite-charnockite (Olarewaju, 1987). Charncokite occurring as individual bodies has been described by Rahaman (1976) as the third type (in terms of occurrence), which is found in Lagun, Awo and Osuntedo areas of Southwestern Nigeria. The charnockite of Osuntedo occur as isolated hills, dark-grey to greenish in colour (Figure 2). Charnockite at Wasimi is porphyritic in texture, and dark grey in colour which often occur as isolated low-lying outcrops (Figure 3). Charncokite has been reported to find various uses most