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Intrinsic activity of aqueous leaf extract of Cymbopogon citratus against plasmodium-mediated tropical disease

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ABSTRACT

Objective: To investigate the intrinsic activity (efficacy) of aqueous leaf extract of Cymbopogon (C.) citratus against malaria, a plasmodium-mediated tropical disease. **Materials and Methods**: Plasmodium (P.) falciparum culture samples from 20 symptomatic adult outpatients were subjected to the antimalarial in vitro test. Parasite quantification by optical microscopy in the performance of in vitro antiplasmodial assays was employed. Roswell Park Memorial Institute (RPMI) 1640 was used as culture medium for cultivation of P. falciparum. Negative control was culture medium with the malarial parasites while treated drug was the leaf extract of C. citratus dissolved in dimethylsulphoxide (DMSO) and prepared into 7 levels concentration (3.125, 6.25, 12.5, 25, 50, 100 and 200 mg/mL.) After culture synchronized with sorbitol 5%, micromalarial culture were divided into control and treated groups then incubated in CO2 candle jar at 370C for 72 h. Each 8 h, the percentage of parasitemia were measured giving the activity of the extract on the growth stages of P. falciparum. Parasitemia was estimated by making the thin blood smear from the erythrocytes layer and stained with Giemsa (10%) for 30 mins. Using probit analysis, the antimlarial activity of the extract was calculated by counting the fifty percent of growth inhibition 50 (IC50). **Results:** The extract inhibited the growth of P. falciparum on mature schizont stage. The fifty percent inhibitory concentration (IC50) of the extract was 3.9 µg/mL after 32 h incubation. **Conclusion**: The leaf extract of C. citratus has efficacious antimalarial effect against P. falciparum in vitro.

Keywords: Cymbopogon citratus, tropical disease, plasmodium-mediated, intrinsic activity

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1. Introduction

Malaria is one of the tropical diseases that is life-threatening due to development of resistance by the most lethal causative parasite[1]. This is a great challenge in malaria controlling program to the most commonly available antimalarials. *Plasmodium (P*.) species mediate and cause malaria. Among the four species of malaria parasites namely *P. falciparum, P. vivax, P. ovale,* and *P. malariae,* the most widespread and dangerous is *P. falciparum,* known to cause severe anaemia, hypoglycemia, kidney failure and brain damage especially in among babies and children[2]. Recognized as important sources of antiprotozoal compounds for the development of drugs against many tropical diseases are plants. Quinine, artemisinin, quassinoids and limonoids are antimalarial natural products present in *Cinchona spp., Artemisia annua,* as well as plants of the *Simaroubaceae* and *Meliaceae* families respectively[3]. Of all the drugs introduced in the past 30 years, natural products are the origin of approximately two-thirds[4].

European scientific methods have shown positive pharmacological activity with most of the herbal remedies, however, a lot of them are devoid of scientific proof and are used superstitiously. For instance, *Xylopia amazonica* and *Zanthoxylum djala-batistae* despite being reported indicating anti-malaria use[5], have been scientifically proven to lack remedy for malaria[6]. This tendency is not restricted to folkloric anti-malarials but cut across other ailments as despite being listed as remedy for anaemia[7], *Khaya grandifoliola* lack scientifically proven anti-anaemic effect[8]. *Cymbopogon citratus, Zingiber officinale,* and *Psidium guajara* sometimes prepared as polyherbal mixtures are among numerous plants acclaimed by traditional healers to have anti-malarial properties. The objective of this study is to investigate the scientific efficacy for the folkloric use of *C. citratus* in the treatment of malaria caused by *P. falciparum.* The English names of *C. citratus* which belongs to the Poaceae family include: Lemon grass, citronella and squinant[9]. The common name in South-eastern Nigeria is “Achara tea”. The use of Lemon grass essential oil as an effective panacea against bacteria flu and colds are perhaps, the most popular of all its numerous utility[9]. It is also a potent anti-inflammatory and antifungal agent[10]. In Egypt and Brazil, hot water extract of *C. citratus* leaf and stem is taken orally as a renal antispasmodic, diuretic, analgesic, anti-inflammatory, antipyretic and sedative while in India, fresh entire plant is said to repel snakes, and inhaled as a fragrance, eaten as a condiment in Thailand[9]. In Indonesia and Malaysia, hot water extract of the entire plant is taken orally as an emmenagogue[9]. *C. citratus* has been shown to contain various phytoconstituents such as flavonoids and phenolic compounds, essential oils and terpenoids which are implicated for its different pharmacological activities[11].

2. Materials and methods

2.1. Plant materials

The fresh leaves of *C. citratus* were collected from Owerri environs in the month of July 2015 and authenticated by Dr. F. N. Osuala of the Department of Pharmacognosy, Madonna University, Nigeria; and air-dried at room temperature (26^0C). The dried leaf was pulverized using an electric blender into powder. Two hundred grams (200 g) of the powdered leaf was extracted by infusing in 2 litres of boiling distilled water for 3 hours[12], and filtered to obtain the aqueous extract. The extract was evaporated to dryness using hot water-bath, yielding a concentrated value of 18.8 g (9.4% w/w). The dried aqueous extract was kept in a sealed container and refrigerated at 4^0C until required.

2.2. Study samples

The *P. falciparum* culture samples used in this study originated from 20 symptomatic adult

outpatients at Laboratory unit of Madonna University Teaching Hospital, Nigeria. The study protocols were approved by the appropriate ethical review boards, and informed consent was obtained from all the study participants. The parasite densities ranged from 0.01 to 0.95% infected red blood cells giving a geometric mean of 0.27%.

2.3. In-vitro culture

The *P. falciparum* parasite isolates were cultured in the presence of serial dilutions of antimalarials (mefloquine [MF], dihydro-artemisinin [DA], quinine [QN], and chloroquine [CQ] at 1.5% hematocrit in RPMI 1640 with 0.5% Albumax 1 [Gibco, Bangkok, Thailand]). This is to establish sensitivities to test isolates with a broad range of antimalarial drug susceptibilities. The plates were frozen after 72 h of culturing, and stores at -20^{0}C.

2.4. In vitro antiplasmodial assessment

In the performance of in-vitro antiplasmodial assays, parasite quantification by optical microscopy is a traditional and reliable technique[6]. To provide a stock solution of 5.0 mg/mL, the extract (1.0 mg) was dissolved in DMSO. Test solutions of the extract were prepared by diluting stock solution in RPMI-1640 culture medium. Antimalarial activity was performed in 96 well microculture plates. To each well in microculture plate, 100 μL treated drugs were added, and 100 μL of parasitized culture (1% haemotocrit, 0.5% parasitemia) were added to each well in microculture plate and incubated in CO_2 candle jar for 72 h at 37 ^{0}C. The percentage of parasitemia was measured every 8 hour for the purpose of observing the activity of the extract on the growth stages of *P. falciparum*. Contents of the wells were harvest and stained after incubation. By making a thin blood smear and stained with *Giemsa* 10% for 30 mins, the growth stages of parasites were monitored. All test were performed in triplicate[13]. Parasitemia was calculated after examination under microscope with magnification 1000 by adding immersion oil, using the formula:

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

The percentage of the growth inhibition of the parasites was calculated using the formula:

$$\% \text{ parasitemia} = \frac{\text{Parasitemia in control} - \text{Parasitemia in treated group}}{\text{Total red blood cells}} \times 100$$

Growth stages percentage inhibition of the parasites at each concentration was determined by the mean of at least three IC_{50} parasite viability in which the fifty percent of growth stages of inhibition was calculated using probit analysis.

2.5 Statistical analysis

Individual inhibitory concentrations (the IC_{50} values) were obtained by probit analysis and non-linear regression using the Graphpad program (Intuitive Software for Science, San Diego CA, USA).

3. Results

3.1. Antimalarial activity

The results of in-vitro antimalarial activity of the aqueous leaf extract of *C. citratus* on *P. falciparum* were obtained. The percentages of parasitemia as well as the percentages of growth stages inhibitory of *P. falciparum* calculated in every 8 h interval to 72 h are shown in Tables I and II, respectively. It showed that the *C. citratus* leaf extract inhibited the growth of *P. falciparum* on mature schizont stage. Antimalarial activity was determined by fifty percent of growth stages inhibitory of *P. falciparum.* The fifty percent inhibitory concentration (IC_{50}) of the extract was 3.9 μ g/mL after 32 h incubation which was calculated using probit analysis (Table III).

Table 1: The percentage of parasitemia after cultivation in 8 h interval to 72 h at different concentrations of *C. citratus* aqueous leaf extract.

Concentration ($\mu g/mL$)	Percentage parasitemia during cultivation								
	8 h	16 h	24 h	32 h	40 h	48 h	56 h	64 h	72 h
0.000	8.17	11.04	12.70	16.22	15.90	16.36	15.70	15.92	16.40
3.125	6.13	8.01	8.04	9.51	11.53	11.20	14.80	13.61	11.21
6.25	5.22	7.33	6.70	6.30	9.00	11.03	13.70	12.40	10.42
12.5	5.04	7.02	5.60	5.80	7.12	11.71	13.02	12.20	9.10
25.0	3.54	5.63	5.07	5.20	6.21	9.60	12.21	11.60	7.80
50.0	2.60	5.30	4.70	4.70	5.10	6.80	10.50	10.22	6.04
100.0	2.23	4.25	4.30	3.40	4.04	5.52	9.20	8.20	5.60
200.0	1.70	3.03	3.70	2.61	3.40	3.80	4.09	5.01	3.92

Table 2: The percentage of growth inhibition of *P. falciparum* after cultivation in 8 h intervals to 72 h at different concentrations of *C. citratus* aqueous leaf extract.

Concentration ($\mu g/mL$)	Percentage growth inhibition of *P. falciparum* during cultivation								
	8 h	16 h	24 h	32 h	40 h	48 h	56 h	64 h	72 h
0.000	–	–	–	–	–	–	–	–	–
3.125	25.0	27.4	37.0	41.4	27.4	32.0	6.0	15.0	32.0
6.25	36.1	34.0	47.4	61.4	44.0	33.0	13.0	22.2	36.4
12.5	38.3	36.4	56.0	64.4	56.0	35.0	17.0	24.0	45.0
25.0	57.0	49.0	60.0	68.2	61.0	41.5	22.0	27.3	52.0
50.0	69.0	52.0	63.1	71.2	68.0	59.0	33.0	36.0	63.2
100.0	73.0	62.0	66.3	79.0	75.0	66.3	42.0	49.0	65.0
200.0	79.2	73.0	71.0	90.0	79.0	77.0	74.0	69.0	76.1

– means no percentage inhibition with negative control.

Table 3: The IC_{50} value against growth of *P. falciparum* after cultivation in 8 h intervals to 72 h of *C. citratus* aqueous leaf extract.

Observation (hour)	$IC_{50}(\mu g/mL)$
8	19.0
16	31.7
24	9.8
32	3.9
40	12.1
48	27.5
56	99.0
64	92.9
72	19.4

4. Discussions

The results obtained in this study showed that aqueous extract of *C. citratus* possess antimalarial effect. The presence of phenolic compounds, flavonoids, essential oils, alkaloids, and terpenoids among others[11,9] corroborate previous studies which have revealed that plant extracts containing such phytochemicals do possess antiplasmodial activities[14, 15]. The presence of phenolic had been reported and implicated in the antimalarial and antiplasmodial activity of husk extract and fractions of *Zea mays*[16], in which eight phenolic compounds have been detected in ethanol husk extract of *Zea mays*[17]. Gallic acid and kaempferol are examples of phenolic compounds implicated in the antiplasmodial activities of plants[18]. Studies on the anti-malarial activity of plant species from countries of the Amazon region such as Brazil have supported the presence of alkaloids as source of potent antiplasmodial substances[19]. The IC_{50} is the concentration of crude treated drug which inhibits the growth of plasmodium by fifty percent. In this investigation, in-vitro antimalarial activity was found in the IC_{50} = 3.9 $\mu g/mL$ of the aqueous leaf extract of *C. citratus* on mature schizont stage after 32 h cultivation. This was at variance with that of the aqueous of the *Azadiratcha indica* leaf extract with concentration inhibition of IC_{50} = 2.0 $\mu g/mL$[20] showing higher potency than *C. citratus.* The synergism of the bioactive compounds present in the extract could explain its in-vitro antiplasmodial activity however, in-vivo, these chemical constituents may have a diminished effect due to low bioavailability, biotransformation and physiological factors in the host organism[21]. Antioxidant potentials of some plants and natural products especially flavonoids which is present in *C. citratus*, have been found to increase schizonticidal activity via modulation of the cellular signaling pathway[22, 16]. This could be one of the mechanisms of action of *C. citratus* as it contains phenolics and flavonoids with antioxidant activity because elevated free radicals levels which are common tendencies of malaria disease are implicated in severe malaria complications[17].

Conclusion

The aqueous leaf extract of *C. citratus* showed intrinsic activity against *P. falciparum* in vitro. This gives credence to the folkloric use of *C. citratus* for the treatment of malaria.

Conflict of interest statement:

We declare that we have no conflict of interest.

Source of support: Nil

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