

ANTIPLASMODIAL EFFICACY OF CYMBOPOGON CITRATUS LEAF CRUDE EXTRACT

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ABSTRACT

Malaria is a potential deadly tropical disease and the most important of infectious diseases in the tropics and sub-tropics. The search for new antimalarial drugs has been necessitated by *P. falciparum* resistance to virtually all antimalarial drugs. In this study, the *in vitro* antimalarial activities of the leaf crude aqueous and ethanolic extracts of *Cymbopogon citratus*, a plant used by traditional healers to treat malaria and other diseases, was evaluated against *P. falciparum*. Six (6) malaria positive fresh blood samples obtained from infected children and adults in Specialist Hospital Sokoto were tested against the plant extracts. Standard microtest technique of schizont maturation and parasite growth assay was used to culture fresh isolates. The student t-test was used to analyze the data. There was no significant ($p \leq 0.05$) reduction in the number of parasitized cells relative to the control. The results of the study showed that ethanol extract of *C. citratus* gave the highest antimalarial activity of 77.20%, with IC_{50} of 13.12 $\mu\text{g/ml}$. This is followed by aqueous extract of *C. citratus* with parasite growth inhibition of 73.01% and IC_{50} of 19.35 $\mu\text{g/ml}$. Comparatively, Ethanol extract of *C. citratus* has high Schizont Growth Inhibitory activity ($48.19 \pm 9.10\%$) than aqueous extract ($42.68 \pm 8.51\%$) but not significantly different, $t(12) = 0.442$, $p = 0.666$ T-Test. The phytochemical investigation of *C. citratus* indicates the presence of Saponins, carbohydrates, Tannins, Phenols, Flavonoids, Alkaloids, Triterpenoids and Cardiac Glycosides. The results of the study also show that *G. latifolium* possess a moderate antimalarial activity. Therefore, the extracts possess promising antimalarial activities which can be exploited for malaria therapy, and also justifies the traditional use of the plants in malaria treatment. Further work is suggested to synthesis and characterizes the active principles from these plants.

Keywords: Antiplasmodial, *Cymbopogon citratus*, crude extracts, *in vitro*.

INTRODUCTION

Malaria parasite is an infectious tropical disease caused by the protozoan parasites, *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium vivax*. The disease is widely spread in the tropical and subtropical regions of the world and is

transmitted by the female anopheles mosquito. World Health Organization (2016) report opined that, there were 212 million new cases of malaria worldwide in 2015 (range 148–304 million).

It is estimated that African Region accounted for most global cases of malaria (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%) with estimated 235,000-639,000 deaths in 2015, mostly among children under five years and pregnant women (WHO, 2016).

In Nigeria, malaria the public health challenges faced by rural and also urban dwellers. It accounts for 25 % of under-five mortality, 30 % of childhood and 11 % of maternal mortality. About 50 % of the Nigerian population will have at least one episode of malaria annually while children below the age of five (about 24 million) will have two to four attacks annually (WHO, 2016). The economic cost of malaria in Nigeria can be as high as 1.3 % of economic growth per annum. This is largely due to rising cost of treatment, loss of productivity and earning due to days lost from illness (WHO, 2016).

From decades, medicinal plants have been used as a source of medicine in virtually all cultures (Hoareau & Dasilva, 1999). In Africa, medicinal plants extract are still widely used in the treatment of many ailments including malaria and about 80 % of African population use traditional medicine for primary health care (WHO, 2002-2005). Several medicinal plants have been used in different part of the world for the treatment of malaria. For example, quinine extracted from the bark of cinchona tree was used as an antimalarial agent as far back as 1632 (Amusan *et al.*, 1996). And this was later developed as antimalarial agents in form of primaquine, quinacrine, chloroquine and other quinine family of drugs (Duker-Eshun *et al.*, 2004).

There is increased and widespread resistance of the malarial parasites especially *P. falciparum* (the major etiological agent for human malaria) to the current standard malaria treatment drugs (WHO, 2016). However, there is urgent need for the development of new novel drugs for the treatment of malaria. This is couple with the fact that most medicinal plants currently used for the treatment of malaria have little scientific data to validate their claimed antimalarial activity (Hoareau & Dasilva, 1999). So, investigation of their antimalarial property is important in order to establish their efficacy as well as their potential as sources of new antimalarial drugs.

MATERIALS AND METHODS

COLLECTION OF EXPERIMENTAL PLANTS

Fresh leaves of *Cymbopogon citratus* were obtained separately from the farm of the ACEPRD University of Jos. Collected plants were identified by a taxonomist at the Department of Botany and Biotechnology, University Jos.

PREPARATION OF LEAVES FOR CRUDE EXTRACTION

The preparation of the crude extract was carried out at the Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. Collected leaves of the plants were washed off dirt and air dried under shade at room temperature. The dried leaves were made into powder using the laboratory grinder.

CRUDE EXTRACTION OF THE COLLECTED LEAVES

Using the maceration protocol described by Pandey & Tripathi (2014) 45.20g of *Cymbopogon citratus* of the powdered leaves were extracted with 95% ethanol and distilled water.

CLINICAL RESEARCH METHODOLOGY

Ethical Consideration

Approval for the study was obtained from the Ethical Committees of University of Jos Health Services (UHS).

Blood Sample Collection

Infected blood samples (Clinical isolates of *P. falciparum* and *P. malariae*) were obtained from the Medical Laboratory Unit of Specialist Hospital Sokoto.

IN VITRO TESTING OF THE PLANT EXTRACTS ON *P. FALCIPARUM*

The *in vitro* study was carried out according to the protocol described by WorldWide Antimalarial Resistance Network (WWARN): *In vitro* Module, WWARN. 2010. Culture of *P. falciparum* erythrocytic stages.

PROCEDURE FOR *IN VITRO* CULTIVATION AND MAINTENANCE OF *PLASMODIUM FALCIPARUM* CULTURE

Preparation of Culture Medium for Cultivation of *Plasmodium falciparum*

RPMI Medium

One packet of RPMI 1640 (containing 25 mM of HEPES buffer, glucose) dissolved in 960 ml of double distilled water. 40 µg/ml of gentamycin sulfate (1.2 ml of Gentamycin/L) was added to prevent bacterial contamination. This solution was passed through a Millipore filter of 0.22 µm porosity and store at 4°C as 96 ml aliquots in glass media bottle.

Extracts Solution

Aqueous extracts of *C. citratus* were first dissolved in distilled water while ethanol extracts were dissolved in ethanol, sonicated for 10 minutes and diluted in distilled-deionised water, and 20mg/ml solution of each was prepared. The 20mg/ml solution was further diluted in 180ml of the malaria culture medium to give 200µg/ml stock solution (Clarkson *et al.*, 2004). Both extracts were tested in 6 serial two-fold dilutions with a final concentration range of 1.55-100µg/ml in 96 wells microtitre plates according to manufacturer's instructions (Becton Dickinson Labwares, USA).

***In Vitro* Assay**

The assay was performed in duplicate. Using a micropipette, 100 µl of distilled water was first distributed into well plates, after which 100µl of culture medium containing extracts of *C. citratus* at various concentrations were added into well plates. One hundred microlitres of parasite culture (isolates) were finally added into each microtitre well plate. The plates were incubated in CO₂ condition at 37°C in candle jar for 24-30 hours. After incubation, the red blood cells were harvested and transferred to a clean microscopic slide to form a series of thick blood films. The films were stained for 10 minutes in 10% Field stain (pH 7.3). Parasite growth was counted in 10 microscopic fields and the mean calculated. The control was considered as 100% growth.

The percentage inhibition with concentration was calculated using the formula: (WHO, 2001b; Ngemenya *et al.*, 2006).

$$\frac{(\% \text{ Parasitemia in Culture wells} - \% \text{ Parasitemia of the Tests wells})}{\% \text{ Parasitemia of the Control}} \times 100$$

The IC₅₀ values, concentration required to inhibit parasite growth by 50% were determined by linear interpolation from the parasite growth inhibition curves (concentration versus percent inhibition) generated from each parasite-extract interaction (Mustofa, Sholikhah and Wahyuono, 2007).

THRESHOLD FOR ANTIMALARIAL ACTIVITY

The threshold for the antimalarial activity of the plant extracts was obtained according to Gessler, Nkunya, Nwasumbi, Heinrich and Tonner (1994). It was classified as follows: extract with IC₅₀ less than 10µg/ml is considered very good, from 10 to 50µg/ml is moderate and over 50µg/ml is considered to have low activity.

PHYTOCHEMICAL SCREENING OF THE CRUDE EXTRACT

The phytochemical screening (qualitative determination) of the bioactive ingredients of the plant extracts were determined using standard conventional protocols described by Pandey & Tripathi (2014) for detecting the major components.

STATISTICAL ANALYSIS

Data are expressed as mean ± SEM. The student t-test was used to statistically analyze the data and values of P ≤ 0.05 were considered significant.

RESULTS

Phytochemical Analysis

The experimental studies on phytochemical analysis and antiplasmodial testing started in 2019. The major aim was to scientifically verify the benefits of two Nigerian medicinal plants *Cymbopogon citratus* for its use by TMPs in malaria treatment by evaluating their efficacy against the *Plasmodium falciparum* parasite. The inventory of new medicinal plants helps in the development of new phytomedicines in malaria treatment.

The phytochemical screening of these medicinal plants showed the presence of saponins, carbohydrates, tannins, phenols, flavonoids, alkaloids, triterpenoids and cardiac glycosides in the extracts of *Gongronema latifolium* (Table 1).

Yield of Extracts

Yield of extracts of *Cymbopogon citratus* are presented in Table 2 and it shows crude extract and solvent extraction of experimental plants. Water solvent yielded more of extracts than ethanol in both plants.

***In vitro* Assay**

There was no significant difference ($p \leq 0.05$) of the reduction in the number of parasitised cells compared to control. The crude extracts were tested mainly on the schizont of *Plasmodium falciparum*. Table 3 shows mean parasite growth at various concentrations. The basic measurement of antimalarial activity used in this study was the reduction in number of parasitised cells in the test cultures after 24 hours incubation.

Table 3 shows the mean percentage inhibition of erythrocytes invasion by *P. falciparum* isolates for the two extracts ranged between 11.25-12.64% at the lowest concentration of 1.55 μ g/ml and 73.01-77.20% at the highest concentration of 100.00 μ g/ml.

There is no significant difference in the extracts of *G. latifolium* ($p \leq 0.05$). The ethanol extract of *C. citratus* gave the highest antimalarial activity of 77.20%, with IC_{50} of 13.12 μ g/ml. This is followed by aqueous extract of *C. citratus* with parasite growth inhibition of 73.01% and IC_{50} of 19.35 μ g/ml. (Table 4).

Table 1

The Major Phytochemicals Presents in the Extract of the selected Plants

Plant species	Extract	%Yield	Major Phytochemical Constituents							
			SAP	CAB	TAN	PHE	FLA	ALK	TRI	CAG
<i>Cymbopogon citratus</i>	Et	4.77	+++	+	-	+++	+++	++	++	++
	Wt	12.22	+++	+++	+++	+++	+++	-	++	+++

Key: Percentage yield is calculated from the formula, %yield = Weight of crude extract (g)/Weight of the sample powder (g) x 100%

Negative, -

Mild positive, ++

Positive, +++

Highly positive, ++++

SAP=SAPONINS, CAB=CARBOHYDRATE, TAN=TANNINS, PHE=PHENOL, FLA=FLAVONIDS, ALK=ALKALOIDS, TRI=TRITERPENIDS, CAG=CARDIAC GLYCOSIDES

Et= Ethanol

Wt= Water

Table 2

Yield of Extracts of *Cymbopogon citratus*

Plant	Solvent	Weight of plant (g)	Weight of solvent (g)	Yield (%)
<i>Cymbopogon citratus</i>	Aqueous	23.0	2.81	12.22
	Ethanol	22.0	1.05	4.77

Key: Percentage yield is calculated from the formula, %yield = Weight of crude extract (g)/Weight of the sample powder (g) x 100%

Table 3

In vitro Schizont Growth Inhibition of *Plasmodium falciparum* isolates by *Cymbopogon citratus* plant extracts.

		Concentration of Extracts (µg/ml)						
Extracts		1.55	3.10	6.20	12.50	25.00	50.00	100.00
Water Extracts (%) Inhibition)	11.25		21.14	31.10	44.8	55.64	61.80	73.01
Ethanol Extract (%) Inhibition)	12.64		24.28	37.43	53.55	63.45	68.75	77.20

Table 4

Antimalarial Activity and Inhibition Concentration (IC₅₀) of Aqueous and Ethanolic Extracts of *Cymbopogon citratus*

Extracts	Solvent	Antimalarial Activity (%)	IC ₅₀ (µg/ml)
<i>Cymbopogon citratus</i>	Aqueous	73.01	19.35
	Ethanol	77.20	13.12

Antiplasmodial Activity of *G. latifolium*

Figure 4 is the growth inhibition curve of *P. falciparum* by aqueous extract of *Cymbopogon citratus* generated from parasite-extract interaction, with IC₅₀ value of 19.35µg/ml. Fig 4 also shows schizont growth inhibition curves generated from each parasite-extract combination/interaction. Inhibition Concentration (IC₅₀) values were determined by linear interpolation from each of the inhibition curves. With regard to concentrations administered, dose-dependent antimalarial activity was clearly shown for the two crude extracts. The percentage inhibitions are higher with increasing concentrations.

Figure 5 also shows the inhibition of *P. falciparum* by ethanolic extract of *Cymbopogon citratus* which produced the IC₅₀ of 13.12µg/ml.

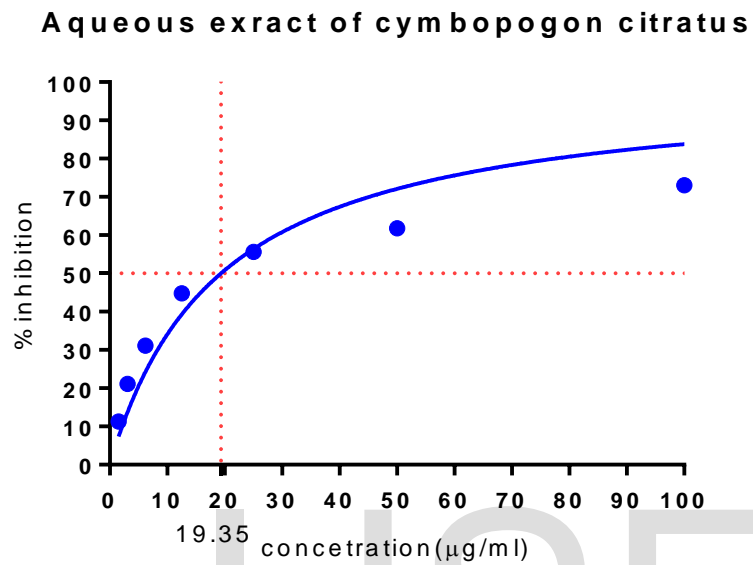


Figure 4 Inhibition of *Plasmodium falciparum* Isolates by Aqueous Extract of *Cymbopogon citratus*

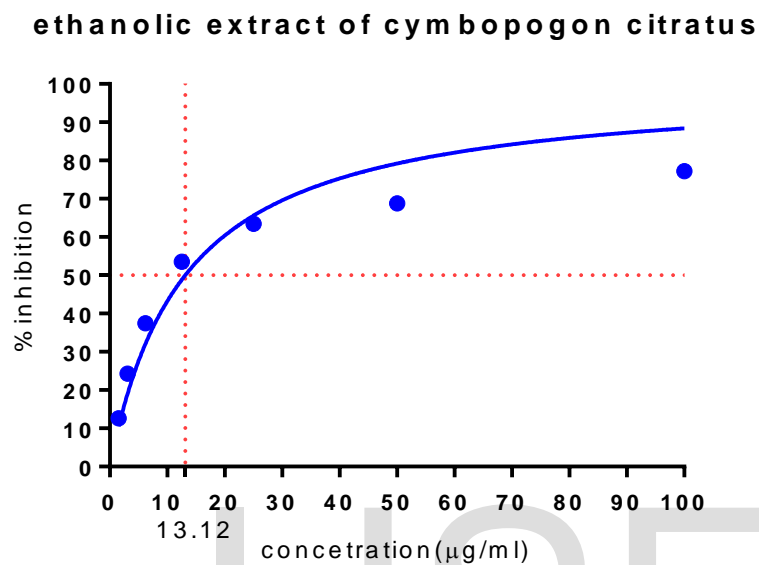


Figure 5 Inhibition of *Plasmodium falciparum* Isolates by Ethanol Extract of *Cymbopogon citratus*

DISCUSSION

Ethanol extract of *Cymbopogon citratus* has high Schizont Growth Inhibitory activity (48.19 ± 9.10 %) than aqueous extract (42.68 ± 8.51 %) but not significantly different, $t(12) = 0.442$, $p = 0.666$ T-Test.

Antiplasmodial efficacy of *Cymbopogon citratus* revealed growth inhibition of *P. falciparum* by aqueous and ethanolic extract of 73.01 and 77.20 with IC_{50} value of 19.35 and 13.12 $\mu\text{g}/\text{m}$ respectively. Inhibition Concentration (IC_{50}) values were determined by linear interpolation from each of the inhibition curves. With regard to concentrations administered, dose-dependent antimalarial activity was clearly shown for the two leaf crude extracts. The percentage inhibitions are higher with increasing concentrations.

The thresholds for the *in vitro* antimalarial activity of the plant extracts were based on the classification according to Gessler *et al.*, (1994) where: extract with IC_{50} less than 10 $\mu\text{g}/\text{ml}$ is considered very good; 10 to 50 $\mu\text{g}/\text{ml}$ considered moderate and over 50 $\mu\text{g}/\text{ml}$ considered to have low activity. Based on this classification, result from this study of the aqueous and ethanol extracts of *Cymbopogon citratus* with IC_{50} of 19.35 $\mu\text{g}/\text{ml}$ and 13.12 $\mu\text{g}/\text{ml}$ respectively, are said to have moderate antimalarial activity. The results of this study is in agreement with the research conducted with Abbas *et al.*, (2017) in Sokoto State Nigeria who reported that crude extract of *Cymbopogon citratus* has moderate antimalarial activity.

The *in vitro* antimalarial activity of *C. citratus* has similarly been reported by Bidla *et al.* (2004), who recorded antimalarial activity of 75.2% at 40 $\mu\text{g}/\text{ml}$ which is comparatively similar than what was recorded in this report: 73.01% (aqueous extract) and 77.20% (ethanol extract) at 100 $\mu\text{g}/\text{ml}$, considering the concentration of 40 $\mu\text{g}/\text{ml}$ used. The finding of this work also agrees with Kiloba (2014) in which *C. citratus* leaves was tested against *P. falciparum* and revealed moderate antimalarial activity.

CONCLUSION

The results of this study have shown that the ethanolic leaf crude extract of *Cymbopogon citratus* exhibited higher antimalarial activity than the aqueous extract. Both extracts possess a moderate antimalarial activity. This result justifies the use of medicinal plants the treatment of malaria. Further work is suggested to synthesis and characterizes the active principles from these plants.

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