Original Research Article

Investigation of the activities of methanol extract of Cleistopholis patens (Annonaceae) leaf and its partitioned fractions against Plasmodium bergheiberghei in mice

Investigation of the methanol extract of*Cleistopholis patens* (Annonaceae) leafand its partitioned fractions against *Plasmodium bergheiberghei* in mice

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ABSTRACT

Aim: The methanol extract and partitioned fractions of *Cleistopholis patens* leaf were evaluated for antiplasmodial activities with a viewto establishing its antimalarial potential and identifying the most active partitioned fraction.

Method: The leaf was collected, authenticated, air-dried and powdered. The extract, prepared by maceration in methanol was tested (0-800mg/kg) against*P. bergheiberghei* in mice using Peter's four-day test. Normal saline and Chloroquine (10mg/kg) were negative and positive control respectively.Percentage parasitemia, percentage chemosuppression, effective doses, survival times and percentage survivors were the parameters used for the assessment. The extract was thereafter suspended in water and successively partitioned into n-hexane, dichloromethane and ethyl acetate and the resulting fractions and the aqueous phase were similarly tested at doses 10-80mg/kg.

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Result: The methanol extract which was comparable (P=. 06) in activity to chloroquine gave 1.05±0.09 percentage parasitemia, 77.45±4.39 percentage chemosuppressionat 40mg/kgand anED_{50} and ED_{90} of 255.83±3.60 and 471.35±15.05 mg/kg. The most active n-hexane fraction eliciteda percentage chemosuppression of 82%, 0.87 percentage parasitaemia at 80mg/kg, moderately high percentage survivor and relatively low ED_{50} and ED_{90} of 28.87±5.29 and 56.30±8.44 mg/kg.

Conclusion: The methanol extract of *C. patens* leaf is active against *P. bergheiberghei*and the antimalarial compounds is likely to beconcentrated in the n-hexane fraction.

. ***Key words:****Cleistopholis patens leaf; antimalarial investigation,*chemosuppression, *partitioned fraction, activity-directed*

1.0. INTRODUCTION

Effective herbal remediesare potential sources of drugs that are used in combating a variety of ailments and diseases, malarial inclusive[1]. The use of infusions of plant barks to treat human malaria since the 17th century, is an encouragement to search for molecules from plants especially those used ethnomedicinally in the treatment of malaria [2]. That the first antimalarial drug, quinine, was obtained from the bark of the Cinchona (Rubiaceae) species and the fact that artemisinin was discovered from *Artemisia annua*in the seventiesencourage further research into other medicinal plants for antimalarial molecules [3; 4]. Artemisinin-combined therapies (ACT) was formally adopted as first-line treatment of uncomplicated malaria for Nigeria in 2005 [5-6]. It is only reasonable that indigenous plants are investigated for chemical compounds that may serve the same purpose in the nearest future.More so, many plants that are used in ethnomedicine as antimalarial have not been investigated for antimalarial potency. *Cleistopholis patens* (Annonaceae), commonlycalled salt and oil tree, is a small to medium-sized tree up to 20-30m high.The extract of the plant alone, as decoctions or infusions or crushed preparations of theleaf,bark or of grounded and roasted seeds, and in some instances, in combination with other plants like *Cymbopogonspp*and*Caricapapaya*have been used in the treatment of fever, sleeping sickness,infective hepatitis, measles, menstrual irregularities, tuberculosis, rheumatic arthritis, headaches, and asvermifuge andpurgative [7-11].Hypolipidemic, haematological, antimicrobial, entomotoxic and anti-arthritic activities have also been reported for this plant.The plant contains alkaloids,terpenoidsand oligorhamnosides [12- 13]. Since *C. patens* have ethnomedicinal claims of anti-fever and antimalarial activities, there is a need to investigate it for its antimalarial potential[14].

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The stem bark and leaf oils of the plant when assessed against chloroquine-resistant strain of *P. falciparum* in an *in vitro*antiplasmodial evaluation elicited anIC_{50} values of 9.19 and 15.19 μg/mL respectively [11]. Also, the leaf methanol extract of the Ghanaian species similarly tested was found to have appreciable activity with an IC_{50}= 8.7ug/mL [15]. On the other hand, the root extract gave an IC_{50} of 21.43 ± 2.45 and 14.68 ± 1.02 μg/mL against CQ-sensitive and CQ-resistant strains of *P. falciparum* respectively [16].

In this work, the methanol extract of the leaf of *C. patens*and its partitioned fractions were investigated for antimalarial activities against chloroquine-sensitive *Plasmodiumbergheiberghei*infected mice with a view to complementing the *in vitro* result and soestablish its antimalarial efficacy. The fraction(s)containing the antimalarial constituents will also be identified.

2.0. METHODOLOGY

2.1. Plant collection and preparation

The leaf of *C. patens* was collected beside the Department of Botany, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria. It was identified and authenticated at the Faculty of Pharmacy Herbarium by Mr. I. I. Ogunlowo of the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife and voucher specimen number FPI 2212was deposited. The leaf was subsequently air dried, powdered and kept in a cool, dry place before further use.

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2.2.Extraction and Partitioning

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The powdered leaf (8kg) was maceratedin two batches (2x 4kg) with methanol (3L)each for 72 hours with intermittentshaking. Thereafter, the extract was filtered and the process was repeated two times for each batch. The filtrates were pooled and concentrated using rotary evaporator to obtain 650g (8.13% yield) of the methanol extract.The extract (422 g) was suspended in 500 mL of distilled water and successively partitioned into n-hexane, dichloromethane and ethyl acetate to obtain their respective partitioned fractions,**CPH** (45 g), **CPD** (205 g), **CPE** (21 g) and **CPA** (143.1 g) after *in vacuo* concentration.

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2.3. Preparation of extract, fractions and drugsfor the assay

The 0.05g, 0.1g, 0.2g, and 0.4g of the extract was solubilized in normal saline (5.0 mL) with the aid of Tween 80 to give doses 100, 200, 400 and 800 mg/kg respectively which was used for the antimalarial experiment. For each of the partitioned fraction,0.005 g, 0.01 g, 0.02 g and 0.04 g was similarly solubilized in 5.0mL of normal saline to give 10, 20, 40 and 80 mg/kg respectively. A 0.005g of chloroquine was similarly dissolved in 5.0 mL of normal saline to give a 10.0 mg/ kg dose as the positive control. Normal saline (0.2mL)was utilized as the negative control.

2.4. Mice

Thirty (30) Swiss albino mice weighing between 18 to 22g (male and female), were purchased from the animal house, Faculty of Pharmacy, OAU, Ife. The mice were housed in aluminum cages in groups of five (5) mice each, fed with grower's marsh and clean water and were acclimatized for 5 days before use. The same process was followed for the preparation of the mice to be utilized in all the experiments.

2.5. Parasite

The rodent parasite *Plasmodium bergheiberghei* NK 65 haboured in a donor mouse was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan. The parasite was maintained by serial intraperitoneal passaging in mice. The mouse with about 30% parasitaemia was subsequently euthanized and blood containing the parasite obtained from the mouse by cardiac puncture. The resulting blood obtained was diluted so that 0.2mL contain 1×10^7 infected erythrocytes. An inoculum of 0.2mL of diluted blood was given to each of the test animals intraperitoneally in all the experiments[17].

2.6. Chemosuppressive Antimalarial Assay

The chemosuppressive antimalarial model was used to evaluate the activities of the extract and the partitioned fractions in *Plasmodiumbergheiberghei* infected mice. The acclimatized and grouped mice (I-VI) were infected with 1×10^7*P. bergheiberghei*on the first day, two hours later, they were treated orally with various volumes (depending on the weight of the animal) of the previously prepared extract andpartitionedfractions. The dose range used are 100, 200, 400 and 800 mg/kg and 10, 20, 40 and 80 mg/kg for the methanol extract and the partitioned fractions respectively. The positive (V) and the negative(VI) control groups received 10mg/kg chloroquine and 0.2mL of normal saline respectively. The above treatment was carried out for four consecutive days (D_0-D_3). On the fifth day, thin blood films were prepared from the tail blood of each experimental animal, fixed in methanol, stained with Giemsa and evaluated. Percentage parasitemia, percentage chemosuppression, survival time and percentage survivor obtained from the evaluation of each slide was used to assess the activities of the different doses of the extract and partitioned fractions.

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2.7. Laboratory Determination of Percentage Parasitemia in Mice

2.7.1.Blood film preparation

Clean slides were arranged and masking tapes were placed at the base of these slides in order to identify the surface on which to make the smear. Drops of blood from each animal's tails were put on each slide. The blood was spread thinly horizontally using another clean slide at 45°. The blood film was allowed to dry and fixed with drops of methanol and allowed to dry.

2.7.2. Staining of blood film

Stain was prepared by diluting a 10mL of Giemsa stain (3%) to 100mL with tap water and mixed thoroughly in a staining tank. The fixed slides were arranged in the solution and left for 30minutes. It was then removed, rinsed, air-dried and neatly packed inside tissue paper and labelled.

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2.7.3. Microscopical examination of stained film

The stained slide was then mounted on the microscopic stage and observed using the oil immersion objective (×100 magnification). Ten fields were viewed and result taken from each. The percentage parasitemia, calculated as a percentage ratio of the number of parasitized and total number of parasitized and unparasitized red blood cells, was determined from an average of ten fields of count on a microscopic slide. The percentage parasitaemia was calculated for each mouse in a group of five and the result was recorded.as average percentage parasitaemia ± standard error of mean (SEM).

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2.8. Analysis of Results

The results were analysed using the parameters percentage parasitemia, percentage chemosuppression, survival time and percentage survivor.

2.8.1. Percentage parasitemia

The percentage parasitemia, calculated as a percentage ratio of the number of parasitized and total number of parasitized and unparasitized red blood cells was determined from an average of ten fields of count on a microscopic slide.

$$\frac{\text{Number of parasitised erythrocytes}}{\text{Number of Parasitised + unparasitised erythrocytes}} \times 100$$

2.8.2. Percentage chemosuppression

The percentage chemosuppression of parasitemia per test dose was calculated relative to the untreated group (negative control) from the percentage parasitemia, using the formula:

$$\frac{\text{Percentage parasitemia in negative control} - \text{Percentage parasitemia in test dose}}{\text{Percentage parasitemia in negative control}} \times 100$$

2.8.3. ED_{50} and ED_{90}

From the percentage chemosuppression, the ED_{50} and ED_{90} were forecasted using Microsoft Office Excel 2019 and Vinstat graph pad software.

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2.8.4. Survival time

The mice were monitored for mortality for 28 days. The number of days each mouse survived from the commencement of the treatment was taken as survival time while the average for the group was recorded as mean ± SEM.

2.8.5. Statistical Analysis

One-way analysis of variance followed by Student New-man Keuls post hoc test was used for comparison to determine the source of significant differences for all values. Values of $p < 0.05$ were considered to be of statistical significance, and data analysis was performed using Vinstatgraphpad software.

3.0. RESULTS AND DISCUSSION

The need to find new effective drugs, which have novel modes of action for the treatment of life-threatening malaria disease is urgent. This is because if the disease is not treated promptly with effective medicines, it may rapidly progress to severe illness and death [18]. The increasing number of cases of resistance to the majority of currently available antimalarial drugs by the *Plasmodium* parasite, even to new ACT drugs has made the management of malaria a big challenge. This is much compounded by increasing resistance by mosquitoes to available insecticides, and the unavailability of effective vaccines and other novel interventions to control the cases [19]. Also, the rural inhabitants are unable to afford very expensive antimalarial drugs like the artemisinin combined therapy (ACTs). All these issues call for an urgent action to counteract thesedevelopments and further affirms the need to resort to ethnomedicine to investigate herbal remedies for their activities against the malarial parasite [20]. Herbal remedies are usually potential sources of medicine, therefore plants used in those remedies and which possess known activities against fever can be tested for antiplasmodial activities [14]. This may eventually lead to the isolation of lead chemicals for the development of new antimalarial drugs [21]*Cleistopholis patens* is widely used in ethnomedicine for the treatment of a myriad of health problems like headache, fever, malaria, infection, hyperlipidemia, rheumatoid arthritis, measles and as anti-fertility [7-11]. This explains its choice for investigation against malarial parasites. In this study, the antimalarial activity of the leaf methanol extract and the partitioned fractions were assessed in chloroquine-sensitive *P. bergheiberghei* infected mice using activity-guided methods.The similarity of the *P. bergheiberghei*to human malaria expressed by Africans is a motivation for utilizing the *in vivo*chemosuppressive antimalarial model in this research [22]. Therefore, the possibility of extrapolating the results to humans is high [23-25].Chloroquine (10mg/kg) was used as the positive control because the plasmodium parasite used for infection of the mice is a chloroquine-sensitive strain [22].

3.1. Antimalarial activity of the methanol extract

The parasitaemia values obtained for all the tested doses of the extract and the positive control were significantly (*P= .001*) different from that of the negative control (Table 1) indicating an intrinsic antiplasmodial activities of the extract and so prove it to be a suitable source of some antimalarial drug molecules [26]. Furthermore, that the activities of the extract at the tested doses were comparable (*P= .06*) to that of the positive control (Table 1) further showed that it has very high antiplasmodial activity and

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may be as effective as the positive control [27].The highest percentage chemosuppression of 77.5 was attained at 400mg/kg while 200 mg/kg and 800 mg/kg gave 76% and 73% suppression respectively. All these activities were comparable (P=. 06) to that of CQ, the positive control which gave 72%. Only at relatively low dose of 100 mg/kg was the extract significantly (*P= .04*) lower in activity with 66% chemosuppression (Table 1). The percentage chemosuppression (65.88 %) at 200 mg/kg reported for *Plumeria alba,*67.72 % for *Morindalucida*at 400 mg and similar values for the methanolic extracts of *Murrayakoenigii* and *Artocarpusaltilis* rightly shows that *C. patens* is an active antimalarial drug [28-31]. The median effective dose ED_{50} (255.83±3.60 mg/kg) and ED_{90} (471.35±15.05 mg/kg) also depicted a relatively high activity of the extract (Table 4). A similarly-active aqueous and ethanol extract of *Moringa oleifera* elicited effective doses, 216.80; 475.58 and 253.46; 426.60 mg/kg respectively in a similar antiplasmodial experiment [15].However, the survival times elicited in mice by the same extract at all the doses tested was comparable (*P= .1*) to the negative control (Table 2) implying that the extract cannot prolong the life of mice beyond the day of drug administration. The lowest dose tested however elicited the highest percentage survivor of 60% (Table 2) in the experiment. The extract at such a dose definitely contain some factors or compounds that promote the survival of a better percentage of the mice after the experiment than in other higher doses.

Table 1: Average percentage parasitemia and percentage chemosuppressionof the methanol extract

Doses (mg/kg)	%PARA±SEM	%CHEMO±SEM
0	4.69 ± 0.25^{b}	0.00 ± 0.00^{a}
100	1.63 ± 0.26^{a}	65.68 ± 9.72^{b}
200	1.16 ± 0.09^{a}	75.45 ± 3.04^{c}
400	1.05 ± 0.09^{a}	77.45 ± 4.39^{c}
800	1.24 ± 0.04^{a}	73.40 ± 2.96^{c}
CQ (10mg/kg)	1.28 ± 0.11^{a}	72.24 ± 7.19^{c}

Keys: Data shows the mean ± SEM, CQ: chloroquine (positive control). Only values with different superscript within the column are significantly different (p<0.05, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test), %PARA: percentage parasitaemia, %CHEMO: percentage chemosuppression.

3.2. Antimalarial activities of the partitioned fractions

In traditional medicine, whole plants or mixtures of plants are used rather than isolated compounds. However, extended studies have shown that crude extract contains a conglomerate of medicinal compounds and often possess greater *in vitro* or/and *in vivo*antiplasmodial activity than isolated constituents at an equivalent dose. Also, it was discovered that the activities of one compound may either mask or potentiate the other. Fractionation of an extract, therefore usually help to eliminate potentially toxic or inactivating compounds while enhancing the potentiating compounds present in the original extract [16]. The partitioned fractions, CPH, CPD, CPE and CPA were tested at 0-80mg/kg. The n-hexane fraction CPH, like the other partitioned fractions gave significantly (*P= .04*) lower parasitaemia levels from

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that of the negative control at all the doses tested. This showed that each of the fractions retain the antimalarial potency of the original extract and the antimalarial compounds in the extract have varying polarities. Furthermore, for CPH, the parasitaemia levels obtained at most of the doses (10-40mg/kg) tested were comparable (*P= .29*) to that of chloroquine, the positive control drug. The highest dose tested, 80mg/kg, gave a significantly (*P= .001*) lower percentage parasitaemia (Fig. 1).

Table 2: Survival time and percentage survivor of the methanol extract.

Dose (mg/kg)	Average survival time (days)	Percentage survivor (%)
0	16.4±2.09[a]	20
100	12.2±2.20[a]	60
200	19.2±2.04[a]	20
400	22.2±2.09[a]	40
800	11.8±4.15[a]	20
CQ (10mg/kg)	18.2±3.90[a]	60

Keys: Data shows the mean ± Standard error of mean, CQ:chloroquine(positive control). Only values with different superscript within the column are significantly different ($p<0.05$, one-way analysis of variance followed by the Student-Newman-Keul's post hoc test).

and higher percentage chemosuppression, which was higher than that of the positive control (Table 3). The n-hexane partition fraction of Stemonocoleus*micranthus* stem bark extract gave a high percentage chemosuppression of 68 % but lesser than that of the ethyl acetate fraction which gave 95% chemosuppression[32].Like CPH, the ethyl acetate partition fraction, CPE gave significantly lower (*P= .001*) % parasitaemia than that of the positive control drug at the highest dose tested.Fraction CPD gave comparable (*P= .29*) parasitaemia values to that of positive control while CPA gave higher (*P= .001*) parasitaemia values than that of the positive control. CPH and CPE may contain constituents that give better activities on the *Plasmodium* than the positive control. For CPA, there was significantly (*P= .001*) higher parasitaemia levels than that of the positive control at all doses tested, particularly, 10mg and 20 mg/kg but those of 40 and 80 mg/kg were significantly (*P= .01*) lower than those of higher doses but comparable (*P= .14*) to that of the positive control .

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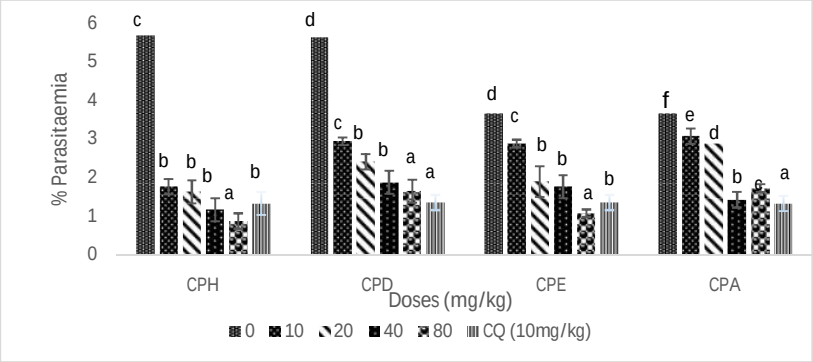

Fig. 1: Percentageparasitaemiaelicited by the partitioned fractions.

Keys: CPH: n- hexanefraction; CPD: dichloromethane fraction; CPE: ethyl acetate fraction; CPA: aqueous fraction.Data show the mean ± SEM, *n* = 5.CQ: chloroquine (positive control). Significant difference ($p < 0.05$, one-way analysis of variance followed by the Student Newman Keul's post hoc test).

Table 3: Percentagechemosuppression of the partitioned fractions

DOSE (mg/kg)	CPH	CPD	CPE	CPA
0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
10	62.56±2.07[b]	37.45±2.75[b]	38.81±2.63[b]	34.63±3.99[b]
20	65.38±3.17[b]	48.81±4.51[b]	59.79±8.63[b]	38.72±0.94[b]
40	75.33±2.67[c]	60.13±6.96[b,c]	62.64±4.98[c]	69.91±3.62[d]
80	81.55±1.64[d]	65.11±6.16[c]	77.19±1.81[c]	63.4±2.20[c]
CQ	71.6±3.22[c]	71.6±3.22[c]	71.6±3.22[c]	71.6±3.22[d]

Keys: CPH: n- hexane fraction; CPD: dichloromethane fraction; CPE: ethyl acetate fraction; CPA: aqueous fraction. Data show the mean ± SEM, *n* = 5. CQ: chloroquine (10 mg/kg) used as the positive control. Significant difference ($p < 0.05$, one-way analysis of variance followed by the Student Newman Keul's post hoc test); from positive control (chloroquine10mg/kg) *: significant difference from negative control.

Comment [C13]: Define what a, b, c, and d stand for in each table. It should be written as a foot note.

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Table 4: Effective doses (ED_{50} and ED_{90})of the extract and the partitioned fractions.

Fraction	ED_{50}	ED_{90}
CPH	25.37±0.41[a]	52.21±0.83[a]
CPD	36.67±1.86[b]	70.69±3.42[b,c]
CPE	32.47±1.69[b]	64.86±4.56[b]
CPA	37.83±1.10[b]	75.39±3.00[c]
Extract	255.83±3.60[c]	471.35±15.05[d]

Keys: CPH: n- hexane fraction; CPD: dichloromethane fraction; CPE: ethyl acetate fraction; CPA: aqueous fraction. Data show the mean ± SEM, *n* = 5.

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Table 5: Survival time and percentage survivor (in parenthesis) of the partitioned fractions

DOSE (mg/kg)	CPH	CPD	CPE	CPA
0	11.6±3.33[a](20)	10.6±1.08[a] (20)	10.6±1.08[a] (20)	11.0 ±1.14[a](20)
10	17.2±1.77[a](20)	11.2± 2.18[a](40)	9.6±1.9[a](40)	11.8±4.32[a](40)
20	13.4±2.02[a](60)	9.8±2.15[a](40)	17.8±2.82[a](60)	20.8±4.93[a](60)
40	15.8±0.73[a](40)	12.4±1.94[a](60)	15.4±5.12[a](40)	18.6±3.23[a](40)
80	14.8±2.82[a](60)	15.0±1.90[a](60)	15.0±1.90[a](60)	17.2±3.73[a](40)
CQ	17.6 ± 4.63[a](60)	11.00 ± 1.27[a](60)	11.0 ± 1.27[a](60)	11.8 ± 1.16[a](60)

Keys: CPH: n- hexane fraction; CPD: dichloromethane fraction; CPE: ethyl acetate fraction; CPA: aqueous fraction. Data show the mean ± SEM, *n* = 5.

3.2.1. Percentage chemosuppression and effective doses of the partitioned fractions

Percentagechemosuppressionelicitedby extract or partition fractions in an antiplasmodial test is a direct measure of their activities on the *Plasmodium* and so their potency as antimalarial agents. From this study, relative to the positive control drug, CPH is the most active as it gave a significantly (*P= .00*) higher percentage chemosuppression at all doses than the other partitioned fractions. This is confirmed by the relatively lower percentage chemosuppression each of them elicited compared to 82% given by CPH at the highest dose tested (Table 3). CPH also gave a significantly (*P= .00*) better activity than chloroquine at 80 mg/kg and a comparable (*P= .001*) activity at 40mg/kg(Fig. 1). CPD and CPE gave comparable (*P= .001*) activities to chloroquine, the positive control drug at 40 and 80mg/kg. However, CPA was comparable (*P= .001*) to chloroquine in activity only at 40mg/kg. The above result indicated that all the partitioned fractions have strong potential as antimalarial agent pointing out to the plant as an antimalarial drug and strongly validating its use in ethnomedicine as such.

Also, the significantly (*P= .0001*) lower effective doses, ED_{50} and $ED_{90}$of CPH (25.37±0.41and 52.21±0.83 mg/kg) when comparedto that elicited by the other partitioned fractions, supported the activity profile shown above, CPD, CPE and CPA being comparable (*P= .002*) to each other but lower in activity to the

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most active CPH(Table 4). The order of activity of the fraction is CPH>CPE>CPD>CPA. This implies that CPH contains more of the antimalarial constituents of the plant and so, it should be further evaluated for the antimalarial compounds of the plant. The effective doses elicited by the partition fractions when compared to that of the methanol extract showed they were more active and that the antimalarial compounds were distributed in the fractions and may have different polarities. Antiplasmodial compounds of different polarities have been isolated from the stem and root of *Gongronemalatifolium*[33].

3.2.2. Survival times and percentage survivor of the partitioned fractions

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Survival times and percentage survivors are complementary to other parameters like percentage chemosuppression and effective doses in assessing the antiplasmodial activities of the extracts of medicinal plants. Survival times is the number of days taken for the mouse to live from the day it started to receive drug against the inoculated parasite while percentage survivors is the percentage of the total number of mice in a group that survived within the average survival time of the group. At all the doses of the methanol extract of C. *patens* tested, the survival times elicited were comparable (*P= .1*) to each other andthe negative control. The ability of the extracts and fractions to arraign enough immunity against the parasite after its suppression in the mice was not strong enough. Hence, the doses tested were not able to prolong the life of the mice beyond the day of drug administration. The percentage survivor, (also called survival rate), is an indirect measurement of the survival of the mice against the effect of the suppressed parasite after series of drug administration to suppress it. The percentage survivor of 60% attained at 100mg/kg was the highest, others were very low. This pattern did not easily correlate with the high % chemosuppression elicited at similar doses (Tables1&2). Such high doses could not produce higher immunity though it produced higher suppression of parasites. In previous similar works, high percentage chemosuppression have been correlated with percentage survivors rather than survival times [34].

Similarly, the survival times for the partitioned fractions were comparable at all the doses including that of the positive control. This implies that neither the extract nor the partitioned fractions could prolong the life of mice better than the negative control (Table 5). However, it was observed that a moderately high, 60% percentage survivor was obtained in all the fractions at 20mg/kg except CPD which incidentally produced 60% at its higher doses of 40 and 80mg/kg. All the fractions elicited percentage survivor of 60% at the highest dose except CPA. Fraction CPA gave its highest percentage survivor and survival times at a relatively lower dose of 20mg/kg (Table 5). In further works, compounds which enhance chemosuppression and those that can increase percentage survivor should be identified while those that can both increase suppression and enhance survivors should be projected as antimalarial compounds.

In summary, the methanol extract of *C. patens* is an active antimalarial agent because of its high chemosuppression of the plasmodium parasite. Its solvent fractions retained the same antiplasmodial activities in the relatively high chemosuppression in the n-hexane fraction, CPH. The moderately high percentage survivor of 60% obtained at the lowest dose of the extract and at 20mg/kg of most of the

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partitioned fractions vividly confirms *C. patens* as a potential candidate for sourcing antimalarial compounds.

4.0. Conclusion

The methanol extract of the leaf of *C. patens* is anantiplasmodial agent with its antimalarial constituents concentrated in the n-hexane solvent fraction.

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.

Comment [C16]: The Animal Research Ethics Committee that approved the research should be stated with the ethical approval number.

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Comment [C17]: Recent references are needed

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Comment [C18]: Follow the same format of referencing. Your references didn't maintain uniformity. The Journal names were abbreviated in some instances and written in full in some instances. Please, unify them following the Journal's style.

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