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Bioactivity guided fractionation of *Icacina trichantha* Oliv. (Icacinaceae) tuber for antimalarial activity against *Plasmodium berghei* infected mice and GC-MS profile of bioactive fraction

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Aqueous methanol extract of the tuber of *Icacina trichantha* was prepared using cold maceration and dried *in vacuo* at 40°C. The extract was purified using solvent-solvent partitioning with *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol. *n*-Hexane fraction was purified using Vacuum Liquid Chromatography, eluting with a gradient of dichloromethane in methanol (9:1, 7:3, 5:5, 0:10, each 500 mL) to obtain four sub-fractions. Acute toxicity study was done using Lorke's method while *in vivo* anti-malarial study was carried out using suppressive model. Phytochemical analysis was carried out using standard procedure and most active sub-fraction was subjected to gas chromatography-mass spectroscopy. The extract at doses of 100, 200 and 400 mg/kg caused a significant ($p<0.001$) increase in percentage suppression of *Plasmodium*: 91.54 %, 94.48 % and 94.58% respectively. Phytochemical analysis of the extract revealed the presence of alkaloids, tannins, flavonoids saponin, glycoside, terpenoids, phenols, steroids, carbohydrates, reducing sugars. The GC-MS analysis showed the presence of eighteen compounds, the most abundant compound includes 9- octadecenoic acid (Z)-, methyl ester (oleic acid, 15.30%), 9, 12-octadecadienoic acid (Z, Z), methyl ester (linoleic acid, 14.34%). These findings suggest scientific evidence in support to the use of *I. trichantha* tuber for the management of malaria.

Keywords: Antimalarial activity, Ethnomedicinal uses, GC-MS analysis, *Icacina trichantha*, *Plasmodium berghei*

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Malaria is well known as a major universal health problem, affecting a large population of the world especially in African countries[1]. Malaria is caused by the parasitic protozoan *Plasmodium falciparum* and other species including *vivax, ovale* and *malariae.* The mosquito which acts as vector is the female *Anopheles funestus, A. mou-cheti, A. gambiae, A. arabiensis* in mammals and *Plasmodium berghei* cause malaria in rodents especially white albino mouse[2]. Malaria causes human mortality and morbidity with a lot of emotional, medical and economic impact in the world[3].

In 2011, 106 countries were at risk of transmission of malaria and approximately 200 million malaria cases were reported in 2012, there were 445,000 deaths and 216 million cases globally in 2016[4]. Pathology of malaria includes anaemia[5], thrombocytopenia[6], acute respiratory distress syndrome[7]. Drugs used in treatment of malaria include quinine, chloroquine, hydroxychloroquine, amodiaquine, pyronaridine, piperaquine, primaquine, artemisinin, artesunate, artemether, arteether, dihydroartemisinin etc[8]. *P. falciparum* is highly resistant to current antimalarial drugs, and majority of antimalarial drugs have various side effects and problems with patient compliance. Hence, there is need for the search for a better, well tolerated and effective antimalarial drugs in most malaria endemic areas[9,10].

Icacina trichantha Oliv. (Icacinaceae) is commonly found in the rain forest region and jungle vegetation of southern Nigeria especially Delta, Edo, Ibadan, Akwa ibom and Enugu State. It is a perennial shrub with scandent growth above with a very large tuber. The Igbos call it *ibugo, ji-muo* and the yourbas call it *gbegbe* (meaning carry away)[11]. The leaves are used by the Yorubas for coronating their chiefs called 'Obas'[12]. *I. trichantha* tuber is extensively used traditionally in treatment of malaria[13], poisoning[14], hepatitis, constipation, induce emesis, rheumatism, toothache and treat soft tumors[15]. Badeku and Jago

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villages of Akinyele L.G.A Ibadan, Oyo State South West Nigeria use the crushed leaves and tuber macerated in local gin to treat malaria. In Ikot Ekpene, Essien Udim, Etim Ekpo, Udung Uko, Ibeno, Itu, Okobo, Uruan, Oron and Ini L.G.A of Akwa Ibom state use the plant in treatment of rheumatism, toothache, abortificent, purgative[16]. It has been reported to show significant antimicrobial and antioxidant activity[16], anti-inflammatory activity[17], antidiabetic activity[18], analgesic effect and antinephrotic, antihepatotoxic and emetic activity[19], antinociceptive activity[20], uterine contractile activity[21]. The study aimed at determination of phytoconstituents and evaluating the antimalarial activity of methanol extracts and fractions of *I. trichantha* tuber *in vivo*.

Methodology

Plant materials

Collection and preparation of plant materials

Sample of *I. trichantha* tuber was collected from Orba, Nsukka, Enugu State Nigeria in March, 2018 by Mr. Felix Nwafor, a taxonomist with the Department of Pharmacognosy and Environmental Medicine of the University of Nigeria, Nsukka, who also identified and authenticated the plant. The voucher specimen: (PCG/UNN/0015) was deposited at herbarium of the above department. The tuber was cleaned and dried in the shade till it was brittle in nature. The dried plant samples were pulverized with an electric blender and stored in amber-coloured bottles prior to extraction.

Extraction of plant material

A certain quantity (1961 g) of the powdered sample was exhaustively extracted with several portions (17,500 mL) of 95% methanol in distilled water by cold maceration for 72 h with frequent agitation and changing the solvent occasionally. The mixture was filtered using muslin and Whatman filters paper no. 1 (Whatman, England). The residue was reextracted for another 72 h twice and filtered. The *I. trichantha* extract (ITE) were dried *in vacuo* at 40ºC. The semi-solid extract was weighed and kept at – 20^0C until use.

Solvent fractionation of crude extract

A certain quantity (100 g) of the methanol crude extract of the tuber was dispersed in 50 mL of 50% methanol and 200 mL of distilled water and successively partitioned with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. First, about 250 mL of *n*-hexane was poured into a separatory funnel and agitated by shaking well. The hexane fraction (HF) was removed by opening the tap and collecting the mixtures separately into another beaker. This process was repeated until the HF was completely extracted to get *I. trichanthan*-hexane fraction (ITHF) (500 mL of *n*-hexane was used). Then, 250 mL of dichloromethane was added to the residuals in the separatory funnel and shaken well. The dichloromethane fraction (DCF) was removed by opening the tap and collecting the mixtures separately into a separate beaker and the process repeated until all the *I. trichantha* dichloromethane fraction (ITDF) was completely extracted (1000 mL dichloromethane was used). To the remaining bulk solution, 250 mL of ethylacetate was added to the separatory funnel and shaken well. The ethylacetate fraction (EAF) was removed by opening the tap and collecting the mixtures separately into a separate beaker and this process was repeated until the *I. trichantha* ethylacetate fraction (ITEF) was completely extracted (2500 mL of ethylacetate was used). Finally, equal volume of distilled water was added to the extract before 250 mL of *n*-butanol was added into the separatory funnel which was shaken. The *n*-butanol fraction (BF) was removed by opening the tap and collecting the mixtures separately into a separate beaker and the process was repeated until the *I. trichantha* water fraction (ITWF) and *n*-butanol fractions (ITBF) were completed extracted (2000 ml of *n*-butanol was used). The *n*-butanol fraction was collected separately from the water fraction (WF). HF, DCMF, EF and water fractions were dried *in vacuo* at 40ºC. BF with the boiling point of 118ºC was mixed with 50 mL of water (100ºC) to form azeotropic mixture which boils below the boiling point of water was used to concentrate the butanol fraction. The fractions were preserved in a refrigerator at 4ºC until required for experiment. The percentage yield of both the crude, fractions and sub fraction of *I. trichantha* tuber extract was also determined from the formula below;

$$\text{Percentage Yield of extract (\%)} = \frac{\text{Weight of Extract}}{\text{Weight of dried pulverized tuber}} \times \frac{100}{1}$$

$$\text{Percentage Yield of fractions (\%)} = \frac{\text{Weight of fraction}}{\text{Weight of methanol extract}} \times \frac{100}{1}$$

$$\text{Percentage Yield of sub fractions (\%)} = \frac{\text{Weight of sub fraction}}{\text{Weight of n} - \text{hexane fraction}} \times \frac{100}{1}$$

Vacuum Liquid Chromatography (VLC)

About 2.5 g of *n*-hexane fraction was dissolved in 50 mL of absolute methanol and titurated with 50 g of silica gel to get a uniform mesh sample. Then the glass column (diameter = 4 cm; length= 150 cm) was packed to two-third with silica gel 100 cm height (100-200 mesh, 500 g: 5699A). For the purpose of this study, solid loading was used. In solid loading of the sample the uniform mesh sample was introduced on the surface of the already well packed adsorbent, which was purified by VLC, using silica gel and eluted with gradient of dichloromethane (DCM) in methanol with different ratios (9:1, 7:3, 5:5, 0:10; 500 mL each) to afford respective sub fractions coded as *n*-HSF_1, *n*-HSF_2, *n*-HSF_3 and *n*-HSF_4 respectively. A moderate vacuum from 20-70 mmHg was used to prevent loss of solvent due to evaporation. The various sub fractions eluted from the VLC were dried at 40ºC and reduced pressure and weighed. The sub fractions were used further for animal studies to screen for the suppressive activity in mouse.

Qualitative phytochemical screening of the extracts and fractions

Phytochemical screening was done using standard procedure as described by Trease and Evans[22]. The methanol extract of *Icacina trichantha* were screened for alkaloids, glycosides, saponin, tannins, terpenoids, steroids, phenols, flavonoids and carbohydrates.

Acute toxicity studies

The acute toxicity parameter (LD_{50}) of the extract was determined using Lorke's method[23]. This method involves two steps and a total of thirteen experimental animals were used. The first step involved the use of nine mice. The nine mice were divided into three groups of three animals per group and administered per oral (p.o.) 10 mg/kg, 100 mg/kg and 1000 mg/kg of methanol extract respectively. Based on the results obtained from the first stage, the remaining animals were grouped into four groups and the second step involved one mouse in each group and specific doses (200 mg/kg, 400 mg/kg, 800 mg/kg, 1600 mg/kg) were administered. The mice were observed for 24 h and deaths were recorded in each case.

The LD_{50} for the crude extract were calculated using the formula:

$$LD_{50} = \sqrt{Minimum\ toxic\ dose \times Maximum\ tolerated\ dose}$$

***In vivo* antimalarial tests**

Experimental animals

Albino mice (6-8 weeks and weight 10.2 - 23.2 g) were procured from the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The mice were allowed to acclimatize for 7 days at the experimental site. They were housed in metallic cages at room temperature of 22±3ºC, relative humidity of 40–50% and 12 h light/ 12 h dark cycle. They were allowed access to feed, with standard pellets (Grand Cereals Ltd, Enugu Nigeria) and water *ad libitum*. *In vivo* antimalarial study on Albino mice was in compliance with the approved protocols (FPSRE/UNN/21/00011) of the University of Nigeria Faculty Research Ethics Committee on the National Code of Conduct for Animal Research Ethics (NCARE)[24].

Parasitological studies

The parasitological study was done *in vivo* for the crude extract, fractions and sub fractions.

Plasmodium preparation and parasite innoculation

Plasmodium berghei was collected from the Department of Parasitology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Blood from the donor mice at peak parasitemia was used. Blood was collected from the tail of the donor mouse and collected into a heparinized syringes. This was diluted serially in phosphate buffered saline (PBS) to make a suspension that contains 1×10^7 infected red blood cell (RBC) in every 0.2 mL suspension. Mice were innoculated intraperitoneally with 0.2 mL blood suspension containing 1×10^7 parasitized erythrocytes on day 0 and were monitored for 3 h before treatment.

Grouping and dosing of animal

The animals were grouped into 15 groups of 5 mice each.

Group 1: infected with 0.2 mL *P. berghei* and treated with 100 mg/kg of ITE.
Group 2: infected with 0.2 mL *P. berghei* and treated with 200 mg/kg of ITE.
Group 3: infected with 0.2 mL *P. berghei* and treated with 400 mg/kg of ITE.
Group 4: infected with 0.2 mL *P. berghei* and treated with 200 mg/kg of ITHF.
Group 5: infected with 0.2 mL *P. berghei* and treated with 200 mg/kg of ITDF.
Group 6: infected with 0.2 mL *P. berghei* and treated with 200 mg/kg of ITEF.
Group 7: infected with 0.2 mL *P. berghei* and treated with 200 mg/kg of ITBF.

Group 8: infected with 0.2 mL *P. berghei* and treated with 200 mg/kg of ITWF.
Group 9: infected with *P. berghei*and treated with 200 mg/kg of *n*-HSF_1.
Group 10: infected with *P. berghei* and treated with 200 mg/kg of *n*-HSF_2.
Group 11: infected with *P. berghei* and treated with 200 mg/kg of *n*-HSF_3.
Group 12: infected with *P. berghei* and treated with 200 mg/kg of *n*-HSF_4.
Group 13: infected with 0.2 mL *P. berghei* and treated with 7 mg/kg of Arthemether/lumefantrine. (Standard antimalarial drug)
Group 14: positive control, infected with 0.2 mL *P. berghei* but not treated.
Group 15: negative control not infected with *P. berghei* and not treated. (10% Tween 80)

Doses were selected based on acute toxicity studies.

The 4 day suppressive test

The infected mice were administered orally 10% v/v tween 80, extract (100, 200 and 400 mg/kg), fractions and subfractions (200 mg/kg) and Arthemether/Lumefantrine 7 mg/kg, respectively. The antiplasmodial activity of *I. trichantha* was evaluated using seventy-five (75) *P. berghei* infected mice according to the method described by Peter *et al.*,[25]. The percentage parasitemia and suppression were determined using this formula;

$$\text{Percentage Parasitemia (\%)} = \frac{\text{Number of parasitized RBC}}{Total\ number\ of\ RBC} \times 100$$

$$\text{\% suppression (\%)} = \frac{\text{Mean parasitemia of negative control} - \text{mean parasitemia of treated group}}{Mean\ parasitemia\ of\ negative\ control} \times 100$$

Determination of effect of *Icacina trichantha* on survival time of mice

The survival time of the mice was calculated for 28 days, immediately after treatment as day 1 till the next 28 days. This is done to ascertain the survival rate of the mice after treatment is given. After the mean survival time was checked, the mice were sacrificed using chloroform anesthesia.

$$\text{Mean survival time MST (days)} = \sum x \div x$$

Where: $\sum x$=Summation of the number of days the individual mice survived in a group.

x= Total number of mice in a group.

Determination of effect of Icacina trichantha on body weight of mice

The weights of the animals were taken under standard conditions. These weights were taken before infection (day 0) and on day 4 (when treatment had stopped).

Determination of effect of Icacina trichantha on rectal temperature of mice

The rectal temperature of the animals was taken before infection; four hours after infection, during treatment and after treatment had stopped for five days. This was carried out to know the antimalarial effect of the plant on increased rectal temperature of the diseased mice.

Determination of effect of Icacina trichantha on packed cell volume (PCV) of mice

Packed cell volume (PCV) was estimated as described by Ochei and Kolhatkar[26]. PCV was measured to predict the effectiveness of the test extract in preventing hemolysis resulting from increasing parasitemia associated with malaria.

$$\text{PCV (\%)} = \frac{Packed\ RBC\ column\ height}{Total\ blood\ volume\ height}$$

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The most potential bioactive non polar subfraction of *n*-hexane of *Icacina trichantha* (*n*-HSF_2), was accurately weighed to 8 mg and was dissolved in dichloromethane as the solvent. GC-MS experiment was done using standard procedure[27].

Statistical analysis

Data were analyzed using Graph pad prism version 5.0 and the results presented as mean ± standard error of mean (SEM) and "n" represents the number of animals per group. One-way analysis of variance (ANOVA) followed by Dunnet multiple comparism *post-hoc* test. $p<0.05$, $p<0.01$ and $p<0.001$ were considered statistically significant.

Materials

Solvents

The solvents used in this study are all of analytical grades and they include: methanol (JHD UN1230, China), dichloromethane (JHD UN1593, China), ethylacetate (JHD UN1173, China), *n*-Hexane (JHD UN1208, China), *n*-Butanol and distilled water.

Equipment and instruments

Equipment and instruments used for this study were obtained from the laboratory units of the Department of Pharmaceutical and Medicinal

Chemistry, Department of Veterinary Medicine, University of Nigeria, Nsukka and Shimadzu Training Centre for Analytical Instruments (STC) Lagos. They include: whatman filter paper no 1 (Whatman, England), electronic weighing balance (PEC MEDICAL, USA), water bath (DK420 Helmreasinn, Germany), micro hematocrit centrifuge (Model SH120-1, England), thermometer (Kris-Aloy International Co., Nigeria), rotary evaporator (Model 349/2, Corning Ltd, England), Refrigerator (Haier thermocool, England), Spectrophotometer (Jeol 400 MHz, Strathclyde Scotland), micro – pipettes (Perfect, USA), microscope (XSZ – 107BN, India), counting chamber (MC Qiujing, China), GCMS-QP2010SE (Shimadzu, Japan).

Reagents and chemicals used for phytochemical analysis

Sulphuric acid (H_2SO_4), chloroform ($CHCl_3$), sodium hydroxide (NaOH), acetic anhydride $(CH_3CO)_2O$, glacial acetic acid, iron (III) chloride ($FeCl_3$), Fehling solutions A and B, Mayer's reagent, Dragendoff reagent, Wagner's reagent, Hagers reagent, silica gel (100-200 mesh, 500 g) and Tween 80.

Results

Qualitative phytochemical analysis

The qualitative phytochemical analysis of ITE of *I. trichantha* tuber revealed the presence of alkaloids, tannins, saponins, glycosides, flavonoids, steroids, terpenoids, phenols, carbohydrates, reducing /free sugar and bound sugar (Table 1). The result also showed that *n*-hexane fractions contained alkaloids, saponins, glycosides, steroids, terpenoids, carbohydrates, reducing sugar.

Antimalarial activity

The data from the antimalarial study (Table 2) revealed that the extract (ITE) produced a significant ($p<0.001$) suppression of parasitemia in the infected mice in a dose dependent manner. All the fractions produced significant ($p<0.001$) suppression of parasitemia. WF produced the highest parasitemia suppression (90.44%) followed by *n*-HF (87.13%), EAF (78.92%) and *n*-BF (66.18%). All the subfractions (*n*-HSF_1 to *n*-HSF_4) also produced significant suppression of the parasite ($p<0.001$) in the range of 90.00 to 76.47% with the *n*-HSF_2 showing the highest effect (90.00%).

Mean Survival Time (MST)

The MST result showed that the fraction *n*-HF ($24.00\pm1.33^{*}$) significantly ($p<0.05$) prolonged the mean survival time of the infected mice (Table 2). The subfraction n-HSF_2 also exhibited moderate prolongation of the survival time when compared to the standard drug ACT.

Body weight and rectal temperature

The fractions *n*-HF and WF revealed an increase in body weight while the subfraction *n*-HSF_2 and *n*-HSF_3 significantly increase ($p<0.05$) in body weight (Table 3). The rectal temperature results shows that the subfraction *n*-HSF_2 and *n*-HSF_4 gives a significant decrease in rectal temperature ($p<0.001$).

Packed Cell Volume (PCV)

Results of PCV (Table 4) showed that the PCV of the mice was lowered on day 4 at the dose of 100 and 200 mg/kg. The plant extract however produced a significant improvement in the PCV of the mice at 400 mg/kg.

GC-MS analysis

The GC-MS analysis (Table 5) showed predominantly the presence of eighteen (18) phytochemical compounds from the non-polar extract *I. trichantha.* From the results we have different classes of

Table 1 — Qualitative phytoconstituents of *Icacina trichantha* extract, its fractions and sub fractions

Phytoconstituent	MCE	*n*-HF	DCMF	ETF	*n*-BF	WF	*n*HSF1	*n*HSF2	*n*HSF3	*n*HSF4
Alkaloids	+	+	+	+	+	+	+	+	+	+
Tannins	+	-	+	+	+	+	-	-	-	-
Flavonoids	+	-	+	+	+	+	-	-	-	-
Saponin	+	+	+	+	+	+	-	+	+	+
Terpenoids	+	+	+	+	-	-	+	+	+	-
Glycosides	+	+	+	+	+	+	+	+	+	+
Phenols	+	-	+	+	+	+	-	-	-	-
Steroids	+	+	+	+	-	-	+	+	+	-
Carbohydrates	+	+	+	+	+	+	-	+	+	+
Reducing sugar	+	+	+	+	+	+	-	+	+	+

Key: += Present, - = Absent. MCE=Methanolic crude extract, *n*-HF= *n*-Hexane fraction, DCMF= Dichloromethane fraction, ETF= Ethylacetate fraction, *n*-BF= *n*-Butanol fraction, WF= Water fraction , *n*-HSF1= *n*-Hexane subfraction DCM: MeOH (9:1), *n*-HSF2= *n*-Hexane subfraction DCM: MeOH(7:3), *n*-HSF3=*n*-Hexane subfraction DCM: MeOH (5:5), *n*-HSF4=*n*-Hexane subfraction MeOH(100%).

Table 2 — Effect of the extract and fractions of *I. trichantha* on the parasitemia level and mean survival time of mice using suppressive model

Treatment	Dose (mg/kg)	Mean Parasitemia	Percentage Parasitemia (%)	Percentage Suppression (%)	Mean Survival Time (Days)
ITE	100	5.75±0.75***	0.62±0.12***	91.54	11.50±0.65
ITE	200	3.75±0.75***	0.36±0.08***	94.48	14.25±1.49
ITE	400	3.68±1.20***	0.34±0.11***	94.58	19.33±0.88
n-HF	200	8.75±2.14***	0.84±0.19***	87.13	24.00±1.33*
EAF	200	14.33±5.36***	1.49±0.58***	78.92	15.00±2.00
n-BF	200	23.00±5.57***	2.62±0.70***	66.18	19.67±1.76
WF	200	6.50±0.87***	0.70±0.10***	90.44	17.00±1.96
n-HSF_1	200	14.00±1.87***	1.51±0.26**	79.41	18.20±1.96
n-HSF_2	200	6.80±0.97***	0.81±0.16***	90.00	22.00±1.52
n-HSF_3	200	16.00±0.27***	1.89±0.30***	76.47	19.75±1.65
n-HSF_4	200	14.25±3.12***	1.63±0.34**	79.04	17.00±2.94
ACT	7	3.20±0.73***	0.32± 0.08**	95.29	24.60±1.33*
Normal		-	-	-	26.00±0.95**
Control		68.00±3.11	10.71±0.63	0.00	19.00±1.87

Results are expressed as Mean ± SEM (n=5),*p<0.05,**p<0.01,***p<0.001, HF= *n*-Hexane fraction, EAF= Ethyl acetate fraction, BF= Butanol fraction, WF= Water fraction, *n*-HSF1= 9:1 DCM: MEOH *n*-Hexane sub fraction, *n*-HSF2= 7:3 DCM: MEOH *n*-Hexane sub fraction, *n*-HSF3= 5:5 DCM: MEOH *n*-Hexane sub fraction, *n*-HSF4= 100% MEOH n-Hexane sub fraction, ACT= Artemether/ Lumefantrine, Control= Parasitized with *Plasmodium berghei* but not treated, Normal= Not Parasited and not treated.

Table 3 — Body weight and rectal temperature of mice treated with methanolic extract, fractions and sub fractions of *Icacina trichantha* tuber

		Body weight (g)			Rectal Temperature (ºC)		
Treatment	Dose (mg/kg)	Day 0	Day 4	Percentage Change (%)	Day 0	Day 4	Percentage Change (%)
ITE	100	16.28±0.77	17.25±1.40	5.96	36.28±0.25	35.75±0.38*	-1.46
ITE	200	15.12±1.17	14.95±0.95	-1.12	35.36±0.43	35.25±0.39*	-0.31
ITE	400	17.06±1.52	15.73±1.12	-7.80	36.06±0.41	36.47±1.17	1.14
n-HF	200	14.72±0.57	16.70±0.60	13.45	35.90±6.46	36.53±0.51	1.75
EAF	200	19.32±1.48	18.60±0.99	-3.73	36.26±0.53	35.77±0.32	-1.35
n-BF	200	15.60±1.37	15.60±2.08	0.00	35.62±1.12	37.13±0.54	4.24
WF	200	15.28±1.63	16.15±1.87	5.69	35.80±0.77	36.03±0.69	0.64
n-HSF1	200	17.44±0.66	18.64±0.62	6.88	35.62±0.32*	36.02±0.28*	1.12
n-HSF2	200	18.56±0.69	19.42±0.74*	4.63	35.50±0.58*	35.46±0.44**	-0.11
n-HSF3	200	18.92±1.04	19.90±1.46*	5.18	35.34±0.32*	35.88±0.39*	1.53
n-HSF4	200	17.72±0.75	18.60±1.02	4.97	35.86±0.36	35.50±0.26***	-1.00
ACT	7	19.56±1.45	18.70±1.34	-4.40	37.46±0.51	35.88±0.58*	-4.22
Normal		17.76±1.46	16.52±1.22	-6.98	37.08±0.33	37.18±0.52	0.27
Control		17.00±1.03	14.85±0.91	-12.65	37.20±0.27	38.00±0.20	2.15

Results are expressed as Mean ± SEM (n = 5), %change = Day 4 – Day 0 ×100 Day 0 1
[k] p<0.05, **p<0.01,*** p<0.001, *n*-HF= *n*-Hexane fraction, EAF= Ethyl acetate fraction, *n*-BF=*n*-Butanol fraction, WF= Water fraction, *n*-HSF1= 9:1 DCM: MEOH *n*-Hexane sub fraction, nHSF2= 7:3 DCM: MEOH n-Hexane sub fraction, *n*-HSF3= 5:5 DCM: MEOH *n*-Hexane sub fraction, *n*-HSF4= 100% MEOH *n*-Hexane sub fraction, ACT= Artemether/Lumefantrine, Control= Parasitized with *Plasmodium berghei* but not treated, Normal= Not Parasited and not treated.

compounds which include: fourteen esters (14), one alcohol (1), one amide (1), one saturated fatty acid (1) and one acyclic alkane (1).

Discussion

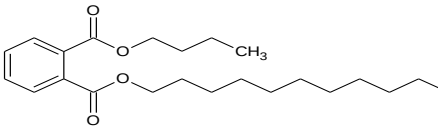
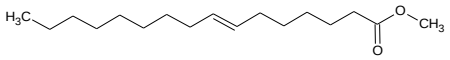
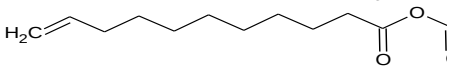
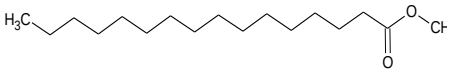
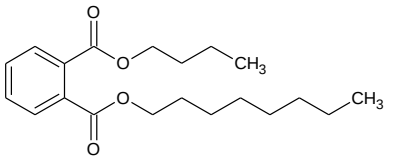
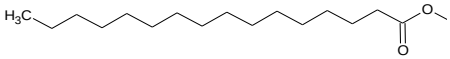
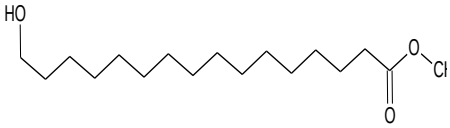
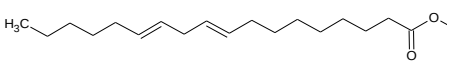
Icacina trichantha (Icacinaceae) tuber extract is used in folk medicine as antioxidant[16], analgesic, anti-inflammatory[17], antidiabetic[18], emetic, antinephrotoxic, antihepatoxic[19], aphrodisiac and in management of poisoning and constipation[19]. From the antimalarial studies, result have shown that *I. trichantha* (tuber) possess a significant antimalarial activity. This observation together with the result of the present study seems to validate the traditional use of

Table 4 — Effect of *Icacina trichantha* on packed cell volume (PCV) of mice in a suppressive antimalarial model

Treatments	Dose (mg/kg)	Packed cell volume (%)		
		Day 0	Day 4	% Change
ITE	100	41.20±0.86	34.00±3.19	-17.48
ITE	200	41.00±0.63	38.50±1.71**	-6.10
ITE	400	38.60±0.98	39.00±2.52**	1.04
ACT	7.0	40.00±0.71	40.00±0.84*	0.00
Control		39.20±0.86	27.75±1.44	-29.21

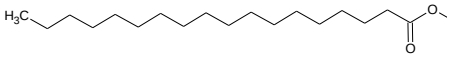
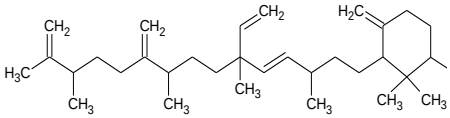
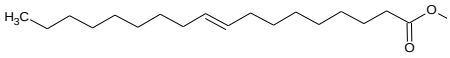
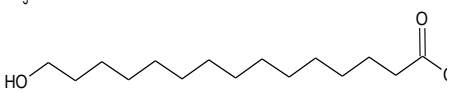
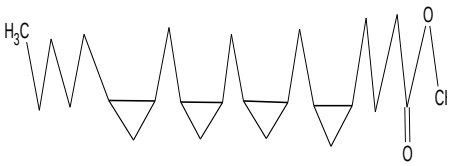
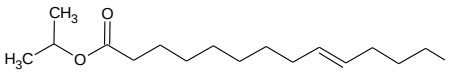
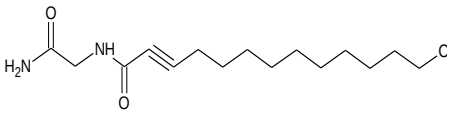
Results are expressed as Mean ± SEM (n =5), * $p<0.05$, ** $p<0.01$, *** $p<0.001$, not significant at $p>0.05$, 100 mg/kg, 200 mg/kg and 400 mg/kg shows the dose of the methanol extract, ACT= Artemether/ Lumefantrine, Control= Parasitized with *Plasmodium berghei* but not treated, Normal= Not Parasitized and not treated

Table 5 — GC-MS analysis of *n*-hexane sub fraction (7:3 DCM: MEOH) of *I. trichantha* tuber

S/N	Retention Time	Name of Compound[a]	Class of Compound	Molecular Weight	Compound	Peak area (%)	Molecular Formular	Compound structure
1.	15.252	Phthalic acid, Butyl undecyl ester	Ester	376	Compound 1	1.53	$C_{23}H_{36}O_4$	
2.	15.514	7-Hexadecenoic acid, methyl ester	Ester	268	Compound 2	1.09	$C_{18}H_{34}O_2$	
3.	15.602	Vinyl 10-undecenoate	Ester	210	Compound 3	0.72	$C_{20}H_{34}O$	
4.	15.651	Hexadecanoic acid, methyl ester	Ester	270	Compound 4	8.21	$C_{17}H_{34}O_2$	
5.	15.950	1,2-Benzenedicarboxylic acid, butyl octyl ester	Carboxylic acid Ester	334	Compound 5	3.95	$C_{20}H_{30}O_4$	
6.	16.126	Hexadecanoic acid, ethyl ester	Ester	284	Compound 6	2.30	$C_{18}H_{36}O_2$	
7.	16.358	Methyl 16-hydroxy-hexadecanoate	Ester	286	Compound 7	2.30	$C_{17}H_{34}O_3$	
8.	16.844	9,12-Octadecadienoic acid (Z,Z), methyl ester	Fatty acid Ester	294	Compound 8	14.34	$C_{19}H_{34}O_2$	
9.	16.882	9- Octadecenoic acid (Z)-, methyl ester Or Oleic acid methyl ester	Fatty acid Ester	296	Compound 9	15.30	$C_{19}H_{36}O_2$	

(*Contd.*)

Table 5 — GC-MS analysis of *n*-hexane sub fraction (7:3 DCM: MEOH) of *I. trichantha* tuber (*Contd*.)

S/N	Retention Time	Name of Compound[a]	Class of Compound	Molecular Weight	Compound	Peak area (%)	Molecular Formular	Compound structure
10.	17.034	Methyl stearate	Ester	298	Compound 10	4.81	$C_{19}H_{38}O_2$	
11.	17.280	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane	Acyclic alkane	452	Compound 11	12.88	$C_{33}H_{56}$	
12.	17.316	(E)-9-Octadecenoic acid ethyl ester	Unsaturated fatty acid Ester	310	Compound 12	6.21	$C_{20}H_{38}O_2$	
13.	18.940	1-Heptatriacotanol	Alcohol	536	Compound 13	8.38	$C_{37}H_{76}O$	
14.	19.698	Butyl 9-tetradecenoate	Ester	282	Compound 14	5.71	$C_{18}H_{34}O_2$	
15.	19.910	15-Hydroxypentadecanoic acid	Saturated fatty acid	258	Compound 15	4.79	$C_{15}H_{30}O_3$	
16.	20.140	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]-,methyl ester	Ester	374	Compound 16	3.93	$C_{25}H_{42}O_2$	
17.	20.260	i-propyl 9-tetradecenoate	Ester	268	Compound 17	1.42	$C_{17}H_{32}O_2$	
18.	20.939	2-Myristynoyl-glycinamide, N-(2-Amino-2-oxoethyl)-2-tetradecynamide	Amide	280	Compound 18	2.15	$C_{16}H_{28}N_2O_2$	

Components listed in order of elution. Compounds identified through NSIT 11 mass spec. li

I. trichantha in treatment of malaria. The *in vivo* model was used to check the involvement of the immune system in combating the infection[28].

The toxicity test (LD_{50}) showed that the tuber of the plant is relatively safe at a dose of 1265 mg/kg. high LD_{50} obtained for any extract indicates that the extract and fraction is relatively safe[29]. Among the fractions, water fractions, *n*-hexane and ethylacetate were found to possess higher antiplasmodial potentials, suggesting the possible localization of the active ingredient in these fractions. Similar observations have been reported in previous studies[30]. Antimalarial treatment with a known compound like artemisinin is associated with a rapid decline in parasitemia[31].

The mean survival time of the various groups that were administered with the fractions relative to the control also helped prove the antimalarial efficacy of the plant. This could be due to suppression of the parasite from multiplying in the bloodstream.

Measurement of packed cell volume (PCV) was done to evaluate the effectiveness of the methanol

extract in preventing hemolysis due to increasing parasitemia level. Blood was used as a diagnostic tool in this study because it is used as a good indices in assessment of haematological parameters[32,33]. The low PCV value observed in 100 and 200 mg/kg of ITE with a corresponding high number of parasite was suggested to be due to excessive destruction of red blood cell by the parasite which could lead to anaemia if left untreated and this suggest that the extract at low doses where not able to prevent hemolysis of the RBC. As observed in this study, it was established that the more malaria parasite is in the blood circulation it will cause severe destruction of the red blood cell as demonstrated by the low PCV. The significant reduction in PCV level indicates a relationship between malaria parasite and anemia[34]. From the study, we could suggest that the potential mechanisms contributing to malaria is multifactorial, there is lysis of parasitized erythrocytes, inadequate reticulocyte production, dyserythroporetic changes, immune mediated haemolysis, phagocytosis, splenic sequestration, bone marrow suppression, effects of inflammatory cytokines, effects of parasite factors and reduction in the lifespan of the erythrocytes[17,35]. The lysis of parasitized erythrocytes will inevitably lead to black urine, increase in body temperature and loss of body weight[36]. Anemia, body weight loss and fever are the general features of malaria infected mice[37]. The increase in body weight of the animals observed for the fraction may be due to constant feeding of the animals.

Rectal temperature determination revealed the plant have an analgesic effect which has been shown in previous studies, the mechanism by which the extract inhibits increase in temperature is not yet known, but a free radical scavenging activity was hypothesized[17].

All the mice used for dichloromethane fraction died on the third day of treatment, thus there was no result of parasitemia for dichloromethane fraction and this could be that at 200 mg/kg dose of the dichloromethane fraction was not enough to suppress the parasite in the red blood cell. Before the mice died it exhibited symptoms such as diarrhea, chills and shivering, reduced activity, pallor, poor appetite, fever/increased rectal temperature, dark urine. All these symptoms showed that the mice have severe malaria. The qualitative phytochemical analysis of the methanol extract, fractions and sub fraction of *I. trichantha* shows that the plant is rich in phytoconstituents. The active compound responsible for the antiplasmodial activity is yet to be identified. Antiplasmodial potentials of *I. trichantha* could be attributed to a single or a combination of its phytoconstituents such as alkaloids, flavonoids, terpenoids, steroids, glycosides, tannins, saponins, reducing sugars, phenols and carbohydrates (Table 1) in high concentrations. The plant owes its medicinal properties to these phytoconstituents[20]. Alkaloids have been implicated in antimalarial activity of many plants[38]. Terpenoid, saponin and steroid have also been found to be detrimental to several infectious protozoans such as *P. falciparum*[39]. Tannins are phytoconstituents used in treatment of inflamed tissues and have been reported to have good potentials in malaria suppression[40]. Flavonoids acts by releasing its anion radicals which circulate to tissues and also by inhibition of membrane bound enzymes such as ATPase and phospholipase A_2 thereby serving as a health promoter[41]. Phenols are the most effective phytoconstituents in protecting the body against different oxidative stressors, phenolic compounds also have anticancer, antiplasmodial and antioxidant potentials[22]. Alkaloids have been shown to have antiplasmodial potentials by inhibiting the actions of plasmodium from lysing the cell membrane[42]. Jeruto *et al*. [43] have reported that flavonoids, steroids and terpenoids have protective effect on RBC due to its antioxidant properties. Eighteen (18) bioactive compounds were predominantly suggested in the sub fraction of *n*-hexane *I. trichantha* tuber. Gas Chromatography-Mass Spectroscopy analysis of the volatile chemical compositions of *I. trichantha* from the sub fraction of *n*-hexane were highly complex containing fatty acids, esters, alcohol, amide, acyclic compounds (Table 5). Out of the eighteen compounds present in *n*-HSF_2, 9,12-octadecadienoic acid (Z,Z), methyl ester (14.34 %), 9- octadecenoic acid (Z)-, methyl ester or oleic acid methyl ester (15.30%), 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl) cyclohexane (12.88%), 1-heptatriacotanol (8.38%) have the highest abundance as shown in their peak area. Thus in the present study, it is possible that the oleic acid and linoleic acid acts in synergy with other identified constituents to exert the antiplasmodial activities exhibited by *I. trichantha* tuber. Most of these constituents have been found to show interesting biological activities against certain illnesses and pathogens, for instance, antibacterial, antioxidant,

antimicrobial[16], antiinflammatory[17], cytotoxic, anticancer activities have been reported. Saturated fatty acids such as oleic acid, a bioactive compound have been scientifically reported to have antimalarial activity[44]. In summary, fatty acids could play some role in the antiplasmodial activity of *I. trichantha* tuber.

Conclusion

The methanol extract and fractions of *Icacina trichantha* possess a good antiplasmodial activity. The crude, fractions and subfractions of the plant can be used for the management of malaria in herbal medicine and can lead to development of lead compound. There was a reduced parasite burden, relative increase in body weight, reduction in rectal temperature and this justify the traditional usage of this species by the local healers in Nigeria for various ailments.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Authors' Contributions

ICA: carried out the animal studies, phytochemical studies, literature search and data analysis; ICA and PFU: participated in writing the manuscript, literature search and data analysis; and NJN: participated in *in vivo* studies and data analysis; All authors discussed the results and contributed to the final manuscript.

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