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Research Paper

Effects of Aqueous Leaf Extract of *Cola acuminata* on Parasitaemia, Haematological and Liver Function Parameters in *Plasmodium berghei* Infected Mice

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Evaluation of the antimalarial activity of the aqueous leaf extract of *Cola acuminata* was carried out on mice infected with *Plasmodium berghei*. Six groups of 10 mice each were used. Groups D, E and F were treated with 50, 150 and 250 mg/Kg body weight of the extract respectively. Group C was treated with 20mg/Kg body weight chloroquine. Groups A and B served as controls. Treatment commenced 3 days after inoculation and lasted 3 days; and 7 days after inoculation, 6 mice from each group were sacrificed; liver function and haematological indices were evaluated. Tail blood was collected from the remaining animals on days 7, 9, 11 and 13 after inoculation for evaluation of parasitaemia. The extract contains alkaloids, phenolics, tannins, flavonoids, saponins, glycosides and terpenoids. Treatment significantly reduced ($p<0.05$) parasitaemia comparing favourably with chloroquine at the highest dose. Infection caused a significant decrease ($p<0.05$) in packed cell volume, haemoglobin, red blood cells, platelets, total protein, and the activity of alkaline phosphatase but caused a significant increase ($p<0.05$) in mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cells and neutrophils and activities of aspartate transaminase and alanine transaminase. These were ameliorated by treatment. Leaves of *Cola acuminata* possess good potentials as antimalarial remedy and improves some of the complications of malaria infection.

Key words: *Cola acuminata, Plasmodium berghei,* antimalarial, haematological indices, liver function indices.

INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans of the genus *Plasmodium* (WHO, 2015). Four types of *Plasmodium* species (*Plasmodium falciparum, malariae, vivax and ovale*) which are all transmitted by the bite of a female *Anopheles* mosquito generally infect humans. *Plasmodium falciparum* causes the most serious form of the disease. A fifth specie, *Plasmodium knowlesi* which is a simian parasite has been shown to infect humans but rarely (WHO, 2014). The disease is prevalent in tropical and subtropical regions and is mostly associated with poverty. There were 214 million cases of malaria worldwide in 2015; 90% of which occurred in Sub Saharan Africa. Out of these, 438,000 resulted in death globally of which 78% were children under the age of five (WHO, 2015). Nigeria and the Democratic Republic of Congo accounted for 35% of the total deaths in 2015. Although several control and treatment strategies exist for malaria, the parasite has developed resistance to many antimalarial medications especially chloroquine and even artemisin in some parts of South East Asia (Caraballo, 2014). This therefore creates the need for continuous search for novel treatment options. In countries where malaria is endemic, including Nigeria; medicinal plants have served as sole source or alternative therapy for malaria for quite a long time.

This practice has its origins in folklore and cultural practices (Jeruto et al., 2015). Two of the most potent antimalarial drugs were obtained fromquinine (obtained from *Cinchona* species) and artemisinin (from *Artemisinia annua)*. Other plants and herbs taken traditionally to treat malaria have also been documented and some have been validated by scientific methods (Keay, 1996). However many others taken to treat or manage malaria have not been documented in terms of efficacy or safety; one of such is *Cola acuminata.*

Cola acuminata belongs to the family *Sterculiaceae*. It has about 125 species native to the tropical rainforests of Africa (Abiodun et al., 2014). *Cola acuminata* fruits commonly called kolanuts are frequently used in social and religious ceremonies in Nigeria. Kolanuts are also used in food and pharmaceutical industries as a source of caffeine and as a component in many formulations (Fabunmi and Arotupin, 2015). The plant's traditional uses include: cancer treatment, an antidote for poisoning, appetite suppressant, increasing alertness, treating migraine and motion sickness and obtaining a state of euphoria (Lowe et al., 2014).*Cola* spp. varieties have been shown to have diverse pharmacological effects including antimicrobial, cytotoxic (Durand et al., 2015) and antioxidant properties (Durand et al.,2015; Fabunmi and Arotupin, 2015). Plants that have these properties sometimes also possess antimalarial potentials. This study therefore aims to investigate if the leaves of *Cola acuminata* has any antimalarial activity and if its components play a role in ameliorating the complications that accompany malaria infection.

MATERIALS AND METHODS

Chemicals and reagents

Chloroquine diphosphate salt was obtained from Sigma Chemical Company, St. Louis, Mo, USA, Giemsa stain was obtained from Anosantec Laboratories, UK and immersion oil was obtained from Panzonar Laboratory Supplies, Canada. All other reagents used were of analytical grade and prepared in all glass distilled water.

Obtaining animals and parasites

Swiss albino mice with an average weight of 22±1.0 g were obtained from the animal breeding unit of the Department of Biochemistry, University of Jos, Plateau State. The mice were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad libitum*. The antimalarial test was carried out using a chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) obtained from the Department of Biochemistry, Federal University of Technology, Minna, Niger State. The parasites were maintained by weekly passing in to naïve mice.

Obtaining plant and extract preparation

Fresh leaves of *Cola acuminata* were collected from a farmland in Isiala Ngwa North Local Government of Abia State and were authenticated at the Forestry Department, Modibbo Adama University of Technology, Yola. Fresh leaves of the plant were dried in the shade at room temperature (25± 2°C) and pulverized to powder using an electric blender (Crush Bull, E139, China). Two hundred gram (200 g) of the powder was macerated in 1500ml of distilled water in dark bottles for 48 hours and thereafter filtered using Whatman filter paper No 1 and concentrated in a water bath at 40ºC. The dried extract was weighed and reconstituted with distilled water into desired doses. The extract was screened for phytochemical constituents as described by Sofowora (1980) and Evans (2005).

Animal inoculation and extract administration

Evaluation of the antimalarial potentials of the aqueous extract of *Cola acuminata* leaf was carried out as described by Nogueira and Virgilio (2010). Sixty naïve mice were divided into six groups (A-F) of 10 mice each. Groups B-F were inoculated with the rodent malaria parasite *Plasmodium berghei* from the same donor mouse. Before inoculation, the percentage parasitaemia and the red blood cell count of the donor mouse was first determined using a haemocytometer and appropriate dilutions of the infected blood with isotonic saline were made. Each mouse was inoculated intraperitoneally on day 0 with 0.2 ml of infected blood containing about 1 x 10^7 *Plasmodium berghei* parasitized red blood cells.

Treatment was withheld for 72 h to allow for establishment of infection and it was commenced when parasitaemia was confirmed in the inoculated animals by screening their tail blood for malaria parasites. A drop of tail blood each from the animals was smeared on glass slides and allowed to dry; the slides were then fixed in methanol; stained with Giemsa and observed using X 100 objective lens. Treatment was given orally once daily for three consecutive days. Groups D, E and F were treated with 50, 150 and 250 mg/Kg body weight of the aqueous leaf extract of *Cola acuminata* respectively. Group C was treated with 20 mg/Kg body weight of chloroquine. Groups A and B served as normal and untreated controls respectively.

On day 7 after inoculation, blood was collected from the tail of each mouse and thin films were made on a microscope slide and examined microscopically to determine the parasitaemia and calculate percentage chemosuppression. On the same day, 6 mice from each group were sacrificed under slight diethyl ether anaesthesia

and blood was collected through cardiac puncture for the evaluation of liver function and haematological indices. Additional smears were taken from the remaining animals in each group on days 9, 11 and 13 post inoculation to track the progress of parasitaemia. Percentage parasitaemia and percentage chemosupression was calculated by the formula below as described by Manser et al. (2013).

$$\text{Percentage parasitaemia} = \frac{\text{No. of Parasitized RBC}}{\text{No. of Parasitized RBC + Non Parasitized RBC}} \times 100 \quad (1)$$

$$\text{Chemo-suppression A} = \frac{B-C}{C} \times 100 \quad (2)$$

Where B = parasitaemia in study group and
C = parasitaemia in control.

Test for liver function indices

Bilirubin was determined by the method of Walter and Gerard, (1970). Serum total protein was determined by the method of Nishi et al. (1985) and serum albumin was determined by the method of Doumas et al. (1971).

Test for haematological parameters

Heamatological parameters including Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), neutrophils (NEU), lymphocytes (LYM) and platelet count were determined using the automated haematological analyzer SYSMEX KX21, a product of SYSMEX corporation, Japan.

Enzyme assays

Alkaline phosphatase activity of serum was determined as described by Wright et al. (1972). Alanine and Aspartate aminotransferaseactivities were assayed by the method described by Huang et al. (2006).

Statistical analysis

The group means ± SEM for each parameter was calculated and significant differences were determined by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% confidence level using SPSS-PC programme package (Version 22.0 SPSS Inc. Chicago).

RESULTS

Phytochemical tests

The aqueous leaf extract of *Cola acuminata*was found to contain alkaloids, phenolics, flavonoids, saponins, tannins, glycosides and terpenoids.

Antimalarial test

Extracts that cause less than 30% chemosupression in *in vivo* antimalarial studies are considered inactive. Those that cause between 30-50% chemosupression are considered partially active. Only those that cause above 50% chemosupression are considered active. Going by this, none of the extracts were active on day 7 post inoculation; although they all gave some level of chemo suppression presented in (Table 1). On day 9 post inoculation, only the group treated with chloroquine was active, the extract produced only partial activity at all doses.

On day 11 post inoculation, only the group treated with 50 mg/Kg body weight extract had partial activity. All the other groups were active. On day 13 post inoculation, all the treated groups were active. The group treated with the highest dose of extract compared favorably with the group treated with chloroquine.

Table 2 shows the effects of administration of the aqueous leaf extract of *Cola acuminata* on the liver function indices of mice. Infection caused a significant decrease ($p<0.05$) in the concentration of total protein which was ameliorated by treatment. Total bilirubin concentration was also significantly elevated ($p<0.05$) in the groups treated with 50 and 150 mg/Kg body weight of the extract compared to controls. There were no significant changes in the other parameters compared to control.

Cellular Enzymes

Figure 1 shows the effects of the aqueous extract of *Cola acuminata* on some serum enzymes in mice. Infection with *Plasmodium berghei* significantly increased ($p<0.05$) the activities of aspartate and alanine transaminases (AST and ALT) compared to control. This was restored towards normal in a dose dependent manner by treatment. Infection however, significantly reduced ($p<0.05$) the activity of alkaline phosphatase (ALP); this was also ameliorated by treatment (Figure 1). Values are means of 6 replicates ± SEM. Bars for each tissue with different letters are significantly different ($p<0.05$).

Table 1. Effects of *Cola acuminata* aqueous leaf extract on parasitaemia in *Plasmodium berghei* (nk 65)-infected mice.

	Parasitaemia (%) (% Chemosupression)			
Treatment	**7°**	**9°**	**11°**	**13°**
Untreated Control	10.20±5.29	13.43±0.76	15.63±0.73	17.80±1.22
Chloroquine (20 mg/Kg b.wt)	6.83±0.37 (21.00)	5.10±0.07 (62.00)	2.03±0.18 (71.00)	0.43±0.24 (99.50)
50 mg/Kg b.wt extract	10.8±0.25 (3.30)	9.23±0.356(31.00)	86±0.44 (50.00)	5.22±0.33 (70.00)
150 mg/Kg b.wt extract	10.03±0.34 (3.80)	8.53±0.225 (40.10)	31±0.26 (65.70)	4.68±0.21 (75.00)
250 mg/Kg b.wt extract	9.03±0.55(12.70)	6.76±0.054 (45.20)	71±0.35 (67.00)	1.81±0.51 (89.00)

Values are means of 4 replicates. ° Days post-inoculation; b.wt., body weight. The figures in brackets show percentage chemo suppression on each. day.

Table 2. Effects of aqueous extract of *Cola acuminata* leaf on liver function indices of mice infected with *Plasmodium berghei.*

Treatment	Albumin (g/dL)	Total protein (mg/ml)	Total bilirubin (mg/dL)	Conjugated bilirubin (mg/dL)
Control	40.86± 12.60^{a}	52.70± 12.20 ^{a}	3.83 ± 0.90 ^{a}	1.80 ± 0.30 ^{a}
Untreated control	38.95± 3.45 ^{a}	44.13± 3.80^{b}	2.96 ± 0.17 ^{a}	2.00 ± 0.28^{a}
Chloroquine (20mg/Kg b.wt)	45.10± 0.95 ^{a}	46.81± 0.80^{b}	3.53 ± 0.14 ^{a}	2.10 ± 0.39^{a}
50 mg/Kg b.wt	36.61± 3.24 ^{a}	42.93± 2.48^{b}	7.25 ± 0.63^{b}	3.14 ± 0.73^{a}
150 mg/Kg b.wt	40.88± 3.71 ^{a}	43.30±2.98 ^{b}	7.30 ± 0.51 ^{b}	3.83 ± 1.87^{a}
250 mg/Kg b.wt	40.70 ± 1.69^{a}	43.73± 1.60^{b}	4.75 ± 1.79^{a}	2.45 ± 1.37^{a}

Values are means of 6 replicates ±SEM. Means in the same column with different superscripts are significantly different ($p<0.05$). B.wt=body weight.

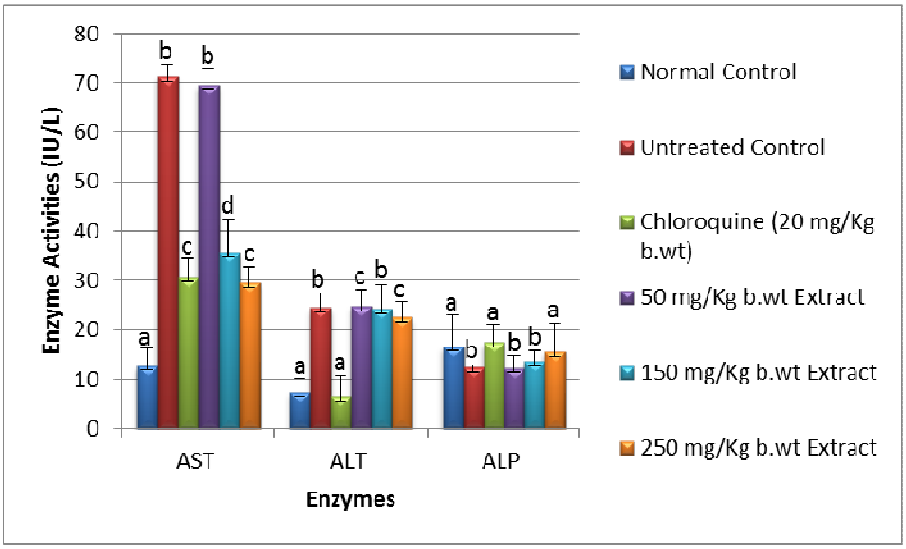

Figure 1. Effects of aqueous extract of *Cola acuminata* leaf on the activities of alanine transaminase, aspartate transaminase and alkaline phosphatase in serum of mice infected with *Plasmodium berghei.*

Red blood cell indices

Infection with *Plasmodium berghei* significantly reduced ($p<0.05$) the packed cell volume, haemoglobin and red blood cells, (Table 3).These were increased towards normal by treatment with chloroquine. Although treatment with the extract increased these parameters in a dose dependent manner, the increase was not significant ($p>0.05$). Infection also caused a significant increase ($p<0.05$) in MCV, MCH, MCHC. This was improved by treatment (Table 4).

White blood cell indices

Infection also caused a significant increase ($p<0.05$) WBC, and neutrophils (Table 5). Treatment with chloroquine and the extract was also able to restore them

Table 3. Effects of aqueous leaf extract of *Cola acuminata* on PCV, Hb and red blood cells of mice infected with *Plasmodium berghei.*

Treatment	PCV (%)	Hb (g/L)	RBC (x10^{12}/L)
Normal Control	36.12±3.07^{a}	10.32±0.72^{a}	7.36±0.46^{a}
Untreated Control	13.67±1.31^{b}	4.76±0.42^{b}	2.20±0.16^{b}
Chloroquine (20 mg/Kg b.wt)	39.48±3.61^{a}	11.72±1.09^{a}	7.43±0.77^{a}
50 mg/Kg b.wt	15.80±5.22^{b}	4.82±1.66^{b}	2.35±0.95^{b}
150 mg/Kg b.wt	16.19±2.90^{b}	5.46±0.76^{b}	2.86±0.70^{b}
250 mg/Kg b.wt	17.56±2.80^{b}	5.74±0.87^{b}	3.11±0.74^{b}

Values are means of 6 replicates ±SEM. Means in the same column with different superscripts are significantly different ($p<0.05$).

Table 4. Effects of aqueous leaf extract of *Cola acuminata* on MCV, MCH and MCHC of mice infected with *Plasmodium berghei.*

Treatment	MCV (fl)	MCH (pg)	MCHC (g/dL)
Normal Control	49.07±2.23^{a}	14.62±0.53^{a}	29.98±0.42^{a}
Untreated Control	63.40±3.97^{b}	23.59±0.70^{b}	38.39±1.35^{c}
Chloroquine (20 mg/Kg b.wt)	53.20±1.20^{ab}	15.86±0.26^{a}	29.64±0.20^{a}
50 mg/Kg b.wt	60.60±2.58^{b}	22.98±2.45^{b}	37.14±2.12^{c}
150 mg/Kg b.wt	59.60±3.33^{ab}	22.32±0.88^{b}	36.04±0.85^{bc}
250 mg/Kg b.wt	58.80±5.56^{ab}	20.50±0.96^{b}	32.92±1.68^{ab}

Values are means of 6 replicates ±SEM. Means in the same column with different superscripts are significantly different ($p<0.05$).

Table 5. Effects of aqueous leaf extract of *Cola acuminata* on white blood cell indices and platelet count of mice infected with *Plasmodium berghei.*

Treatment	WBC (x10^{9}/L)	Platelet count (x10^{9}/L)	Neutrophils (%)	Lymphocytes (%)
Normal Control	13.20±1.14^{a}	471.60±51.14^{a}	11.42±0.70^{a}	75.54±1.63^{a}
Untreated Control	46.21±3.58^{b}	222.20±52.67^{b}	17.71±1.16^{b}	60.78±2.34^{a}
Chloroquine (20 mg/Kg b.wt)	13.54±1.60^{a}	468.80±74.22^{a}	12.26±1.27^{a}	72.42±0.69^{a}
50 mg/Kg b.wt	38.57±8.74^{b}	246.12±62.63^{b}	16.69±0.41^{b}	63.56±3.96^{a}
150 mg/Kg b.wt	36.69±5.12^{b}	257.83±59.37^{b}	16.47±0.39^{b}	65.78±5.03^{a}
250 mg/Kg b.wt	36.52±4.98^{b}	411.20±147.97^{a}	16.30±1.41^{b}	65.96±8.56^{a}

Values are means of 6 replicates ±SEM. Means in the same column with different superscripts are significantly different ($p<0.05$).

towards normal. This is more evident in the groups treated with chloroquine and the group treated with the highest dose of the extract. However, infection significantly reduced ($p<0.05$) platelets compared to control. This was also ameliorated by treatment.

DISCUSSION

The phytochemical analysis of the aqueous leaf extract of *Cola acuminata* revealed the presence of tannins, alkaloids, phenolics, glycosides, terpenoids and flavonoids. This agrees with the findings of (Sonibare et al*.,* 2009; Atanda et al*.,* 2011; Dewole et al., 2013; Mbolo and Udoh, 2014 and Durand et al., 2015). The determination of percentage inhibition or chemosupression of parasitaemia is the most reliable parameter in antimalarial screening; and results of this study clearly exhibit the antimalarial activity of the aqueous leaf extract of *Cola acuminata* especially at higher doses which was comparable to the standard drug, chloroquine. Although its mechanism of antimalarial activity has not been elucidated; previous studies have attributed the antimalarial activity of plants to their phytochemical components. Alkaloids have long been known to be a component of antimalarial agents (Christenzen and Kharazmi, 2001); and several studies have reported alkaloids from different plants to have antimalarial action (Ancolio et al*.,* 2002; Oliveira et al*.,* 2009 and Pivatto et al*.,* 2014). The alkaloids contained in the aqueous leaf extract of *Cola acuminata* may have contributed to the observed antimalarial activity. Flavonoids have also been shown to have antimalarial activity (Liu et al*.,* 1992; de Monbrison et al*.,* 2006; Ntie-

Kang et al., 2014). In addition to these, phenolic compounds have also been shown to have antimalarial activity (Ovenden et al*.,* 2011; Builders et al*.,* 2014 and Tjahjandarie et al. 2014). Reddy et al. (2007) also reported the antimalarial activity of phenolic and tannin rich fractions of *Punica granatum* L. These phytochemicals which have been detected in the aqueous leaf extract of *Cola acuminata* acting singly or synergistically through diverse mechanisms may have been responsible for the antimalarial activity seen in this study (Table 1). In addition, Flavonoid and phenolic compounds have been reported to act as primary antioxidants or free radical scavengers which can help to counteract oxidative damage induced by infection with the malaria parasite. The presence of these compounds in the aqueous leaf extract of *Cola acuminata* may help in reducing the complications induced by oxidative stress resulting from the infection (David et al*.,* 2004; Reddy et al*.,* 2007). Balogun et al. (2014), also reported the augmentation of the antioxidant system by extracts of *Clerodendrum violaceum* leaf as a means of ameliorating the deleterious effects of reactive oxygen species (ROS) produced *in vivo* during malaria infection through antioxidant species present in the extract. Therefore, aqueous leaf extract of *Cola acuminata* may have exerted the antimalarial effect observed in this study through parasite clearance by the various phytochemicals present in the plant extract.

When infected mosquitoes bite a host and transmit malaria parasites, the host liver is among the organs affected in the early stage. The parasite incubates in the liver before infecting the red blood cells. Their stay in the liver leads to significant alterations in host hepatocyte physiology and morphology e.g. alteration in blood flow through the organ as parasitized red blood cells adhere to endothelial cells and blocking the sinusoids and obstructing the intrahepatic blood flow. There's also hepatocyte necrosis, bile stasis due to impairment of bilirubin transport and increased haemolysis of parasitized and non-parasitized red blood cells which leads to jaundice. All these changes occurring in the liver affects its permeability and consequently, will affect function indices and enzyme activities within the liver too (Viriyavejakul et al*.,* 2014).

Some of these changes were observed in this study: infection led to increase in the activities of ALT and AST in the serum (Figure 1); the increase was probably through altered membranes of the liver. Treatment was able to restore these activities towards normal. Total protein concentration was also decreased by infection probably due to impaired biosynthetic capacity of the liver (Table 2). The changes in the liver function indices and some enzymes in this study confirm the reported effects of malaria on the liver and the results obtained show that the aqueous leaf extract of *Cola acuminata* can help ameliorate some of these disturbances. Changes in blood and blood cell counts are an established aspect of malaria infection. These changes can lead to malarial aneamia, thrombocytopenia and leukocytosis although the severity of these changes can vary (Kotepui et al*.,* 2015). Some of these reported changes have also been confirmed in this study: infection caused a significant decrease in packed cell volume, haemoglobin, red blood cell count (Table 3) and platelets (Table 5); but increased white blood cell count and neutrophils. Since white blood cells including neutrophils function mainly to fight infection and defend against foreign bodies by phagocytosis and production of antibodies in the immune response, this increase can contribute in fighting the malaria infection. The significant increase in platelet count at the dose of 250 mg/kg body weight suggests stimulation of the bone marrow where the cells are produced. This increase could be beneficial in the treatment of malaria because malaria infection causes a decrease in the platelet count as a result of sequestration of the platelets in the spleen (Ogboi et al*.,* 2011).The results obtained in this study show that aqueous leaf extract of *Cola acuminata* has promising potentials in malaria management by direct parasite clearance. Some phytochemical constituents present in the leaf extract may also help to ameliorate some complications of malaria infection.

Conclusion

This study has clearly exhibited the antimalarial activity of the aqueous leaf extract of *Cola acuminata.* The activity is more at higher doses and compared favourably to the standard drug, chloroquine. Treatment with aqueous leaf extract of *Cola acuminata* also improved some derangements associated with malaria. There is, thus, a need for further investigation to harness the potentials of this plant in the management of malaria.

AUTHOR`S DECLARATION

We declare that this study is an original research by our research team and we agree to publish it in the journal.

REFERENCES

Abiodun OA, Oyekanmi AM, Oluoti OJ (2014). Biochemical and Phytochemical Properties of *Cola acuminata* varieties. Am. J. Expl. Agric. 4(11):1280-1287.

Ancolio, C., N. Azas, V. Mahiou, E. Ollivier, C. DiGiorgio, A. Keita, P. Timon-David, and G. Balansard (2002).Antimalarial activity of extracts and alkaloids isolated from six plants used in traditional medicine in Mali and Sao Tome. Phytother. Res.16(7):646-9.

Atanda OO, Olutayo A, Mokwunye FC, Oyebani AOAdegunwa MO (2011).The quality of Nigerian Kola Nuts. Afr. J. Food Sci.5:904-909.

Balogun EA, Zailani AH, Adebayo JO (2014). Augmentation of antioxidant system: Contribution to antimalarial activity of *Clerodendrum violaceum*leaf extract. TANG Vol 4, No 4.

Builders M, Alemika T, Aguiyi J (2014). Antimalarial Activity and

Isolation of Phenolic Compound from *Parkiabiglobosa*.
J. Pharm. Biol.Sci.9(3):78-85.
Caraballo H (2014). Emergency Department management of Mosquito-borne illness: Malaria, Dengue and West Nile virus. Emerg. Med. Pract. 16(5):1-24.
David AF, Philip JR, Simon IC, Reto B, Solomon N (2004). Antimalarial drug discovery: Efficacy models for compound screening. Nat. Rev.3:509-520.
Christenzen SB, Kharazmi A (2001). Antimalarial natural products isolation characterization and biological properties, Bioactive Compounds from Natural Sources Isolation Characterization and Biological Properties, pp 379–432.
deMonbrison F, Maitrejean M, Latour C, Bugnazet F, Peyron F, Barron D, Picot S (2006). *In vitro* antimalarial activity of flavonoid derivatives dehydrosilybin and 8-(1;1)-DMA-kaempferide.Acta. Trop. 97(1):102-7.
Dewole EA, Dewumi DFA, Alabi JYT, Adegoke A, (2013). Proximate and Phytochemical of *Cola nitida* and *Cola acuminata*. Pak. J. Biol. Sci. 16: 1593-1596.
Doumas BT, Watson WA, Biggs HG. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. Clin.Chim.Acta. 31(1):87-92.
Durand D, Hubert A, Nafan D, Haziz DS, Pacŏme AN, Farid B, Adolphe A, Joachim DG, Lamine B (2015). Antimicrobial, Antioxidant, Cytotoxic and Phytochemical Assessment of *Cola acuminata* used in Benin. Int. J.Pharm. Sci. 7(6):102-108.
Evans WC (2005). Trease and Evans Pharmcognosy (14th Ed) WB Saunders Company Limited, London.Pp 357-358.
Fabunmi TB, Arotupin DJ (2015). Antioxidant Properties of Fermented Kolanut husk and Tests of Three Species of Kolanut: *Cola acuminata*, *Cola nitida* and *Cola verticillata*. Br.Biotechnol J.8(2):1-13.
Huang XJ, Choi YK, Im HS, Yarimaga O, Yoon E, Kim HS (2006). Aspartate aminotransferase (AST/GOT) and Alanine aminotransferase (ALT/GPT) detection techniques. Sensors (Basel), 6(7):756-782.
Jeruto P, Nyangacha RM, Mutai C (2015). *In vitro* and *in vivo* antiplasmodial activity of extracts of selected Kenyan medicinal plants. Afr. J. Pharm. Pharmacol. 9(16):500-505.
Keay RWJ (1996). Trees of Nigeria. Ibadan, Oxford Science Publication and vector control divivsion, Abuja, Nigeria. pp 13.
Kotepui M, Piwkham D, PhunPhech B, Phiwklam N, Chupeerach C, Duangmano S (2015). Effects of malaria parasite density on blood Cell parameters. Plos One 10(3):e0121057.
Liu KC, Yang SL, Roberts MF, Elford BC, Phillipson JD (1992). Antimalarial activity of Artemisia annua flavonoids from whole plants and cell cultures. Plant Cell Rep.11(12):637-40.
Lowe HIC, Watson CT, Badal S, Patrice PP, Toyang NJ, Bryant J (2014). Promising Efficacy of the *Cola acuminata* Plant: A Mini Review.Adv. Biol.Chem, 4:240-245.
Manser M, Olufsen C, Andrews N, Chiodini P (2013). Estimating the parasitaemia of *Plasmodium falciparum*: experience from a national EQA scheme. Malaria Journal. DOI10.1186/1475-2875-12-428.
Mbolo CI Udoh P (2014). Susceptibility of *Proteus mirabilis*, *Staphylococcus aureus* and *Candida albicans* to extracts of *Cola acuminata*. Global Adv. Res. J.Microbiol. 3:078-082.
Nishi HH, Kestner J, Elin RJ (1985). Four Methods for Determining Total Protein compared by using Purified Protein Fractions from Human Serum. Clin. Chem. 31(1):95-98.
Ntie-Kang F, Onguéné AP, Lifongo LL, Ndom JC, Sippl W, Mbaze LM (2014). The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. Malaria Journal.DOI: 10.1186/1475-2875-13-81.
Ogboi SJ, Akpulu SP, Joshua IA, Usman J (2011). Effects of cerebral malaria on platelet count, platelet factor – 3 and platelet aggregate availability in children. Adv. Bio. Res. 2(2):72- 74.
Oliveira AB, Dolabela MF, BragaL FC, Jácome RP, Fernando PV, Marinete MP (2009). Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids.Anais da Academia Brasileira de Ciências 81:4 doi.org/10.1590/S0001-37652009000400011.
Ovenden SP, Cobbe M, Kissell R, Birrell GW, Chavchich M, Edstein MD (2011). Phenolic glycosides with antimalarial activity from *Grevillea* "Poorinda Queen".J. Nat. Prod. 28;74(1):74-78.
Pivatto M, Baccini LR, Sharma A, Nakabashi M, Danuello A, Claudio VJ, Celia RSG, Bolzani VS (2014). Antimalarial activity of piperidine alkaloids from *Sennaspectabilis* and semisynthetic derivatives. J. Braz. Chem. Soc. 25:10 doi.org/10.5935/0103-5053.20140195.
Reddy MK, Gupta SK, Jacob MR, Khan SI, Fereirra D (2007). Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punicagranatum* L. Planta Med. 73(5):461-467.
Sonibare MA, Soladoye MO, Esan OO, Sonibare OO (2009). Phytochemical and Antimicrobial Studies of Four Species of *Cola* Schott &Endl. (Sterculiaceae).Afr. J. Trad.Compl. Altern. Med. 6(4): 518–525.
Sofowora A (1980). The present status of knowledge of the plants used in traditional medicine in West Africa: A medical approach and a chemical evaluation. J. Ethnopharma. 2:109-118.
Tjahjandarie TS, Pudjiastuti P, Saputri RD, Tanjung M (2014). Antimalarial and antioxidant activity of phenolic compounds isolated from *Erythrina crista-galli* L. J. Chem.Pharm. Res. 6(4):786-790.
Viriyavejakul P, Vasant K, Chuchard P (2014). Liver changes in severe Plasmodium malaria: histopathology, apopotosis, and nuclear factor kappa B expression. Malaria Journal 13:106. DOI:10.1186/1475-2875-13-106.
Walter M, Gerard H (1970). Ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma.Microchem. J.15:231–236.
Wright PJ, Leathwood PD and Plummer DT (1972). Enzymes in rat urine alkaline phosphatase. Enzymologia 42:317-327.
WHO (2014).World Malaria Report 2014.World Health Organisation, Geneva, Switzerland, ISBN-9789241564830 p 2.
WHO (2015).World Malaria Report 2015. World Health Organisation, Geneva, Switzerland, ISBN-9789241565158.