Research Article **IJTCM (2020), 5:31**

International Journal of Traditional and Complementary Medicine
(ISSN:2474-1361)

Phytochemicals extracted from *Cola nitida* leaf possess antimalarial effects and improve derangements in haematological indices of *Plasmodium berghei*- infected mice.

Zailani, A. H., Iliyas, M. B., Benjamin, L., Ibrahim, B. A., Ubah, B., *Lamiya, A.

Department of Biochemistry, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria

ABSTRACT

Classical antimalarial drugs such as quinine and artemisinin are both plant derived suggesting that plants are a promising source of bioactive components that can help in combating the scourge of malaria. *Cola nitida* leaf has been reported to possess antimalarial activity and also contain pharmacologically active phytochemicals including alkaloids, flavonoids, tannins and phenolics. The effects of these phytochemicals in mice infected with *Plasmodium berghei* was evaluated in this study. For each phytochemical, 7 groups (A-G) of eight mice each were used. Groups A and B served as normal and infected controls respectively. Group C was treated with 20mg/Kg body weight of chloroquine and served as the treated control. Groups D, E and F were administered 12.5, 25 and 50 mg/kg body weight of the different phytochemicals while group F was treated with 50mg/Kg body weight of the phytochemicals only without parasite inoculation thus serving as extract control. Treatment commenced 72hrs after inoculation and was done once orally for four consecutive days after which parasitaemia was evaluated. All the phytochemicals were found to exhibit antimalarial activity in a dose dependent manner. The mean survival time of all the experimental groups were also prolonged in a dose dependent manner compared to that of untreated control. Similarly, all the phytochemicals improved the altered haematological indices towards normal. These phytochemicals of *Cola nitida* exhibited significant antimalarial activities and thus can be further studied in the search for novel antimalarial drugs.

Keywords: Antimalarial; phytochemical; *in vivo*; *Cola nitida*; haematological indices.

***Correspondence to Author:**
Lamiya, A.
Department of Biochemistry, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria

How to cite this article:
Zailani, A. H., Iliyas, M. B., Benjamin, L., Ibrahim, B. A., Ubah, B., Lamiya, A.Phytochemicals extracted from *Cola nitida* leaf possess antimalarial effects and improve derangements in haematological indices of Plasmodium berghei- infected mice. International Journal of Traditional and Complementary Medicine 2020, 5:31.

eSciPub LLC, Houston, TX USA.
Website: https://escipub.com/

1.1 INTRODUCTION

Malaria is one of the most deadly infectious diseases that continue to pose a threat to millions of lives [1] especially in the tropical and subtropical regions of the world [23]. The disease is caused by a single-celled obligate Protozoa of the genus *Plasmodium* [4] of which *Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale* and *Plasmodium malariae* are the major species that cause malaria and are mostly found in sub-Saharan Africa. *Plasmodium falciparum* has been reported to be the most virulent among all the species and is responsible for most of the malaria infections in this region [5]. It has been reported that 54% and 90% of the world's and African population are at risk of malaria respectively, with pregnant women and children under 5 years as the most vulnerable groups [3]. The causes of the rise in the cases of malaria are multifactorial but most especially are due to resistance of the parasite to the available classic drugs [6]. Thus, there is a necessity for the development of more potent novel antimalarial drugs. The classical antimalarial drugs suchas quinine and artemisinin, are both plant derivatives obtained from *Cinchona* species and *Artemisia annua* respectively, suggesting that other effective antimalarial drugs might be plant-derived [7]. Plants found in sub-saharan Africa including *Cola spp* have been reported to be used in orthodox medicine for the treatment and management of infections including malaria [8 9]. *Cola nitida* is a tree that grows up to 18m tall, usually less, slightly buttressed to 1m high with a short bole above to 2m girth, bearing a crown of slender ascending branches. It is indigenous to Guinea and Ghana but Its cultivation has gained ground in Nigeria [9]. *Cola nitida* leaf extracts have been reported to contain alkaloids, flavonoids, phenolics, saponins and tannins which may be responsible for the activity of this plant against infections [8 9]. This work therefore aims to evaluate the efficacy of some of these phytochemicals found in *Cola nitida* leaves to determine which one(s) contributed to the reported antimalarial activity of *Cola nitida* leaf.

2.1 MATERIALS AND METHODS

2.1.1 Collection and identification of plant material

Fresh leaf samples of *Cola nitida* was collected from Mubi, Mubi Local Government of Adamawa State, Nigeria. The leaves were identified and authenticated at the Department of Plant Sciences, Modibbo Adama University Technology Yola, Adamawa State.

2.1.2 Experimental Animals

Two hundred and twenty four (224) Swiss albino mice (about 6 to 8 weeks old) were obtained from the animal breeding unit of the University of Jos, Plateau State. The average body weight of mice (22.02±1.37g) was measured using a Shimadzu (UX4200H) top pan animal balance to the nearest 0.1g. The animals were then housed in well ventilated plastic cages, and acclimatized (in the Biochemistry laboratory) for 7 days prior to the commencement of the experiment with free access to rat pellets and tap water *ad libitum*.

2.1.3 Parasite Strain

Chloroquine sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Science,Ahmadu Bello University Zaria, Kaduna State Nigeria.The parasites were maintained weekly by serial passage of blood from the donor-infected mice to healthy uninfected mice via intraperitoneal (IP) injection [7].

2.1.4 Chemicals and reagents

Chloroquine diphosphate salt, immersion oil, and Giemsa strain were obtained from Sigma Chemical Company St. Louis, Mo, USA. Assay kits for enzymes and liver function indices were obtained from Randox Laboratory Ltd, UK.

2.2.1 Preparation of plant sample

Whole fresh leaves of *Cola nitida* were washed with water and dried in the shade at room temperature and ground to powder using an electric blender (Mazeda Mill, MT 4100, Japan). It was

then packed in a sealed plastic bottle until extraction.

2.2.2 Extraction of alkaloids

Alkaloids were extracted gravimetrically according to the method described by [10].

2.2.3 Extraction of flavonoids

Flavonoids were extracted according to the method described by [11].

2.2.4 Extraction of phenolics

Phenolics were extracted according to the method described by [12].

2.2.5 Extraction of Tannins

Extraction of tannins from *Cola nitida* leaf extract was done according to the method of [13]

2.2.6 Parasite inoculation

The mice were inoculated from the same donor mouse. 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. They were then monitored for 72 hours after which infection was confirmed by observing tail blood microscopically prior to commencement of treatment.

2.2.7 Extract and chloroquine administration

Experimental groups to receive extract or the standard drug (chloroquine) started receiving treatment 72 hours after infection. The administration of the extracts as well as the standard drug was carried out orally using intra-gastric tube. Treatment was done once daily for 4 days.

2.2.8 Experimental design

For each phytochemical, the mice were divided into seven groups (A-G) of eight mice per group.

Group A was not inoculated and not treated and served as normal control; group B was inoculated but untreated and served as infected control. Group C was treated with 20mg/Kg body weight of chloroquine (treated control). Groups D, E and F were inoculated with *Plasmodium berghei* and administered 12.5, 25 and 50 mg/kg body weight of the different phytochemicals respectively while group F was treated with 50mg/Kg body weight of the phytochemicals only without parasite inoculation thus serving as extract control.A week after treatment, five (5) mice from each group were sacrificed and blood collected to evaluate the effect of treatment on haematological parameters which is usually greatly affected by malaria infection. The remaining three (3) mice in each group were monitored for up to thirty (30) days.

2.2.9 Parasitaemia count

Parasitaemia count was carried out on days 4, 6, 8, 10, 14, 21, 26 and 28 after commencement of treatment. A drop of tail blood from each of the animals was smeared on glass slides and allowed to dry; the slide was then fixed in methanol; stained with Giemsa and observed using X100 objective lens. The number of parasitized red blood cells seen per film was counted and recorded using the formula:

$$\frac{\text{parasitized RBC}}{\text{parasitized RBC} + \text{nonparasitized RBC}} \times 100. \qquad (1)$$

Where RBC= Red Blood Cells

PRBC= Parasitized Red Blood Cells

The percentage parasitaemia suppression of the extracts was then compared with that of the untreated control and parasitaemia suppression was calculated using the following formula below as described by 14:

$$\%\ \text{chemo-suppression}\ A = \frac{B - C}{C} \times 100, \qquad (2)$$

2.3.1 Determination of mean survival time (MST)

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse. The mean survival time (MST) was calculated as follows:

$$\text{MST} = \frac{\text{sum of days of survival of animals/group}}{\text{total number of animals in the group}}. \qquad (3)$$

2.3.2 Haematological analysis

A week after treatment, five (5) animals from each sub-group of the four phytochemical

groups were sacrificed under diethyl ether anaesthesia, and the blood was collected by cardiac puncture into clean EDTA containers for hematological analysis. Determination of white blood cell (WBC) and its differential count, red blood cell count (RBC), platelet (thrombocyte) counts**,** packed cell volume (PCV), hemoglobin concentration (HGB), the mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and the mean corpuscular hemoglobin concentration (MCHC) indices were done using an automated electronic blood analyser "Abacus 380".

2.4.1 Statistical analysis

The group data was expressed as mean ± standard error of mean (SEM) and the significant differences were determined using Statistical Package for Social Sciences (SPSS V. 25).

3.1 RESULTS

Table 1 shows the result of the effects of some phytochemicals extracted from *Cola nitida* leaf and chloroquine on percentage parasitaemia and chemosuppression in *Plasmodium berghei* (NK-65) infected Mice. Percentage parasitaemia increased continuously with time (days 4, 6, 8, 10, 14, 21 and 28) in the negative control group. All the treatment groups decreased percentage parasitaemia in a dose dependent manner with time, with the treated control having the lowest percentage parasitaemia followed by group treated with alkaloids on days 10, 14, 21 and 28. On days 6,8,10 and 28 all the treatments were active (chemosurpression> 50%). But on day 4, only the treated and highest doses of the phytochemicals were active.

Table 2 shows the result of the effects of some phytochemicals extracted from *Cola nitida* leaf and chloroquine on mean survival time of mice infected with *Plasmodium berghei* (NK-65).The results revealed that treatment of the infected mice with all the phytochemical extracts increased the mean survival time of the infected mice in a dose dependent manner though not as long as that of chloroquine with alkaloids and flavonoids exhibiting the highest mean survival time of 30 days and tannins and phenolics with mean survival times of 29 days at the highest dose.

Table 3 shows results of the effects of some phytochemicals extracted from *Cola nitida* leaf on Packed Cell Volume, Haemoglobin and Red Blood Cells of *Plasmodium berghei* (NK-65) infected Mice. Infection of the mice with the parasite decreased the level of these haematological parameters significantly ($P<0.05$). However, treatment with all the phytochemical extracts significantly ($P<0.05$) improved the haematological parameters towards normal in a dose dependent manner with doses of 50mg/kg b.w.t of all the phytochemicals having no significant difference ($P>0.05$) with the normal.

Table 4 shows results of the effects of some phytochemicals extracted from *Cola nitida* leaf on Mean Corpuscular Volume, Mean Corpuscular Heamoglobin and Mean Corpuscular Haemoglobin Concentration of *Plasmodium berghei* (NK-65) infected Mice. Infection of the mice with the parasite was found to increase these haematological parameters significantly ($P<0.05$). All the phytochemicals were found to significantly ($P<0.05$) improve the sehaematological parameters towards normal in a dose dependent fashion with doses of 50mg/kg b.w.t of all the phytochemicals having no significant difference with the normal.

Table 5 shows results of the effects of some phytochemicals extracted from *Cola nitida* leaf on White Blood Cell count, lymphocytes and Platelets of *Plasmodium berghei* (NK-65) infected Mice. Infection of the mice with the parasite significantly ($P<0.05$) increased white blood cell and lymphocytes count, and significantly ($P<0.05$) decreased platelets. Treatment of the infected mice with all the phytochemical extracts significantly ($P<0.05$) improved these haematological parameters towards normal with the dose of 50mg/kg b.w.t of all the phytochemicals having no significant difference from the normal.

4.1 DISCUSSION

Plants have been a source of novel therapeutic substances and continues to play a pivotal role in primary health care in most parts of the world most especially in the treatment and management of malaria; two of the most important antimalarial drugs, artemisinin and quinine were sourced from *Artemisia annua* and *Cinchona officinalis,* respectively[15]. Some phytochemicals have been found to elicit antimalarial activity individually or in synergy with other phytoconstituents to elicit their biological activities[16].[8] reported that *Cola nitida*leaf extracts exhibit antimalarial activity *in vivo* and that the extracts contain alkaloids, flavonoids, phenolics, saponins and tannins. [9] reported similar findings in the leaf extract of *Cola nitida.* These phytochemicals have also been reported in several literature to be the major constituents of plant materials that exhibited antimalarial activities [4 15 17.]

Table 1: Effects of some phytochemicals extracted from *Cola nitida* leaf on parasitaemia and chemosuppression in *Plasmodium berghei* (NK-65) infected Mice. Parasitaemia (%) **(% chemosuppression**)

PHYTOCHEMICALS	**TREATMENT**	**Day 4**	**Day 6**	**Day 8**	**Day 10**	**Day 14**	**Day 21**	**Day 28**
	Negative control	15.10 -	22.25 -	38.75 -	87.60 -	100 -	100 -	100 -
	Standard control 20mg/kg b.w.t	5.15 **(65.89)**	3.25 **(85.39)**	1.00 **(97.42)**	0.00 **(100)**	0.00 **(100)**	0.00 **(100)**	0.00 **(100)**
Alkaloids	12.5mg/kg b.w.t	10.85 **(28.14)**	10.30 **(53.70)**	9.98 **(74.24)**	9.30 **(89.38)**	8.85 **(91.15)**	7.80 **(92.20)**	5.55 **(94.45)**
	25mg/kg b.w.t	7.78 **(48.47)**	6.70 **(68.89)**	5.80 **(85.03)**	4.94 **(94.36)**	3.80 **(96.20)**	2.20 **(97.80)**	2.00 **(98.00)**
	50mg/kg b.w.t	6.08 **(59.73)**	5.55 **(75.05)**	4.80 **(87.61)**	3.80 **(95.66)**	2.50 **(97.50)**	1.60 **(98.40**)	0.09 **(99.10)**
Flavonoids	12.5mg/kg b.w.t	10.30 (**31.9**)	10.10 (**54.6**)	9.97 (**74.2**)	9.50 (**89.15**)	8.80 (**91.20**)	7.60 (**92.40**)	5.85 (**94.15**)
	25mg/kg b.w.t	7.80 (**48.34**)	6.90 (**68.98**)	5.75 (**85.16**)	4.98 (**94.32**)	3.90 (**96.20**)	2.50 (**97.50**)	2.00 (**98.00**)
	50mg/kg b.w.t	6.05 (**59.93**)	5.50 (**75.28**)	4.85 (**87.48**)	3.80 (**95.66**)	2.50 (**97.50**)	1.67 (**98.35**)	0.90 (**99.10**)
Phenolics	12.5mg/kg b.w.t	10.80 **(28.48)**	10.16 **(54.84)**	9.80 **(74.84)**	9.68 **(88.95**)	8.50 **(91.50)**	7.80 **(92.20)**	5.60 **(94.4)**
	25mg/kg b.w.t	7.90 **(47.68)**	6.90 **(69.33**)	5.80 **(85.11)**	5.00 **(94.29)**	3.98 **(96.02)**	3.00 **(97.00)**	2.40 **(97.60)**
	50mg/kg b.w.t	6.30 **(58.28)**	5.80 **(74.22)**	4.80 **(87.67)**	3.30 **(96.23)**	2.51 **(97.49)**	1.38 **(98.60)**	0.91 **(99.90)**
Tannins	12.5mg/kg b.w.t	10.85 **(31.13)**	10.30 **(54.89)**	9.98 **(74.71)**	9.30 **(89.04)**	8.85 **(91.40)**	7.80 **(92.40)**	5.55 **(94.20)**
	25mg/kg b.w.t	7.78 **(48.34)**	6.70 **(69.00)**	5.80 **(85.03)**	4.94 **(94.29)**	3.80 **(96.08)**	2.20 **(97.00)**	2.00 **(97.50)**
	50mg/kg b.w.t	6.08 **(59.27)**	5.55 **(75.11)**	4.80 **(87.48)**	3.80 **(95.89)**	2.50 **(97.50)**	1.60 **(98.31)**	0.90 **(99.02)**

Values are means of 5 replicates. Figures in brackets are the percentage chemosuppression on each day.

Table 2: Effects of some phytochemicals extracted from *Cola nitida* leaf on mean survival times (MST) of *Plasmodium berghei* (NK-65) infected Mice.

PHYTOCHEMICALS	TREATMENT	MEAN SURVIVAL TIME (days)
	Negative control	10.00
	Standard control 20mg/kg b.w.t	30.00
ALKALOIDS	12.5mg/kg b.w.t	23.00
	25mg/kg b.w.t	24.00
	50mg/kg b.w.t	30.00
FLAVONOIDS	12.5mg/kg b.w.t	21.00
	25mg/kg b.w.t	26.00
	50mg/kg b.w.t	30.00
PHENOLICS	12.5mg/kg b.w.t	23.00
	25mg/kg b.w.t	23.00
	50mg/kg b.w.t	29.00
TANNINS	12.5mg/kg b.w.t	24.00
	25mg/kg b.w.t	26.25
	50mg/kg b.w.t	29.00

Table 3: Effects of some phytochemicals extracted from *Cola nitida* leaf on PCV, HB and RBC of *Plasmodium berghei* (NK-65) infected Mice.

PHYTOCHEMICALS	TREATMENT	PCV(%)	HB(g/dL)	RBC($X10^{12}$/L)
	Normal control	54.99± 1.04^{e}	12.26 ± 0.32^{e}	8.51 ± 0.35^{c}
	Negative control	27.57± 0.10^{a}	8.85 ± 0.82^{a}	5.71 ± 0.17^{a}
	Standard control 20mg/kg b.w.t of extract	50.96± 0.64^{d}	11.11 ± 0.57^{c}	7.05 ± 0.50^{b}
ALKALOIDS	12.5mg/kg b.w.t of extract + MP	41.79±0.53^{b}	8.93±0.41^{a}	5.98±0.64^{a}
	25mg/kg b.w.t of extract + MP	44.72±0.31^{c}	10.05±0.85^{b}	7.01±0.59^{b}
	50mg/kg b.w.t of extract + MP	48.38±0.51^{d}	12.31±0.29^{d}	7.68±0.31^{bc}
	50mg/kg b.w.t of extract without MP	47.71±0.40^{d}	12.50±0.18^{d}	7.64±0.28^{bc}
FLAVONOIDS	12.5mg/kg b.w.t of extract + MP	39.06±0.26^{b}	9.38±0.18^{a}	6.88±0.18^{b}
	25mg/kg b.w.t of extract + MP	42.04±0.38^{c}	11.03±0.25^{b}	7.83±0.15^{c}
	50mg/kg b.w.t of extract + MP	48.11±0.18^{d}	12.59±0.19^{c}	8.60±0.10^{c}
	50mg/kg b.w.t of extract without MP	50.65±0.28^{e}	12.95±0.15^{c}	8.16±0.68^{c}
PHENOLICS	12.5mg/kg b.w.t of extract + MP	39.58±0.45^{b}	9.31±0.19^{a}	6.09±0.01^{a}
	25mg/kg b.w.t of extract + MP	42.39±0.56^{c}	10.97±0.35^{b}	7.71±0.16^{bc}
	50mg/kg b.w.t of extract + MP	48.15±0.12^{d}	12.09±0.61^{b}	7.99±0.06^{c}
	50mg/kg b.w.t of extract without MP	50.86±0.24^{e}	11.33±0.46^{b}	7.72±0.26^{bc}
TANNINS	12.5mg/kg b.w.t of extract + MP	34.72± 1.02^{b}	9.20 ± 0.29^{b}	5.70 ± 0.22^{a}
	25mg/kg b.w.t of extract + MP	42.70± 1.00^{c}	10.14 ± 0.19^{b}	7.30 ± 0.18^{b}
	50mg/kg b.w.t of extract + MP	50.55± 0.19^{d}	11.00 ± 0.30^{c}	8.35 ± 0.09^{c}
	50mg/kg b.w.t of extract without MP	52.31± 0.46^{d}	11.38 ± 0.33^{e}	8.30 ± 0.29^{c}

Values are means of 5 replicates± SEM. Means in the same column with different superscripts are significantly different (P<0.05).
Key: PCV: packed cell volume, HB: haemoglobin, RBC: red blood cells.

Table 4: Effects of some phytochemicals extracted from *Cola nitida* leaf on MCV, MCH and MCHC of *Plasmodium berghei* (NK-65) infected Mice.

PHYTOCHEMICALS	TREATMENT	MCV(fl)	MCH(pg)	MCHC(g/l)
	Normal control	0 ± 0.51^{ab}	12.48 ± 0.50^{ab}	21.92 ± 0.44^{b}
	Negative control	0 ± 0.51^{e}	17.88 ± 0.31^{d}	26.04 ± 0.27^{c}
	Standard control 20mg/kg b.w.t of extract	0 ± 0.51^{a}	14.04 ± 0.57^{c}	19.68 ± 0.51^{a}
ALKALOIDS	12.5mg/kg b.w.t of extract + MP	79.60±0.51^{c}	12.95±0.70^{a}	23.26±0.56^{c}
	25mg/kg b.w.t of extract + MP	72.80±0.86^{b}	12.95±0.70^{a}	21.43±0.44^{bc}
	50mg/kg b.w.t of extract + MP	71.00±0.77^{ab}	12.81±0.23^{a}	18.93±0.31^{a}
	50mg/kg b.w.t of extract without MP	71.20±0.86^{ab}	12.45±0.47^{a}	19.87±0.62^{a}
FLAVONOIDS	12.5mg/kg b.w.t of extract + MP	78.20±0.49^{c}	14.95±0.53^{b}	22.40±0.26^{c}
	25mg/kg b.w.t of extract + MP	74.40±0.51^{b}	14.00±0.88^{b}	21.28±0.39^{bc}
	50mg/kg b.w.t of extract + MP	70.60±0.51^{a}	11.96±0.52^{a}	19.62±0.28^{a}
	50mg/kg b.w.t of extract without MP	70.40±0.25^{a}	12.43±0.50^{a}	20.12±0.64^{ab}
PHENOLICS	12.5mg/kg b.w.t of extract + MP	79.00±0.63^{d}	12.89±0.29^{a}	24.63±0.42^{e}
	25mg/kg b.w.t of extract + MP	74.20±0.37^{c}	11.95±0.13^{a}	21.09±0.42^{cd}
	50mg/kg b.w.t of extract + MP	71.40±0.51^{b}	11.77±0.32^{a}	18.87±0.35^{a}
	50mg/kg b.w.t of extract without MP	71.40±0.51^{b}	11.98±0.23^{a}	20.17±0.23^{bc}
TANNINS	12.5mg/kg b.w.t of extract + MP	0 ± 0.86^{d}	13.04 ± 0.22^{abc}	22.36 ± 0.79^{b}
	25mg/kg b.w.t of extract + MP	0± 0.80^{c}	12.14 ± 0.33^{a}	21.28 ± 0.56^{b}
	50mg/kg b.w.t of extract + MP	0 ± 0.40^{b}	12.16 ± 0.18^{a}	19.08 ± 0.31^{a}
	50mg/kg b.w.t of extract without MP	0± 0.45^{b}	13.54 ± 0.30^{bc}	21.48 ± 0.52^{b}

Values are means of 5 relplicates ± SEM. Means in the same column with different superscripts are significantly different. **Key**. MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration.

Table 5: Effects of some phytochemicals extracted from *Cola nitida* leaf on WBC, LYM and PLT of *Plasmodium berghei* (NK-65) infected Mice.

PHYTOCHEMICALS	TREATMENT	WBC($X10^9$L)	LYM(%)	PLT($X10^9$/L)
	Normal control	11.98 ± 0.16^{a}	55.46 ± 5.14^{a}	607.40 ± 14.75^{e}
	Negative control	18.16 ± 0.28^{c}	80.18 ± 0.61^{c}	381.60 ± 8.68^{a}
	Standard control 20mg/kg b.w.t of extract	12.32 ± 1.11^{a}	60.26 ± 6.06^{a}	576.80 ± 3.68^{d}
ALKALOIDS	12.5mg/kg b.w.t of extract + MP	17.29±0.18^{C}	75.39±0.35^{b}	460.80±15.33^{b}
	25mg/kg b.w.t of extract + MP	14.07±0.32^{b}	72.53±0.50^{b}	533.00±5.95^{c}
	50mg/kg b.w.t of extract + MP	12.32±0.14^{a}	62.21±0.35^{a}	562.00±12.93^{cd}
	50mg/kg b.w.t of extract without MP	12.06±0.08^{a}	61.65±0.21^{a}	584.80±7.59^{de}
FLAVONOIDS	12.5mg/kg b.w.t of extract + MP	17.68±0.31^{c}	76.34±0.19^{b}	455.60±18.43^{b}
	25mg/kg b.w.t of extract + MP	14.40±0.18^{b}	71.85±0.35^{b}	522.60±12.40^{c}
	50mg/kg b.w.t of extract + MP	11.89±0.25^{a}	61.91±0.44^{a}	567.00±33.11^{d}
	50mg/kg b.w.t of extract without MP	12.02±0.29^{a}	61.02±0.34^{a}	572.80±22.13^{d}
PHENOLICS	12.5mg/kg b.w.t of extract + MP	17.65±0.24^{c}	75.70±0.25^{b}	421.40±15.24^{b}
	25mg/kg b.w.t of extract + MP	13.91±0.35^{b}	73.26±0.36^{b}	542.04±10.40^{c}
	50mg/kg b.w.t of extract + MP	12.24±0.13^{a}	61.60±0.54^{a}	573.40±5.12^{d}
	50mg/kg b.w.t of extract without MP	11.96±0.21^{a}	61.02±0.48^{a}	575.60±8.28^{d}
TANNINS	12.5mg/kg b.w.t of extract + MP	18.41 ± 0.36^{c}	74.31 ± 1.17^{bc}	459.00 ± 18.00^{b}
	25mg/kg b.w.t of extract + MP	15.28 ± 0.35^{b}	73.48 ± 0.60^{bc}	535.20 ± 5.09^{c}
	50mg/kg b.w.t of extract + MP	12.50 ± 0.31^{a}	64.27 ± 0.66^{ab}	553.40 ± 13.51^{d}
	50mg/kg b.w.t of extract without MP	14.03 ± 0.37^{b}	62.87 ± 3.35^{a}	586.20 ± 10.40^{d}

Values are means of 5 replicates ±SEM. Means in the same column with different superscripts are significantly different ($p<0.05$). **WBC**=White blood cells, **LYM**=Lymphocytes and **PLT**=Platelet count.

Although all the phytochemicals studied demonstrated the ability to reduce parasitaemia in a dose dependent manner, alkaloids exhibited the highest ability to clear parasites with percentage parasitaemia of 0.09% at the dose of 50mg/kg b.w.t. Nevertheless, the parasite clearance ability was not 100% even on the last day as in the standard control. Parasite clearance/antimalarial activities of various phytochemicals (including tannins, flavonoids, alkaloids and phenolics) from different herbs with antimalarial activities have been found to follow different mechanisms [18] in exerting their activities which these phytochemicals must have probably employed for the activity they exhibited. Alkaloids for example have been reported by [19] to target the apicoplast, an organelle in the *Plasmodium* parasite, while others such as benzylisoquinoline alkaloids inhibits protein synthesis in the parasite. The mechanism of action of flavonoids remains unclear although it has been suggested that flavonoids impede the influx of myoinositol and L-glutamine in erythrocytes that are infected [19]. While some flavonoids have been reported to increase the level of oxidation of erythrocytes and inhibit protein synthesis in malaria parasites and also inhibit fatty acid biosynthesis (FAS II) in *Plasmodium*species [20]. Theantioxidant activities of flavonoids, phenolics and tannins [21] [8] may have augmented the exhibited antimalarial activity by counteracting the oxidative damage induced by the malaria parasite. Due to the observed parasite clearance and improvement in haematological indices, all the phytochemical treated groups had an extended mean survival time.

Infected mice were found to have reduced PCV, haemoglobin and RBC and increase in RBC indices levels (Tables 3 and 4). This agrees with the report of [22] [23] [7] which can be attributed to increase in haemolysis of parasitized red blood cells and increase in fragility of the normal cells. All the phytochemical treatment groups had improved PCV, haemoglobin and RBC and its

indices towards normal. This observation is in line with the results obtained for percentage chemosuppresion and mean survival time of the treated animals. Thus, as the parasitaemia reduced, the effect on the blood also reduced and the blood indices improved. Results of the extract control group shows that these phytochemicals are relatively safe since there was no significant difference (P>0.05) between this group and the normal group with regards to PCV, haemoglobin, RBC and its indices. The number of leukocytes in the blood is often an indicator of disease, and thus the WBC count is an important subset of the complete blood count [24]. Lymphocyte is a type of white blood cell that fights against invading pathogens including *Plasmodiumspp.* In this study, infection of mice was found to increase both WBC and lymphocytes substantially (P<0.05) when compared with the normal. This can be due to immune response to resist the parasite infection by the host mice. [23] also reported a similar finding. All the phytochemicals improved both WBC and LYM in dose dependent manner, thus suggesting that these phytochemicals possesses immunomodulatory activities and also clear the parasites.

Thrombocytopenia is a major complication of malaria [25 26]. This is characterized by low platelets in the blood. Results from the untreated control group confirmed this reduction in the platelet count which was significantly improved by all the phytochemicals used in treatment in a dose dependent manner with the dose of 50mg/kg b.w.t of all the phytochemicals having no significant difference from the normal control. The ability of these phytochemicals to reduce percentage parasitaemia significantly at this dose might be the reason for this improvement in platelet count which agrees with the findings of [27] that thrombocytopenia usually disappears with the treatment of malaria infection. The results of this study suggest that all these phytochemicals possessantimalarial activity in dose dependent manner thereby supporting the folkloric claim of the use of this plant and other plants containing these phytochemicals in the treatment or management of malaria [8 9 17 7].

5.1 CONCLUSION

All the phytochemicals of *Cola nitida* leaf used in this study individually exhibited antimalarial activity. This can be due to the plasmocidal or immunomodulatory activities of the phytochemicals. All the phytochemicals were also able to restore altered haematological indices probably through haematopoietic properties and plasmocidal activity elicited by the phytochemicals. These effects were better at higher doses. Thus, these phytochemicals are promising antimalarial agents.

REFERENCES

1. Abah, A. E. and Temple, A. (2015). Prevalence of malaria among asymptomatic primary school children in angiama community, Bayesa state of Nigeria, *Trop. med. and surge.* 4:203.
2. Kunihya, I. Z., Samaila A. B., Pukuma M. S. and Qadeer M. A. (2016).Prevalence of malaria infection and malaria anaemia among children attending Fedral Medical Center Yola, Adamawa State of Nigeria.The Int. J. of eng. and sci. 5(7): 8-14.
3. Kweyamba, P. A., Zofou, D., Efange, N., Assob, J-C.N., Kitau, J. and Nyindo, M.(2019).*Invitro* and *in vivo* studies on anti-malarial activity of *Commiphora Africana* and *Dichrostachyscinerea*used by the Maasai in Arusha region, Tanzania. *Malar. J. .18:119* https://doi.org/10.1186/s12936-019-2752-8
4. Bassey, S.E. and Izah, S. C. (2017).Some determinant factors in malaria prevalence in Nigeria.*J. of Mosquito Res*. 7(7); 48-58.
5. Muluye1, A. B., Desta1, A. G., Abate, S. K. and Dano, G. T. (2019). Anti-malarial activity of the root extract of *Euphorbia abyssinica*(*Euphorbiaceae*) against *Plasmodium berghei* infection in mice. *Malar. J.* 18:261. https://doi.org/10.1186/s12936-019-2887-7
6. Rout, S. and Mahapatra, R. K. (2019) *Plasmodium falciparum*: multidrug resistance,*Chemical boil. & drug. des.*, 93(5):737–759.
7. Ounjaijean, S., Kotepui, M. and Somsak, V. (2019).Antimalarial Activity of *Tinosporabaenzigeri*against *Plasmodium berghei*-Infected Mice. *J. of trop. Med.*. 2019;6. https://doi.org/10.1155/2019/5464519
8. Zailani, H. A., Ibe, I.J. and Utor, O.J. (2016). Effects of aqueous leaf extract of *Cola nitida* on Parasitaemia, *In vitro* antioxidant and biochemical

parameters in *Plasmodium berghei* infected Mice. *J. of Health and pharmacol.* 4(3):21-28.
9. Eromosele, O. J. and Kehinde, O. M.(2018).Phytochemical Study of Underutilized Leaves of *Cola acuminata and Cola nitida. Am. Res. J. of Bios ci. 4(I1):pp: 1-7.*
10. Harborne, J. B. (1973). Phytochemical methods. Chapman and Hall ltd., London; 46-188
11. Yahaya, M. M. (2015). Isolation and Purification of Flavonoids from the Leaves of locally produced *Carica papaya*.*Int. J. of sci. and technol. Res.*4 (12): 282- 283.
12. Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. (1998). antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem; 46(10): 4113-7.
13. Marka A. O. S. and Goodchild, A. V., (1996).Quantification of tannins. A laboratory manual; international center for Agricultural research in the dry areas (ICARDA), Allepo, Syria,: 25
14. Dikasso, D., Mekonnen, E. and Debella, A. (2006). *In vivo* antimalarial activity of hydroalcoholic extracts from Asparagus africanus Lam. in mice infected with *Plasmodium berghei.* Ethiop J Health Dev. 20:112e118.
15. Laryea, M. K. and Borquaye, L. S. (2019).Antimalarial Efficacy and Toxicological Assessment of Extracts of Some Ghanaian Medicinal Plants.*J. of Parasitol. Res.* .2019:9, Article ID 1630405, https://doi.org/10.1155/2019/1630405
16. Kaur, H. and Mukhtar, H. M., Singh, A. andMahajan, A. (2018).Antiplasmodial medicinal plants: a literature review on efficacy, selectivity and phytochemistry of crude plant extracts," *J. of Biologically Act. Prod. from nat.* ; 8(5): 272– 294,
17. Gebrehiwot, S., Shumbahri, M., Eyado, A. and Yohannes, T. (2019).Phytochemical Screening and *In vivo* Antimalarial Activity of Two Traditionally Used Medicinal Plants of Afar Region, Ethiopia, against *Plasmodium berghei* in Swiss Albino Mice. *J. of Parasitol. Res.* : 8 Article ID 4519298. https://doi.org/10.1155/2019/4519298
18. Okello, D. and Kang, Y.(2019).Exploring antimalarial herbal plants across communities in Uganda Based on Electronoc Data.*Evidence-based complementary and alternative medicine.*Pp 1-27. Article ID 3057180. https://doi.org/10.1155/2019/3057180.
19. Chinwuba, P. Akah, P. A. and Iiodigwe, E. E. (2015).*In vivo* antiplasmodial activity of the ethanol stem extract and fractions of *Citrus sinensis* in mice,*Merit Res. J. of Med. and Med. Sci.* 3(4):140–146.
20. Freundlich, J. S., Anderson, J. W., Sarantakis, D. (2005). Synthesis, biological activity, and X-ray crystal structural analysis of diaryl ether inhibitors of malarial enoyl acyl carrier protein reductase. Part 1: 4′-substituted triclosa derivatives. *biorgan. & Med. Chem. Lett.*15(23):5247–5252.
21. Builders, M.I, Wannang, N.N, Ajoku, G.A, Builders, P.F, Orisadipe, A, Aguiyi, J.C. (2010).Evaluation of antimalarial potential of *Vernonia ambigua-Kotschy* and *Peyr* (*Asteraceae*).*Int. J. of Pharmacol.* 18(11):1-9.
22. Meraiyebu, A., Akintayo, C.O. and Nenchi, D.Y. (2012).Evaluation of PCV and Hemoglobin Variations among Malaria Positive and Malaria Negative Patients, At the ECWA Community Health Centre Bukuru, Jos, Nigeria.*IOSR. J. of Pharm*.2: 65-69.
23. Francis, U., Isaac, Z., Yakubu, A., Enosakhare, A., Felix, E. (2014). Haematological Parameters of Malaria Infected Patients in the University of Calabar Teaching Hospital, Calabar, Nigeria. *J. of Haematol. and Thromboembolic dis.* 2: 171.
24. Bruce-Chwatt, L.J. (1982). Qinghaosu: a new antimalarial. British Medical Journal (Clinical Research Ed.) 284, 767–768 PATH Malaria Vaccine Initiative (MVI). p. 2.
25. Ahktar, K. P., Saleem, M. Y., Asghar, M., Alis.,Sarwar, N. and Elahi M. T. (2012). Resistance of *Solanum* species to *Phytophtorainfestans* evaluated in the detached leaf and whole plant assays; *Pak. J. of bot.*, 44(3): 1141- 1146
26. Chandra, H. and Chandra, S. (2013). Role of hematological parameters as an indication of acute malarial infection in Uttrakhand state of India; Mediterr. J. of Haematol. and Infect. dis. DOI: 10.4084/MJHID.2013.009. PMC3552839.
27. Ifeanyichukwu MO, Esan A. (2014). Evaluation of blood cells and platelets in Plasmodium falciparum malaria infected individuals. Int. J. Hematol. Disord. 1:49–54.