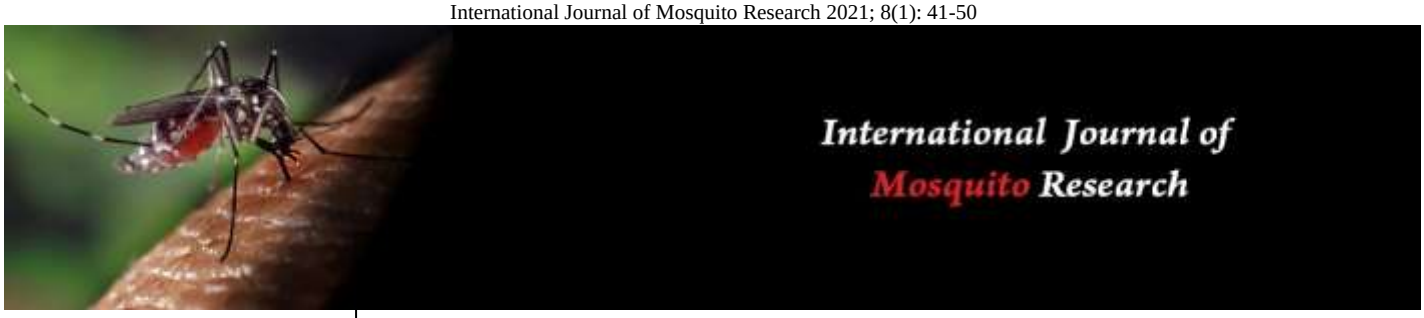

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Effect of extracts from *Carica papaya, Psidium guajava* and *Vernonia amygdalina* on metals level (Zinc and Magnesium) in blood samples of plasmodium infected rats

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Abstract
The incidence, mortality and morbidity rate of malaria infection is on the increase across the globe affecting over 300 million people per population causing over 1 million related deaths annually. Evidence exists indicating that malaria parasites depletes essential metals including Zinc and Magnesium among others required for the normal functions of the body during infection. More worrisome is the compounded burden of malaria by the recent emergent of resistant strains of plasmodia to conventional anti-malaria drugs including chloroquine, quinine and artemisinin derivatives among others. Intriguely, medicinal plants including *Carica papaya, Psidium guajava and Vernonia amygdalina* among others have been used since ancient times in the treatment of malaria infection which though have been speculated to affect the levels of essential metals including Zinc and Magnesium among others but the molecular mechanisms underlying the efficacies of these plants remained elusive. This study therefore investigated the effects of aqueous leaf extracts of *Carica papaya, Psidium guajava, Vernonia amygdalina* and the composite on essential metals levels (Zn and Mg) of plasmodium berghei infected rats treated with 200 mg/kg body weight ethanol leaf extracts of these plants for 20 days using atomic absorption spectrophotometer. *Vernonia amygdalina* caused statistical significant Zinc and Magnesium induction in the plasmodium berghei infected treated rats whilst 10-25% Zinc induction was observed in the composite treated rats. 5% - 31% magnesium induction was observed in *Carica papaya, Psidium guajava* and the composite treated rats. These findings may be relevant in the quest for alternative drugs for the treatment and chemoprevention of malaria.

Keywords: *Carica papaya, Psidium guajava, Vernonia amygdalina,* Zinc, magnesium, plasmodium berghei

Introduction
Zinc is an important element in human body that is needed for proper growth, maintenance of immune system; wound healing and thyroid function [1]. Deficiencies of zinc result in growth retardation, loss of appetite and impaired immune functions. The World Health Organization has noted that this is largely related to inadequate intake or absorption of zinc from the diet even though excess loss of zinc during diarrhoea may also contribute [2, 3]. Although severe Zinc deficiency is rare but evidence suggests that about one third of the world's population is affected with estimates ranging from 4% to 73% according to region [4]. Zinc deficiency ranks eleventh out of the 20 leading risk factors of disease resulting in disability and death worldwide. In the developed countries, more than 130,000 healthy life years are lost annually because of zinc deficiency [4] whilst in developing countries especially in Africa zinc deficiency ranks fifth among the ten leading risks factors of disease resulting in over 28 million Disability-Adjusted Life Years (DALYs) [4].
It has been speculated that Zinc deficiency may increase susceptibility to Plasmodium falciparum infection because of changes in the immune functions [5, 6]. Further evidence from animal studies have shown that mildly zinc deficient mice died from a normally non-lethal strain of P. yoelii and zinc supplements decreased markers of oxidative stress during infection with P. berghei infected mice [6, 7]. Human study has further shown that zinc concentrations are depressed during the acute phase response in children [8].
Moreover, Magnesium is a cofactor in more than 300 enzymes systems that regulate diverse biochemical reactions in the body including protein synthesis, nerve conduction,

oxidative phosphorylation and glycolysis among others [9, 10]. It has been speculated that Variations in the concentration of Magnesium caused by P. falciparum malarial infection may be associated with various errors of metabolism resulting in morbidity and eventually mortality among children. Study has shown that high *in vitro* growth of Plasmodium falciparum was reduced by 35 and 43% through high concentrations (5Mmol/L) of magnesium in RPMI medium and magnesium-free medium whilst high physiologic magnesium plasma levels has been shown to significantly increase the survival time of NMRI mice infected with P. berghei strain indicating that magnesium is an important cofactor in plasmodium infection [11].

Meanwhile, Medicinal plants have been used in the treatment and prevention of malaria in various parts of the world. Quinine extracted from the bark of the cinchona tree was the only antimalaria agent used as early as 1632 and up to 19th century [12]. Primaquine, quinacrine and particularly Chloroquine produced after the First World War have been designated the drug of choice for treatment of malaria especially in Nigeria for its cost effectiveness and efficacies [13, 14].

Although the weak anti-plasmodia activity of *Carica papaya* has been reported by some researchers but recent studies have shown that aqueous leave extracts of *Carica papaya* has potential of reducing parasitaemia at an activity second to that of Sulfadoxine-Pyrimethamine in Plasmodium berghei-infected mice and thus has been widely used in the treatment of malaria and splenomegaly in Africa especially Nigeria [15, 16, 17]. Its antimalaria activity has been adduced to its total antioxidant increments potential with inhibition of the onset and development of anaemia [18].

Furthermore, *Psidium guajava* which belongs to the family *Myrtaceae* and a native to tropical America has been used across the globe for the treatment of different ailments. For instance, in South Africa, decoctions leaves of *Psidium guajava* are used traditionally to treat diabetes mellitus and hypertension [19]. Whilst in Brazil, West Africa, Latin America and Caribbean countries decoction leaves and fruits are used in the treatment of anorexia, gastrointestinal and other infectious diseases due to its anti-inflammatory activities [20, 21].

More importantly, *Psydium guajava* has been used for malaria treatment in different part of the world including KwaZulu-Natal Province of South Africa, North Indian Buchpora, South Indian Eastern Ghats and the North-eastern Nigeria [22, 23, 24] whilst recent ethno botanical survey showed that *Psydium guajava* leaves were used to control mosquitoes in rural communities of Bagamoyo district in Tanzania [25].

Moreover, *Vernonia amygdalina* found in savannah regions, central and south tropical Africa has been used for medicinal purposes in the treatment of different ailments including malaria, diabetes Mellitus, pneumonia, anaemia and gastrointestinal disorders among others across the globe [26, 27].

Malaria is a life threatening protozoan disease of humans and it is worrisome that its incidence is rising annually worldwide especially in Africa. Malaria is one of the major causes of deaths in children and pregnant women, poses social-economic burden globally especially in endemic African countries. More than one million children die annually from malaria infection. It is estimated that a child dies every 30 seconds from malaria in Africa whilst 70% of these deaths occur in children less than 5 years of age [28]. In Nigeria, there is estimated 25% to 30% mortality in children under five, an estimated 300,000 related deaths each year due to malaria [29]. There is a growing evidence that malaria causes 0ne out of three deaths in children and one out of ten deaths in adults especially pregnant women [30]. Studies have shown that malaria limits international trades and development, the major cause of absenteeism from work and school in Africa thus reducing productivity, labour supply; increases illnesses and deaths [31]. In Nigeria, over $1 billion is spent annually in treating malaria at the expense of other infrastructural developmental project resulting in abject poverty [32].

More worrisome are the recent surveys showing the emergent of chloroquine and other anti- malaria drugs resistant strains of malaria parasites especially in Kenya, Madagascar and Tanzania [33, 34, 35].

Intriguely, medicinal plants have been used in the treatment and prevention of malaria in various parts of the world. Surprisingly, not much attention has been given to these Indigenous medicinal plants especially in Nigeria to showcase their efficacies in conferences both locally and internationally as alternative drugs for malaria treatment in spite of our rich flora diversity.

More compounding are the varied controversial and conflicting theories in circulation on the impact of malaria on essential metals especially zinc, magnesium and supplements status of malaria infected patients complicated by the emergent of drug resistant strain of malaria parasites and limited data to justify the various claims. Most worrisome, is the little or no attention given in the past by other researchers to correct these anomalies so as to establish the mechanisms underlying the efficacies of these plants? Thus, there is urgent need to investigate these controversies in order to fully comprehend the impacts of these medicinal plants on patient's essential metal status and to further create insights into the mechanisms underlying the efficacies of these plants in the treatment of malaria. It was based on this present insight that this study was designed to determine the levels of zinc (Zn) and magnesium (Mg) in *Carica papaya, Vernonia amygdalina and Psidium guajava* and their concentrations in the blood samples of plasmodium berghei infected rats to bridge this gap and to contribute our quota to the existing body of knowledge.

Materials and methods

Animal preparation

The rats that were used in this study were 8–14 weeks old non-pregnant Swiss albino rats with average weight of 25 ± 2 obtained from Gombe State University. These were allowed to acclimatize in the animal house of Gombe state University; and were fed with standard rat's feed for five days before the commencement of the experiment.

Parasites

The parasites used in this study are chloroquine-sensitive strain of *Plasmodium berghei* NK 65, maintained in rats, from the National Institute of Medical Research (NIMR), Lagos, Nigeria which served as the donor rats in this study.

Preparation of herbal extracts

The plants that were used in this study are '*Carica papaya, Psidium guajava and Vernonia amygdalina* identified by Mr Ibrahim Mohammed of Federal college of Horticulture Dadin-kowa, Gombe, Gombe State. One hundred grams of mature

fresh leaves *of Carica papaya, Psidium guajava and Vernonia amygdalina* were allowed to drip dry. The leaves of each plant were grounded in a clean porcelain mortar and then macerated in cold distilled water for 24 hours separately. The extract of each plant was recovered by passing it through a fine mesh of muslin cloth and allowed to settle. The supernatant pawpaw crude extract (PCE), Guava crude extract (GCE) and Bitter leave crude extract (BCE) respectively were stored in a refrigerator at 2-4ºC before use. A measured aliquot of each extract was evaporated to dryness in order to determine the concentration. Each extract was diluted with a mixture of Tween 80 and ethanol in sterile distilled water, so as to enable the administration of doses of interest per kilogram of the extracts [36].

***In vivo* schizontocidal activity determination of donor rats**

On Day 0 of the test, the percentage parasitaemia of the donor rats were determined using a Giemsa-stained thick blood smear of the donor mice on a slide and microscope using oil emersion objective.

***In vivo* schizontocidal activity and heavy metal level determination of the tests and control rats**

Prior to the infection of the test mice with malaria parasites and crude herbal extracts administration, the percentage parasitaemia of the test and control rats were determined to ascertain that they were free from malaria infection using a Giemsa-stained thick blood smear of each tests and control mice collected by cardiac puncture from the retro-orbital plexus vein on a slide and microscope using oil emersion objective. Similarly, the blood of the tests and control rats were collected by cardiac puncture from the retro-orbital plexus vein for the determination of the basal levels of essential metals (Zn and Mg) using Atomic Absorption Spectrophotometer (AAS).

Determination of the median lethal dose (LD_{50}) of *Carica Papaya, Psidium guajava and Vernonia Amygdalina*

The median lethal dose of each herbal crude extracts was determined in two stages using Lorke's method as follows:

In the first stage, nine mice were randomly divided into three groups of three each. Group one was administered orally with 10 mg/kg body weight of each of the crude herbal extract and group with 100 mg/kg body weight whilst group three was administered orally with 1000 mg each of the herbal crude extracts and the rats were kept under observation for 24 hours for behavioural change and possible mortality.

In the second stage, three rats were randomly selected and administered orally with 2000 mg/kg body weight; 3000 mg/kg body weight and 5000 mg.kg body weight each of herbal crude extracts of *Carica Papaya, Vernonia Amygdalina and Psidium guajava* and were observed for 24 hours for any physiological or behavioural change and possible mortality. Thereafter the LD50 of each herbal extract was calculated using the following formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality

Rats' infection

Subsequently, the blood sample of the donor rats were collected by cardiac puncture and from the retro-orbital plexus vein and were diluted with physiological saline (Normal saline) to give a concentration of 10^8 parasitized erythrocytes per ml. The rats were randomly shared into seven groups of twenty rats each. 0.2 ml of 10^8 parasitized erythrocytes/ml was injected intraperitoneally into each of the experimental rat and was kept under observation for 72 hours.

***In vivo* schizontocidal activity determination of the infected test rats**

The percentage parasitaemia of the infected test rats were determined after 72 hours to ascertain that the test rats were initially malaria infected before administering herbal extracts using a Giemsa-stained thick blood smear of the infected mice collected by cardiac puncture from the retro-orbital plexus vein on a slide and microscope using oil emersion objective.

Crude herbal extracts administration

Sequel to *in vivo* schizontocidal activity determination of infected test rats, group one to three were administered orally with 200 mg/ml of pawpaw crude extract, guava crude extract and bitter leaf crude extract per kilogramme body weight of each rat respectively. Group four was administered orally with 200 mg/ml of the mixture and equal volume of pawpaw crude extract, guava crude extract and bitter leaf crude extract (Composite) per kilogramme body weight of each rat whilst the fifth group which served as control group were given equal volume of placebo orally as the test groups. The rats were monitored for three weeks from the date of herbal administration. Subsequently, blood samples were taken from the retro-orbital plexus vein on day 5, 10, 15 and 20 for the determination of zinc and magnesium respectively.

Sample preparation, acid digestion and processing

Two ml of whole blood sample was taken from each rat into a labelled plain sterile container and was allowed to stand for 15 – 20 minutes at room temperature for clot retraction. These were centrifuged at a speed of 5000 rotation per minute (RPM) on a centrifuge for 10 minutes. The supernatant of each sample was obtained by careful separation using a Pasteur pipette into another clean and sterile plain container labelled accordingly. Each sample was then diluted 1:100 with double distilled water and was kept at 2-4^{0}C before use.

In addition, the dried crude herbal extract of *Psidium guajava, Carica Papaya* and *Vernonia Amygdalina* were digested with concentrated Nitric - hydrochloric acid mixture before analysis as follows; freshly prepared mixture of 75 ml of concentrated Nitric acid and 25 ml of concentrated hydrochloric acid was added to 1.0 gram of each dried crude herbal extract in a sterile clean conical flask and boiled gently over a water bath maintained at 95 ^{0}C until the samples were completely dissolved. The solution of each extract was filtered using Whatman filter paper to remove any particulate matter retained. Each digested sample was made up to 100 ml with double distilled water and kept 2-4^{0}C before use [37].

Determination of essential metals levels (Zinc and Magnesium)

The concentrations of Zinc and Magnesium of each rat sample and that of Pawpaw, guava and bitter leaf crude extracts were determined using a Perkin Elmer, Analyst 800 Atomic Absorption Spectrophotometer (AAS).

Principle: free gaseous atoms in their ground state when

heated absorb energy at a specific wavelength to attain exited state. The amount of energy absorbed is directly proportional to the concentration of analyte in the sample in accordance to Beer Lambert's law [38].

Statistical analysis

The results obtained from these experiments were subjected to statistical analysis using instant and graph pad prism software to test the significant level of each parameter at $P < 0.05$.

Results

Table 1: levels of Mg and Zn in the various leaf extracts

Leaf extract	Magnesium	Zinc
Guava	2.84	0.18
Bitter leaf	8.88	0.36
Pawpaw	8.20	0.13
Normal control	0.28	0.17

Table 2: Mean concentration of Zinc at Day 5, 10, 15 and 20 in varius groups treated with different leafs extracts.

Days	Pawpaw (ppm)	Guava (ppm)	Bitter leaf (ppm)	Composite (ppm)	Normal control (ppm)	Negative control (ppm)	Standard drug (ppm)
Day 5	0.08	0.13	0.19	0.13	0.13	0.03	0.23
Day 10	0.10	0.15	0.22	0.18	0.15	0.02	0.21
Day 15	0.14	0.16	0.21	0.20	0.13	0.05	0.24
Day 20	0.15	0.16	0.22	0.24	0.14	0.02	0.27

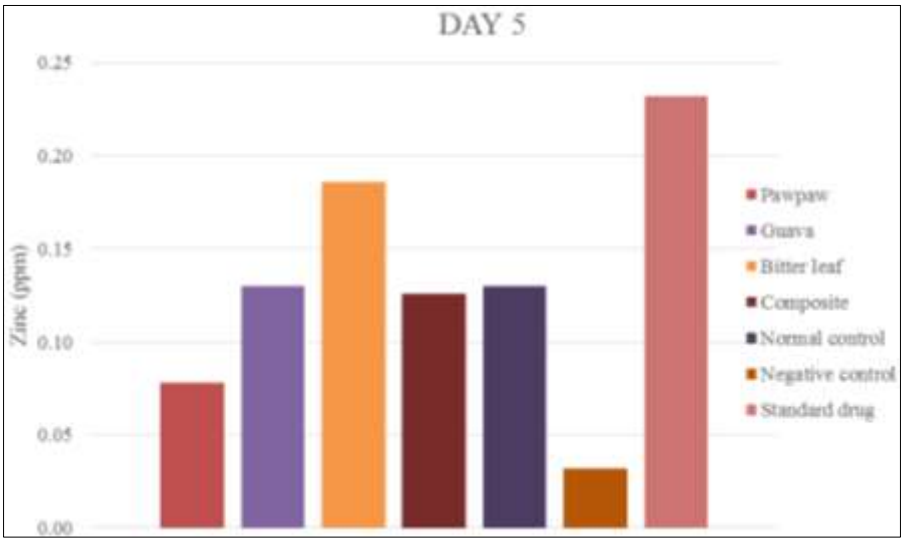

Fig 1: Graphical representation of the mean concentration of Zn at Day 5 in various group of plasmodium infected rats treated with 200 mg/kg body weight of Pawpaw, bitter leaf, guava leaf extracts and the composite respectively. Bitter leaf extracts treated group and standard drug treated group showed statistical significant induction in Zinc level ($P < 0.05$) compared to normal control whilst no significant Zinc induction was observed in the pawpaw, guava and the composite treated groups.

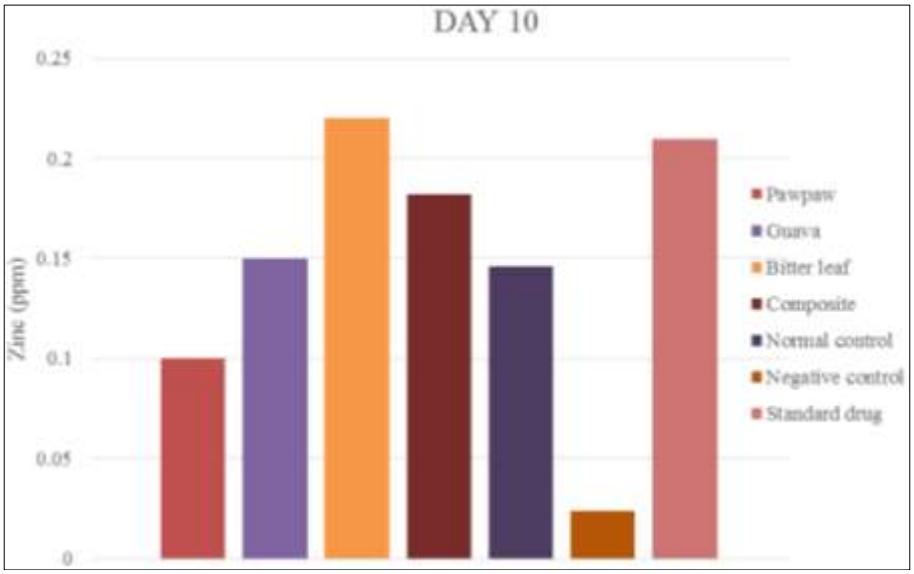

Fig 2: Graphical representations of the mean concentration of Zn at Day 10 in various group of plasmodium infected rats treated with 200 mg/kg body weight of Pawpaw, bitter leaf, guava leaf extracts and the composite respectively. Bitter leaf extracts treated group and standard drug treated group showed statistical significant induction in Zinc level ($P < 0.05$) compared to normal control whist 20-25% Zinc induction was observed in the composite treated group.

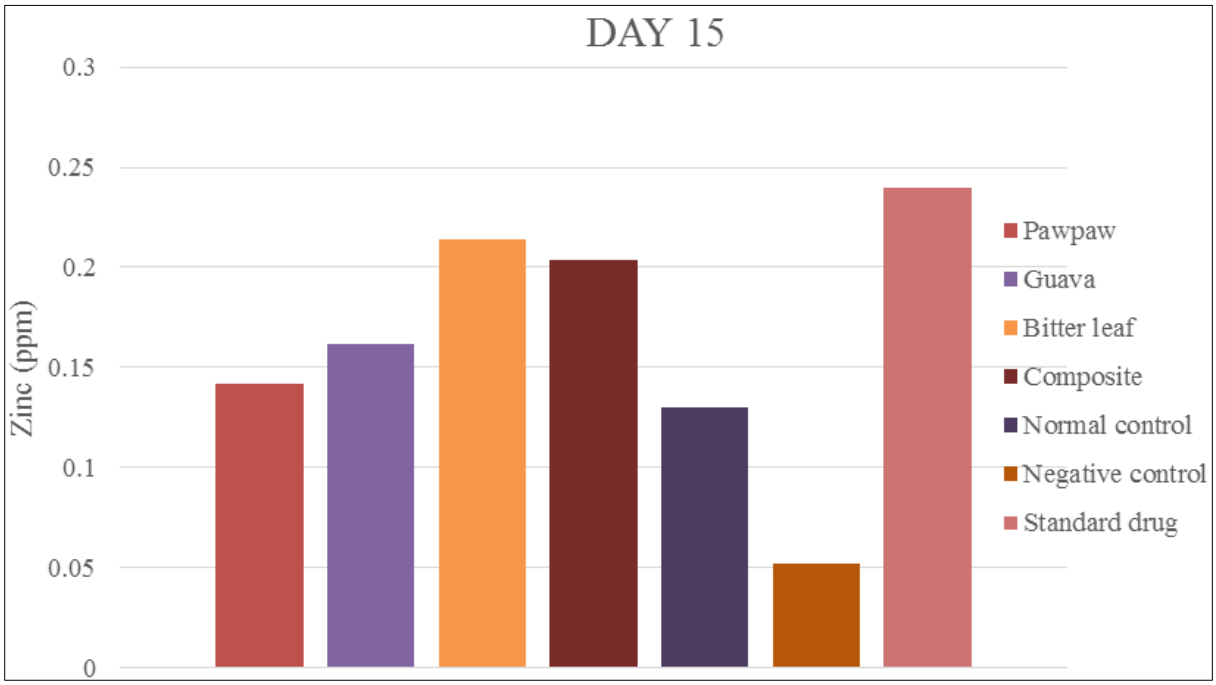

Fig 3: Graphical representations of the mean concentration of Zn at Day 15 in various group of plasmodium infected rats treated with 200 mg/kg body weight of Pawpaw, bitter leaf, guava leaf extracts and the composite respectively. Bitter leaf extracts treated group, composite treated group and standard drug treated group showed statistical significant induction in Zinc level ($P < 0.05$) compared to normal control whist 10-25% Zinc induction were observed in pawpaw and guava extracts treated groups.

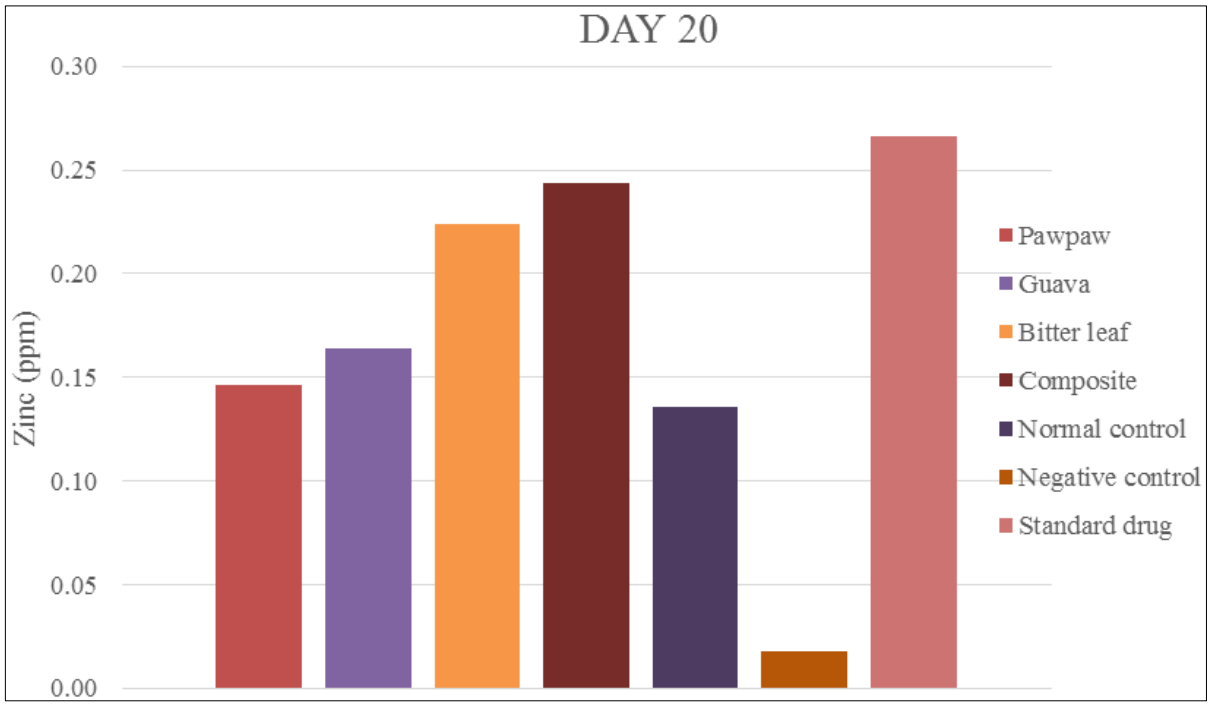

Fig 4: Graphical representations of the mean concentration of Zn at Day 20 in various group of plasmodium infected rats treated with 200 mg/kg body weight of Pawpaw, bitter leaf, guava leaf extracts and the composite respectively. Bitter leaf extracts treated group, composite treated group and standard drug treated group showed statistical significant induction in Zinc level ($P < 0.05$) compared to normal control whist 10-20% Zinc induction were observed in pawpaw and guava extracts treated groups.

Table 3: Mean concentration of Magnesium at Day 5, 10, 15 and 20 in varius group treated with different leafs extracts.

Days	Pawpaw (ppm)	Guava (ppm)	Bitter leaf (ppm)	Composite (ppm)	Normal control (ppm)	Negative control (ppm)	Standard drug (ppm)
Day 5	0.186	0.242	0.270	0.206	0.196	0.132	0.282
Day 10	0.218	0.260	0.302	0.232	0.198	0.120	0.336
Day 15	0.206	0.270	0.274	0.262	0.196	0.156	0.340
Day 20	0.244	0.274	0.296	0.258	0.180	0.128	0.312

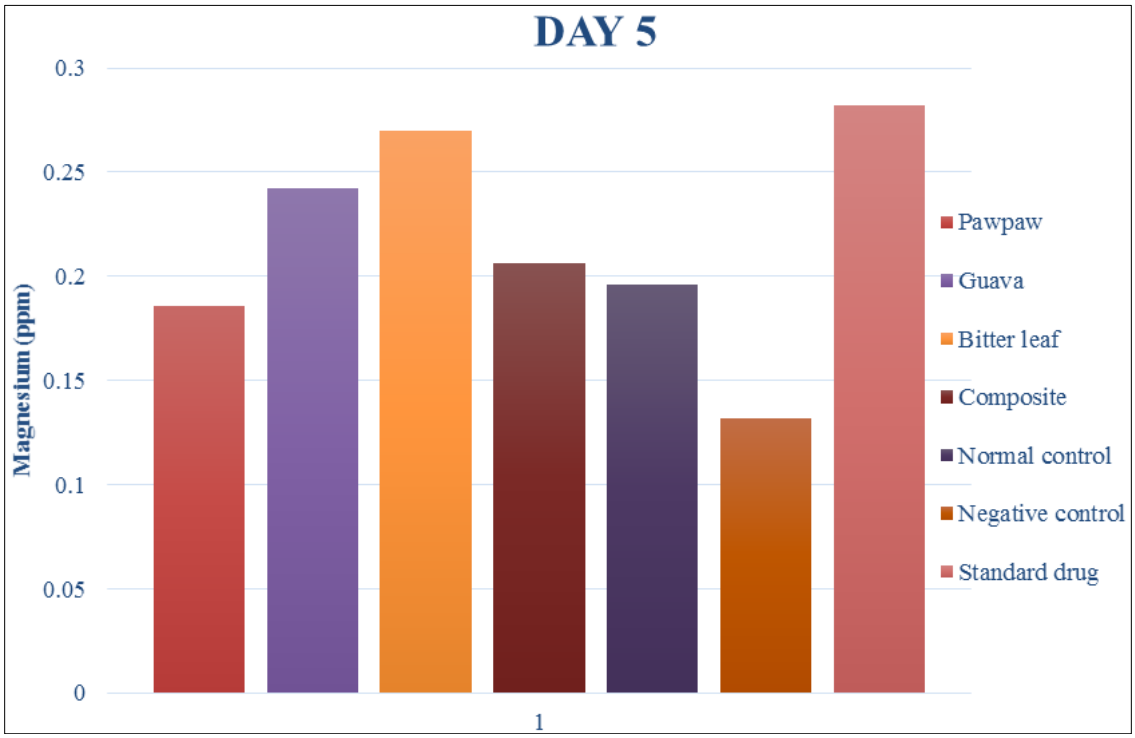

Fig 5: Graphical representations of the mean concentration of Mg at Day 5 in various group of plasmodium infected rats treated with 200 mg/kg body weight of Pawpaw, bitter leaf, guava leaf extracts and the composite respectively. Bitter leaf extracts treated group, guava extracts treated group and standard drug treated groups showed statistical significant induction in Mg level ($P < 0.05$) compared to normal control whist 5% Mg induction was observed in the composite extracts treated group.

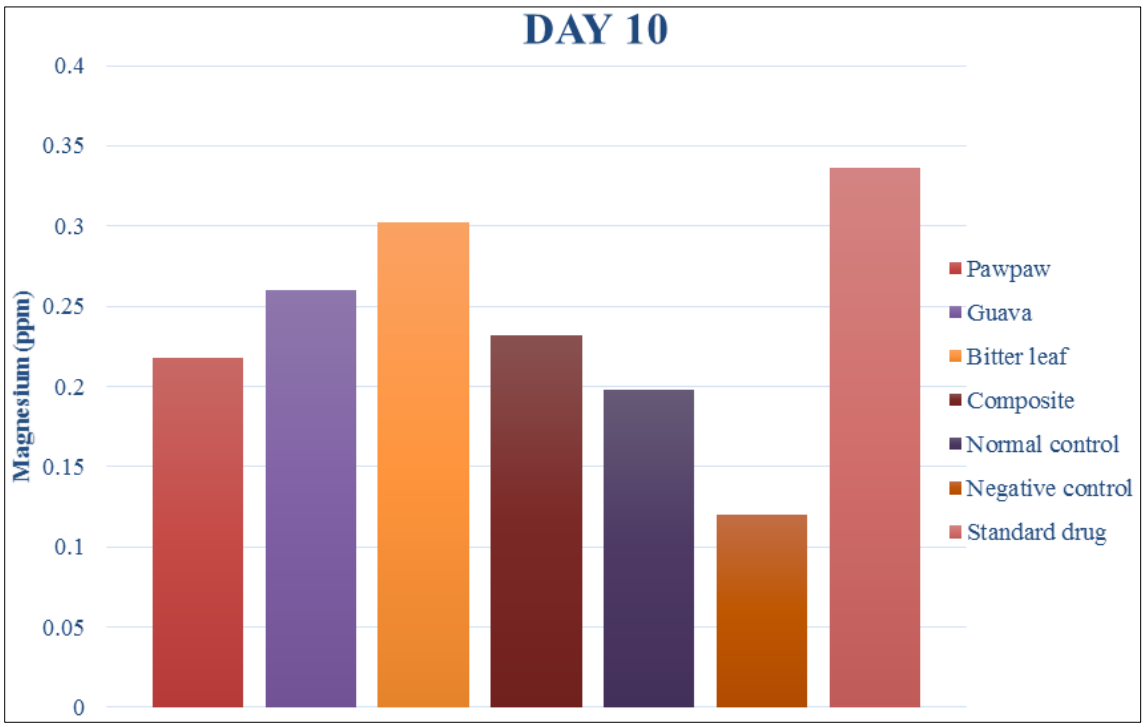

Fig 6: Graphical representations of the mean concentration of Mg at Day 10 in various group of plasmodium infected rats treated with 200 mg/kg body weight of Pawpaw, bitter leaf, guava leaf extracts and the composite respectively. Bitter leaf extracts treated group and standard drug treated group showed statistical significant induction in Mg level ($P < 0.05$) compared to normal control whilst 2.0%, 3.1% and 31% Mg induction were observed in pawpaw, composite and guava extracts treated groups respectively.

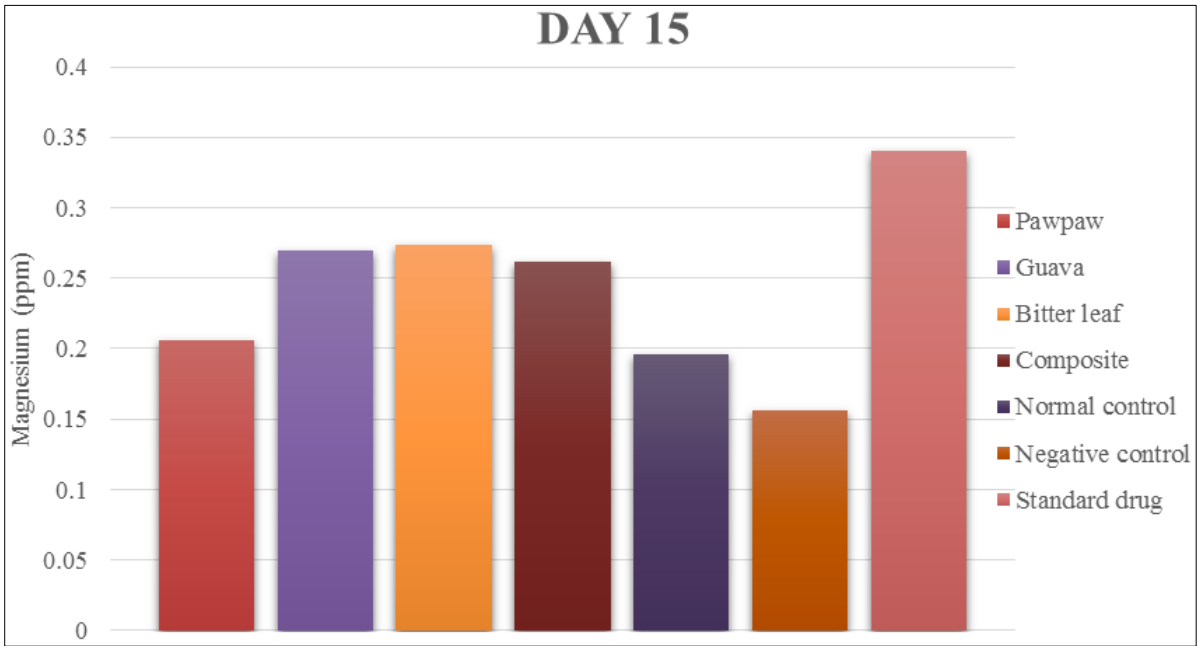

Fig 7: Graphical representations of the mean concentration of Mg at Day 15 in various group of plasmodium infected rats treated with 200 mg/kg body weight of Pawpaw, bitter leaf, guava leaf extracts and the composite respectively. Standard drug treated group showed statistical significant induction in Mg level ($P < 0.05$) compared to normal control whist 5% -7% Mg induction were observed in the guava, bitter leave and composite extracts treated groups.

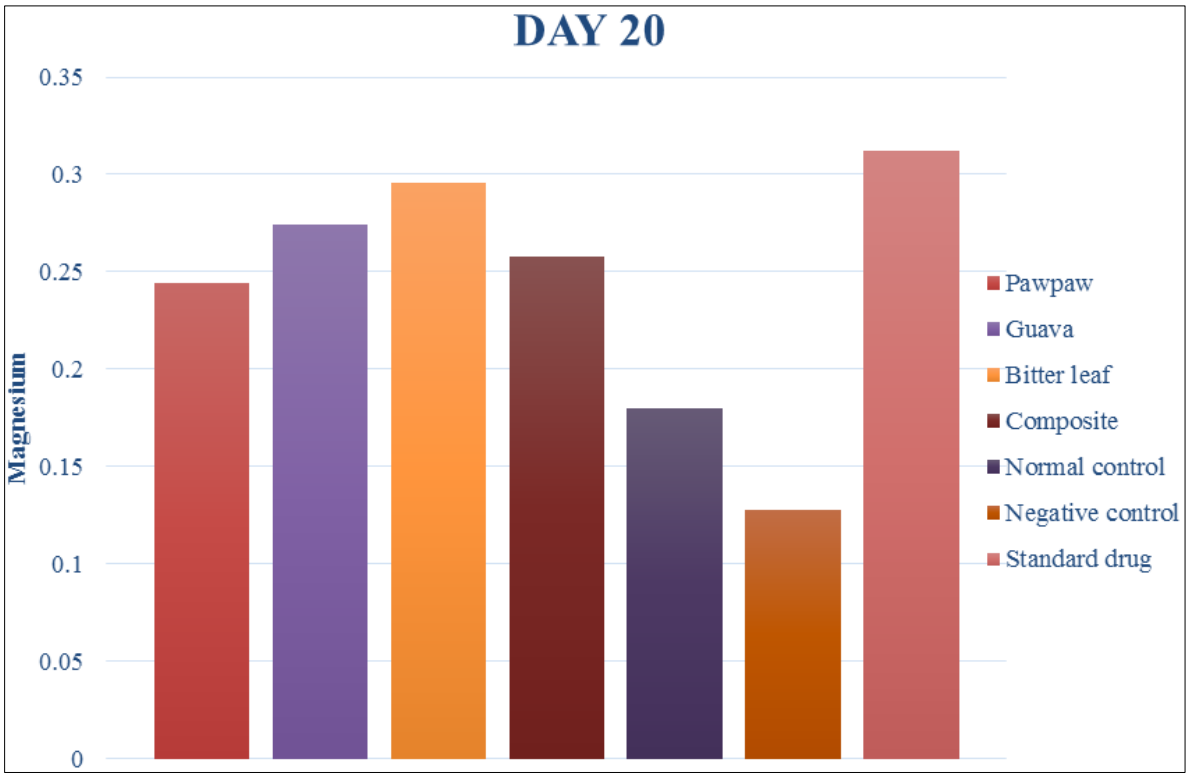

Fig 8: Graphical representations of the mean concentration of Mg at Day 20 in various group of plasmodium infected rats treated with 200 mg/kg body weight of Pawpaw, bitter leaf, guava leaf extracts and the composite respectively. Standard drug treated group and bitter leaf treated groups showed statistical significant induction in Mg level ($P < 0.05$) compared to normal control whist 6% -10% Mg inductions were observed in the pawpaw, guava and composite extracts treated groups.

Discussion

Assessment of the effect of pawpaw, guava and bitter leaf extracts on Zinc status of plasmodium infected rats

The incidence of malaria infection is on the increase globally affecting over 300 million people per population resulting in over 1 million related deaths annually [39, 40]. Zinc is an essential metal necessary for the production of cells that help the body to fight diseases, maintenance of membrane integrity and physical growth. Thus, Zinc deficiency constitutes a major health problem as one of the leading cause of illnesses and diseases [41]. Zinc deficiency has been shown to increase the susceptibility to plasmodium falciparum infection because of the changes in immune function whilst malaria infection itself has been shown to causes Zinc depletion [8]. One of the

molecular mechanisms that have been linked to Zinc depletion by plasmodium infection is that the weakly bound Zinc to infected erythrocytes are easily depleted which are essential for the pathogenicity and survival of the parasite especially for the rupture of the host cell parasites [42]. Therefore, Zinc supplementation has been used to reduce the mortality associated with plasmodia infection based on this presumption [43].

Invariably, medicinal plants have been used since ancient times in the treatment of malaria infection [44]. More importantly, attention has been focussed recently on pawpaw, bitter leaf and guava among others as novel agents that might be of potential benefits in the treatment of malaria infection. Although, there are considerable theories in circulation with respect to the plasmodia efficacies of these plants [43] but the molecular mechanisms underlying their anti-plasmodia activities remained controversial. Thus, to establish the molecular mechanism underlying the anti-plasmodia efficacies of these plants, the response of Zinc to 200 mg/kg body weight of ethanol leaf extracts of pawpaw, bitter leaf and guava leaf extracts in plasmodia infected rats were investigated. Bitter leaf extracts treated group and standard drug treated group showed statistical significant induction in Zinc level at Day 5 ($P < 0.05$). There was 20-40% Zinc induction in the composite treated group whilst non statistical significant difference in Zinc induction was observed in the standard drug treated group and bitter leaf extracts treated group. Surprisingly, this research work is the first of its kind to investigate the effects of these medicinal plants extracts on the level of Zinc.

If we assume uniform and equal rate of metabolization of 200 mg/kg body weight of each of the leaf extracts administered to all the treated rats, then it could be reasonably argued that perhaps one of the anti-plasmodia molecular mechanisms of this plants is via induction of Zinc and thus, bitter leaf extracts is more potent, more efficient and more efficacious anti-plasmodia agent compared to guava and pawpaw leaf extracts. Although, 20-40% Zinc induction was observed in the composite treated group but could not justify its potency and efficacy in the treatment of malaria infection. We speculated that the Zinc induction might have occurred from the activity of the leaf extracts of bitter leaf present in the mixture.

Intriguely, the non-statistical significant difference observed in Zinc induction between Vernonia leaf extracts and standard drug treated groups probably suggests equal anti-plasmodia efficacies of bitter leaf and the current conventional drugs (Arthemetal Lumefantrine) used in the treatment of malaria infection. This is major discovery that is absolutely relevant and must be considered in the quest for alternative and a substitute drug for the treatment and chemoprevention of malaria infection now and in the nearest future.

Assessment of the effect of pawpaw, guava and bitter leaf extracts on Magnesium status of plasmodium infected rats

Magnesium is an essential metal with variety of biological functions including cell proliferation, modulation, and progression and as cofactor in many enzymatic reactions [9, 10]. There are many theories in circulation with respect to the effect of malaria on magnesium levels during infection. It has been reported that high physiological concentration of magnesium plasma level significantly aid the longer survival time of plasmodium infected mice [11]. Whilst other researchers have shown that malaria infection depletes magnesium levels in malaria infection and the severity of the malaria varies with the concentration of magnesium associated with it [45]. The mechanism underlying the depletion of magnesium during malaria infection has been linked to errors of metabolism [46].

Invariably, medicinal plants have been used since ancient times in the treatment of malaria infection [44]. More importantly, attention has been focussed recently on Pawpaw, bitter leaf and guava leave extracts among others as novel agents that might be of potential benefits in the treatment of malaria infection. Although, there are considerable theories in circulation with respect to anti-plasmodia efficacies of these plants [43, 45] but the molecular mechanisms underlying their activities remained elusive. Thus, to establish the molecular mechanism underlying the anti-plasmodia efficacies of these plants, the response of Magnesium to 200 mg/kg body weight of ethanol leaf extracts of pawpaw, guava, bitter leaf and the composite in plasmodia infected rats were investigated. Bitter leaf extract treated group and standard drug treated group showed statistical significant induction in Magnesium levels ($P < 0.05$). 30-45% Magnesium induction in the Pawpaw, guava and the composite extracts treated groups were observed whilst non statistical significant difference in Magnesium induction was observed in the bitter leaf extracts treated group compared to standard drug treated group.

Surprisingly, this research work is the first of its kind to investigate the effects of these medicinal plants extracts on the level of Magnesium.

If we assume uniform rate of metabolization of 200 mg/kg body weight of each of the leaf extracts administered to all the treated rats, then it could be reasonably argued that perhaps one of the anti-plasmodia molecular mechanisms of these plants is via induction of Magnesium. Thus, bitter leaf extracts is presumably more potent, efficient and efficacious anti-plasmodia agent compared to guava, pawpaw leaf extracts and the composite. Although there was 30-45% magnesium induction in pawpaw, guava and the composite treated groups, this level of induction is not suffice to account for their anti-plasmodia efficacies and thus need further research.

More interestingly, the non-statistical significant difference observed in Magnesium induction between bitter leaf extracts and standard drug treated groups probably suggests equal anti-plasmodia efficacies of bitter leaf and the current conventional drugs (Arthemetal Lumefantrine) used in the treatment of malaria infection. This is major discovery that is absolutely relevant and must be considered in present quest for alternative and substitute drug for the treatment and chemoprevention of malaria infection now and in the nearest future.

Conclusion

It is obvious from the results of this study that though bitter leaf and to some extent guava leaf extracts induced the levels of these metals investigated but bitter leaf is presumably believed to be the most potent of all the plants investigated. The administration of 200 mg leaf extracts of bitter leaf extracts caused statistical significant increment in the levels of Zinc and Magnesium in the plasmodium berghei infected rats. The presence of Magnesium and Zinc in high concentration in the leaf extracts of bitter leaf could probably account for these increment and need further investigation. We therefore recommend that the use of pawpaw, guava and especially

bitter leaf extracts be encouraged both in the treatment of malaria infection and as supplement.

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