GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Anti plasmodial evaluation of the methanol and aqueous extracts of *Gnetum africanum* in wistar albino mice

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GSC Biological and Pharmaceutical Sciences, 2024, 29(02), 364–373

Publication history: Received on 01 October 2024; revised on 26 November 2024; accepted on 27 November 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.29.2.0418

Abstract

The methanol and aqueous extracts of different parts of *Gnetum africanum* plant were studied for their *in vivo* anti-plasmodial activity against chloroquine resistant *Plasmodium berghei* Nk 65 in Swiss albino mice. A four day curative standard test was used employing the rodent malaria parasite, *Plasmodium berghei* NK 65. The mice were divided into groups. Five mice were used for each of the seven test/treatment groups. The aqueous extract of the stem of *Gnetum africanum* showed the highest antiplasmodial activity. The extract significantly suppressed the parasite. There was a significant difference ($p<0.05$) observed when the level of parasitemia of the animals treated with the aqueous extract of the stem bark of *Gnetum africanum* was compared with the untreated, chloroquine standard control and other treatment groups. The aqueous leaf extract of *Gnetum africanum* had the lowest suppression on the parasite as none of the animals in this group survived to the end of the 14 days study. In as much as some the animals in the *Gnetum africanum* stem methanol (4.00±0.00%) and *Gnetum africanum* leaf methanol (3.20±0.00%) survived till the end of the 14 days study, the parasite load was increasing in both groups, thereby indicating that there was no significant antiplasmodial activity in these groups. Since *Gnetum africanum* showed good antiplasmodial activity, it is concluded that this plant has potentials in fighting malaria.

Keywords: Antiplasmodial; Acute toxicity; Swiss Albino mice; *Gnetum africanum*; *Plasmodium berghei*

1. Introduction

Malaria has proved to be one of the most debilitating illnesses of all time and is the most common parasitic disease in sub-Saharan Africa (Ene et al., 2018). Malaria is the single most important cause of ill health, death and poverty in Sub-Saharan Africa (Kilama, 2005). The disease is a major obstruction to social and economic development in Africa, causing enormous misery and suffering through the pain of fevers and the anguish of bereavement, with one African child dying every 40 seconds (Kilama, 2005; Uwimana et al., 2021).

The prevalence of malaria in this region is due to the fact that mosquito, *Anopheles gambiae* Giles which are found throughout tropical Africa is the most efficient vector of human malaria in the Afrotropical Region (Kilama, 2005).

Globally, malaria deaths declined steadily from 864 000 in 2000 to 586 000 in 2015 and to 576 000 in 2019. In 2020, malaria deaths increased by 10% compared with 2019, to an estimated 631 000. Estimated deaths declined in 2022 to 608 000. The percentage of total malaria deaths in children aged under 5 years decreased from 87% in 2000 to 76% in 2015. Since then, there has been no change. (WHO, 2023),

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About 96% of malaria deaths globally were in 29 countries. Four countries accounted for just over half of all malaria deaths globally in 2022 – Nigeria (31%), the Democratic Republic of the Congo (12%), Niger (6%) and the United Republic of Tanzania (4%). (WHO, 2023).

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans (a group of single-celled microorganisms) belonging to the *Plasmodium* type (WHO, 2014). The disease is most commonly transmitted by an infected female *Anopheles* mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood (WHO, 2014). The parasites travel to the liver where they mature and reproduce.

Until recently, four malaria parasites were known to infect humans, the most common being *P. falciparum* and *P. vivax*. However, a fifth parasite, *P. knowlesi*, has emerged as a significant concern for malaria control, especially in SouthEast Asia. This zoonotic parasite, initially found in monkeys, has a human fatality rate of 1–2%, and is known for causing severe and rapid onset of disease. Since large infection clusters were discovered in Malaysia in 2004, *P. knowlesi* has spread across nearly all of South-East Asia and globally through travel and tourism. Although the number of *P. knowlesi* cases declined globally in 2022 by 24.2% (to a total of just 2768 cases), the number increased significantly in Indonesia and Thailand, and *P. knowlesi* caused all malaria deaths in Malaysia and Thailand in that year (WHO, 2023).

Climate variability, such as changes in temperature and rainfall, can impact the behaviour and survival of the malaria-carrying *Anopheles* mosquito. Extreme weather events such as heatwaves and flooding may lead to increases in the transmission and burden of the disease (Nissan et al., 2021; WHO, 2023).

Infection with these protozoa is caused by mosquito bite. An infected female Anopheles mosquito is the most common transmitter of malaria. First, sporozoites enter the bloodstream, and migrate to the liver. They infect liver cells, where they multiply into merozoites, rupture the liver cells, and return to the bloodstream. The merozoites infect red blood cells, where they develop into ring forms, trophozoites and schizonts that in turn produce further merozoites. Sexual forms are also produced, which, if taken up by a mosquito, will infect the insect and continue the life cycle (Schlagenhauf-Lawlor, 2008).

The emergence of multidrug-resistant plasmodium is a worldwide problem. Drug resistance is usually first recognized clinically as a recrudescence, when parasites reappear in the circulation after a period of latency following drug treatment. Recrudescent parasites, however, are not always resistant to the drug used for treatment, it acts this way either because the dose was inadequate or because the parasite has developed resistance to the drug (Ittarat et al., 2003; Rasmussen et al., 2021).

Poor compliance and erratic absorption of drugs can lead to lower plasma concentrations and inadequate exposure to therapeutic concentration of drug. Thus, despite adequate drug treatment, recrudescence may still occur (Yeka et al., 2019).

Taken together, these threats are undermining gains in the global fight against malaria. In 2022, the global tally of malaria cases reached 249 million – well above the estimated number of cases before the COVID-19 pandemic, and an increase of five million over 2021 (WHO, 2023).

Many studies have been carried out and more ongoing with the aim of reducing the incidence of drug resistance in malaria and other parasitic diseases (Ene et al., 2008). Therefore, for the purpose of providing an effective weapon for evaluating new chemotherapeutic agents targeted at chloroquine resistant malaria, we attempt in the present study to experimentally develop a drug that has efficacy against chloroquine resistant malaria.

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. Traditional medicinal plants play an important role in medical system in Nigeria and plant materials remain an important resource to combat serious diseases in the world. Pharmacognostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents (Feachem et al., 2010). Since many drugs, e.g quinine and artemisinin were isolated from plants and because of the increased resistance of many pathogens, e.g malaria parasites, against established drugs, investigation of the chemical compounds within traditional plants is necessary. It is believed strongly that if the herbs used to treat malaria by our ancestors in Africa hundreds of years ago were not effective, malaria would have destroyed Africa. More so, Missionaries that came to Africa would not have met a single person on the continent of Africa (Feachem et al., 2010; Ezeji-Chigbu et al., 2018). In view of the problems associated with antimalarial drug resistance, new drugs or drug combinations are urgently required today for treatment of malaria. Preferably, the new drugs should have novel modes of action or be chemically different from the drugs in current use. Plants have always been considered to be a possible alternative and rich source of new drugs and most of the

antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates (Feachem et al., 2010). Due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the populations depend on traditional medical remedies mainly from plants (WHO, 2014; Zinhi et al., 2005).

Gnetum africanum is a vine gymnosperm species found native throughout tropical Africa. Though bearing leaves, the genus Gnetum are gymnosperms, related to pine and other conifers. *Gnetum africanum*, is a climbing vine in the tropical rainforest of West and Central Africa. This vine will grow in all seasons and typically spreads along forest floors. The vine grows in two ways: through rhizomes, or through new shoots that grow where the stem has been cut (Chaw, et al., 2000).

Gnetum africanum tends to grow best in shaded areas.and through the years been domesticated and grown for subsistence and commercial use among the people of the southern part of Nigeria. The leaves are highly valued as a nutritious green vegetable and today, as an article of considerable cross-border trade, extending to Europe and America. (Tekwe et al., 2003)

The leaves of the vine are sold in markets throughout the year and may be used in soups and stews or eaten raw. The leaves may further be used as a remedy for nausea, sore throats, or as a dressing for warts. The stem of the plant may also be eaten for medicinal purposes, including the reduction of pain during childbirth. (Soltis et al., 2002)

Gnetum africanum is a good source of protein and is rich in essential and non-essential amino acids. It is high in glutamic acid, leucine, and aspartic acid, with low levels of histidine, and cysteine, while there appears to be trace amounts of tryptophan in the plant. The content of amino acids found in Gnetum africanum is similar to recommended levels by the FAO. It has also been found that the levels of iodine are also high in the vine. Fibre levels average approximately 33.4 g/100 g of dried *Gnetum africanum* leaves, while recommended daily intake of fibre is 30g. Medicinally, *Gnetum africanum* is used in the treatment of a variety of illnesses. In Nigeria the leaves are used for the treatment of enlarged spleen, for sore throat and as a cathartic (Ndam et al., 2000). *Gnetum africanum* has been noted to be anti-inflammatory, anticarcinogenic and antioxidant(Ali, et al., 2011) .

Many researches have been carried out and more ongoing with the aim of reducing the incidence of drug resistance in malaria parasitism without much success (Ene et al., 2008). Therefore, for the purpose of providing an effective weapon for evaluating new chemotherapeutic agents targeted at chloroquine resistant malaria, we attempt in the present study to experimentally develop a more plasmodium sensitive drug using chloroquine resistant *Plasmodium berghei* (Ezeji-Chigbu et al., 2018).

Despite the war against the scourge of malaria, the African region continues to shoulder the heaviest malaria burden. To worsen the situation, the emergence of multidrug-resistant *Plasmodium spp* recognized clinically as recrudescence, when parasites reappear in the circulation after a period of latency following drug treatment, has been a major setback. Although major research efforts have been made to understand the mechanisms of drug resistance in malaria parasites, a definitive explanation remains elusive. Therefore, it has become necessary that medicinal plants which offer more potency in the treatment of malaria and also has minimal or no side effects on the biochemical parameters in the human system be used in the treatment of this disease.

Gnetum africanum has been identified with so many pharmacological activities that have made it attractive in herbal market. Potentially it could be a source of lead for development of a number of effective and essential modern drugs. Therefore, this study seeks to examine the antiplasmodial activities *Gnetum africanum* as a substitute to regular anti-malarial drugs to fight this global scourge (Ali et al., 2011).

Traditional remedies are being used more frequently, and they are being referred to by other names, such as complementary and alternative medicine (CAM) or alternative medicine, as the world seems to be rediscovering its significance. Many researches have been carried out with the aim of reducing the incidence of drug resistance in malaria and other parasites without much success. For the purpose of providing an effective weapon for evaluating new chemotherapeutic agents targeted at chloroquine resistant malaria, we attempt in the present study to assess the possibility of experimentally developing chloroquine-resistant *Plasmodium berghei* using chloroquine sensitive *Plasmodium berghei* NK65 (Ene et al., 2018).

In view of the fact that many researches have been carried out and more ongoing with the aim of reducing the incidence of drug resistance in malaria parasitism without much success (Ene et al., 2008). Therefore, for the purpose of providing an effective weapon for evaluating new chemotherapeutic agents that target on chloroquine resistant malaria, we

attempt in the present study to experimentally develope a more plasmodium sensitive drug using chloroquine resistant *Plasmodium berghei*.

2. Materials

2.1. Identification/Collection of Samples

The plants *Gnetum africanum* were harvested in large quantities from Amaiyi Obohia Ahiazu Mbaise and Trans Egbu, Owerri Municipal L.G.A respectively and were identified by a plant taxanomist Dr. D. I Edet from the Department of Forestery and Wildlife, Federal University of Technology, Owerri, Nigeria and deposited at the University herbarium with voucher number FUTO/FW/HERB/2022/075, for reference purposes.

2.2. Plant Extraction Process

The plant parts (leave, stem bark and root) were harvested in large quantities in June, 2022; washed thoroughly in running tap water. The leaves, stembark and root were cut into pieces (about 3cm). All parts including the leaves and seeds were dried separately under shade at room temperature for about seven weeks before grounding into a powder form using an electric blender. Using a soxholet extraction, 30 g each were extracted separately in deionised water and methanol, for 6 hours. Aqueous and methanol filtrates (infusions) were concentrated using water bath at 100^{o}C and 45^{o}C respectively and stored at -4^{o}C until required. However, the percentage yield of each extract was determined.

2.3. Animals

Thirty five Swiss albino mice were used for the antiplasmodial studies.

2.3.1. Antiplasmodial Activity Study

70 Swiss albino mice of both sexes were purchased from Nano farms, Owerri, Imo State, and transported to Federal University of Technology Owerri (FUTO); where the research was carried out. The mice were acclimatized for 2 weeks, after which they were innoculated with *Plasmodium berghei* NK 65 through passaging (David et al., 2004; Ene et al., 2018).

2.3.2. In vivo Culture of the Plasmodium berghei Using Albino Mice.

The *Plasmodium berghei* infected red blood cells (RBC) were diluted with phosphate buffer saline (PBS) pH 7.2 in such a way that each 0.2 ml had approximately 10 x 10^{7} infected red blood cells (parasite per kg body weight). This diluted infected red blood cells were intraperitoneally injected into clean/healthy mice. The mice (both infected and uninfected had free access to standard laboratory mice feedstuff (Vital starter) and water, and were later kept under standard laboratory condition with subsequent check. The mice were weighed and grouped (five per cage based on their similar body weights. The mice were infected by injecting 0.2 ml of diluted infected RBC intraperitoneally. Parasitemia was confirmed in the animals after 24 hours of infection.

2.3.3. In vivo Treatment of the Infected of the Infected Albino Mice

Table 1 Antiplasmodial Groups and Treatments

Groups	Dose (mg/kg bw)	Treatment
1	100	*Gnetum africanum* leave (methanol extract)
2	100	*Gnetum africanum* stem bark (methanol extract)
3	100	*Gnetum africanum* leave (aqueous extract)
4	100	*Gnetum africanum* stem bark (aqueous extract)
5	10	Chloroquine standard control
6	1.6	Artemisin-based Combination Therapy (ACT) standard control
7	-	Normal Control

A four (4) day curative standard test of David et al. (2004); Peter and Anatoli, (1998, Ene et al., 2008a) were used employing the rodent malaria parasite, *Plasmodium berghei*. The mice were divided into groups. Five mice was used for each of the 12 test/treatment groups.

For the preliminary studies, Five mice were used for each of the 11 test/treatment groups. Forty eight hours (48 hrs) after infection with the malaria parasite; the plant extracts were administered to the experimental groups (group one to group nine) at a dose level of 100 mg/kg body weight daily for four days (Ene *et al.* 2008a). The drugs were administered based on their average body weight. Artesunate was administered to the artesunate standard control group at a dose of 1.6 mg/kg body weight. Chloroquine (CQ) was administered to the CQ standard (control) group at the standard dose of 10 mg/kg body weight. Artemisin-based Combination Therapy ACT was administered to the ACT standard (control) group at 1.6 mg/kg body weight for four days. The negative controls were not treated. The intraperitoneal route was used for all drug administration. The extracts were dissolved in normal saline to the indicated suitable dose level in the solution.

For the main study, the screening of the fractions for anti-malarial activity; five Swiss albino mice were used per fraction. After confirmation of parasitemia, fractions derived from plant extract were administered intraperitoneally at a dose of 10 mg/kg for 4 days. ACT was administered to the control group at the standard dose of 1.6 mg/kg for 4 days, and the negative control group was left untreated. The fractions were dissolved in 0.3% (v/v) Tween 80 in normal saline. The parasitemia was monitored in all the groups for 14 days (for the animals that survived) using thick and thin smears of blood films made from tail vein of mice (David et al., 2004).

Treatments were performed daily for 4 consecutive days starting 48hrs after infection, with each animal receiving a total of 4 intraperitoneal doses (David et al., 2004).

To assess the level of parasitemia, a blood smear was made from the tail snip of the mice. The smears were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 15 min, washed under running water, allowed to dry and then examined under the microscope using 100 x (under immersion oil) to access the parasitemia level. The percentage parasitemia was calculated according to the method adopted by Ene et al., 2008 as:

$$\text{Parasitemia}(\%) = \frac{\text{Number of parasite in treated}}{500} \times 100$$

Where the number of parasites in the untreated is assumed to be = 500

3. Results

An analysis of the percentage yield of the methanol and aqueous extracts of the leaves and stembark of *Gnetum africanum* showed that different amounts of extracts were produced by the plant parts in different solvents.

Analysis of the percentage yield of the methanol extracts of different parts of *Gnetum africanum*, more extracts were gotten from the leaves of the plant when compared with the stem, with *Gnetum africanum* leaves producing the highest amount of extracts (20.86%) as shown in Table 2. below.

Altogether, the aqueous solvent yielded more extracts than methanol. The percentage yield of extracts showed that for aqueous extracts of different parts of *Gnetum africanum*, more extracts were gotten from the stem of both plants when compared with the leaves. With *Gnetum africanum* stem producing the highest amount of extracts (40.86%) as shown in table 3. below.

Table 2 Percentage Yield Of Extracts From Different Parts Of *Gnetum africanum* Using Methanol

Samples	Methanol (ml)	Sample weight(g)	Weight of extract (g)	Percentage yield of extract (%)
G. africanum stem	500	50	6.63	13.26
G. africanum leaf	500	50	10.43	20.86

Table 3 Percentage Yield of Extracts from Different Parts of *Gnetum africanum* Using Deionized Water**.**

Samples	**Deionised H_2O (ml)**	**Sample weight(g)**	**Weight of extract (g)**	**Percentage yield of extract (%)**
G. africanum stem	500	50	20.39	40.78
G. africanum leaf	500	50	7.61	15.22

In this study carried out with the aqueous and methanol extracts of the stem and leaves of *Gnetum africanum.* The aqueous extract of the *Gnetum africanum* stem bark extract (1.20±0.00) showed the highest antiplasmodial activity. The *Gnetum africanum* stem bark aqueous extract showed the highest suppression of the plasmodium parasite when compared to other *Gnetum africanum* samples, this extract showed a significant decrease in parasitemia ($p<0.05$) when compared to the *Gnetum africanum* stem methanol (4.00±0.00) and *Gnetum africanum* leaf methanol (3.20±0.00). The aqueous leaf extract of *Gnetum africanum* had the lowest suppression on the the parasite as none of the animals in this group survived to the end of the 14 days study. In as much as some the animals administered with the *Gnetum africanum* stem methanol (4.00+0.00) and *Gnetum africanum* leaf methanol extracts (3.20+0.00) survived till the end of the 14 days study, the parasite load was increasing in both groups, thereby indicating that there was no significant antiplasmodial activity. The comparisons were made between days 0, 4, 8, and 14. There was however, no statistically significant difference ($P>0.05$) observed when the percentage parasitemia of the infected mice treated with chloroquine was compared with that of infected mice not treated. All the mice treated with the chloroquine standard control did not survive till Day 14 while the ACT standard control suppressed the parasite significantly ($p<0.05$) as shown in Table 4.1k below.

Although the parasite load was not completely cleared in all the *Gnetum africanum* test groups, it was drastically reduced in the aqueous extract of the *Gnetum africanum* stem bark extract as stated in Table 3. However, some of the test animals started dying within 10 days of extract administration, as the parasitemia rose again after being suppressed, when treatment with the extract was withdrawn, especially in the group treated with *Gnetum africanum* stem methanol extract and *Gnetum africanum* leaf methanol extract as opposed to the group on ACT which survived the 14 days of the study.

Table 4 *In vivo* Antiplasmodial Effect of Methanol and aqueous Extracts of *Gnetum africanum* plant parts on chloroquine resistant *Plasmodium berghei* NK 65 (Day 0 to Day 5)

Drug/treatment	Dose (mg/kg bw)	Mice	Day 0	Day 1	Day 2	Day 3	Day4	Day 5	Day 6	Day7
G. africanum Stem (aqueous)	100	5	1.56±0.17^{a}	1.20±0.24	1.92±0.58	1.84±0.55	1.45±0.34^{a}	2.07±0.31	2.10±0.42	2.40±0.85
G. africanum leaf (aqueous)	100	5	1.60±0.32^{a}	1.08±0.30	1.72±0.54	1.90±0.42	2.80±0.00^{c}	3.80±0.00	4.40±0.00	3.00±0.00
G. africanum Stem (methanol)	100	5	1.76±0.33^{a}	1.48±0.23	1.60±0.28	1.72±0.46	2.16±0.30^{b}	3.40±0.43	3.20±0.28	3.30±0.14
G. africanum leaf (methanol)	100	5	1.76±0.17^{a}	1.36±0.38	1.44±0.46	1.70±0.59	1.28±0.11^{a}	1.12±0.27	1.35±0.47	1.47±0.31
Chloroquine Std Control	10	5	1.68±0.44^{a}	1.84±0.61	2.00±0.42	1.84±0.26	1.35±0.19^{a}	2.15±0.19	1.67±0.12	1.67±0.12
ACT Std Control	1.6	5	1.84±0.09^{a}	1.56±0.55	1.88±0.30	1.60±0.24	1.32±0.22^{a}	1.68±0.11	1.52±0.11	1.56±0.22
No extract/drug (Negative control)	-	5	1.64±0.38^{a}	2.08±0.23	2.64±0.17	3.05±0.34	3.80±0.28^{d}	4.80±0.00	-	-

All values were compared with untreated on days 0, 4,8, and 14 at P<0.05; CQ =Chloroquine, STD = Standard; Aq = Aqueous, Met = Methanol; Values represent mean ± standard deviation of five (n=5) determinations and values with different superscripts vertically different statistically (P<0.05)

Table 4 contd: *In vivo* Antiplasmodial Effect of Methanol and aqueous Extracts of *Gnetum africanum* plant parts on chloroquine resistant *Plasmodium berghei* NK 65 (Day 8 to Day 14)

DRUG/ TREATMENT	DOSE (mg/kg bw)	MICE	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14
G. africanum Stem (aqueous)	100	5	2.50±0.71^{c}	2.10±0.42	1.80±0.28	1.60±0.28	1.40±0.00	1.40±0.00	1.20±0.00^{b}
G. africanum leaf (aqueous)	100	5	3.00±0.00^{c}	2.80±0.00	2.00±0.00	-	-	-	-
G. africanum Stem (methanol)	100	5	2.70±0.42^{c}	2.00±0.00	2.20±0.00	2.40±0.00	3.00±0.00	3.60±0.00	4.00±0.00^{d}
G. africanum leaf (methanol)	100	5	1.93±0.31^{b}	1.80±0.57	2.00±0.00	2.00±0.00	2.80±0.00	3.00±0.00	3.20±0.00^{c}
Chloroquine StdControl	10	5	2.00±0.00^{b}	2.20±0.00	2.20±0.00	2.20±0.00	2.20±0.00	2.20±0.00	2.20±0.00^{c}
ACT Std Contrl	1.6	5	1.56±0.30^{a}	1.40±0.24	1.16±0.17	0.80±0.20	0.50±0.14	0.00±0.00	0.00±0.00^{a}
No extract/drug (Negative contrl	-	5	-	-	-	-	-	-	-

All values were compared with untreated on days 0, 4,8, and 14 at P<0.05; CQ =Chloroquine, STD = Standard; Aq = Aqueous, Met = Methanol; Values represent mean ± standard deviation of five (n=5) determinations and values with different superscripts vertically different statistically (P<0.05)

The weight of the parasitized animals treated with *Gnetum africanum* Stem (methanol), *Gnetum africanum* Stem (methanol) and *Gnetum africanum* leaf (methanol) extracts showed a decrease in body weights. The Chloroquine standard control, Untreated group and groups treated with Gnetum africanum aqueous leaf extracts, died off before the Day 14, as shown in Table 5, Hence, no weight was shown.

Table 5 Percentage Body Weight Change of the Experimental Animals used in the Preliminary *In vivo* Antiplasmodial Studies

DRUG TREATMENT	DOSE (mg/kg bw)	MICE	Initial weight of Mice in Day 0 (g)	Final weight of Mice in Day 14 (g)	% Change In Body Weight Of Mice (%)
G. africanum Stem (aqueous)	100	5	22.70±0.66	21.23±0.00	-6.48
G. Africanum leaf (aqueous)	100	5	18.99±0.52	-	-
G. africanum Stem (methanol)	100	5	22.75±0.67	16.39±0.00	-27.96
G. Africanum leaf (methanol)	100	5	21.84±0.62	14.59±0.00	-33.20
Chloroquine Std Control	10	5	18.99±0.55	-	-
ACT Std Control	1.6	5	22.96±0.55	27.16±0.23	18.29
No extract/drug (Negative control)	-	5	19.16±0.39	-	-

STD = Standard, Aq = Aqueous, Met = Methanol; Values represent mean ± standard deviation of five (n=5) determinations.

4. Discussion

Methanol and aqueous solvents were used in this study because they are the major solvents the natives who used this plant for medicinal purposes use. The percentage extracts of the methanol and aqueous extracts were different, this might actually be due to the polarity of the solvents. The yield was however low when compared with the mass (g) of the ground plant parts used for the soxholet extraction process. Soxholet extraction method has been reported to yield more extracts than the crude method and some other methods of plant extraction (Ene et al., 2018)

The *Gnetum africanum* was used for study because it has been shown to possess medicinal properties. In addition, *Gnetum spp*. have high contents of vitamin A, dietary fiber, minerals (potassium, calcium, iron, zinc, phosphorus) and high chlorophylls, which are related to chlorophyllin synthesis, antioxidant activity, anti-diabetes activity, and health benefits against various diseases. Therefore, *Gnetum. spp.* is considered a candidate plant with high nutritional potential providing a wide range of essential nutrients and health benefits, and can be used safely as raw material for food, nutraceuticals, and medicinal products (Anisong et al., 2022). In recent years, substantial interest has been placed on the chemical and pharmacological properties of herbal plants, especially in traditional medicine (Igwe et al., 2007).

The present study reavealed that in view of the percentage yield of extracts from different parts of *Gnetum africanum.* Using methanol, for methanol extraction of different parts of *Gnetum africanum*, more extracts were gotten from the leaves of the plant when compared with the stem. While, the aqueous solvent, yielded more extracts than methanol, the percentage yield of extracts showed that for aqueous extracts of different parts of *Gnetum africanum*, more extracts were gotten from the stem of the plant when compared with the leaves. Phytochemicals occur in various parts of plants. Their functions are diverse which include provision of strength to plants, attraction of insects for pollination and feeding, defense against predators, provision of colour, while some are simply waste products (Ibegbulem et al., 2003)

In this study carried out with Aqueous and methanol extracts of the stem and leaves of *Gnetum africanum.* The *Gnetum africanum* stem bark aqueous extract showed the highest suppression of the plasmodium parasite when compared to other *Gnetum africanum* samples (1.20±0.00), this extract showed a significant decrease in parasitemia ($p<0.05$) when compared to the *Gnetum africanum* stem methanol (4.00±0.00) and *Gnetum africanum* leaf methanol (3.20±0.00). All

the mice treated with the chloroquine standard control did not survive till Day 14 while the ACT standard control suppressed the parasite significantly ($p<0.05$). The aqueous leaf extract of *Gnetum africanum* had the lowest suppression on the the parasite as none of the animals in this group survived to the end of the 14 days study. In as much as some the animals in the *Gnetum africanum* stem methanol (4.00±0.00) and *Gnetum africanum* leaf methanol (3.20±0.00) survived till the end of the 14 days study, the parasite load was increasing in both groups, thereby indicating that there was no significant antiplasmodial activity. The comparisons were made between days 0, 4, 8, and 14. There was however, no statistically significant difference ($P>0.05$) observed when the percentage parasitemia of the infected mice treated with chloroquine was compared with that of infected mice not treated. All the mice treated with the chloroquine standard control did not survive till Day 14 while the ACT standard control suppressed the parasite significantly ($p<0.05$).

Although the parasite load was not completely cleared in all the *Gnetum africanum* test groups, it was drastically reduced in the aqueous extract of the *Gnetum africanum* stem bark extract. However, some of the test animals started dying within 10 days of extract administration, as the parasitemia rose again after being suppressed, when treatment with the extract was withdrawn, especially in the group treated with *Gnetum africanum* stem methanol extract and *Gnetum africanum* leaf methanol extract as opposed to the group on ACT which survived the 14 days of the study.

5. Conclusion

It can be concluded that from this study that *Gnetum africanum* possesses antiplasmodial properties and the metabolites responsible for its antimalarial effect are higher in the aqueous extract of the *Gnetum africanum* stem bark. Furthermore, while medicinal plants continue to gain acceptance as alternative panacea for quick and accessible healthcare delivery, it is necessary to concentrate efforts in screening such medicinal plants to expose any possible adverse effect and possible potential for toxicity, apart from their efficacy in the control of some of these health challenges. This would be most done effective way by using scientific methods to separate the active components from these plants and integrate them as major components of these contemporary medications. Because plant materials contain a large number of secondary metabolites, many of which have specific capacities that are still poorly characterized or even unidentified, therefore, these screenings are crucial.

Further studies should be carried out on these plants to ascertain its efficacy in ameliorating other disease conditions. Also, researchers should work harder to screen the medical plants that are readily available so that local users can work together to prevent potentially harmful materials from entering the body of these end users, especially given the great acceptance of medicinal plants in the delivery of healthcare.

Compliance with ethical standards

Acknowledgments

The authors are grateful to Nmadike G. N. Ezeji-Chigbu for supplying the funding for this research work, Dr. Emeka S. Asiwe, for providing the laboratory for the execution of part of the research.

Disclosure of conflict of interest

We declare that we have no conflict of interest.

Funding

This research was individually funded.

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