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Gongronema latifolium Benth. Leaves (Uteze) Ameliorate Malaria Infection in *Plasmodium berghei*-Infected Mice

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ABSTRACT: Herbs are vital sources of bioactive compounds. This study evaluates the antimalarial, antioxidant, phytochemical and nutritional properties of *Gongronema latifolium* Benth. (Uteze) leaves. Pulverized dry leaves sample were macerated in hexane, methanol, ethanol and distilled water to obtain their respective extracts. The antimalarial efficacy of these extracts was assessed in male Swiss albino mice infected intraperitoneally with chloroquine-sensitive *Plasmodium berghei* NK65, using the chemo-suppressive, prophylactic and mean survival time (MST) assays. Phytochemical and proximate analyses were assessed by standard protocols. The antioxidant capacity of the extracts was analyzed using DPPH radical scavenging and ferrous ion chelating assays. From the results, the plant had significant ($p < 0.05$) antimalarial activity compared to the infected untreated control. The chemo-suppressive activity of the extracts declined accordingly; ethanol (46.34%) > methanol (43.16%) > aqueous (32.24%), while their prophylactic activity declined in the order of aqueous (40.25%) > ethanol (38.99%) > methanol (26.94%). Only MST in the four-day chemo-suppressive assay was significant ($p < 0.05$) compared to the infected untreated control. Tannins, alkaloids, flavonoids, saponins, phenolics, cardiac glycosides and proanthocyanidins were detected in reasonable quantities in the plant. The plant also had antioxidant activity as the aqueous extract (IC_{50} 48.50 ± 0.42) scavenged DPPH radicals better, while methanol extract (IC_{50} 21.15 ± 0.33) chelated ferrous ion radicals more compared to other extracts. Meanwhile, the percentage nutritional composition of the plant was moisture (6.23 ± 0.01), total ash (2.22 ± 0.02), crude fat (16.32 ± 0.04), crude fibre (1.04 ± 0.01), crude protein (9.33 ± 0.58) and nitrogen free extract (64.85 ± 0.63). In conclusion, the antimalarial activity of *Gongronema latifolium* Benth. (Uteze) leaves could be due to its rich phytochemical and nutritional properties.

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Keywords: *Gongronema latifolium*, Antimalarial, *Plasmodium berghei*, Phytochemical, Antioxidant.

Malaria, a parasitic protozoan infection, accounts for thousands of deaths in sub-Saharan Africa, Asia and parts of the Americas where it remains endemic. Over 229 million cases were recorded in 2019 with a mortality figure of around 409,000, mostly pregnant women, children under 5 years of age and immune-compromised individuals owing to their weak immune state (WHO, 2020). Global estimates in 2019 alone indicate that around 274,000 children died of malaria infection (WHO, 2020). About 94% of cases are found in Africa and Nigeria accounts for 23% of this disease burden. Other endemic countries in the sub-Saharan belt include Democratic Republic of Congo (11%), Tanzania (5%), Burkina Faso, Mozambique and Niger (4% each) (WHO, 2020). The increasing global poverty levels, especially in developing countries like Nigeria (WHO, 2020; NBS, 2021), dilapidated social infrastructure including healthcare, decrepit sanitary conditions, poor budgetary allocations to health by governments and households, and high cost of medications have fueled the perils associated with this disease. This has led to a sizeable portion of the population in developing countries, Nigeria inclusive, depending on medicinal herbs for the management of disease conditions (WHO, 2003). *Gongronema latifolium* Benth., an edible spicy vegetable of the Asclepiadaceae family (Iwu, 2014) has been applied well enough in African pharmacopoeia. The climbing perennial herbaceous shrub, which measures up to 5m when stretched is widespread in tropical Africa and can be found from Senegal East to Chad and South to Democratic Republic of Congo as well as in the rain forest zones of Nigeria, Guinea-Bissau, Western Cameroon, Sierra Leone, Cote d'Ivoire, Ghana, etc., (Chattopadhyay, 1999; Owu *et al*., 2012). Also, it has middling presence in America, Northern and South Eastern Asia (Nelson, 1965; Agbo *et al*., 2005). Some common names of *G. latifolium* include Amaranth globe, Swallow apple and Sodom apple. Locally, it is called Uteze, Urianene (Edo), Utazi (Igbo), Madumaro, Arokeke, Aunjeadiye, Iteji, Itaji (Yoruba), and Utasi (Ibibios, Efik and Quas) (Aigbokhian, 2014). Elsewhere in Africa, the plant is referred to as kurutu nsurogya (Akan-asantes of Ghana), Gasub

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(Serers of Senegal), ndondo-polole, tawabembe and ra-bilong (by Kissis, Mende and Temnes, respectively of Sierra Leone) (Balogun *et al*., 2016). The fruits, seeds, leaves, root and stem have been harnessed for treatment and management of diseases (Morebise, 2015). For instance, the people of Southsouth and Southwest of Nigeria have been credited to use the plant in the treatment of malaria, diabetes, hypertension, anorexia, constipation and nausea (Morebise and Fafunso, 1998; Morebise *et al*., 2006). It has also been reported that the leaves of *G. latifolium* are used as a vermifuge and stomachic, as well as in the treatment of asthma, dyspepsia and fowl cough in some localities in Nigeria (Oliver-Bever, 1986; Iwu, 1988; Owu *et al*., 2012). Elsewhere in Africa, Balogun *et al*. (2016) reported that *G. latifolium* is used in treating cough, intestinal worms, dyspepsia, dysentery and malaria in communities around the West African plane. Furthermore, both Egba *et al*. (2014) and Akpan and Effiong (2015) affirmed that *G. latifolium* is associated with immuno- and haematological-modulatory effects in experimental animals. Therefore, owing to the vast potentials inherent in this plant, this study was aimed at ascertaining the antimalarial activity, antioxidant, phytochemical and nutritional properties of *Gongronema latifolium* Benth. (Uteze) leaves and its efficacy in ameliorating malaria infection in *Plasmodium berghei* infected male Swiss albino mice.

MATERIALS AND METHODS

Drugs and Chemicals: Phosphate buffered saline (Silver Health Diagnostics, Nigeria), giemsa stock (Trust Chemical Lab., India), immersion oil (Scisco Res. Lab., India), normal saline, chloroquine, gallic acid, quercetin and tannic acid ferrous chloride, ferrozine and citric acid, vitamin C, DPPH, EDTA, ethanol, methanol and Hexane (Sigma Aldrich, Germany). All reagents were of analytical grade and procured from certified suppliers.

Plant Material: Fresh *Gongronema latifolium* Benth. (Uteze) leaves were obtained within November from local gardens in Edo State, South-South Nigeria. The plant was identified and authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, where voucher No. (UBHa302) was assigned. The leaves sample was cleaned of debris and air-dried under shade at room temperature. The dried material was coarsely pulverized using mortar and pestle. The pulverized powdered sample was stored at room temperature in an air-tight plastic container until ready for extraction.

Extraction of Plant Material: Extraction was done by first weighing four portions of 400 g each (a portion for each solvent) of the pulverized leaves sample of *G. latifolium* using a sensitive weighing balance (S. Mettle, Switzerland). They were each macerated in 1.5 L of hexane, methanol, ethanol and distilled water in separate jars for 72 h under repeated stirring. Thereafter, the mixture was filtered using Whatman filter paper No. 1 (Whatman, England), while the ensuing residues were again macerated twice for the same duration in similar solvents (Cannell, 2006). Filtrates from the three rounds of maceration of each solvent portion were combined and evaporated at 45 °C *in vacuo* to dryness using a rotary evaporator (Buchi, Germany). The dried extracts were preserved under refrigerated condition in air-tight glass containers until further use.

Experimental Animals: Male Swiss albino mice, sixty in number and weighing 20 ± 2 g, which were between six to eight weeks old and acquired from the Animal House of Igbinedion University, Okada, Edo State, were used for the study. They were housed in well-ventilated plastic cages ladened with softwood shavings and chips as beddings, and acclimatized for one week before the commencement of study. Each animal received *ad libitum* water and rat chow throughout the experimental period. Guidelines for the handling of laboratory animals as prescribed by the Institute for Laboratory Animal Research (ILAR, 2011) were followed in this study.

Malaria Parasites: The chloroquine-sensitive *Plasmodium berghei* NK65 strain, obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria, was used in this study. It was maintained via weekly intraperitoneal passage to naive mice (Fidock, *et al*., 2004).

Preparation of Inoculum: A standard inoculum of parasitized red blood cells-PRBCs was prepared from a donor mouse at 30% parasitaemia. The PRBCs were obtained through cardiac puncture under anesthesia and diluted in normal saline (0.9 %) in a ratio of 1:3, to give 1×10^7 PRBCs standard inoculum. Each mouse was inoculated with 0.1 mL of the standard inoculum, which was capable of causing infection in naive mice (Fidock, *et al*., 2004).

Grouping and Dosing of Animals: Male Swiss albino mice, thirty for each experimental model, were divided into five groups comprising five mice each. Group I (Negative control) was infected and treated with distilled water. Group II (Positive control) was infected, but treated with the reference drug, chloroquine (10 mg/kg). Groups III, IV and V were

infected and treated with 800 mg/kg of methanol, ethanol and aqueous extracts, respectively. The oral gastric tube was used to administer the various extracts, while infection with *P. berghei* was done through the intraperitoneal route (ip).

Experimental Models

Four-day Chemo-suppressive Antimalarial Test: The chemo-suppressive activity of the extracts was tested using the modified method of Fidock *et al*., 2004. Inoculation and treatment of mice with the various extracts were done on the same day (D_0), while observing a two hour interval in both procedures. Thereafter, the treatments were continued for the next three days (i.e., D_0 to D_3). On D_4 i.e., the 5th day, thin blood smears were made on microscopic slides with frosted end (Fischer Scientific, USA) from the tail of each mouse and monitored for parasitaemia under the microscope (Olympus, Japan).

Prophylactic Antimalarial Test: The modified method of Fidock *et al*., 2004 was used to evaluate the prophylactic activity of the extracts. Administration of the extracts was first done for four consecutive days (D_0 to D_3). Thereafter, on D_4 (fifth day), 0.1 mL 10^7 PRBCs was injected into each mouse. After 72 h of infection (D_7), thin blood smears were prepared from tail-blood of each mouse on a microscopic slide with frosted end and parasitaemia monitored under a microscope.

Determination of Parasitaemia: Parasitaemia was determined by counting the number of PRBCs in random fields of microscope view, under x100 objective lens-oil immersion. Percent parasitaemia and percent suppression were accordingly calculated (Fidock, *et al*., 2004; Kalra *et al*., 2006).

$$\%\ \text{Parasitaemia} = \frac{\text{NP}}{\text{TN of RBC}} \text{X } 100$$

$$\%\ \text{Suppression} = \frac{(\%\ \text{PNC} - \%\ \text{PTG})}{\%\ \text{PNC}} \text{X } 100$$

Where NP = number of parasitized RBCs; TN = Total Number; PNC = parasitaemia of negative control; PTG = parasitaemia of treated group;

Determination of Mean Survival Time (MST): The MST of infected but treated mice as against their infected but not treated counterparts was determined by monitoring the daily mortality and number of days of survival from the time of infection to death of the animal. It was then recorded for each mouse in the treatment and control groups throughout the experimental period. MST was calculated accordingly;

$$\text{MST (days)} = \frac{\text{Sum of DSA in a group}}{\text{TNA in the group}}$$

Where DSA = sum of days of survival of animals; TNA = total number of animals

Phytochemical Screening: Qualitative phytochemical estimations were conducted on the various extracts to identify their phytoconstituents in accordance with standard protocols (Stahl, 1973; Sofowora, 1982; Harborne, 1998; Evans, 2002). About 5 g of the samples were boiled with 75 mL of distilled water for 30 min. The solution was filtered hot and allowed to cool. The filtrates obtained were used to assay for the presence of tannins, alkaloids, flavonoids, phenolic, saponins and cardiac glycosides. Also, the total amounts of phenolic (Folin and Ciocalteau, 1927), flavonoid (Miliauskas *et al*., 2004), proanthocyanidin (Sun *et al*., 1998) and tannin (Polshettiwar *et al*., 2007) were quantitatively determined in the various extracts.

Determination of Antioxidant Capacity: The ability of the various extracts to scavenge 2, 2–diphenyl–1–picrylhydrazyl (DPPH) and chelate ferrous ion radicals were used as a measure of their antioxidant capacity. The modified methods of Brand-Williams *et al*. (1995) and Dinis *et al*. (1994), respectively, were adopted for these experiments.

Determination of Nutritional Content: The methods of AOAC, 2000 were employed in the determination of the proximate/nutritional content of the dry plant sample. The water loss on drying, total ash, crude fat, crude fibre, crude protein and Nitrogen Free Extract (NFE) were determined.

Data Analysis: The data were expressed as mean ± SEM. Data were analyzed using windows SPSS version 21.0. The differences between measured means were compared using one-way ANOVA followed by Turkey's HSD multiple comparison test for difference in significance. Statistical significance was set at p values < 0.05.

RESULTS AND DISCUSSION

Chemo-Suppressive Antimalarial Activity of Gongronema latifolium Leaves Extracts: The four-day chemo-suppressive antimalarial activity of crude extracts of *G. latifolium* leaves; methanol, ethanol and aqueous are presented in Table 1. From the results, the extracts were able to significantly ($p < 0.05$) suppress the growth of *P. berghei* in infected but treated mice

when compared with the infected untreated control (PBS- pH 7.4) mice. The activity of the extracts declined accordingly ethanol > methanol > aqueous. Although the ethanol extract had the highest ameliorative effect, its activity was non-significant ($p > 0.05$) compared to the reference drug, chloroquine.

Prophylactic Antimalarial Activity of Gongronema latifolium Leaves Extracts: The prophylactic antimalarial activity of the crude extracts of *G. latifolium* leaves; methanol, ethanol and aqueous extracts, against *P. berghei* infection in mice is presented in Table 2. From the results obtained, the extracts had significant ($p < 0.05$) prophylactic antimalarial activity in infected but treated mice when compared with the infected untreated control (PBS- pH 7.4) mice. The prophylactic activity of the extracts increased accordingly methanol > ethanol > aqueous. However, chloroquine, with a significant ($p > 0.05$) antimalarial activity performed better than the extracts.

Table 1: Four-day Chemo-Suppressive Activity of *Gongronema latifolium* leaves Extracts against *P. berghei* Infection in Mice

Treatment	Dose (mg/kg)	% Parasitaemia	% Chemo-Suppresion
Methanol extract	800	4.33 ± 0.27	43.16[ab]
Ethanol extract	800	4.09 ± 0.78	46.34[ab]
Aqueous extract	800	5.16 ± 0.92	32.24[ab]
PBS (pH 7.4)	-	7.62 ± 0.13	0.00
Chloroquine	10	0.33 ± 0.35	95.67[a]

Values are expressed as mean ± SEM; n = 5. Where a = significant (p < 0.05) compared to infected untreated control (PBS- pH 7.4), b = significant (p < 0.05) compared to reference drug, chloroquine. p < 0.05.

Table 2: Prophylactic Activity of *Gongronema latifolium* Leaves Extracts against *P. berghei* Infection in Mice

Treatment	Dose (mg/kg)	% Parasitaemia	% Chemo-Suppresion
Methanol extract	800	4.61 ± 0.33	26.94[ab]
Ethanol extract	800	3.85 ± 0.12	38.99[ab]
Aqueous extract	800	3.77 ± 0.45	40.25[ab]
PBS (pH 7.4)	-	6.31 ± 0.24	0.00
Chloroquine	10	0.54 ± 0.81	91.44[a]

Values are expressed as mean ± SEM; n = 5. Where a = significant (p < 0.05) compared to infected untreated control (PBS- pH 7.4), b = significant (p < 0.05) compared to reference drug, chloroquine. p < 0.05.

Effect of Gongronema latifolium Extracts on Mean Survival Time (MST): The MST results for methanol, ethanol and aqueous extracts of *G. latifolium* leaves in the four-day chemo-suppressive and prophylactic tests are presented in Figures 1 and 2, respectively.

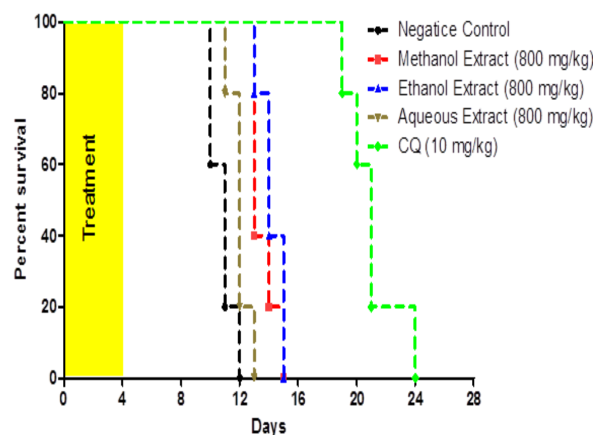

Fig 1: Chemo-Suppressive Activity of *Gongronema latifolium* leave extracts against *P. berghei*-infected Mice

From the result of the four-day chemo-suppressive study (Figure 1), only mice in the group administered ethanol extract lived significantly ($p < 0.05$) longer (MST = 14.25 days) than those in the infected untreated control group (MST = 10.80 days).

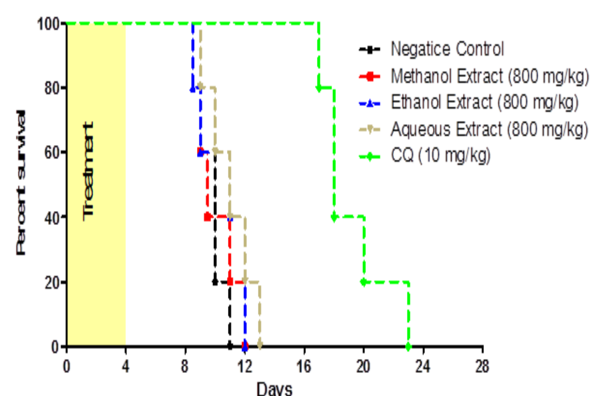

Fig 2: Prophylactic Activity of *Gongronema latifolium* leave extracts against *P. berghei*-infected Mice

However, mice in the methanol and aqueous extracts administered groups lived non-significantly ($p > 0.05$) longer (MST = 13.50 and 12.00 days, respectively) than the infected untreated control mice. Meanwhile, for the prophylactic study (Figure 2), methanol (MST = 10.00 days), ethanol (MST = 10.50 days) and aqueous (MST = 11.00 days) extracts did not prolong significantly ($p > 0.05$) the survival time of treated mice compared to mice in the infected untreated control group (MST = 9.80 days). Nonetheless, in both experimental models, chloroquine prolonged significantly ($p < 0.05$) the lives of mice (MST = 21.00

and 19.20 days, respectively) when compared with the extract treated groups and the infected untreated control group.

Phytochemical Content of the Extracts of Gongronema latifolium Leaves: The qualitative and quantitative phytochemical composition of extracts of *G. latifolium* leaves are presented in Table 3 and Figure 3, respectively. From the results obtained, tannins, phenolic, saponins, flavonoids, alkaloids and proanthocyanidins were identified in varying quantities in the extracts. But, saponins were not detected in the hexane extract, alkaloids in the ethanol and aqueous extracts, while cardiac glycosides were only present in the ethanol extract. Meanwhile, tannins, phenolics and proanthocyanidins were present in relatively large amounts in the hexane extracts compared to the other extracts (Figure 3).

Table 3: Phytochemical Evaluation of Extracts of *Gongronema latifolium* leaves

Phytochemicals	Hexane	Methanol	Ethanol	Aqueous
Phenolics	+	+	+	+
Flavonoid	+	+	+	+
Saponins	–	+	+	+
Alkaloid	+	+	–	–
Tannins	+	+	+	+
Cardiac glycoside	–	–	+	–

+ Positive; – not detected

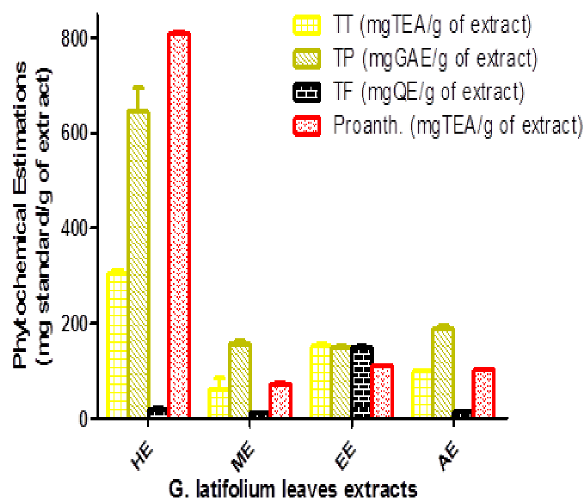

Fig 3: Quantitative Phytochemical Estimations of various Extracts of *Gongronema latifolium*.

All determinations were in triplicate. Values are presented as mean ± SEM. TAE: tannic acid equivalent, GAE: gallic acid equivalent, QE: quercetin equivalent, AAE: ascorbic acid equivalent, HE: hexane extract, ME: methanol extract, EE: ethanol extract and AE: aqueous extract.

Antioxidant Capacity of Extracts of Gongronema latifolium: The antioxidant activity of hexane, methanol, ethanol and aqueous extracts of *G. latifolium* leaves against DPPH and ferrous iron radicals are presented in Figure 4. The results revealed that the plant had antioxidant activity against both ion radicals. While the aqueous extract had the highest DPPH radical scavenging activity (IC_{50} 48.50 ± 0.42), the methanol extract had the highest ferrous iron radical chelating activity of IC_{50} 21.15 ± 0.33. However, these antioxidant activities were non-significant ($p > 0.05$) compared to the reference antioxidants ascorbic acid (IC_{50} 12.81 ± 0.11) and EDTA (IC_{50} 4.03 ± 0.25), respectively.

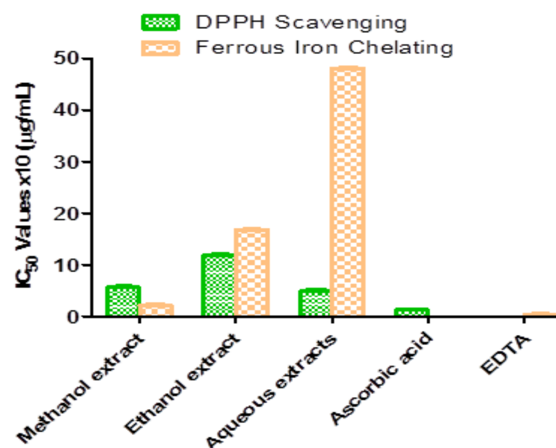

Fig 4: DPPH-radical Scavenging and Ferrous Ion Chelating Activities of the Extracts of *Gongronema latifolium* leaves. IC_{50} value of triplicate determinations (n = 3/group).

Nutritional Content of Gongronema latifolium Leaves: The percentage proximate composition of *G. latifolium* leaves indicate reasonably good nutritional content (Figure 5). The plant leaves were very high in nitrogen free extract (NFE), crude fat and crude protein, but low in crude ash and fibre. Moisture was also reasonably present.

Vegetables are good sources of spices for most African delicacies, but some are even more important owing to their rich medicinal values. An example of such vegetables is *Gongronema latifolium* Benth. (Uteze) leaves which has wide applications in traditional folklore medicine, thus, endearing it to many homes. The efficacy of this plant against *Plasmodium berghei*-induced malaria in Swiss albino mice was evaluated in this study using the chemo-suppressive, prophylactic and mean survival time (MST) tests. The inoculation of naïve mice with parasitized red blood cells (PRBCs) led to the induction of malaria as the parasites proliferated in the

blood of the host which was visible under the microscope days after infection; thus, confirming the susceptibility of Swiss albino mice to malaria infection from *P. berghei* NK65.

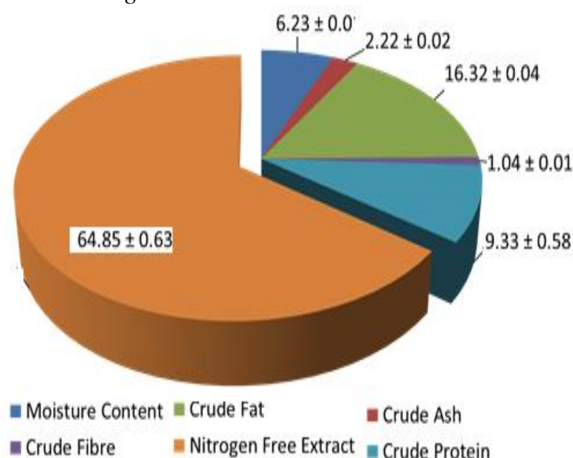

Fig 5: Nutritional Content of *Gongronema latifolium* Leaves. Data represent percentage mean ± SEM of triplicate determinations.

However, parasitaemia was significantly ($p < 0.05$) suppressed in the extracts and reference drug treated groups compared to the infected untreated control (Table 1). The aqueous extract demonstrated better chemo-suppressive activity compared to other extracts, but this was non-significant compared to the reference drug, chloroquine. The order of activity of the extracts was ethanol > methanol > aqueous. Furthermore, the extracts possessed significant ($p < 0.05$) prophylactic antimalarial activity (Table 2) when compared with the infected untreated control, although with reduced efficacy when compared with the chemo-suppressive study. Also, activity increased with increased polarity of the extracting solvent, viz; aqueous extract > ethanol extract > methanol extract. Meanwhile, the extracts were able to prolong the mean survival time (MST) of animals in the treated groups beyond those of the infected untreated control (Figures 1 and 2). While this was significant ($p < 0.05$) in the chemo-suppressive study, MST was non-significant ($p > 0.05$) in the prophylactic study as compared with the infected untreated control. However, chloroquine was able to prolong MST significantly ($p < 0.05$) in both experiments. The bioactivities of medicinal plants have been ascribed to a vast array of phytochemicals present in them (Rathor, 2021; Yu *et al*., 2021). These phytochemicals have been found to contain functional groups which participate in biochemical processes that ultimately lead to the elimination of these disease causing organisms (Rathor, 2021; Yu *et al*., 2021). These phytoconstituents belong to several classes of compounds which include flavonoids, tannins, saponins, phenolics, cardiac glycosides, alkaloids, proanthocyanidins, etc. They are believed to function via mechanisms which include pro/anti-oxidation, immune-modulation, enzyme modulation, DNA intercalation, etc. (Perron and Julia, 2009; Samy *et al*., 2011). These phytoconstituents were present in relatively good amounts in the leaves of *G. latifolium* (Table 3 and Figure 3). It was also noticed that the plant extracts had antioxidant potentials (Figure 4) when compared with reference antioxidant molecules such as ascorbic acid and EDTA. The extracts were able to scavenge DPPH radicals as well as chelate ferrous ion radicals. Although, the results were non-significant ($p < 0.05$) compared to the standard molecules, the aqueous extract was a better scavenger of DPPH radicals, while methanol extract chelated ferrous iron radicals more than other extracts tested. Thus, it may be inferred that the antimalarial activity noticed in this study could be as a result of the presence of these phytochemicals which have demonstrated antioxidant activities. These findings are in tandem with those espoused in similar studies (Samy *et al*., 2011; Singh *et al*., 2017; Orumwensodia and Uadia, 2020). Also, the plant *G. latifolium*, was found to contain useful classes of food such as carbohydrate, protein and fat in reasonable amounts (Figure 5). These macromolecules are necessary requirements for the syntheses of important biomolecules needed for proper healthy body functioning (Nelson and Cox, 2017). Their presence in this plant could also explain the plant's therapeutic potentials as they could help to replenish drained store of these molecules during malaria infection, which has been credited with low levels of glucose, lipids and proteins (Singh *et al*., 2017).

Conclusion: The antimalarial activity of *Gongronema latifolium* Benth. (Uteze) leaves has been demonstrated in this study. This activity could be due in part to phytochemicals present in the plant as well as its rich nutritional content; thus, supporting its usage in folklore medicine.

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