

Antiplasmodial Activities of Some Locally Prepared Herbs Against *Plasmodium falciparum* Obtained from Asymptomatic School Children in Zaria, Kaduna State, Nigeria

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ABSTRACT

The efficacy of current or any intended antimalarial can only be resolute by cultivation and susceptibility studies. The aim of this research was to cultivate *Plasmodium falciparum in vitro* and comparing the antiplasmodial effects of standard antimalarial medications including herbaceous preparation. Asymptomatic pupils attending some schools in Zaria, Kaduna state, Nigeria were recruited into this research and blood samples were collected from them. Microscopy was done after thin and thick blood films were prepared and stained. The antiplasmodial activities of antimalarial drugs as well as herbal preparation were determined after the successful culturing of red blood cells in the *Jatropha curcas* medium. The phytochemical constituents of the herbs that made up the concoction were determined. The incidence of asymptomatic *Plasmodium* infection amongst school kids was 17.5%. There was no statistical association of location, gender and age with the obtained prevalence. Presence of alkaloids was observed in every plant screened while the same was also observed for tannins except in *Enantia chloranta*. Steroids and phlobatanins were observed in *Citrus aurantifolia* while there was presence of saponins in all the plant extracts except *Cymbopogon citratus*. All the plants except *Enantia chloranta* contained flavonoids. There was presence of terpenoids in all screened plants except *Azadirachta indica* and *Cymbopogon citratus* while cardiac glycosides were found in every plant except *Magnifera indica* and *Enantia chloranta*. Results obtained from the *in vitro* cultivation of the *Plasmodium falciparum* with Artemether/lumefantrine, amodiaquine and herbal concoction showed average percentage parasite inhibition of 80%, 37.8% and 38.6% respectively. This implies that Artemether/lumefantrine was capable of inhibiting the growth of the parasite best. The herbal concoction also inhibited growth (38.6% inhibition). There is need for additional investigation on a wider variety of plants to explore their antiplasmodial activities since there is evidence that it works, and it is quite available and affordable.

Keywords: Prevalence, *Plasmodium falciparum*, Phytochemical, Herbal Concoction, *Jatropha curcas*

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Introduction

Malaria is caused by infection with protozoan parasites of the *Plasmodium* species. *Plasmodium falciparum* is widespread in Africa while *P. vivax*, *P. ovale*, and *P. malariae* infections are uncommon and geographically restricted (Howes *et al.*, 2015). The transmission of the parasites is by Anopheles mosquitoes with *An. gambiae sensu stricto*, *An. funestus*, and *An. arabiensis* being the foremost common in Africa (Sinka *et al.*, 2010). Malaria is endemic in large parts of Sub-Saharan Africa. It had been estimated in 2017 that 219 million cases of malaria occur worldwide and 92% of worldwide cases were within the African region (WHO, 2020). Asymptomatic *Plasmodium* parasitaemia is *Plasmodium* parasitaemia of any density, within the absence of fever or other acute symptoms in individuals who haven't received recent antimalarial treatment. It's been identified as a reservoir for malaria transmission also as a precursor within the progression to symptomatic disease. The clinical consequences of asymptomatic *Plasmodium* parasitaemia in children aren't fully understood and it's generally assumed that in endemic areas, asymptomatic parasitaemia results from the event of partial immunity (Njama-Meya *et al.*, 2004). The methods for cultivation of the erythrocytic stages of *P. falciparum* reported by Trager and Jensen (1976) are usefully applied in nearly all aspects of research on malaria; chemotherapy, drug resistance, immunology and vaccine development. Therefore, species that infect humans are cultured *in vitro*, of those four *Plasmodium* species; *P. falciparum* remains the sole species that all stages are cultured. The commercially prepared culture Rosewell Park Memorial Institute (RPMI) 1640 medium to which serum and erythrocytes are added for cultivation of *Plasmodium falciparum* species were found to be less commonly used in Nigeria probably because of its cost and storage problem. (Nassar *et al.*, 2014). It's therefore necessary to hunt optional methods for cultivating *Plasmodium spp* to avoid the aforementioned problem of

(RPMI). This led to the utilization of *Jatropha* plant extract medium for cultivation. *Jatropha curcas*, a drought resistant large shrub belongs to the genus *Euphorbiaceae* and has oil producing seeds (Openshaw, 2000).

Herbal plants are medicinal plants which are useful to humans in combating diseases from the dawn of civilization. In this study, the antiplasmodial activities of a concoction prepared from neem, *Magnifera indica*, *Citrus aurantiifolia*, *Cymbopogon citratus* and *Enantia chlorantha* leaf extracts in various concentrations were determined. The aim of this research hence is to research the antiplasmodial activities and phytochemical constituents of some locally sold herbs against *Plasmodium falciparum* obtained from school children in Zaria, Kaduna state.

Materials and Methods

Study Area

The study area was Zaria, Kaduna state, Nigeria. Zaria, a major city in Kaduna state in Northern Nigeria is found on latitude 11°04'N and longitude 7° 42'E and is an ancient city in Kaduna state. It is a largely heterogenous city with a population of about 1,490,000 of individuals drawn from different ethnic mix during where Hausa and Fulani predominate. It is the gateway to many states within the north and thus provides the unique and multicultural population ideal for the conduct of the study (Oladimeji and Ojiboo, 2012).

Study Population

The study was conducted among school children between the ages of 2-15 years attending schools selected randomly across two government areas (LGAs) in Zaria metropolis.

Study Design

A cross sectional, school-based study where equal numbers of samples were obtained from children with consenting parents attending selected schools, employing a simple randomized sampling technique. The study population was drawn from

five schools. Two of the faculties, LEA primary school Wusasa and Ahmad Gyallesu primary school are located in Zaria LGA while LGEA primary school Kwangila, LEA primary school Zango, and UBE Layin-Zomo are situated in Sabon-Gari LGA. These schools serve an outsized population of youngsters seeking knowledge. Children that weren't within the defined age group, showed symptoms of malaria like chills and fever also as those whose parents declined consent were excluded from the research. Ethical approval was granted by Kaduna State Ministry of Health and Human Services ethics board (REF-MOH/ADM/744/VOL.1/795).

Determination of Sample Size

The sample size was arrived at by means of the equation by Sarmukaddam and Gerrard (2006) and a prevalence of 14.1% (Inabo and Umaru, 2011) at 95% confidence interval

$$n = Z^2 Pq / L^2 = 1.96^2 \times 0.141 \times 0.859 / 0.05^2 = 0.4512 / 0.0025 = 180.48 \sim 180 \text{ samples}$$

The calculated minimum size was 180; however, 200 blood samples were collected for the assay.

Collection of Samples

Venous blood samples were collected from 200 children aseptically into Ethylene Diamine Tetra ethanoic acid (EDTA) vacutainer tubes through venipuncture with the help of professional and authorized medical personnel. The samples were transported in cold packs to the Department of Microbiology, Ahmadu Bello University Zaria for analysis.

Preparation and examination of thick and thin films for trophozoites and parasite density

Thin and Thick blood films were prepared and stained with 5% Giemsa stain for the microscopic examination of malaria parasites as described by (Cheesbrough, 2000). Thin film was for the identification of trophozoites and parasite species, whilst thick film showed parasite density. Parasite

density per microliter of blood (parasitemia) was derived from the thick film taking the amount of leucocytes per microliter of blood as 8000 and expressed as follows:

$$\text{Parasite density}(\mu\text{l}) = \frac{\text{Parasite count} \times 8000}{\text{Number of WBCs counted} (200)}$$

Phytochemical Analyses

Chemical tests were conducted for the occurrence of tannins, saponins, phlobatanins, flavonoids, steroids, terpenoids, cardiac glycosides and alkaloids on the aqueous extract as well as on the dry milled specimen using set procedures to spot the constituents as described by Sofowara (1993), Trease and Evans(1989) and Harborne (1973).

Preparation of *Jatropha curcas* medium.

Leaves of *Jatropha curcas* were obtained from Ahmadu Bello University, main campus, Zaria, Nigeria. These were taxonomically identified and authenticated with authentication number (ABU 01911) by a Taxonomist within the Department of Plant Science, Ahmadu Bello University Zaria, Kaduna state, Nigeria. The *Jatropha curcas* medium was constituted containing 10ml of the *Jatropha curcas* leaf extract, 20mls of water, 2mls of a 0.2g/ml glucose solution, 1ml saline and 1ml PBS 1X. To the medium was also added 12ml of liver extract, 5ml of 10% human serum, 0.2ml uninfected human red blood cells as well as 15µl Gentamicin. The pH of the new medium was adjusted to 7.4±0.2 using NaHCO₃ solution (0.002g/ml). The fresh medium was stored at -21°C (Nassar *et al.*, 2014).

In-vitro Assay for antiplasmodial activity of locally made concoction (agbo)

Ingredients used for the locally made concoction includes yellow wood (*Enantia chlorantha*) bark, lime leaves (*Citrus aurantifolia*), lemon grass (*Cymbopogon citratus*), mango (*Magnifera indica*) leaves, and neem leaves (*Azadirachta indica*) with authentication numbers (ABU 01846), (ABU 01440), (ABU 06899), (ABU 01944) and (ABU 900151) respectively. Each plant was dehydrated in

shade, chopped and milled to a fine powder. A hot water infusion (10g in 100ml hot water) was constituted and the solution filtered through Whatman paper No 1. The extracts (100µl at 10mg/ml) were serially diluted with sterile water and dispensed into the 96 well microtitre plate (Ahmad *et al.*, 2014).

Preparation of Drug and Dosing of the Culture Plate Wells

Stock solutions of Artemether/Lumefantrine as well as Amodiaquine were prepared in 100% ethanol. These solutions were filtered with 0.22µm membrane filter (Millipore) aseptically and further dilution of the drug with ethanol was made to get concentrations 0.2, 0.4, 0.8, 1.6, 3.2µmol/ml (Jensen and Trager, 1980). The diluted drugs (10µl of each) were dispensed in sterile flat bottomed microtitre plate allowing the ethanol to evaporate while the powdered form adhered to the bottom of the plate. For every preparation, a fresh microtitre plate was used. The 96-well micro titre plate consisted of 8 rows (A-F), where Row A was drug free while row B-F had various drug concentrations (Basco, 2007).

Examination of Post culture Blood slides and determination of IC50

After incubation, for 36 hours, microtiter plate was placed in a semi vertical position about (45° inclined) for 30minutes. The supernatant was removed, thick blood film made from each well; air-dried, stained with 5% Giemsa and observed microscopically. Parasite growth was counted in 10 microscopic fields, the average calculated and parasite density determined. The percentage inhibitory concentration was calculated using the formula: (Ngemanya *et al.*, 2006).

$$\text{Percentage inhibition} = 100 - \frac{\text{Number of schizont in test wells}}{\text{Number of schizont in control well}} \times 100$$

The IC50 values, concentration required to inhibit parasite growth by 50% decided by linear interpolation from the parasite growth inhibition curves (concentration versus percent inhibition) derived from each parasite-extract interaction (Mustofa *et al.*, 2007).

Data analysis

The data obtained were analyzed with SPSS (statistical package for social sciences) version 2.0 software program. Pearson's chi-square χ^2 was employed to work out association of variables set significance at 95% confidence interval and $P < 0.05$ was considered insignificant. Means of parametric variables were analyzed using student *t* tests.

Results

The results showed that 35 children representing a prevalence of 17.5% were positive for *Plasmodium falciparum*. The prevalence observed among children from LEA Primary school Zango, was 27.5% (11/40) which was the highest, while those that attended LEA Primary School Wusasa had the least prevalence of 12.5% and this variation was not statistically significant ($\chi^2 = 600.000^a$, $p < 0.05$) (Table 1).

Children between the ages of 6-10 had the highest prevalence of 20% (17/85) while those between the ages 11-15 had the least prevalence of 15.2%. This variation also had no statistical significance ($\chi^2 = 400.000^a$, $p < 0.05$) (Table 2). Furthermore, *Plasmodium* parasitaemia among males and females was almost the same as a prevalence of 17.3% (18/104) was obtained; while 17.7% of the females tested were positive (Table 2).

Alkaloids were present in all the plant extracts; tannins were present in all except in *Enantia chloranta*. Phlobatanins and steroids were only present in *Citrus aurantifolia*. Saponins were present in all the plant extracts except *Cymbopogon citratus* while flavonoids were present in all the plants except *Enantia chloranta*. Terpenoids were present in all except *Azadirachta*

indica and *Cymbopogon citratus* while cardiac glycosides were present in all except *Magnifera indica* and *Enantia chlorantha*. (Table 3).

The IC₅₀ value of Artemether/Lumefantrine, Amodiaquine and Herbal concoction and 2.3, 7.1 and 4.4 nM were obtained respectively (Table 4).

The effect of Artemether/Lumefantrine on *Plasmodium falciparum* showed average parasite densities of 500, 156.25, 0, 125, 93.75 and 125 parasites per ml (Fig 1) and parasite inhibition of 68.75%, 100%, 75%, 81.25% and 75% (Fig 2). The effect of Amodiaquine on *Plasmodium falciparum* showed average parasite densities of 1812.5, 750, 1125, 1156.25, 1450.75 and 1156.25 parasites per ml (Fig 1) and percentage inhibition of 59%, 38%, 36%, 20% and 36% (Fig 4).

The antiplasmodial efficacy of the locally sold herbs (agbo) which is a concoction of *Azadirachta indica*, *Magnifera indica*, *Citrus aurantifolia*, *Cymbopogon citratus*, and *Enantia chlorantha* was also assessed over varying concentrations of 0.2, 0.4, 0.8, 1.6 and 3.2 µg/ml and average parasite densities of 2000, 1281.25, 1343.75, 1437.5 and 758 parasites per ml (Fig 1) and percentage inhibition of 36%, 33%, 33%, 29% and 62% respectively (Fig 5).

Table 1: Distribution of *Plasmodium falciparum* infection among apparently healthy school children in relation to schools in Zaria, Kaduna state.

School	Number examined	Number Positive (%)	Chi-square	p-value
LPSW	40	5(12.5)	600.000 ^a	0.000
AGPSZ	40	6(15)		
LPSK	40	7(17.5)		
LPSZ	40	11(27.5)		
UBELZ	40	6(15)		
Total	200	35		

Key: p<0.05=No significant association. LPSW: LEA Primary School Wusasa; AGPSZ: Ahmad Gyallesu Primary School Zaria; LPSK: LEA Primary School Kwangila; LPSZ: LEA Primary School Zango UBELZ: UBE Layin Zomo

Table 2: Distribution of *Plasmodium falciparum* infection among apparently healthy school children in relation to age and sex in Zaria, Kaduna state

Host Variable	Number examined	Number positive (%)	Chi-square	p-value
Age (years)				
2-5	69	11(15.9)	400.000 ^a	0.000
6-10	85	17(20)		
11-15	46	7(15.2)		
Total	200	35		
Sex				
Females	96	17(17.7)	200.000 ^a	0.000
Males	104	18(17.3)		
Total	200	35		

Key: p<0.05=No significant association.

Table 3: Phytochemical Constituents of Selected plants

Phytochemical Constituent	<i>Azadirachta indica</i>	<i>Magnifera indica</i>	<i>Citrus aurantifolia</i>	<i>Cymbopogon citratus</i>	<i>Enantia chloranta</i>
Tannins	+	+	+	+	-
Phlobatanins	-	-	+	-	-
Saponins	+	+	+	-	+
Flavonoids	+	+	+	+	-
Steroids	+	-	+	-	-
Terpenoids	-	+	+	-	+
Cardiac Glycosides	+	-	+	+	-
Alkaloids	+	+	+	+	+

Key: + = Present; -=Absent

Table 4: In vitro Susceptibility of *Plasmodium falciparum* isolates to antimalarial drugs and herbal concoction

Drug	IC ₅₀ nM
Arthemether/ Lumefantrine	2.3
Amodiaquine	7.1
Herbal Concoction	4.4

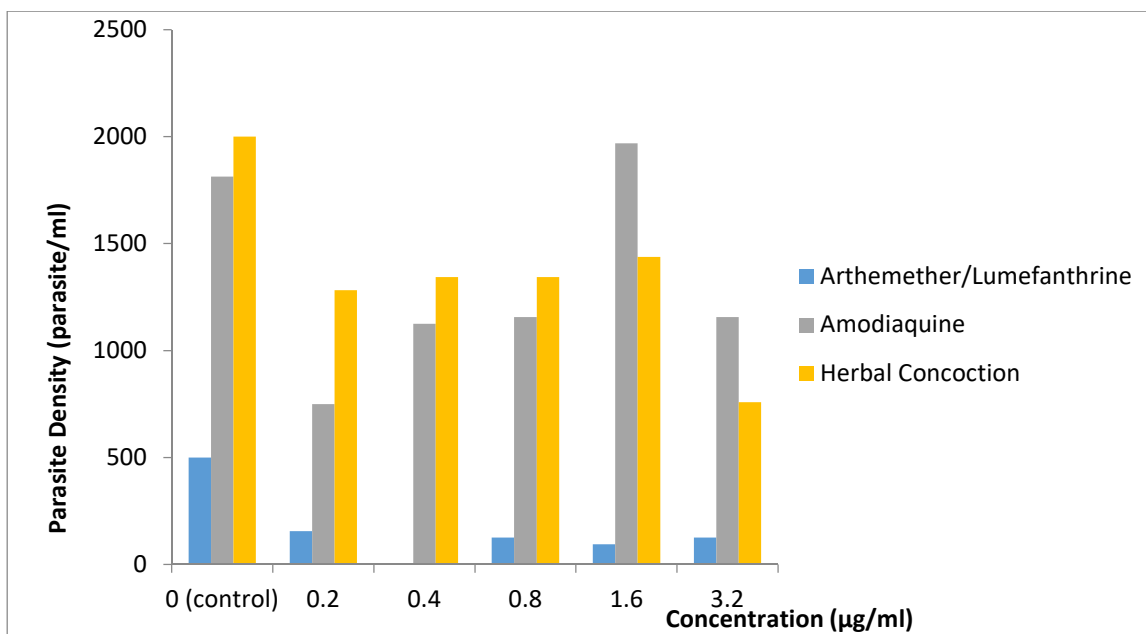


Fig 1: Parasite density of *Plasmodium falciparum* after cultivation on *Jatropha curcas* leaf extract medium and inoculation with Arthemether/Lumefantrine, Amodiaquine and Herbal concoction.

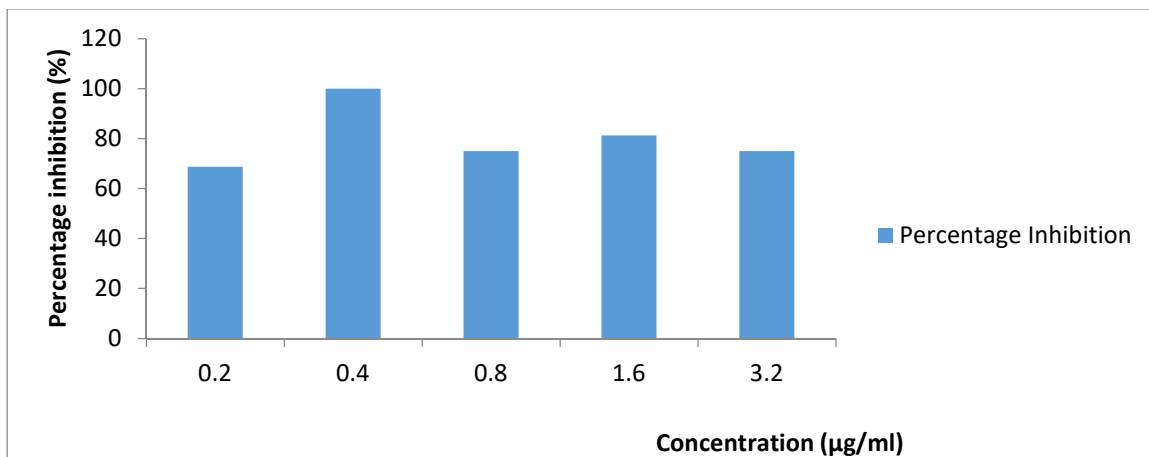


Fig 2: Percentage parasite inhibition of *Plasmodium falciparum* by Artemether/lumefantrine

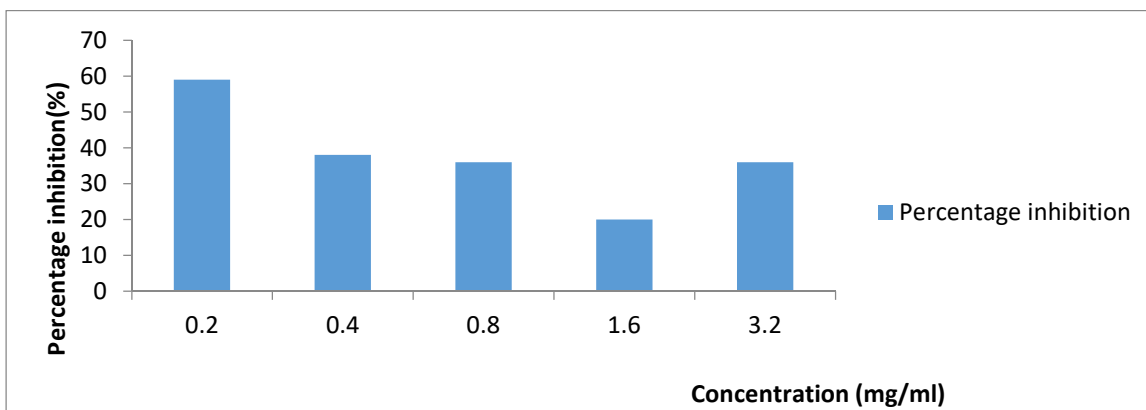


Fig 3: Percentage parasite inhibition of *Plasmodium falciparum* by Amodiaquine.

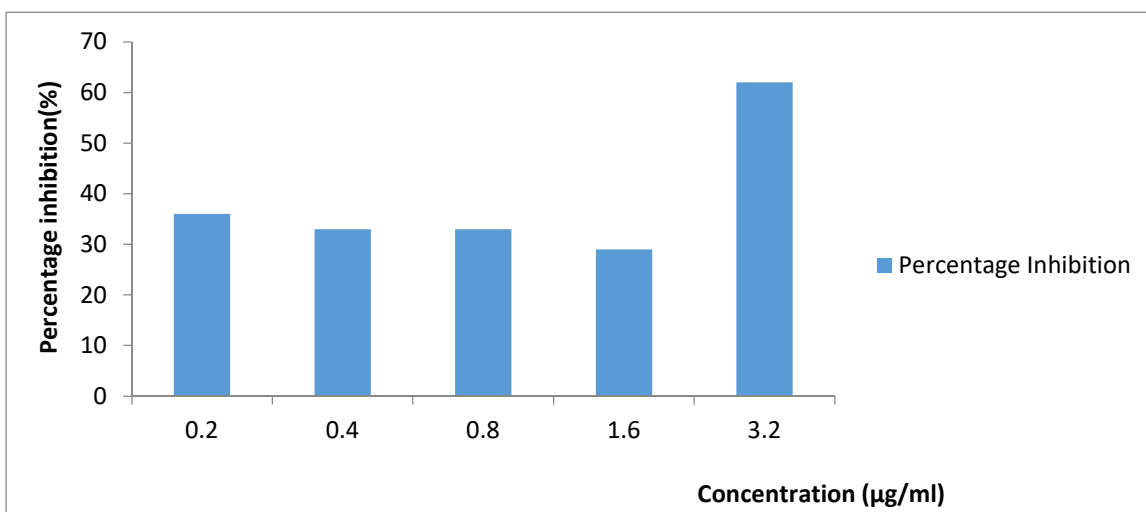


Fig 4: Percentage parasite inhibition of *Plasmodium falciparum* by Herbal Concoction

Discussion

The overall prevalence of asymptomatic *Plasmodium falciparum* parasitaemia among school children in Zaria is 17.5%. This percentage is above the 14.1% reported during a study involving children in orphanages in Zaria and Kaduna, Nigeria (Inabo and Umaru, 2011). Higher prevalence of *Plasmodium falciparum* infection has been reported in sub-Saharan African cities; 33.8% in Kenya (Bousema *et al.*, 2004) and 30% in Uganda (Nankabirwa *et al.*, 2013). Furthermore, studies among children in Bayelsa, Nigeria reported 63.3% (Abah and Temple, 2015) while another research conducted in Osogbo, Nigeria reported 25.6% (Ojurongbe *et al.*, 2011). The high asymptomatic Plasmodium falciparum infection prevalence reported during this study among children contrasts reports from Colombia (4.24%), Lao People's Democratic republic (6.3%) and Ethiopia (2.28%) (Cucunuba *et al.*, 2008; Akiyama *et al.*, 2016 and Lemu Golassa *et al.*, 2015).

The low prevalence obtained during this research might be as a result of improved awareness on preventive measures against malaria within the country. Subjects recruited during this research are school children; which indicates that they need some sort of education and are acquainted with certain awareness programs in class. The dissimilarity may additionally be as a result of contrast in geographical locations like our study being conducted in Zaria, Kaduna state, Nigeria which is essentially savannah region while research conducted in Bayelsa state- a mangrove tropical rain forest; Totoro, Eastern Uganda with a tropical monsoon climate and short dry season; or additional factors like lack of proper use of insecticide treated nets in areas where high percentages were recorded, diversity in sampling season and variation in socio economic level which will account for access to propagation sites for mosquitoes. The marginally higher prevalence observed in children between the ages 6-10 years could also be attributed to immunity thanks to

subsequent re-exposure to *Plasmodium falciparum* as observed by Bloland *et al.* (1999). The incidence of asymptomatic *Plasmodium* parasitaemia with regards to gender showed almost no difference thanks to almost an equivalent prevalence in both males and females. The association of some socio-demographic factors like age, gender and site wasn't significant statistically ($p < 0.005$).

The phytochemical screening of leaf extracts of lime showed the presence of tannins, flavonoids, phlobatanins, saponins, steroids, terpenoids, cardiac glycosides and alkaloids. These findings are in agreement with a study administered in Uyo, Akwa-Ibom state, Nigeria where *Citrus aurantifolia* was analyzed for phytochemical constituents while testing for its antiplasmodial effects (Ette *et al.*, 2019). It is evident that *C. aurantifolia* has been useful against malaria parasite which support its use in the treatment of malaria.

E. chlorantha (bark and leaf) which is employed for sore treatments, fevers, coughs etc by traditional healers contains saponins, alkaloids and terpenoids consistent with a study conducted in Ibadan, Nigeria and agrees with our findings (Adebiyi and Abatan 2013).

Cymbopogon citratus was seen to have alkaloids, tannins, flavonoids and Cardiac glycosides during this study which is in conformity with Sha'a (2014) during a study carried out in Jos, Nigeria but doesn't agree totally with findings obtained from a research conducted by Ojo and Anibijuwon *et al.*, 2010 which showed absence of cardiac glycosides and flavonoids. Leaf extracts of *Magnifera indica* are highly antiplasmodial in concurrence with Ohashi *et al.*, 2018 and Bidla *et al.*, 2004; Ibrahim *et al.*, 2012. Wauthoz and Balde, 2007 suggested that the foremost important constituent in mango related antiparasitic activity is mangiferin having the C-glucosyl Xanthone structure. Our findings show occurrence of alkaloids, tannins, saponins, flavonoids, and terpenoids in *M. indica* which is

in acceptance with Ohashi *et al.*, 2018. Kimbi *et al* (1998) reported little or no antimalarial activity of neem and *Magnifera lucida* in studies carried out by them, however, Makinde and Obih (1985) outlined that boiled water extract of *A.indica* appeared to have schizonticidal activity against chloroquine sensitive *Plasmodium berghei*. Hence there is a possibility that the disparity can be accounted for by strain of parasites or species where *P. falciparum* could also be sensitive to *A. indica* as revealed in this research. It isn't unusual that some plants that are usually employed in the cure of fever or malaria in certain places could also be inert or lethal in mice while being very active *in vitro*. There is a presence of alkaloids, steroids, tannins, saponins, flavonoids, and cardiac glycosides in *A. indica* according to this research. This agrees with Al-Hashemi and Hossain, (2016) in their research administered in Oman during which they screened the biological activities of various neem crude extracts.

Plasmodium falciparum when treated with Arthemether/Lumenfanthrine at concentrations 0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml recorded a percentage parasite inhibition of 68.75, 100, 75, 81.25 and 75% respectively. The mean parasite inhibition was 80% which indicates the effectiveness of the drug. However, it might be expected that a sustained or exponential rise is observed in the increase in parasite inhibition by this drug, but that wasn't precisely the case during this research. With concentration 0.4mg/ml, a 100% inhibition was observed, upon increase to 0.8mg/ml a decrease to 75% parasite inhibition occurred. Doubling of the concentration to 1.6mg/ml yielded an 81.25% inhibition which later dropped to 75% upon increase to three .2mg/ml. This unexpected outcome is presumably to be due to resistance to the drug, which has been a serious concern as regards the treatment of malaria. Consistent with Nosten and Brasseur (2002), the choice of those organism's resistance (or less susceptibility) to a particular drug is

dependent on the concentration that the parasites are exposed to, because in a lot of cases, the mutant parasite will initially be only less susceptible, not entirely resistant. Therefore, a better concentration should eliminate them. An average parasite inhibition of 37.8% was recorded when Amodiaquine was tested on *Plasmodium falciparum*. An initial reduction in the percentage inhibition was recorded upon increase in concentration. However, after increase to 3.2mg/ml, there was a rise in the inhibitory rate. This pattern could also be explained as resistance by the parasite as substantiated by variety of studies that shows that there's reduced susceptibility of *Plasmodium falciparum* to Amodiaquine and is best utilized in combination with an Artemisinin derivative (Gupta *et al.*, 2002 and Folarin *et al.*, 2011). Nosten and Brasseur (2002) also corroborate these claims while stating that "resistance to Amodiaquine follows resistance to chloroquine and therefore the useful therapeutic lifetime of the mixture (Amodiaquine and Artemisinin derivative) is perhaps short". An average percentage parasite inhibition of 38.6% was recorded when *Plasmodium falciparum* was treated with the formulated herbal concoction. There was initially a decreased percentage parasite inhibition; however, upon increase at 1.6mg/ml, there was a pointy increase. It's speculated that this pattern could also be explained by the unknown pharmacokinetic or pharmacodynamic characteristics of the components of the herbal concoction. Furthermore, the increased parasite inhibition observed after a raise in concentration could also be attributed to a synergetic effect of the active ingredients present in the herbal concoction. This agrees with the findings of Gathirwa *et al.*, (2008) whose research demonstrated the usefulness of mixing numerous plants to treat malaria. The discrepancy between the drug performance of Arthemether/ lumenfanthrine and herbal concoction was statistically significant.

Conclusion

The study established that a large number of children in school have asymptomatic *Plasmodium falciparum* infection and were harboring the parasite as confirmed by the result. This is often a sign that apparently healthy looking children have the disease and buttresses the very fact that malaria is endemic and of public health importance.

There are biologically active nutrients (phytochemicals) present in many plants locally utilized in the treatment of malaria that play several roles within the body of consumers including aiding the treatment of malaria. The phytochemical constituent in the prepared herbal concoction tend to have a complementary effect on one another i.e, in whichever plant a phytochemical constituent is lacking, another plant augments it.

Plasmodium falciparum can be cultivated in *Jatropha curcas* plant extract medium with materials that are available and really affordable. Artemether/Lumenfanthrine is the best drug against malaria parasite (*Plasmodium falciparum*) in this study albeit herbal medication has also been very effective.

Competing Interest

The authors declare no competing interests

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