

Invitro Analysis of the Effect of Carica Papaya Leaves Extract on Plasmodium Falciparum in Malumfashi, Katsina State

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Abstract

Original Research

Malaria is a parasitic infectious vector-borne disease and major public's health problem with haematological parameters. The Aim of this research was to determine the effect of Carica papaya leaves on Plasmodium falciparum. The antiplasmodial activity of the C. papaya was performed in 96-well tissue culture plates. Serial dilutions of test samples dissolved in sterile methanol were placed in microtiter plates and diluted with culture medium. Parasitized erythrocytes were added to the wells to give a final volume of 110 μ l. Chloroquine was used as standard. The plates were incubated at 37 $^{\circ}$ C with 5%CO₂. After incubation for 24 hours, Giemsa-stained thin blood films were prepared and the percentage of inhibition of parasite was determined under microscope. The percentage inhibition at each concentration was determined and the mean calculated. At 100 (μ g/ml) the water based extract, ethanol extract and Chloroquine standard produced a mean of 0.00, 0.00 and 0.62 parasitaemia and at 50 (μ g/ml) the water based extract, ethanol extract and Chloroquine produced a mean of 0.37, 0.34 and 0.62. While at Concentration level of 25 (μ g/ml) the water based extracts and ethanol extract produced a mean of 1.05, 0.93 and 0.62. At 12.5 (μ g/ml) the water based extract, ethanol extract and chloroquine produced a mean of 1.25, 1.06 and 0.62 respectively against the control 3.5. This trend signifies a decline in Plasmodium falciparum proliferations as the concentration increases from 12.5 to 100 (μ g/m L). Also ethanol based extract have more anti-malarial effect on P. falciparum followed by water base extract at 100 (μ g/m L). The Government should support research and provide vaccine against malarial parasites for its citizen.

Keywords: Invitro, Carica papaya leaves, plasmodium falciparum, antiplasmodial activity.

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INTRODUCTION

Malaria is a parasitic infectious vector-borne disease and major public health problems that causes suffering and premature death in tropical and subtropical continents (Arora, 2012). This treatable and preventable disease has reached epidemic

proportions in many geographical parts of this world and continues to spread (WHO, 2002). Children under five years and pregnant women are the most at risk of malaria in Africa. One million people die from malaria fever annually and ninety nine percent are children (WHO, 2003). In those children who

survive, malaria drains vital nutrients from them, impairing their physical and intellectual development (WHO, 2002). The anti-malarial potential of medicinal plants such as *quinine*, obtain from *cinchona* species and *artemisinin* obtained from *Artemisia annua* (Dery *et al.*, 1999). Selection of plant to be screened for anti-malarial activity is done on the basis of traditional reputation of *Carica papaya* (*C. papaya*) for its efficacy in the treatment of malaria parasites (Dery *et al.*, 1999).

The needs to use *Carica papaya* leaves extract for antiplasmodial activities are due to the emergence of drug-resistant of malarial parasites: can be considered in two discrete phases: first produces the resistant mutant, and the subsequent selection process that leads to its preferential transmission and spread. Resistance arises from spontaneous mutations or gene duplications, which are independent of drug pressure. Once formed, however, resistant mutants have a survival advantage in the presence of anti-malarial drugs and, conversely, a survival disadvantage in the absence of at least certain anti-malarial drugs.

The conventional drugs for the treatment of malarial parasites might not be available in some of the villages and might not be affordable to some rural dwellers; the chemotherapy is cost and not accessible to some rural dwellers and some drugs shows resistant to malarial parasites particularly some strains of *Plasmodium falciparum* (Wang *et al.*, 2014). Artemisinin combination therapy (ACT) is known to be the drugs of choice for malaria for the past ten years and the morbidity and mortality of malaria have fallen greatly due to the use of ACT in extremely endemic areas (Wang *et al.*, 2014), more so the medicinal effect of ACT has reduce drastically due to its rampant and routine use, which trigger the emergence of resistance to its derivatives in the treatment of *Plasmodium falciparum* (Wang *et al.*, 2014), the emergence and re-emergence of resistance to malarial parasites signify a serious threat to malarial fever and its haematological consequence: in tackling the menace caused by malarial parasites the standard preventive measure need to be observe. The spread of parasites resistance to anti-malarial chemotherapy from Asia to Africa was earlier

occurred with Chloroquine and Sulphadoxine (Fansider) thus, suggesting the need to Strengthened surveillance of a new anti-malarial chemotherapy in Nigeria and Africa at large.

Historical accounts of traditional medicine shown, that different plants were used as early as 5000 to 4000 BC in China and 1600 BC by Syrians, Babylonians, Hebrews and Egyptians (Dery *et al.*, 1999). Considerable indigenous knowledge system, from the earliest times, is linked to the use of traditional medicine in different countries (Farnsworth, 1994).

Papaya plant is a large, single-stemmed herbaceous perennial tree having 20–30 feet height. The leaves are very large (up to 2½ feet wide), palmate lobed or deeply incised with entire margins and petioles of 1-3 feet in length. Stems are hollow, light green to tan brown in color with diameter of 8 inches and bear prominent of scars (Arvind *et al.*, 2013).

The fruits are big oval in shape and sometimes called pepo-like berries, since they resemble melon by having a central seed cavity (Figure 2.5). Fruits are borne auxiliary on the main stem, usually singly but sometimes in small clusters. Fruits weigh from 0.5 up to 20 lbs, and are green unlike ripe, turning yellow or red orange. Flesh is yellow orange to salmon (pinkishorange) at maturity. The edible portion surrounds the large central seed cavity. Individual fruits mature in 5-9 months, depending on cultivator and temperature. Plants begin bearing fruits in 6-12 months (Arvind *et al.*, 2013).

Papaya plants are dioecious or hermaphroditic, producing only male, female or bisexual (hermaphroditic) flowers. Papaya as are sometimes said to be “trioecious” meaning that separate plants bear either male, female, or bisexual flowers. Female and bisexual flowers are waxy, ivory white, and borne on short peduncles in leaf axils, along the main stem. Flowers are solitary or small cymes of 3 individuals. Ovary position is superior. Prior to opening, bisexual flowers are tubular, while female flowers are pear shaped. Since, bisexual plants produce the most desirable fruit and are self-pollinating; they are preferred over female or male plants. A male papaya is distinguished by the smaller

flowers borne on long stalks. Female flowers of papaya was pear shaped, when unopened whereas, bisexual flowers are cylindrical (Arvind *et al.*, 2013).

Synonyms and Common Names

There are almost 40 vernacular names of *C. papaya* L. such as *Arbre de melon*, *Chamburo*, *Doeum lahong*, *Fan Kua*, *Gedang*, *Houng*, *Kepaya*, *Lapaya*, *Mapaza*, *Papaya*, *Thimbaw*, and *Wan Shou Kuo*, popularly known as *Gwanda* in Hausa language.

The objectives of the study were:

- To determine the prevalence of *Plasmodium falciparum* among the subjects in the study area.
- To culture *Plasmodium falciparum*.
- To determine the anti Plasmodial activity of Both water and ethanol based extracts of *C. papaya* leaves.
- To compare the activity of papaya leaves on *Plasmodium falciparum* parasites and that of chloroquine.

Ho: It hypothesized that *Carica Papaya* leaves extract has no significant anti *Plasmodium falciparum* activity.

MATERIALS AND METHOD

STUDY AREA

The study was carried out in Malumfashi L.G.A. where about 25% of the population are infected with *P. falciparum*, Malumfashi L.G.A. is bordered with Gwargo L.G A of Kano state by east, while at the north Malumfashi is bounded with Kankara local government area, in the South is bounded with Kafur local government area and in west it bounded by Bakori local government area. The town has an average temperature of between 18- 38°C. The warmest temperature usually occurs in dry season in the month of March - April. The highest rain fall is within month of July- August, latitude of 11.79 ° N, and longitude of 7.62 ° E, Elevation of 608 m. The

population of the area under study is about 182,920 people living in Malumfashi municipal according to 2006 National Population Commission. Malumfashi L.G.A. the inhabitant of this area are mainly Hausa Fulani, Hausa and Fulani, their main occupations are farming, rearing of animals and commerce (NPC, 2006).

PLANT MATERIALS AND PREPARATION OF EXTRACT

C. papaya leaf was collected in and around Malumfashi area Katsina state, Nigeria. It was taken to Botany department Bayero University Kano for authentication, before processing; it was identified and herbarium number given as "BUKHAN 12". *C. papaya* leaves were washed with tap water and shade dried at room temperature. An electric blender was used to powder the dried plant materials (leaves). The powder 500g of the leaf was extracted with 1.5L of organic solvent of absolute ethanol for 8h using a Soxhlet apparatus. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of plant residue was dissolved in 100ml of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml respectively. Another 1gram of the plant extract was dissolved in 100mls of distilled water and considered as 1% stock and was treated as above, (as in ethanol extract) so as to obtain ethanol based extract and water based extract.

Parasite Sample Collection

A total number of 200 samples collected for this study, the sample size generated by statistical programs, using the standard epidemiological formula invented by Hinderson 1982.

Venous Blood Sample Collection: Prior the collection of blood consent form were given to the volunteers to document their willingness and the detailed procedure also the benefits of this research were explained to them, moreover ethical clearance

was obtained from the ministry of health Katsina State. Blood sample collected by standard veins punctured; using 70% v/v Alcohol a vein was cleaned. A vein: visible from the skin surface was located and 4ml of blood drawn with needle and syringe was delivered into a tube containing Citrate Phosphate Dextrose Adenine (CPDA), Rapid Diagnostics Test (RDT) was done prior to determination of parasitaemia. Malaria positive samples collected were stored at 4°C. Vaccine carrier was used for the transportation of samples (Cheesebrough, 2003).

PREPARATION OF CULTURE MEDIUM FOR CULTIVATION

Serum preparation

Blood group B Rhesus positive (Rh +ve) was collected and centrifuge in a tube without anticoagulant at 1000 rpm for 20 minutes also was kept at 4°C, it was separated aseptically and kept in aliquots. The serum was inactivated by keeping it at 56°C in water bath for 30 minutes (Cheesebrough, 2003).

3.4.2 Preparation of erythrocytes (RBCs) for culture

Blood group O Rhesus positive was collected and centrifuge in a tube without anticoagulant at 1000 rpm for 20 minutes. Plasma and Buffy coat was removed with sterile Pasteur pipette, normal saline added to it and centrifuge again at 1500 rpm for 10 minutes and supernatant was removed. This process was repeated thrice. Equal volume of complete medium was added to the RBCs to obtain 50% haematocrit and store at 4°C until used (Cheesebrough, 2003).

3.4.3 Estimation of the percentage of erythrocytes infected (parasitaemia) with *Plasmodium falciparum*

Thick blood film: Using a pipette the blood sample was drawn, about two drops of blood dispensed into a clean grease free slide, a large circle of the thick

film was made approximately 15x15mm and the blood spread using a tip of Pasteur pipette to cover evenly the area of thick film 15x15mm while mixing the blood. This method gives a much higher concentration of parasites when they are few in number (Cheesebrough, 2003).

Thin Blood Film: Another drop of well mixed blood was placed, in front of that thick film, a spreader was placed at a suitable angle i.e. 45° (steep angle) angles in front of the blood and allowed the blood to touch and spread, to the edge of spreader, the spreader along the slide was pushed, drawing blood behind it, the whole blood has been smeared, to have a good film with body and tail, the smears were labeled with a pencil and kept air dry. Protected from insect and dust,. This method permits the study of *Plasmodium falciparum* morphology and density, also the condition of cells (Cheesebrough, 2003).

Staining Giemsa Method: For thin and thick film, Romanowsky stain was used, it requires dilution of 1:10 in pH 7.2 buffered water before used. Thin film was fixed in a methanol (absolute methyl alcohol) for two minutes and the diluted giemsa stain used to flood both the thick, thin film and left to stain for at least 10 minutes in staining rack, the slides were rinsed in buffered Distilled water and drain in the staining rack, allowed air dry. An area of stained thin blood film, where erythrocytes were evenly distributed was examined systematically using x100 objective (under oil immersion). Starting from the top section of the film, typical film with both parasitized RBCs and uninfected RBCs was seen, RBCs both parasitized RBCs and uninfected RBCs were all counted and recorded. In this method 1000 erythrocytes were examined and the percentage of parasitaemia was calculated by multiplying, the number of parasitized RBCs times 100 and dividing by the total number of RBC's index (WHO, 2002).

$$\% \text{ of parasitaemia} = \frac{\text{number of parasitized RBCs} \times 100}{\text{Total RBCs counted (1000)}}$$

Initiation of culture

Suspension (50%) of infected RBCs with complete media (with 15% serum) was prepared. Approximately amount of uninfected RBCs was added to get an initial parasitaemia of about 1% and Culture was kept at CO₂ incubator at 37⁰c (Dario *et al.*, 2009).

Cultures of parasites

Parasites were grown in human erythrocytes in a settled layer of cells in RPMI-complete. Parasites from culture are added to the freshly washed erythrocytes to give a starting parasitemia between 0.5-1.0percent. The cultures were provided with appropriate atmosphere: incubated at 37⁰c using the candle-jar method with 5% CO₂ and 24hours medium changes, requiring subculture by addition of fresh erythrocytes every three days (Dario *et al.*, 2009).

In vitro antiplasmodial assay

The antiplasmodial activity of the extract and test compounds was performed in 96-well tissue culture plates as described (Kalimuthu *et al.*, 2012) with modifications reported ((Dario *et al.*, 2009). Twofold serial dilutions of test samples dissolved in sterile methanol were placed in microtiter plates and diluted

with culture medium (RPMI 1640 plus 10% human serum). A suspension of parasitized erythrocytes (0.5-1% parasitaemia) containing mainly trophozoites were added to the wells to give a final volume of 200 μ L. Chloroquine was used as standard and uninfected and infected erythrocytes were included as negative controls and positive controls. The plates were incubated at 37⁰C using Candle jar method with 5%CO₂ and after 24 and 48h the culture medium was replaced with fresh medium.

After incubation for 24 hours, Giemsa-stained thin blood films were prepared for each well and the percentage of inhibition of parasite growth was determined under microscope by comparison of the number of schizonts with three or more nuclei (merozoites), with that of control wells. The percent inhibition at each concentration was determined and the mean of the least three LC₅₀ values of parasite viability was calculated using a Statistical tool (Dario *et al.*, 2009).

RESULTS

Prevalence of Malaria in the Study Area

Out of 200 samples collected and examined, 50(25%) were confirmed to be positive for *Plasmodium falciparum* parasite. However, 150(75%) were negative (Table 4.1).

Table 4.1: Prevalence of *Plasmodium falciparum* among the study Subjects.

No. of Participants	Frequency	Percentage (%)
No. of positive samples	50	25%
No. of negative samples	150	75%
Total	200	100%

Prevalence of *Plasmodium falciparum* according to the age of the subjects.

The study comprises of 200 subjects aged 0-12 years. Those within the age range of 3-5years were found to have the highest (52%) malarial parasitaemia,

followed by age group of 9-12 years (20%), while the age groups of 6-8years and 0-2 years accounted for 18% and 10% respectively.

From 108 males examined, 29 (58%) were positive, while 21 (42%) of the 92 females were positive for

Malaria parasite (Table 4.2).

Table 4.2: Prevalence of *Plasmodium falciparum* according to sex of the subjects

Age group (years)	Males		Females	
	No. examine	No. positive (%)	No. examine	No. positive (%)
0-2	11	2	9	2
3-5	43	15	37	11
6-8	23	5	19	4
9-12	31	7	27	4
TOTAL	108(100%)	29 (58%)	92 (100%)	21 (42%)

Antiplasmodial effect of the extracts.

The anti Plasmodial effect of ethanol based and water based of *C. papaya* against *Plasmodium falciparum* shows no growth at 100 µg/ml concentration of the extract (zero parasitaemia). But at 50µg/ml there were insignificant growth to less than 1 percent but as the concentration reduced to 25 µg/ml parasites

were able to tolerate the action of the extract and grow with mean of 1.05% and 0.938% of parasitaemia. Finally, the 12.5µg/ml water based extract and ethanol based extract produced a mean of 1.25% and 1.06% parasitaemia against the positive control (without extract) which enhance the proliferation of *Plasmodium falciparum* up to the mean of 3.5% parasitaemia in 24 hours (Table 4.3).

Table 4.3: Antiplasmodial effects of ethanol-based and water-based extract of *C. papaya* against *Plasmodium falciparum* (positive control mean = 3.5%).

		Concentrations. (µg/ml)			
		100	50	25	12.5
Water based extract	Mean±sd (% parasitemia)	0.00±0.00	0.375±0.49	1 ± 0.51	1.25±1.42
	p-value	0.001	0.002	0.014	0.001
Ethanol based extract	Mean±sd (% Parasitemia)	0.00±0.00	0.344±0.08	0.938±0.08	1.06±0.20
	p-value	0.001	0.001	0.071	0.001
Chloroquine	Mean±sd	0.625±0.087	0.625±0.087	0.625±0.087	0.625±0.087

standard	(% parasitemia)				
	p-value	0.043	0.043	0.043	0.043

Discussion

In this study, it was observed that children between the ages of 3 - 5 years had the highest prevalence of *Plasmodium* infections compared with the other age groups. This may be due to the fact that at that age, their immunity to parasitic infections has not been fully developed. Although it has been established that residual immunity derived from mothers could be very effective in younger children but environmental conditions and inability of children of this age in the study area to ward-off environmentally induced mosquito attacks predisposed them to malaria attack. The prevalence of *Plasmodium* infections has been found to reduce within the ages 6-8 and 9-12 years. This could be attributed to the fact that children of this age have developed immunity against *Plasmodium* parasite. Immunity produced after malarial infection is specific and the immunity last only till original infection remain active. This is called concomitant immunity otherwise previously called premium/infection immunity (Arora and Arora, 2012).

The prevalence of parasitic infections among the different age groups in the present study was not significant ($P < 0.05$) indicating that the occurrences of these infections on these age groups were the same. The present study has shown that *Plasmodium* infections were more common in the male than in the female subjects. The present result conforms to the recorded higher prevalence of *Plasmodium* infection in males than in females in the hospital. However, studies have shown that females have better immunity to parasitic diseases and this was attributed to genetic and hormonal factors such as Glucose-6-Phosphate Dehydrogenase (G6PD) traits which confer some protection against *Plasmodium falciparum* (Arora and Arora, 2012).

This study determine the effect of *C. papaya* leaves

on *Plasmodium falciparum*, the findings depicts the effect of the extracts at various concentration in $\mu\text{g/mL}$, that is 100, 50, 25 and 12.5 of water based extract of *C. papaya* leaves and ethanol based extract respectively, in which at 100 $\mu\text{g/ml}$ the water based extract and ethanol based extract produce a mean of 0.00 and 0.00% parasitaemia against the control which produces 3.406 % parasitaemia. For 50 $\mu\text{g/mL}$, water-based and ethanol-based extracts produced a mean of 0.375% and 0.344% parasitaemia respectively. While for 25 $\mu\text{g/ml}$, the water based extract and ethanol based extract produced a mean of 1.05% and 0.938% of parasitaemia. Finally, the 12.5 $\mu\text{g/mL}$ water based extract and ethanol based extract produced a mean of 1.25% and 1.06% parasitaemia against the positive control which enhance the proliferation of *Plasmodium falciparum* up to the mean of 3.406% parasitaemia in 24 hours. This vividly signifies the fact that ethanol extract of *C. papaya* have more impact on *Plasmodium falciparum* that is, it inhibit the level of *Plasmodium falciparum* proliferation more when compared to water based extract. This findings is in consistent with findings of Udobre *et al.*(2016); Bejon *et al.*(2008); Newton and Krishna (1998) and Olowe *et al.*(2015).

Kovendan *et al.*(2012) reported that Plant extracts of *C. papaya* showed moderate to good antiplasmodial effects at these concentrations (25, 50 and 100 $\mu\text{g/mL}$) of ethanol leaf extracts exhibited promising inhibitory activity against the CQ sensitive strain with values 40.75%, 36.54%, and 25.30%. However this study found reduction of 100% at 100 $\mu\text{g/ml}$, 90.3% at 50 $\mu\text{g/ml}$, 70% at 25 $\mu\text{g/ml}$ and 64.28% reduction at 12.5 $\mu\text{g/ml}$ indicating more antiplasmodial effect than his finding, this could be due to difference in soil nutrient and phytochemical constituent of the leaves extract.

A study conducted by Sha'a *et al.* (2011) revealed 78.1% and 74.3% anti-malarial activity with ethanol and water extract respectively using extract of *Vernonia amygdaline*. Similar study by Abosi and Raseroka (2003) reported 67% reduction of parasitaemia in a 4 day culture. However this study found reduction of 100% at 100µg/mL, 90.3% at 50µg/mL, 70% at 25µg/mL and 64.28% reduction at 12.5µg/mL indicating more activity of *C. papaya* than *Vernonia amygdaline*.

Another studies conducted by Mgbemena, (2010) reported that Neem leaves exhibit a prophylactic effect at 13.40% on plasmodium species, while lemongrass shows suppression of malarial parasites at 43.20 % and at 48.09%. However this study found reduction of 100% at 100µg/mL, 90.3% at 50µg /mL, 70% at 25 µg /mL and 64.28% reduction at 12.5µg/ml indicating more activity of *C. papaya* than neem leaves and lemon grass this could be due to solvent used in their extraction because methanol has higher volatility than ethanol (Mgbemena, 2010).

Sadiq *et al.* (2017) found out that Acacia's leaves, pod and bark extract have the following value in inhibiting trophozoites maturation to Schizont: 1.29, 4.16, and 4.28 respectively However this study found reduction of 100% at 100µg/mL, 90.3% at 50µg/ml, 70% at 25µg/mL and 64.28% reduction at 12.5µg/mL indicating contrary activity of *C. papaya* than Acacia.

Another experiment by Fang *et al.* (2019) discovered that antimicrobial peptides (AMPs) are emerging as a new source of antimalarials. In their study, an AMP LZ1 derived from snake cathelicidin was identified with antimalarial activity of 61% reduction in parasites number. However this study found reduction of 100% at 100µg /ml, 90.3% at 50µg/mL, 70% at 25µg /mL and 64.28% reduction at 12.5µg/mL indicating more activity of *C. papaya* than AMP LZ1, this could be due to the differences in constituent: papain, chymopapain etc versus AMP LZ1 which are peptides that includes 15 amino acid residues with a linear structure and contains only four amino acids, including lysine, arginine, valine, and tryptophan.

Wang *et al.* (2015) reported that Artesunate inhibition rate for *Plasmodium falciparum* was 20% at 0.0025µmol/µl, 60% at 0.05µmol/µl) and finally 100% at 0.2µmol/µl concentrations. They also reported inhibition rate for pyronaridine as 20% at 0.1µgmol/µl and 80% inhibition rate at 0.2µmol/µl. Similarly they reported inhibition rates for piperazine against *Plasmodium falciparum* as 20% at 0.4µmol/µl and 100% inhibition rates at 51.2µmol/µl. Furthermore, Wang *et al.*, (2015) reported that Chloroquine has inhibition rates of 20% at 0.8 umol/µl and 100% at 12.8 umol/µl for *Plasmodium falciparum*. This signifies the relationship between drugs concentration and the degree (%) of inhibition. However this study found reduction of 100% at 100µg/mL, 90.3% at 50µg/mL, 70% at 25µg/mL and 64.28% reduction at 12.5µg/mL. This indicates more antiplasmodial activity of artesunate, Piraquine and pyronaridine than *C. papaya* this could be due to differences in phytochemical constituents and the differences within the strains of *Plasmodium falciparum*.

SUMMARY

These results confirm that extracts from leaves of these plants which displayed good activities against *Plasmodium falciparum* are suitable for their traditional use in the treatment of malaria.

The current tools for combating malaria such as chloroquine can result in a major reduction in *Plasmodium falciparum*. In other to determine the anti-malarial activities of both water and ethanol based extracts of *Carica papaya* i.e. comparing the anti-malarial activities of water leaf extract and ethanol extract the result vividly signifies the fact that ethanol extract of *C. papaya* have more impact on *P. falciparum* i.e. it decline the level of *plasmodium* proliferation more when compare to water Extract, meanwhile water based extract produce a decline ranking mean of 0.00, 0.375, 1.05 and 1.25 at concentrations (µg/ml) 100, 50, 25 and 12.5 respectively and ethanol based extract shows the least mean of *P. falciparum* 0.00 (no growth), 0.344, 0.938 and 1.063 at concentration (µg/ml) 100, 50, 25 and 12.5 respectively which signified low level of *P.*

falciparum when compare to water based extract. Hence ethanol extract have more anti-malarial activities when compare to water based extract of *C. Papaya*.

Conclusion

From the result of this research, of the 200 children (0-12yrs) sample size, 50 children (25%) were infected with malaria parasite, out of which children between the age of 2-5 were observed to have the highest percentage (29%) of the infection followed by 6-10 and 11-15 years respectively as shown in Table 2, with *P. falciparum* having the highest prevalence among the causative agents as shown in Table 2.

The findings of the study revealed that all the culture of chloroquine, positive control as well as water and ethanol extract of *C. papaya* were displayed. The ethanol and water based extracts have some level of anti-malarial activity on *Plasmodium falciparum* when compared with the control, however the Anti-malarial activity of ethanol based extract of *C. papaya* leaves have more impact on *Plasmodium falciparum* parasites when compared with anti-malarial activity of water based extract, meanwhile if the concentration increases from 12.5 to 100 (μ g/ml) the mean level of *Plasmodium falciparum* proliferation rate also decrease.

The study revealed that both the chloroquine standard, ethanol based extract of *C. papaya* as well as water based extract of *C. papaya* have significant impact on *Plasmodium falciparum* parasites, however, chloroquine standard has more anti-malarial impact on *Plasmodium falciparum* parasites then follow by ethanol based extract of *C. papaya* and lastly water based extract of *C. papaya*.

Recommendations

The following recommendations were therefore made based on the finding of the study:

- i. Due to the increasing global spread of multi-drug resistant malaria parasite showed that: there is a need for new chemotherapeutic agents to combat the malady of malarial

parasites, there is need for the search of new anti-malarial drugs.

- ii. Development of new active and safe drugs for the community is therefore an urgent need. Towards this goal, research into new anti-malarial drugs from natural products is paramount though; traditional healers use parts of many plants for the treatment of several pathologies, including malaria
- iii. These results confirm that extracts from leaves of these plants which displayed good activities against *Plasmodium falciparum* are suitable for their traditional use in the treatment of malaria.
- iv. Based on this research, the best solvent to be used is absolute ethanol, it yield more anti-malarial activities than water as a solvent.
- v. The extract should be used at 100 μ g/ml, for best anti-malarial activities.
- vi. The extract has a lethal dose of 50 μ g/ml, it should not be given in excess/over dosage.
- vii. From the literature *C. papaya* contains *papain*, *chemopapain*, *pectin*, *caratenoid*, *choline*, *monoamides*, *lipase* and many more, more research should be done to establish the active ingredients responsible for lethal activities against malarial parasites

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