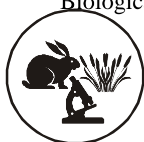

BEST JOURNAL 20(1): 29 - 45
Date received: 08/01/2023
Date accepted: 16/04/2023

ANTI-MALARIAL ACTIVITIES OF HAXANE EXTRACTS OF *Senna occidentalis* (COFFEE WEED) LEAVES AND *Syzygium aromaticum* (CLOVE) FLOWERS BUD AGAINST *Plasmodium falciparum*

Hotoro, A. S[1*], Dantata, A. A[2]. Hotoro, S. Y[3], Hotoro, S. S[4] and Biliyaminu, A[5]
[1]College of Health Science and Technology Kano, School of Health Technology Bebeji.
[2]Department of Biological Sciences, Bayero University, Kano, Nigeria.
[3]College of Health Science and Technology Kano, School of Health Technology Kano.
[4]Hospital Management Board Kano, Sir Muhammad Sunusi Specialist Hospital Kano.
[5]Department of Biology, College of Education, Gumel, Jigawa State.
Corresponding author: *alsosanihotoro@gmail.com

ABSTRACT
Malaria is a major health concern in Subtropical Africa and treatment options is a great challenge. This study has screened the phytochemicals and determined in vitro anti-malarial activities and acute toxicity study of leaves of Senna occidentalis and flower buds of Syzygium aromaticum hexane extracts against Plasmodium falciparum using standard procedures. The results of phytochemical screening of the extracts have showed presence of alkaloids, flavonoids, saponins, tannins and anthraquinones in both selected plant materials while; steroids were only detected in S. occidentalis. The test for anti-malarial activity of S. occidentalis showed strong activity against P. falciparum at concentrations of 50, 25 and 12.5mg/ml with percentage growth inhibition and mean infected RBC count (SD) of 87.0 (1.3), 67.7 (3.3) and 50.0 (4.3) respectively, while S. aromaticum showed strong activity only at concentration of 50mg/ml with percentage growth inhibition and mean infected RBC count (SD) of 81.1(1.7). From the acute toxicity study, the results of phases I and II showed no mortality in any of the experimental groups of rats after 24hours and up to four weeks after oral administration of both extracts at concentrations ≤5000 mg/kg, no signs of delay toxicity were recorded. It can be concluded that, the most potent extract is S. occidentalis and oral administration of both extracts at doses ≤5000 mg/kg is experimentally safe.

Keywords: ***Anti-malarial activity, Phytochemicals, Senna occidentalis, Syzygium aromaticum***

INTRODUCTION

Malaria is a common and serious tropical disease caused by a protozoan parasite transmitted through the bite of female Anopheles mosquitoes, vertical/congenital, transfusion of infected blood and organs transplant (WHO, 2018). In humans, the disease is caused by one of five species of *Plasmodium; P. falciparum, P. vivax, P. malariae P. ovale*, and *P. knowlesi* (Visser *et al.*, 2014). In 2018 *P. falciparum* accounts for the majority of the infections, and deaths from malaria (WHO, 2019). The most easily recognizable symptoms in non-severe malaria are chills, fever, and sweating which are frequently observed in patients experiencing the first episode of malaria, a stage during which they have no immunity (Martins *et al.*, 2015). Falciparum malaria is the most common cause of severe malaria in pregnancy and can also cause other complications, such as anemia and low birth weight babies (WHO, 2020). However, semi-immune patients may experience several other symptoms such as headache, myalgia, arthralgia, weakness, abdominal pain and others which can be helpful in the clinical diagnosis of non-severe malaria (Martins *et al.*, 2015) and there is an evidence that antibody can confers hormonal immunity against malaria infections (WHO, 2019).

Malaria can be diagnosed microscopically using thick and thin films of blood, the method of choice for confirming the clinical diagnosis of malaria and identifying the specific species responsible for the disease. Parasite in thick and thin blood films are best stained at pH 7.1-7.2 using a Romanowsky stain containing azure dyes and eosin (WHO, 2017). The thick film is a concentration method that may be used to detect the presence of the organisms and the thin film is most used for establishing species identify. Serological procedure are available but they are used primarily for epidemiological survey or for screening blood donor (WHO, 2019). Malaria is widely distributed in tropics and subtropics of Africa, Asia and Latin America. Malaria can be prevented using insecticide treated mosquito nets, removing the breeding ground of insect vector and killing adult mosquitoes by insecticides (WHO, 2019). Malaria can be treated in many parts of the world, chloroquine resistance stains of *P. falciparum* are present other agents includes mefloquine, quinine, guanidine, pyrimethamine-sulfadoxine, artemeter and doxycycline were employed (WHO, 2017).

The vast majority of malaria patients live in endemic, mainly tropical countries. Although malaria has been eradicated from North America and Europe, there has been resurgence in many parts of the tropics despite massive control efforts (WHO, 2019). Moreover, the malaria parasites are developing resistance to the current therapies and the mosquito vectors to insecticides, also poor sanitation increases the breeding ground of insect vectors (mosquito) WHO, 2019).

Quinine and derivatives of artemisinin are the two most common plants products useful in clinical practice. In the case of artemisinin, chemical modifications of the natural compound have led to a series of highly potent anti-malaria (Mekonnen, 2015). The constant evolution of the malaria parasite (plasmodium) has rendered the most commonest available anti-malarial treatments ineffective, especially with the recent reports about the increasing resistance of *Plasmodium falciparum* to artemisinin-based compounds(Christian *et al.*, 2015). Anti-malarial resistance has also undermined malaria control efforts and continues to be a threat (WHO 2010). Drug resistance has increased the burden of malaria in Africa and there is deep concern that this parasite will soon develop total resistance to such treatments. This has led researchers to look for other alternatives, among which is the evaluation of medicinal plants (Christian *et al.*, 2015). Hence, there is need to explore and utilize the naturally endowed rich biodiversity of communities through research that could translate to benefits for mankind. Therefore, such studies on medicinal and beneficial plants could provide useful leads for the synthesis of important active compounds (Christian *et al.*, 2015).

Historically, communities in tropical regions have used local plants as means of preventing and treating malaria (Okigbo, 2009; Willcox, 2004). There were over 1200 plant species used in the treatment malaria throughout the world (Willcox, 2004). Similarly, medicinal plants are widely used to treat malaria (Katuura *et al.,* 2007). Some plants which are widely used as anti-malarials have been shown to be significantly active *in vitro* and *in vivo* against *Plasmodium* species (Willcox *et al.*, 2011). For this reason, herbal medicine continues to play an essential role in covering the basic health needs in many developing countries, including Nigeria (Kuglerova *et al.*, 2011). Many existing anti-malarial drugs have been produced from the active compounds of plants such as quinine from the *Cinchona* bark and artemisinin from *Artemisia annua* (Asteraceae) (Bloland *et al.*, 2000; Willcox, 2004).

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The fresh flower of *Syzygium aromaticum* (clove) flower bud was obtained from Kasuwar Rimi, Kano (latitude 11°59’45.6”N and longitude 8°31’33.3”E) and *Senna occidentalis* leaves was collected from the Botanical garden of Biological sciences, Bayero University, Kano (latitude 11°58’50.5”N and longitude 8°28’51.5”E). The *Syzygium aromaticum* flower bud and *Senna occidentalis* leaves were identified and authenticated at the Herbarium of Plant Biology Department, Bayero University Kano, Nigeria and were kept and acknowledged with the accession numbers of BUKHAN 342 and BUKHAN 73 respectively

Senna occidentalis **leaves**

Syzygium aromaticum **flower bud**

Extraction of the Plant Materials

The modified protocol used by Basniwal (2005) was adopted, the *Syzygium aromaticum* flower bud and of *Senna occidentalis* leaves were gently separated and washed thoroughly with tap water at room temperature to facilitate drying. The plant materials were dried under shade for two weeks. These were then pounded using pestle and mortar to obtain a fine powder. One hundred grams of each plant materials were percolated in 1000 ml of hexane for five days with constant hand shaking at regular intervals, using successive cold maceration. The percolates were then filtered and the solvent was evaporated using Thermostat water bath (HH 6) brand at 40°C. The extracts were stored in a refrigerator at 4°C until needed.

Phytochemical Screening of the Hexane Extracts

Small portions of the hexane extracts were subjected to the phytochemical test following the modified methods of Trease and Evans (1989), Sofowora (1993) and Harborne (1998), methods to tests for alkaloids, tannins, flavonoids, steroids, saponins and anthraquinones.

Collection of Test Organisms

Test organisms (*Plasmodium falciparum*) were obtained from the parasitology laboratory of Sir Muhammad Sunusi Specialist Hospital Kano located at latitude 12°0’0.43”N and longitude 8°31’0.19”E.

The organisms were isolated from the infected blood samples of patients attending General Outpatient Department unit with malaria infection, after confirming microscopically by placing a drop of blood sample on a clean grease free slide to form a head and tail film, the film was fixed using absolute ethanol, allow to air dried and stained with 10% Giemsa solution for 30mins. It was then observed using oil immersion objective lens in compound light microscope (Cheesbrough, 1987).

Preparation of the Culture Medium for Cultivation of Malarial Parasites

Cultivation of malarial parasites was performed using modified Candle Jar Method developed by Trager and Jensen (1976). RPMI 1640 medium (containing 25mM of HEPES buffer, glucose) was used. One packet (about 10.4g) of RPMI 1640 was dissolved in 960ml of double distilled water, 40 µg/ml of gentamycin sulfate was added. This solution was passed through a Millipore filter of 0.22 µm porosity and sterilized in an autoclave at 121°C for 15 mins. This was then stored at 4°C in refrigerator as 96ml aliquots in glass media bottle.

Washing Medium (incomplete medium), Serum Preparation and formation of the Complete Medium

The washing medium was achieved by adding 4.2 ml of 5 % sodium bicarbonate to the 96 ml of stock RPMI 1640 medium but the medium is still incomplete. To obtained complete medium, blood group O Rhesus positive was collected from the blood bank of Sir Muhammad Sunusi Specialist Hospital in a plain container and kept at 4°C and then centrifuged at 10,000 rpm for 20 mins, at 4°C. The serum was separated aseptically and kept in aliquots, then inactivated by keeping it at 56°C water bath for half an hour. After inactivation the serum, 10 ml of the inactivated serum was added to 90 ml of the incomplete medium to get a complete enriched medium for culture. Furthermore, this inactivated serum could be stored in deep freezer at -20° C / -70° C for up to six months.

Preparation of Infected Erythrocytes (RBCs) for Culture

Parasite used for the study were field specimens obtained from the patient/subjects positive of malaria. The parasite was confirmed to be *P. falciparum* based on the slide smear observation of the positive slide by the hospital laboratory scientist. After that the confirmed infected blood sample were collected and transferred into centrifuge tubes containing anticoagulant (EDTA) and centrifuged at 1500 rpm for 10 min at room temperature. Plasma and fats were removed with the sterile Pasteur pipette. After this washing medium was added, centrifuged at 1500 rpm for 10min and the supernatant was removed. This washing process were repeated three times after which equal amount of complete medium was added to the sediments (parasitized red blood cells) and stored at 4°C.

Continuous Culture of the *Plasmodium falciparum*

The parasite was then obtained for continuous culture after centrifugation of the original blood specimen (infected blood sample) obtained. The infected RBCs obtained were washed following the modified Trager and Jensen, 1976 protocol. For initiation of culture, suspension (50 %) of infected cells with complete medium (with 15% inactivated serum) was prepared. Appropriate amount of uninfected cells were added to get an initial parasitemia of 3 to 5 % and diluted with complete medium to get 5 % cell suspension. The culture was kept in a Candle Jar incubator at 37°C.

The culture was monitored after every 24 hours, the medium was removed using a sterile Pasteur pipette without disturbing the parasitized cells that settled down. The parasitized cells were then mixed without frothing, a drop of blood was placed on the slide and a thin film was made.

Fresh complete media (with 10 % serum) was added, mixed properly and kept back in the incubator (Fairlamb, 1985) and the film was stained and examined microscopically following the methods described by Cheesbrough, 1987.

Preparation of Different Concentrations of the Test Extracts

Following the method of Ekwenye and Elegalam (2005) a concentration of 100 mg/ml of both extracts were prepared by dissolving 0.1 g of each extract in 1 ml of dimethyl sulfoxide (DMSO) to form stock solutions of 100 mg/ml. Then, from the stock solution of each extract, concentrations of 50, 25, 12.5, 6.25 and 3.125 mg/ml were prepared by double dilution procedure using serial dilution methods (Aneja, 2005).

Preparation of Drug (positive) Control

Stock solution 80mg/ml of artemether was prepared in 70 % dimethyl sulfoxide (DMSO) according to the procedure of Noedl *et al.* (2002). Subsequent dilutions (serial dilution) were made using incomplete RPMI 1640 medium as a diluent to yield a desired two-fold concentration for the artemether. Fifty microliter of the solution was transferred to 96 well micro culture plates (NUNC) brand. The plate was dried in an incubator at 37ºC and stored at 4ºC in sterile plastic container before use in accordance with the procedure of Russell *et al.* (2003).

Anti-malarial Activities of the Hexane Extracts against *P. falciparum*

The anti-malarial activities of the *Syzygium aromaticum* (clove) flower bud and *Senna occidentalis* (coffee weed) leaves extracts were evaluated *in vitro* against *Plasmodium falciparum* grown on RPMI 1640 medium at various concentrations of both extracts. The amount of the medium, crude extracts and infected red blood cells sample (test organisms) were in ratio of 8:1:1 in the culture vials (WHO, 2015). The DMSO was used as negative control (drugs free well) accordingly, and artemether was used as positive control. All vials were kept in a Candle Jar incubator and were incubated at 37°C. The percentage parasitemia were determined microscopically using thin blood smear after 72 hours intervals of the extracts and parasites contact. The percentage parasitemia of both crude extracts treated (tests culture) at various concentrations were recorded and the percentage growth inhibition (IC_{50}) were calculated using percentage parasitemia of negative control and that of the test vials culture at various concentrations (Benoit, 1996).

Acute Toxicity Assay of the Hexane Extracts

The LD_{50} of the crude extracts were determined using Lorke method (1983). The study was carried out in two phases of thirteen (13) experimental rats (*Rattus norvegicus*) each. The first 13rats were labeled as A using rings for the *Senna occidentalis* (coffee weed) leaves crude extract and the second 13rats were labeled as B using rings too for the *Syzygium aromaticun* (clove) flower bud hexane extract. The experimental rats were deprived of food for 16 – 18 hours prior to administration of the crude extracts. In phase 1 of the experiment, three group of three rats per group were used each for both extract, each crude extract was administered orally per body weight using feeding straw in geometrically increasing doses (10, 100 and 1000 mg/kg). The treated rats were observed for four hours post administration for signs of toxicity. After 24 hours, when no mortality occurred, phase 2 of the experiment of both extracts was initiated. In phase 2, four groups of one rat each, were administered orally as mentioned above in geometrically increasing doses (1500, 2250, 3250 and 5000 mg/kg). The rats were then observed for signs of toxicity for the first four hours and mortality for 24 hours.

The arithmetic means of the lowest dose that killed the rats and the highest dose that did not kill were taken as the mean lethal dose (LD_{50}) of the both extracts, as calculated using the following formula.

LD_{50} = Maximum non-lethal × Minimum lethal dose for both animal species (Lorke, 1983).

Data Collection and Analyses

Determination of Percentage Parasitemia and Growth Inhibition (%GI)

The percentage of parasitemia was determined using the methods adopted by (Kalra *et al*., 2006). From the test(s) vials, thin smears were prepared on slides. The slides were allowed to dry and then fixed with absolute ethanol. After fixing, the slides were allowed to dry and then stained with 10 % Giemsa in methanol for 30 mins. After 30mins the slides were rinsed with water and then allowed to air dry. To estimate the percentage of red blood cells infected with *Plasmodium falciparum,* the slides were carefully observed under microscope using ×100 oil immersion objective lens at five different fields on each slide. The percentage parasitemia was calculated using the following formula.

$$\%\ \text{Parasitemia} = \frac{\textbf{Parasitised RBC}}{\textbf{Total number of RBC}} \times 100$$

The percentage of growth inhibition (% GI) of each concentration of the crude extracts was determined using the following equation:

$$\%\ \text{GI} = \frac{a-b}{a} \times 100$$

Where "a" stands for mean % parasitemia of negative control (drugs free well) vials and "b" stands for mean % parasitemia of treated vials; for comparing growth inhibition (GI) in treated vials and negative control vials. One way-ANOVA was used to analyze the data to determine significance difference among the selected concentrations at 72 hours for each plant hexane extracts. The data were found to be normally distributed hence, two way-ANOVA was used to determine significance interaction between the two plant hexane extracts and their concentrations as computed using R version 3.4.0 statistics software.

RESULTS

Percentage Yield and Appearance of the Hexane Extracts

The mass, percentage yield and appearance of the hexane extracts as presented in Table 1 revealed that, the *Senna occidentalis* (coffee weed) leaves extract appears dark green in color and had the highest yield of 10.2 g and 25.5 % as compared with the *Syzygium aromaticum* (clove) flower bud hexane extract, which appears dark brown in color and had the lowest yield of 8.6 g and 21.6 %.

Table 1: Percentage Yield and Appearance of the *Senna occidentalis* (coffee weed) and *Syzygium aromaticum* (clove) Flower Bud Hexane Extracts

Plants	Mass (g)	Percentage Yield (%)	Hexane Extracts Appearance
S. occidentalis	10.2	25.5	Dark green in color
S. aromaticum	8.6	21.5	Dark brown in color

Qualitative Phytochemical Screening of the *Senna occidentalis* (coffee weed) and *Syzygium aromaticum* (clove) Flower Bud Hexane Extracts

The phytochemical screening for the selected plant extracts was conducted following the modified methods of Harborne (1998), Sofowora (1993) and Trease and Evans (1989). The result presented in Table 2; confirmed the presence of alkaloids, flavonoids, saponins, tannins, anthraquinones and steroids in both plants extracts, except for steroids which was absent in *Syzygium aromaticum* (clove) flower bud extract. And all the tests were carried out in triplicates (Table 2).

Table 2: Phytochemical Screening of Hexane Extracts of *Senna occidentalis* (leaves) and *Syzygium aromaticum* (flower bud)

Plants spp.	Alkaloids	Flavonoids	Saponins	Tannins	Anthraquinones	Steroids
Senna occidentalis	+	+	+	+	+	+
Syzygium aromaticum	+	+	+	+	+	-

KEY: + = Present and - = Absent.

Anti-Malarial Activity of Hexane Extracts of *Senna occidentalis* (coffee weed) Leaves and *Syzygium aromaticum* (clove) Flower bud against *P. falciparum*

Comparison of anti-malarial activity of *S. occidentalis* and *S. aromaticum* hexane extracts at different concentrations against *P. falciparum* revealed lower infected RBC (1.3 and 1.7) at concentration of 50mg/ml than concentration of 3.125 mg/ml (9.3 and 9.0), while higher infected RBC was recorded from negative control (11.3 and 11.0) for *S. occidentalis* and *S. aromaticu*m respectively. The percentage growth inhibition as calculated using mean infected RBC count and mean RBC count shows a significant difference at different concentrations at $p < 0.05$ with *Senna occidentalis* having the highest growth inhibition (87.0 %) as compared to *Syzygium aromaticum* (81.1 %) (Table 3). The comparison of the efficacy of *S. occidentalis* and *S. aromaticum* hexane extracts against *P. falciparum* is presented in Figure 1.

Table 3: Anti-malarial Activity of *S. occidentalis* leaves and *S. aromaticum* flower bud Hexane Extracts at Different Concentrations against *P. falciparum* with Respect to their Percentage Growth Inhibition

Parameters	Concentration (mg/ml)					
	50	25	12.5	6.25	3.125	Control
S. occidentalis						
Mean RBC (SD)	103 ± (5.0)	105 ± (4.6)	87.3 ± (20)	117 ± (27.2)	122 ± (14)	115 ± (13.5)
Mean infected RBC (SD)	1.3 ± (0.6)	3.3 ± (0.6)	4.3 ± (0.6)	7.0 ± (1.0)	9.3 ± (0.6)	11.3 ± (1.2)
Mean % Parasitemia	1.28	3.19	5.11	6.26	7.41	9.88
% Growth inhibition	87.0	67.7	50.0	36.6	25.0	-
S. aromaticum						
Mean RBC (SD)	100 ± (14.2)	109 ± (15.0)	117 ± (8.6)	114 ± (22.7)	117 ± (13)	128 ± (9.7)
Mean infected RBC (SD)	1.7 ± (0.6)	5.3 ± (0.6)	6.3 ± (1.5)	8.7 ± (0.6)	9.0 ± (1.0)	11.0 ± (1.0)
Mean % Parasitemia	1.63	4.98	5.42	7.91	7.85	8.63
% Growth inhibition	81.1	42.3	37.2	8.3	9.0	-

Key: RBC = Red Blood cells, SD = Standard Deviation

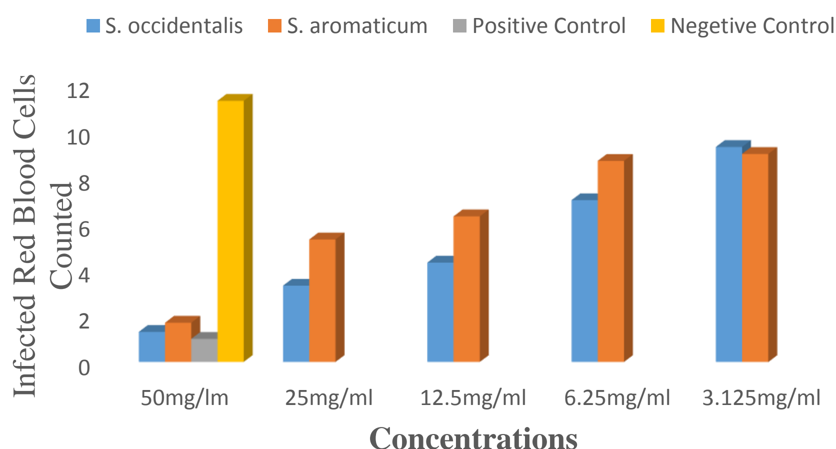

Figure 1: Comparison of the Efficacy of *S. occidentalis* and *S. aromaticum* hexane extracts against *P. falciparum* at Different Concentrations with respects to their mean Infected Red Blood Cells count (Growth inhibition)

Acute Toxicity Study of hexane extracts of *Senna occidentalis* (coffee weed) Leaves and *Syzygium aromaticum* (clove) Flower bud

The acute toxicity of the *Senna occidentalis* (coffee weed) Leaves and *Syzygium aromaticum* (clove) flower bud hexane extracts were conducted following the protocol adopted by Lorke (1983). The result showed no mortality in any of the experimental three groups in phases I after 24 hours of oral administrations of both extracts at doses of 10, 100 and 1000 mg/kg to each three groups. This initiates the phase II of the experiment were both extracts administered orally in geometrical increasing doses of 1500, 2250, 3250 and 5000 mg/kg to the second four groups of one rat each, after 24 hours no mortality were also recorded. The oral median dose (LD_{50}) for the crude extracts of the *Senna occidentalis* (coffee weed) Leaves and *Syzygium aromaticum* (clove) flower bud were therefore estimated to be greater than 5000 mg/kg and no sign of behavioral changes were also observed up to four weeks (Tables 4).

Table 4: Acute Toxicity Study on Hexane Extracts of *Senna occidentalis* (leaves) and *Syzygium aromaticum* (clove) flower bud

Treatment	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24h *Senna occidentalis*	Mortality recorded after 24h *Syzygium aromaticum*
Phase 1	I	3	10	0/3	0/3
	II	3	100	0/3	0/3
	III	3	1000	0/3	0/3
Phase 2	I	1	1500	0/1	0/1
	II	1	2250	0/1	0/1
	III	1	3250	0/1	0/1
	IV	1	5000	0/1	0/1

Key: Numerator = Number of animals died and Denominator = Number of animals tested.

DISCUSSION

Senna occidentalis (coffee weed) leaves and *Syzygium aromaticum* (clove) flower bud evaluated in this study were identified through an ethno-botanical approach whereby plants are selected based on their use in local traditional settings to treat/control malaria associated symptoms. Other methods are used to identify plant species for biological evaluation such as random approach, chemotaxonomic approach and phytochemical approach (Frabicant and Fransworth 2001).

The qualitative pharmacological analyses of *Senna occidentalis* leaves hexane extract revealed the presence of alkaloid, flavonoid, saponins, tannins, anthraquinones and steroids. These phytochemical results are in line with that of Nuhu and Aliyu (2008), Olurundare *et al*. (1992) and Tona *et al.* (2004), while they differ with that of Bin-Hafeez *et al.* (2001). This might be due to different solvent (water) used in extraction of the plant materials which confirms that polarity affects the extraction of some secondary metabolites. These phytochemicals are known to be biologically active, with one or more therapeutic uses and thus aid the anti-malarial activity of the selected plant extracts (Odeja *et al.,* 2014). *Syzygium aromaticum* flower hexane extract however, revealed the presence of alkaloid, flavonoid, saponins, tannins, anthraquinones however, only steroids were found to be absent. These phytochemicals result corroborate the work of Ayoola *et al.* (2008) who also reported the presence of alkaloid, flavonoid, saponins, tannins and anthraquinones in the crude extract of *S. aromaticum*. Scrutiny of past research on *S. aromaticum*, shows that not much has been reported on the anti-malarial activity. Literature related to anti-malarial activity and phytochemical constituent of *S. aromaticum* is scanty. This might be attributed to the fact that *S. aromaticum* is a tree indigenous to West Africa and therefore research on the plant is scanty and claims by traditional herbalists on the usefulness of the plant as medicine mostly centered on the use of the stem bark, root and leaf not the flower.

The anti-malarial activity of *Senna occidentalis* in this study showed IC_{50} at concentrations of 50, 25 and 12.5 mg/ml. This is in line with the research demonstrated by Sharma *et al*. (2000) and Bin-Hafeez *et al*. (2001) which showed that at concentration of 50 and 25 mg/ml *S. occidentalis* hexane extract inhibits the growth of *P. falciparum* with the high percentage of 93 and 71.1 %. It is likely that, this activity could be attributed to the presence of phytochemicals with anti-malarial properties like alkaloid, flavonoid, saponins, tannins, anthraquinones and steroids in this plant (Oliveira, 2009). According to the anti-malarial activity observed by Choudhary and Nagori (2013) the concentration of 25mg/ml was found to be more effective with percentage growth inhibition of 63%. However, this work differs with that of Chukwujekwu *et al.* (2006) in which the anti-malarial activity showed only at concentration of 6.25mg/ml with %GI of 57.6 %. The research conducted by Sheeba *et al*. (2009) showed more or less anti-malarial activity at all concentrations. This might be due to the different season, weather conditions or used species of plasmodium.

The extract of clove flower (*S. aromaticum*) showed strong anti-malarial activity against *P. falciparum* at only concentration of 50 mg/ml from the result obtained, this is in agreement with the result of previous study by Oshomoh and Idu (2012) who reported that extract from the clove flower (*S. aromaticum*) has appreciable activity against *P. falciparum* at concentration of 50mg/ml with % GI of 85.1 %.

The finding of this study corroborated the report of Ayoola (2008) who equally reported that *S. aromaticum* is active on *P. falciparum* at higher concentration of 100mg/ml. According to the investigation carried out by Oshomoh (2015), *S. aromaticum* hexane extract was found to be active on *P. falciparum* at various concentrations of 50, 25, 12.5 and 6.25 mg/ml, and this is in disagreement with the result of the present study. The variation might be as a result of using different solvent in extraction (water) or due to the absent of strong secondary metabolite with anti-malarial property (steroids).

From the results of acute toxicity study of *Senna occidentalis* (coffee weed) leaves and *Syzygium aromaticum* (clove) flower hexane extracts no mortality was recorded in all experimental groups within 24 hours and up to four weeks after oral administration of 5000 mg/kg of each plant extract. According to the toxicity classes of Hodge and Sterner (2005) said that, any compound with oral LD_{50} (rat) of 5000 mg/kg or less could be considered as practically harmless. This is in conformity with the findings by Stephen and Duffy (2001), their findings also showed that both the plant extracts were found to be experimentally safe. This work is a line with that of Shiavone *et al*. (2008). However differ with that of Raffi and Mark (2009) with mortality recorded at phase I, group III (1000 mg/kg) as well as phase II at all groups. Hence oral administrations of *Senna occidentalis* (coffee weed) leaves and *Syzygium aromaticum* (clove) flower hexane extracts at doses of less than or equal to 5000mg/kg would be not experimentally safe (Gadanya, 2011).

CONCLUSIONS

From the research result, it was confirmed that, hexane extracts of; *Senna occidentalis* (coffee weed) leaves and *Syzygium aromaticum* (clove) flower have an extracts with secondary metabolites like alkaloids, flavonoids, saponins, tannins, anthraquinones and steroids; only steroids were found to be absent in *S. aromaticum.* In addition, the inhibitory effects of *Senna occidentalis* (coffee weed) leaves and *Syzygium aromaticum* (clove) flower hexane extracts against *P. falciparum* at concentrations of 50, 25 and 12.5 mg/ml of *S. occidentalis,* were confirmed, while in the case of *S. aromaticum*, inhibitory effect was only at 50mg/ml. The presence of phytochemicals in these extracts might have been responsible for the anti-malarial activities possessed by the plants, which supports the uses of these plants to treat malaria associated symptoms in local/traditional settings. The hexane extracts of the two selected plants showed that *Senna occidentalis* was more potent than *Syzygium aromaticum.* Acute toxicity showed that, *Senna occidentalis* (coffee weed) leaves and *Syzygium aromaticum* (clove) flower hexane extracts are experimentally harmless.

REFERENCES

Abdulrazak, N., Asiya, U. I., & Usman, N. S. (n.d.). Anti-plasmodial activity of ethanolic extract of root and stem back of *Cassia sieberiana* DC on mice, 96–101. https://doi.org/10.5455/jice.20141231014333

Achan, J., Talisuna, A. O., Erhart, A., Yeka, A., Tibenderana, J. K., Baliraine, F. N. & Alessandro, U. D. (2011). Quinine , an old anti-malarial drug in a modern world : role in the treatment of malaria. *Malaria Journal*, *10*(1), 144. https://doi.org/10.1186/1475-2875-10-144

Ajayi, E. I. O., Adeleke, M. A., Adewumi, T. Y., & Adeyemi, A. A. (2017). Antiplasmodial activities of ethanol extracts of Euphorbia hirta whole plant and Vernonia amygdalina leaves in Plasmodium berghei-infected mice. *Integrative Medicine Research*. https://doi.org/10.1016/j.jtusci.01.008.

Aneja, K.R.(2005). *Experiment in Microbiology, Plant Pathology and Biotechnology*. New Age Publishers, p. 69. ISBN 81-224-1494-X.

Ayoola, G. A., Lawore, F. M., Adelowotan, T., Aibinu, I. E., Adenipekun, E., Coker, H. A. B. and Odugbemi, T. O. (2008). Chemical analysis and antimicrobial activity of the essential oil of *S. aromaticum* (clove). *African Journal of Microbiology Research* **2**: 162 - 166.

Baird, J., Kevin, (2005). Effectiveness of antimalarial drugs. *New England Journal of Medicine*; **352**(15):1565-77 93

Basniwal, P.K. (2005) Supercritical fluid extract: A new millstone in extraction technology, *Ind J Pharm Sci*. **67**(5), 532-9.

Basco, L.K., Ramiliarisoa, O., Le Bras, J., (1995). *In vitro* activity of atovaquone against the African Isolates and clones of *Plasmodium falciparum. Am. J. Trop. Med.* **53**(4): 388-391.

Beldjoudi, N., Mambu, L., Labaïed, M., Grellier, P., Ramanitrahasimbola, D., Rasoanaivo, P., & Frappier, F. (2003). Flavonoids from *Dalbergia ouvelii* and their Antiplasmodial Activity. *Journal of Natural Products*, **66**(11), 1447-1450.

Benoit, F., Valentin, A., Palliser, Y., Diafouka, F., Marion, C., Kone-Bamba, D., Kone, M., Yabo, A.& Bastide, J.M. (1996) In vitro antimalarial activity of vegetal extracts used in West African Traditional medicine. *Am J. Trop. Med. Hyg*. **54**(1):67-71.

Bin-Hafeez, B., Ahmad, I., Haque, R., Raisuddin, S. (2001). Protective effect of *Cassia occidentalis* on Cyclophosphamide-induced suppression of humoral immunity in mice. *J. Ethnopharmacol.* **75**(1): 13-18.

Bloland, P. B., Lackritz, E. M., Kazembe, P. N., Were, J. B., Steketee, R., & Campbell, C. C. (2000). Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *Journal of Infectious Diseases*, **167**(4), 932-937.

Bourgaud, F., Gravot, A., Milesi, S., & Gontier, E. (2001). Production of plant secondary metabolites: a historical perspective. *Plant Science*, **161**(5), 839-851.

Bruce, N. C. (2008). Alkaloids. *Biotechnology Set, Second Edition*, 327-361.

Choudhary P. K. and Nagori B. P. (2013) Evaluation of in Vitro Antimalarial Activity of Cassia occidentalis. *World Journal of Pharmacy and PharmaceuticalSciences* **3**(2): 2241-2248.

Christian, A. G., Ahunna, A. G., Nwakaego, E. M., & Chimsorom, K. (2015). Antimalarial potential of the ethanolic leaf extract of Pseudocedrala kotschyi. *Journal of Acute Disease*, *4*(1), 23–27. https://doi.org/10.1016/S2221-6189(14)60077-9

Chukwujekwu J. C., Coombes P.H., Mulholland D. A. and van Staden J. (2006) Emodin, an antibacterial anthraquinone from the roots of Cassia occidentalis. *South African Journal of Botany* **72**(2): 295-297.

Chung, I. M., Ghimire, B. K., Kang, E. Y., & Moon, H. I. (2010). Antiplasmodial and cytotoxic activity of khellactone derivatives from *Angelica purpuraefolia* Chung. *Phytotherapy Research*, **24**(3), 469-471.

Croteau, R., Kutchan, T. M., & Lewis, N. G. (2000). Natural products (secondary metabolites). *Biochemistry and Molecular Biology of Plants*, 1250-1318.

Cunha-Rodrigues Margarida, Miguel Prudencio, Maria, M. Mota and Werner Haas., (2006). Antimalarial drugs – host targets (re)visited. *Biotechnology Journal*, 1, 321–332

Daily, J. P. (2006). Antimalarial drug therapy: the role of parasite biology and drug resistance. *The Journal of Clinical Pharmacology*, **46**(12), 1487-1497.

Dettrakul, S., Surerum, S., Rajviroongit, S., & Kittakoop, P. (2009). Biomimetic transformation and biological activities of globiferin, a terpenoid benzoquinone from *Cordia globifera*. *Journal of Natural Products*, **72**(5), 861-865.

Dharani, N., Rukunga, G., Yenesew, A., Mbora, A., Mwaura, L., Dawson, I., & Jamnadass, R. (2010). *Common antimalarial trees and shrubs of East Africa*. World Agroforestry Centre.

Dondorp, A. M., Yeung, S., White, L., Nguon, C., Day, N. P., Socheat, D., & von Seidlein, L. (2010). Artemisinin resistance: current status and scenarios for containment. *Nature Reviews Microbiology*, **8**(4), 272-280.

Ekpenyong, E., & Eyo, J. (2006). Plasmodium infection in man: A review. *Animal Research International*, **3**, 573–580.

Ekwenye, U. N. and Elegalam, N. N. (2005). Antibacterial activity of ginger (*Zingiber officinale*, Roscoe) and garlic (*Allium sativum*, L.) extracts on *Escherichia coli* and *Salmonella typhi*. *Int. J. Mol. Med. Adv. Sci.* **1**:411-416

Eloff, J. N. (1998). A sensitive and quick method to determine the minimum inhibitory concentration of plant extracts for malaria. *Plant Medicine* 64: 711-713.

Fabricant, D. S., & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives*, *109* (Suppl 1), 69.

Fairlamb, A.H., Warhurst, D.C., Peters W. (1985). An improved technique for the cultivation of *Plasmodium falciparum* in vitro without daily medium change. *Annals of Tropical Medicine and Parasitology* **79**, 379-390.

Fentahun, S., Makonnen, E., Awas, T., & Giday, M. (2017). In vivo antimalarial activity of crude extracts and solvent fractions of leaves of Strychnos mitis in Plasmodium berghei infected mice. *BMC Complementary and Alternative Medicine*, 1–12. https://doi.org/10.1186/s12906-016-1529-7

Figueiredo, J.N., Raz, B., Sequin, U. (1998) Novel quinonemethides from Salaciakraussii with in vitro Antimalarial activity. *J. Nat. Prod.* **61**(6): 718-723.

Francisco, L., Lima, E. S., Vasconcellos, M. C. De, Suzany, E., Aranha, P., Costa, D. S., … Pohlit, A. M. (2013). In vitro and in vivo antimalarial activity and cytotoxicity of extracts , fractions and a substance isolated from the Amazonian plant Tachia grandiflora (Gentianaceae), *108*(June),501–507. https://doi.org/10.1590/0074-0276108042013017

Freitas, L. A., Russo, C. A. M., Voloch, C. M., Mutaquiha, O. C. F., Marques, P., & Schrago, C. G. (2015). Diversification of the Genus Anopheles and a Neotropical Clade from the Late Cretaceous, 1–12. https://doi.org/10.5061/dryad.bp6gv

Gadanya, A.M. (2011). Biochemical and Toxicological studies on "Gadagi" tea in rats. Ph.D Thesis. Department of Biochemistry, Bayero University Kano, Pp. 139-142.

Garner, P. (2013). Artemisinin Combination Therapy: A good antimalarial, but is the dose right? *PLoS Medicine*, **10**(12), e1001565.

Gboeloh, L. B., Okon, O. E., & Udoh, S. E. (2014). Antiplasmodial Effect of Anthocleista vogelii on Albino Mice Experimentally Infected with Plasmodium berghei berghei (NK 65), *2014*. https://doi.org/10.1155/2014/731906

Grotewold, E. (Ed.). (2008). The science of flavonoids. *Springer*.

Harbone, J.B. (1998). *Phytochemical Methods, A guide to plants analysis*, 3rd Edn. Chapman and Hall Int. Edn, New York.

Hastings, I. M., & Hodel, E. M. (2014). Pharmacological considerations in the design of anti-malarial drug combination therapies - is matching half-lives enough? *Malaria Journal*, *13*(1), 62. https://doi.org/10.1186/1475-2875-13-62

Hay, S. I., Sinka, M. E., Okara, R. M., Kabaria, C. W., Mbithi, P. M., Tago, C. C., & Godfray, H. C. J. (2010). Developing global maps of the dominant anopheles vectors of human malaria. *PLoS Medicine*, *7*(2), 1–6. https://doi.org/10.1371/journal.pmed.1000209

Heather, N., Honekopp, J. and Smailes, D. (2009). Progressive stage transition does means Getting better. A further test of the transtheoretical model in recovery from alcohol problems. *Addiction* **104**: 949-958.

Hobbs, C., & Duffy, P. (2011). Drugs for malaria: something old, something new, something borrowed. *F1000 Biology Reports*, **3**(24), 1-9

Hodge, A. and Sterner, B. (2005) .Toxicity classes. In:Canadian center for occupational Health and safety. Copy right @1997-2010. Retrieved from (http://www.ccohs.ca/oshanswers/chemicals/ id50.htm) 0n 3/5/2010.

Irungu, B. N., Adipo, N., Orwa, J. A., Kimani, F., Heydenreich, M., Midiwo, J. O., & Erdelyi, M. (2015). Antiplasmodial and cytotoxic activities of the constituents of Turraea robusta and Turraea nilotica. *Journal of Ethnopharmacology*, *174*, 419–425. https://doi.org/10.1016/j.jep.08.039

Kano state ministry for health, Malaria report (2015).

Kano state ministry for health, Malaria report (2017).

Kalra, B. S., Chawla, S., Gupta, P. and Valecha, N. (2006) "Screening of anti-malarial drugs: an overview," *Indian Journal of Pharmacology*, vol. **38**(1): 5–12.

Katuura E., Waako P., Ogwal-Okeng J. and Bukenya-Ziraba R. (2007). Traditional treatment of malaria in Mbarara District, Western Uganda. *African Journal of Ecology,* **45**: 48-51.

Kar, N. P., Kumar, A., Singh, O. P., Carlton, J. M., & Nanda, N. (2014). A review of malaria transmission dynamics in forest ecosystems, *7*(1), 1–12. https://doi.org/10.1186/1756-3305-7-265

Kefe, A., Giday, M., Mamo, H., & Erko, B. (2016). Antimalarial properties of crude extracts of seeds of Brucea antidysenterica and leaves of Ocimum lamiifolium. *BMC Complementary and Alternative Medicine*, 1–8. https://doi.org/10.1186/s12906-016-1098-9

Kiplagat, D. M., Akala, H. M., Liyala, P. O., Wangui, J. M., Odhiambo, R. A. O., & Omolo, J. O. (2016). Antiplasmodial activity of flavan derivatives from rootbark of Cassia abbreviata Olive. *Journal of Saudi Chemical Society*, *20*, S140–S144. https://doi.org/10.1016/j.jscs.2012.10.002

Kshirsagar N., Gogtay N., Rajgor D., Dalvi S. and Wakde M. (2000). An unusual case of multidrug-resistant *Plasmodium vivax* malaria in Mumbai (Bombay), India. *Annals of Tropical Medicine and Parasitology,* **94**: 189-190.

Kuglerova M., Tesarova H., Grade J.T., Halamova K., Wanyana-Maganyi O., Van Damme P. and Kokoska L. (2011). Antimicrobial and antioxidative effects of Ugandan medicinal barks. *African Journal of Biotechnology,* **10**: 3628-3632.

Lambros, C., Vanderberg, J.P. (1976) Synchronisatton of *Plasmodium falciparum* erythrocytic stage in culture. *Journal of Parasitology* **65**: 418-420.

Laurent, D., Jullian, V., Parenty, A., Knibiehler, M., Dorin, D., Schmitt, S., Lozach, O., Lebouvier, N., Frostin, M., Alby, F., Maura, S., Doerig, C., Meijer, L. & Savant, M. (2006). Antimalarial potential of xestoquinone, a protein kinase inhibitor isolated from a Vanuatu marine Sponge *Xestospongia sp. Bioorg. Med. Chem.* **14**(13): 4477-4482.

Lorke, D. (1983). A new approach to tropical acute toxicity testing. *Arch. Toxicol.* 53: 275-287.

Martins, A. C., Ara, F. M., Braga, B., Guimar, M. G. S., Nogueira, R., & Arruda, R. A. (2015). Clustering symptoms of non-severe malaria in semi-immune Amazonian patients, 1–20. https://doi.org/10.7717/peerj.1325

Mekonnen, L. B. (2015). In vivo antimalarial activity of the crude root and fruit extracts of Croton macrostachyus (Euphorbiaceae) against Plasmodium berghei in mice. *Journal of Traditional and Complementary Medicine*, *5*(3), 168–173. https://doi.org/10.1016/j.jtcme.2014.07.002

Miller, J. S., Cao, S., Hou, Y., Brodie, P. Randrianaivo, R., Rakotobe, E. & Kingston, D. G. (2011). Antiproliferative compounds of *Cyphostemma greveana* from a Madagascar dry forest. *Chemistry & Biodiversity*, **8**(4), 643-650.

Ngasala, B. E., Malmberg, M., Carlsson, A. M., Ferreira, P. E., Petzold, M. G. & Blessborn, D. (2011). Efficacy and effectiveness of artemether-lumefantrine after initial and repeated treatment in children< 5 years of age with acute uncomplicated *Plasmodium falciparum* malaria in rural Tanzania: a randomized trial. *Clinical Infectious Diseases*, **52**(7), 873-882.

Noedl, H., Wensdorfer, W. H., Miller, R. S. and Wangsrichanalai, C. (2002). Histidine rich protein II: a *Novel approach to malaria drug sensitive testing. Antimicrobial agents and chemotherapy,* **46**(6): 1658-1664.

Nuhu, A.A. and Aliyu, R. (2008). Effects of *Cassia occidentalis* aqueous leaf extract on biochemical markers of tissue damage in rats. Trop. J. Pharm. Res. **7**(4): 1137-1142.

Nzila, A. (2006). The past, present and future of antifolates in the treatment of *Plasmodium falciparum* infection. *Journal of Antimicrobial Chemotherapy*, **57**(6), 1043-1054.

Odeja O. O., Obi G., Ogwuche C. E., Elemike E. E. and Oderinlo O. O. (2014) phytochemical screening, Antioxidant and Antimicrobial activities of *Senna occidentalis* (L.) leaves. *International Journal of Herbal Medicine* 1: 6.

Oketch-Rabah, H. A., Mwangi, J. W., Lisgarten, J., & Mberu, E. K. (2000). A new antiplasmodial coumarin from *Toddalia asiatica* roots. *Fitoterapia*, **71**(6), 636-640.

Okigbo, P. N. (2009). Nigeria Malaria control Database : An Overview of 100 Years ' Research, *6*(12), 6–7. https://doi.org/10.131/journal.pone.

Okorie, P. N., Mckenzie, F. E., Ademowo, O. G., Bockarie, M., & Kelly-, L. (2011). Nigeria Anopheles Vector Database : An Overview of 100 Years ' Research, *6*(12), 6–7. https://doi.org/10.1371/journal.pone. 0028347

Oliveira, F. Q., Andrade-Neto, V., Krettli, A. U., & Brandao, M. G. L. (2009). New evidences of antimalarial activity of *Bidens pilosa* roots extract correlated with polyacetylene and flavonoids. *Journal of Ethno pharmacology*, **93**(1), 39-42.

Olorundare, E.E., Emudianugbe, T.S., Khaar, G.S., Kuteyi, S.A. and Irobi, D.N. (1992). Antibacterial Properties of Leaf Extract of *Cassia alata*. *Biol. Res. Com.* **4**:113-117.

Omoya, F. O. (2016). The in vivo assessment of antiplasmodial activities of leaves and stem bark extracts of Mangifera indica (linn) and Cola nitida (linn). *International Journal of Infectious Diseases*, *45*, 373. https://doi.org/10.1016/j.ijid.2016.02. 801.

Oshomoh, E.O. and Idu, M. (2012). Antimicrobial activities of ethanol and aqueous crude extracts of *Hymenocardia acida* stem against selected Dental Caries Pathogens. *Pharmacognosy Journal,* **4**(29): 55-60.

Osisanya, J.O.S., Gould, S., Warhurst, D.C. (1981) A simplified culture technique for *Plasmodium falciparum*. *Annuals of Tropical Medicine and Parasitology* **75**:107-109.

Raffi, k. and Mark, S. (2009).Plant poisoning, glycosides-cardiac. Continually Updated Clinical Reference. Retrieved from (http://www.answers.com/topic/glycosides) on 21/3/2010.

Russell, B. M., Udomsangpeth, R., Ricckmann, K. H., Kotecka, B. M., Coleman, R. E., and Sattabongkot, J. (2003). Simple *in vitro* essay for the determining the sensitivity of *Plasmodium vivax* isolate from fresh human blood to anti-malarial in areas where *P. vivax* is endemic. *Anti-malarial agent and chemotherapy,* **47**(1): 170-173.

Saifi, M. A., Beg, T., Harrath, A. H., Altayalan, F. S. H., & Al Quraishy, S. (2013). Antimalarial drugs: Mode of action and status of resistance. *African Journal of Pharmacy and Pharmacology*, **7**(5), 148-156.

Sanne de Ridder, Frank van der Kooy, Robert Verpoorte, (2004). *Artemisia annua* as a self-reliant treatment for malaria in developing countries. *Journal of Ethnopharmacology,* **120:**302-314

Sinka, M. E., Bangs, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway, J., & Hay, S. I. (2010). The dominant Anopheles vectors of human malaria in Africa , Europe and the Middle East : occurrence data , distribution maps and bionomic précis. *Parasites & Vectors*, *3*(1), 117. https://doi.org/10.1186/1756-3305-3-117

Shanks, G., Dennis Daniel, M., Gordon Francis, W., Klotz Gladys, M., Aleman, Aggrey J., Oloo, Deborah Sadie, and Trevor R., Scott. (1998). Efficacy and safety of Atovaquone/Proguanil as suppressive prophylaxis for *Plasmodium falciparum* malaria. *Clinical Infectious Diseases* **27**(3):494–499

Sheeba, M., Emmanuel, S., Revathi K. and Ignacimuthu S. (2009) wound healing activity of *Cassia occidentalis* L in Albino Wistar rats. IJIB 8(1): 1-6.

Shiavone, A., Gup, K. and Tassone, S. (2008). "*Effects of a natural extract of chestnut wood on digestibility, performance traits and nitrogen balance of broiler chicks*". *Poult. Sci.* **87** (3):521 – 7.

Somsak, V., Chachiyo, S., Jaihan, U., & Nakinchat, S. (2015). Protective Effect of Aqueous Crude Extract of Neem (Azadirachta indica) Leaves on Plasmodium berghei -Induced Renal Damage in Mice, *2015*. https://doi.org/10.1155/2015/961205

Sofowora, A. (1989). *Medicinal Plants and Traditional Medicine in Africa* 2nd edition. Spectrum Books Limited, Ibadan, Nigeria. Pp134-156.

Sharma, N., Trikha, P., Athar, M. & Raisuddin, S., (2000). *In vitro* inhibition of carcinogen induced mutagenicity by *Cassia* occidentalis and *Emblica officinalis*. *Drug Chem. Toxicol.* **23**(3): 477-484.

Srinivasa, B. Reddy, T. Sujith, M. Sathish Kumar, A. Narendra Babu, N. Rama Rao, J. Manjunathan, A. (2012). Malarial drugs got resistance. *International Journal of Pharmaceutical and Biomedical Research* **3**(4); 213-215

Steele, B. Reddy, T. Sujith, M. Sathish Kumar, A. Narendra Babu, N. Rama Rao, J. Manjunathan, A. (1999). Malarial drugs got resistance. *International Journal of Pharmaceutical and Biomedical Research* **3**(4); 213-215

Stephen, J.and Duffy, M.D.(2001). "Black tea" Retrieved from(htt://en.wikipedia.org/wiki/black- tea) on 12/2/2010.

Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., De Bruyne, T. & Apers, S. (2004) In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. *J Ethnopharmacol*; **93**:27–32.

Trease, G.E. and Evans, W.C. (1989). *A textbook of Pharmacognocy*, 13th edn. Bailliere-Tyndall Ltd., London.

Trager, W., Jensen J.B, Doherty J. (1979) *Plasmodium falciparum:* continuous cultivation in a semiautomated apparatus. *Exp Parasitol*; **48**: 36-41.

Trager, W., Jensen, J.B. (1976) Human malaria parasites in continuous culture. *Science* **193**, 673-675.

Visser, B. J., Wieten, R. W., Kroon, D., Nagel, I. M., Bélard, S., Vugt, M. Van, & Grobusch, M. P. (2014). Efficacy and safety of artemisinin combination therapy (ACT) for non-falciparum malaria : a systematic review.

Wahyuni, T. S., Ekasari, W., Widyawaruyanti, A., Hirasawa, Y., Morita, H., & Zaini, N. C. (2009). Artopeden A, a new antiplasmodial isoprenylated flavone from *Artocarpus champeden*. *Heterocycles*, **79**(1), 1121-1126.

Weathers, P. J., Jordan, N. J., Lasin, P., & Towler, M. J. (2014). Simulated digestion of dried leaves of Artemisia annua consumed as a treatment (pACT) for malaria. *Journal of Ethnopharmacology*, **151**(2), 858–863. https://doi.org/10.1016/j.jep.2013.11.043

Wells, T. N. (2011). Natural products as starting points for future antimalarial therapies: going back to our roots. *Malaria Journal*, *10* (Suppl 1), S3.

Willcox, S. (2004). Medicinal plants: a source of antiparasitic secondary metabolites. *Molecules*, **17**(11), 1277-1279.

White, N. J. (2008). Qinghaosu (artemisinin): the price of success. *Science*, **320**(5874), 330-334.

Weiss, C.R., Moideen, S.V., Croft, S.L. & Hougton, P.J. (2000). Activity of extracts and isolated Naphthoquinones from *Kigelia pinnata* against *Plasmodium falciparum. J. Nat. Prod.* **63**(9): 1306-1309.

Wells, T. N. (2011). Natural products as starting points for future antimalarial therapies: going back to our roots. *Malaria Journal*, **10** (1): S3.

Wernsdorfer, W. H. & Kouznetsov, R. L. (1980). Drug-resistant malaria--occurrence, control, and surveillance. *Bulletin of the*

Wink, M. (2010). Medicinal plants: a source of antiparasitic secondary metabolites. *Molecules*, **17**(11), 12771-12791.

W. H. O. (2010). Full-text. *European Region, 3*, 2-6.

W. H. O. (2017). Full-text. *European Region, 3*, 6-13.

W. H. O. (2018). Full – text on Malaria Report of children under five globally.

W. H. O. (2019). Full – text on World Malaria Report, 4th December

Wiser. (2011). Major protozoan pathogens infecting humans. *Protozoans and Diseases,* 2, 5-11.

W. H. O. (2020). Malaria Report Full-text. *European Region, 4*, 5-10.

Zwenger, S. & Basu, C. (2008). Plant terpenoids: applications and future potentials. *Biotechnology Molecule Biology Review*, **3**(1), 1-7.