IN VITRO ANTIPLASMODIAL ACTIVITY OF EXTRACTS AND FRACTIONS OF *LUFFA CYLINDRICA*

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Abstract
Malaria is one of the major important health challenges in developing countries and *Luffa cylindrica* is a medicinal plant use in Nigeria folk medicine to treat malaria. This study was embarked upon to investigate the *in vitro* antiplasmodial activity of the extracts (hexane, ethylaceatate, methanol and aqueous) and fractions of *Luffa cylindrica* on *Plasmodium falciparum* strain. Phytochemical screening was carried out on the extracts using standard procedures *P. falciparum* were cultivated and maintained in fresh O^+ human erythrocytes at 4 % haematocrit in complete medium (RPMI 1640 with Albumax II). The ring stage synchronized *P. falciparum* (*Pf3D7*) strain, 1% parasitemia, 2% hematocrit were incubated in a 96-well microplate for 96 h with different concentrations of plant extracts (0.05, 5 and 5 mg/ml) and fractions of the most potent extract (0.01, 0.1 and 1 mg/ml). 0.4% dimethy sulfoxide (DMSO) was used as negative controls; while WR-194,965 was used as positive control. Alkaloids, saponins, phenolics and flavonoids were among the secondary metabolites present in the extracts. The results on antiplasmodial study were obtained by the microtiter plate using SYBR Gold I fluorescence assay and of the four extracts tested, the highest antiplasmodial (IC_{50} of 2.75 ± 0.21 mg/ml) activity was observed with methanolic extract of the leaves of *Luffa cylindrica* and was later subjected to reverse-phase flash chromatography towards antiplasmodial activity guided purification of metabolites and isolation of the active fractions. Neighboring fractions were combined judiciously to ensure optimum purity and 10 pools were obtained. Among the 10 pools evaluated, the most potent fractions against the parasite growth were fractions F7-F10 with an IC_{50} of 2.23 ± 0.34, 2.30 ± 0.45, 2.31 ± 0.64 and 2.20 ± 0.62 mg/ml respectively and was comparable with WR-194,965 (2.31 ± 1.06). Our findings, therefore, confirm the acclaimed use of *L. cylindrica* as antimalarial in folk medicine of Nigeria.

Keywords: antiplasmodial, *Luffa cylindrica*, *Plasmodium falciparum*, chloroquine, artemisinin

Introduction

Malaria is one of the major important health challenges in Nigeria and the globe. About 500 million people are affected by malaria each year; mostly from sub-Saharan Africa and cause about 2.3 million deaths every year (WHO, 2013). The drugs resist *Plasmodium falciparum* cause the most virulent form of malaria in humans and it is declared as a public health disaster causing increased morbidity and mortality. The development and spread of drug resistant strains of the causative agent *P. falciparum* have mitigated the effectiveness of the commercially available anti-malarial drugs like chloroquine and the newly introduced artemisinin (Zofou *et al*., 2011; Inbaneson *et al*., 2012). The use of chloroquine which was the first line of treatment for malaria has been discontinued in some Africa countries like Kenya and Nigeria due to overwhelming presence of *P. falciparum* strains (Dianne *et al*., 2003). This creates the urgent need for new antimalarial drugs. Plants have always been considered to be a possible alternative and rich source of new drugs, and most of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plants-derived compounds as template. *Luffa cylindrica* commonly referred as sponge gourd provides a rich source of structurally diverse secondary metabolites. In Nigeria, it is locally called "Kankan oyibo" by Yoruba tribe, "Ahia mmla" by Igbo tribe, "Ihion by Edo tribe and "Soosoo" by the Hausa tribe. Several studies have demonstrated that *L. cylindrica* contain excellent source of chemical components such as alkaloids, flavonoids, saponins, phenolics, terpenes and tannins that have exhibited different biological activities (Salman *et al*., 2013**,** Sharma *et al*., 2014**,** Etim *et al*., 2018; Saliu *et al*., 2019). Ethno botanical survey also revealed that *L. cylindrica* is used to treat malaria (Partap *et al*., 2012; Azeez *et al*., 2013) by traditional health practitioners but studies to substantiate this claim is lacking. In this vein, the present study was carried out to investigate the *in vitro* antiplasmodial activity of *L. cylindrica* leaves using different solvent.

Materials and Methods

Collection of plant material and authentication

Leaves of *Luffa cylindrica* were collected from Zulle Farms, Suleja, Niger State, Nigeria and authenticated at the National Institute for Pharmaceutical Research and Development, (NIPRD), Abuja, where a voucher specimen (NIPRD/H/6650) was deposited at the herbarium of the institute.

Extract Preparation

Fresh leaves of *L. cylindrica* were washed with water to remove dirt and air-dried to a constant weight for three weeks. Leaves were milled into powder with an electrical blender (Mazeda Mill, MT 4100, Japan). 500 g of the dried leaf powder was extracted sequentially with n-hexane ethylacetate, methanol and distilled water by maceration method. The extracts were filtered using Whatman No 1 filter paper, concentrated in a rotary evaporator (RE-300B model, product of Henan Touch Science, China) and then with a water bath at 45°C. The yield of each extract thus obtained was recorded. Portions of each extract were weighed and diluted in dimethyl sulfoxide (DMSO) to concentrations of 5% w/v. Solutions of each extract were transferred to a 96-well plate where a series of 10-fold dilutions were made into concentrations of, , and 0.5, 5 and 50 mg/ml. The extracts were then stored in a refrigerator at -4^0C till further use.

Reference drugs

WR-194,965 was used as the reference antimalarial drug for positive controls for *P. falciparum (Pf3D7)* strain while 0.4% DMSO was used as negative control.

Phytochemical screening

Phytochemical screening of the each crude extract was carried out adopting the standard procedures and tests of Trease and Evans (1989); Sofowora (1993) for phytochemical analysis to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, phlobatannins and cardiac glycosides.

Fractionation of most active crude extract

The extract that exhibited the most effective antiplasmodial activity was subjected to reverse-phase flash chromatography in Wright laboratory at McMaster University;

Hamilton, Canada. Fractionation of the methanolic extract began with C18 reverse-phase flash chromatography using Teledyne Isco CombiFlash Rf 200 automated chromatography system with column pre-packed with C18-functionalized silica gel. The sample was initially pre-absorbed onto a solid support in order to improve resolution during chromatography in which 4 gram of C18-reverse-phase chromatography was added to a solution of the methanolic extract (1 g wet weight) in methanol (50 ml) and the suspension was concentrated under reduced pressure with a rotary evaporator (bath temperature 28-30ºC). The resulting powder was then transferred to an empty cartridge for chromatography. A portion of the methanolic extract (1g) was subjected to C18 reverse-phase chromatography on a 26 gram C18 column and a gradient of $MeOH/H_2O$ from 10% to 100%. The eluent was collected in approximately 125 test tubes, but tubes were pooled to give 10 fractions (F1-F10) which were concentrated by rotary evaporation followed by Genevac (chamber temperature of 30ºC) in pre-weighed test tubes. Each fraction was re-suspended in DMSO (1.0 ml) to give the investigated concentrations (0.001, 0.01, 0.1 and 1mg/ml) used for the antimalarial testing.

Parasite cultivation

P. falciparum strain (*Pf3D7*) are cultivated *in vitro* in fresh O^+ human erythrocytes at 4% haematocrit in complete medium at 37ºC under reduced O_2 (gas mixture 5% O2, 5% CO2, 90% N_2) and was obtained from the Institute of Infectious Diseases, McMaster University, Canada.

Synchronization of *Plasmodium falciparum* parasite development using sorbitol

The cell culture was centrifuged at 1800 rpm for 5 minutes. After centrifugation, the supernatant was discarded and the pellet retained. Then, 10 pellet volume of 5% sorbitol solution was added. This solution was mixed and kept at 37ºC for 7 minutes. The sorbitol solution containing the cells was taken out of the incubator and centrifuged at 1800 rpm for 5 minutes. The supernatant (sorbitol) was discarded and the synchronized culture was suspended in fresh cRPMI and transferred to the Petri dishes. The parasite culture was incubated at normal cultures conditions (37ºC under reduced O_2). After one hour, a thin blood smear was prepared and stained slides were examined under a microscope at 100 X magnification for the parasites stages identification and parasitemia (Lambros and Vanderberg, 1979).

Antiplasmodial assay

The *in vitro* antiplasmodial activity of *L. cylindrica* extracts and the fractions from the most active crude extract against *Pf3D7*strain was carried out in Tim Gilberger laboratory, Hamburg, Germany using SYBR gold fluorescence assay which is a modified method of SYBR green fluorescence assay of Johnson *et al.* (2007) which measures the malarial growth or inhibition by quantifying DNA with a nucleic acid-binding florescent dye. Synchronized culture at 2% hematocrit and 1% parasitemia was aliquoted with test drugs (plant crude extracts, WR-194,965 at concentrations of 0.05, 5, 50 mg/ml respectively and fractions of active extract (0.001, 0.01, 0.1 and 1 mg/ml) to 96-well flat bottom tissue culture to a final volume of 100 µl. After 96 h of incubation, wells tested were supplemented with equal volumes of lysis buffer (Tris-20 mM, EDTA-5 mM, Saponin-0.008%, Triton-X 100 – 0.08%) containing 1X SYBR Gold I dye. After the addition of lysis buffer, the plates were incubated for another one hour. After this period, the plates were read using 96-well fluorescence plate reader (Victor, Perkin-Elmer), with excitation and emission wavelengths of 485 and 530 nm respectively. In order to validate the SYBR gold data, thin blood smears of treated and untreated wells were prepared and stained (Niharika *et al*., 2015). Negative control (DMSO group) was maintained with fresh red blood cells and 2% parasitized *P. falciparum* diluted with 2% haematocrit while positive control was maintained with parasitized red blood cells treated with WR-194,965. Average percentage suppression of parasitaemia was calculated using the expression:

Average % suppression of parasitaemia =

$$\frac{\text{Average \% parasitaemia in control} - \text{Average \% parasitaemia in test}}{\text{Average \% parasitaemia in control}} \times 100$$

The fluorescence readings were plotted against drug concentration, and fifty percent (50%) inhibitory (IC_{50}) values were determined using excel custom function software.

Statistical analysis
Data were analyzed by analysis of variance (ANOVA) procedures followed by Duncan Multiple Range test and statistical difference were achieved when $P < 0.05$.

Results
The phytochemical screening of the leaf extracts of *Luffa cylindrica* reveals that, the extracts contain variety of secondary metabolites (Table 1). Alkaloids were present in all the extracts (n-hexane, ethylacetate, methanol and aqueous) while cardaic glycosides were present only in the hexane, methanol and aqueous extract. Phenolics and saponins were present in the aqueous and methanolic extracts, flavonoids in methanol and ethylacetate extracts while tannins were present only in the methanolic extracts. Other secondary metabolites screened were phlobatannins, terpenes and anthraquinones but were absent in all the extracts (Table 1). The variation of the mean inhibition rate on the growth of *Pf3D7* strain of *P. falciparum* according to the different concentrations of extracts of *L. cylindrica* leaves is shown in Table 2. The assay indicated that WR-194,965 and the three organic extracts (hexane, ethylacetate and methanol) were all active against the parasite by inhibiting the growth of *Pf3D7* strain of *P. falciparum*. The inhibition of the parasite using a red blood assay by the WR-194,965, hexane, ethylaetate and methanol extracts was dose-dependent (Table 2). The methanol extract appeared to be more potent with IC_{50} value of 2.75 mg/ ml while the ethylacetate extract also showed a promising inhibitory effect of the parasite growth (wit IC_{50} 2.99 mg/ml), followed by the hexane extract (Table 2). Additionally, at 5 and 50 mg/ml investigated, both the methanol and ethylacetate extracts exhibited a significant higher ($p < 0.05$) inhibitory effect against *Pf3D7* strain when compared with the reference antimalarial compound (WR-194,965). The chromatographic trace for the fractionation of the methanolic extract of *L. cylindrica*, showing the colours of each fraction as it came off the column and the tubes that were pooled together into fractions F1-F10 at UV absorbance of 214 and 254 nm is presented in Figure 1. The colours of F1, F4 and F5 were dark yellow, F2 and F3 were light yellow, F6 and F7 were yellow brown, F8 and F9 were brown-green while F10 was green and yellow-green. Table 3 depicts the antiplasmodial activity of the fractions from methanolic crude extract of *L. cylindrica* leaves fractionated by reverse-phase flash chromatography. Fractions F1 and F3-F10 caused a significant reduction ($p < 0.05$) in the parasite growth with over 70% reduction at the highest concentration (1 mg/ml) investigated while fractions F7-F10 caused over 80% significant reduction ($p < 0.05$) in the parasite growth at concentrations 0.1 and 1.0 mg/ml and with IC_{50} values of which was similar to the reference antimalarial compound.

Table 1: Secondary Metabolites of *Luffa cylindrica* Leaf Extracts

Phytochemical	Extracts			
	Aqueous	**Methanol**	**Ethylacetate**	**Hexane**
Saponins	+	+	-	-
Tannins	-	+	-	-
Anthraquinones	-	-	-	-
Terpenes	-	+	+	+
Phenolics	+	+	-	-
Flavonoids	-	+	+	-
Alkaloids	+	+	+	+
Phlobatannins	-	-	-	-
Glycosides	+	+	-	+

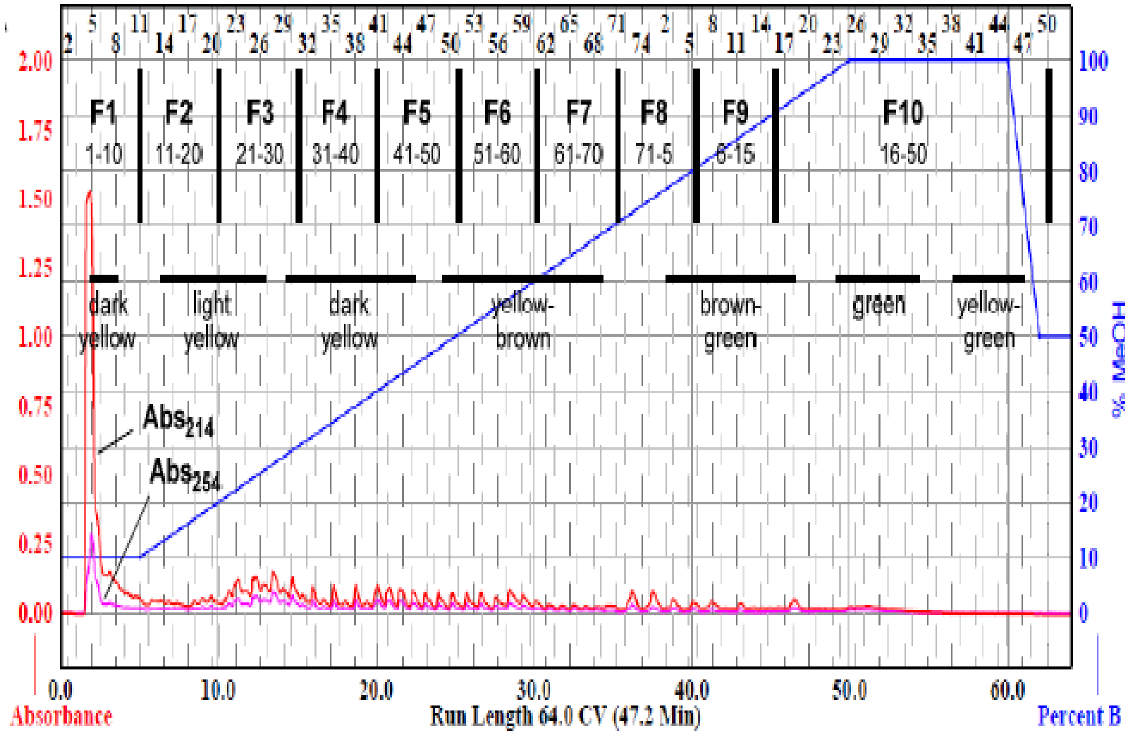
Values are means ± SEM of three replicates

Table 2: IC_{50} values of *Luffa cylindrica* leaf extracts against *Plasmodium falciparum*

Extracts	0.5 mg/ml	5 mg/ml	50 mg/ml	IC_{50} mg/ml
	% Inhibition			
Aqueous	9.05 ± 0.45[a]	13.01 ± 0.17˝	13.01 ± 0.17˝	-
n. Hexane	25.06 ± 0.39˘	29.00 ± 0.16˘	88.00 ± 0.26˘	25.97 ± 0.11˝
Ethylacetate	13.04 ± 0.33˘	79.05 ± 0.23˘	85.02 ± 0.31˘	2.99 ± 0.31˘
Methanol	15.02 ± 0.21˘	86.01 ± 0.21˝	88.97 ± 0.24˝	2.75 ± 0.21˘
DMSO	2.05 ± 0.11˝	2.07 ± 0.11˘	2.06 ± 0.11˝	-
WR-194,965	70.05 ± 0.22˘	75.08 ± 0.12ˈ	77.05 ± 0.10˝	3.45 ± 0.24˝

Values are means of three replicates ± SEM. Values with different alphabet superscript down the column are significantly different at $p < 0.05$.

DMSO – Dimethyl sulfoxide



RediSep Column: C18-26 g
Flow Rate: 35 mL/min

Wavelength 1 (red): 214nm
Wavelength 2 (purple): 214nm

Solvent A: H_2O
Solvent B: MeOH

Figure 1: Chromatographic trace for the fractionation of methanolic extract of *L. cylindrica* by C18 reverse-phase chromatography using the Teledyne CombiFlash Rf 200 system

Table 3: *In vitro* inhibition of parasite growth by fractions of methanolic extract of *luffa cylindrica*

Fractions	0.001 mg/ml	0.01 mg/ml	0.1 mg/ml	1 mg/ml	IC_{50} mg/ml
	% Inhibition				
F1	20.04 ± 0.26[a]	19.00 ± 0.23[a]	11.95 ± 0.42[a]	76.99 ± 0.32[a]	NA
F2	20.05 ± 0.35[a]	13.99 ± 0.42[b]	23.02 ± 0.33[b]	42.99 ± 0.42[b]	NA
F3	20.99 ± 0.22[a]	21.00 ± 0.32[c]	27.99 ± 0.32[c]	85.99 ± 0.32[c]	NA
F4	20.99 ± 0.42[a]	12.95 ± 0.37[b]	26.95 ± 0.36[c]	87.00 ± 0.42[c]	NA
F5	14.025 ± 0.31[b]	22.99 ± 0.42[c]	23.99 ± 0.42[b]	89.01 ± 0.33[c]	2.48 ± 0.21[a]
F6	23.015 ± 0.31[c]	24.99 ± 0.42[d]	49.99 ± 0.42[d]	88.02 ± 0.23[c]	2.41 ± 0.44[a]

F7	20.99 ± 0.42^{a}	29.95 ± 0.22^{e}	88.02 ± 0.32^{e}	88.02 ± 0.33^{c}	2.23 ± 0.34^{a}
F8	26.02 ± 0.33^{d}	45.02 ± 0.44^{f}	89.02 ± 0.41^{e}	80.02 ± 0.34^{a}	2.30 ± 0.45^{a}
F9	23.02 ± 0.23^{c}	39.99 ± 0.42^{g}	85.02 ± 0.31^{f}	81.02 ± 0.33^{a}	2.31 ± 0.64^{a}
F10	24.02 ± 0.33^{c}	51.00 ± 0.43^{h}	89.02 ± 0.32^{e}	83.02 ± 0.41^{d}	2.20 ± 0.62^{a}
DMSO	2.05 ± 0.01^{e}	2.07 ± 0.01^{i}	2.06 ± 0.01^{g}	2.05 ± 0.01^{e}	NA
WR-194,964	21.22 ± 0.42^{a}	42.32 ± 0.33^{f}	85.04 ± 0.21^{e}	78.12 ± 0.35^{a}	2.31 ± 1.06^{a}

Values are means of three replicates ± SEM. Values with different alphabet superscript down the column are significantly different at $p < 0.05$.

DMSO – Dimethyl sulfoxide

Discussion

A number of assays for antimalarial activity have been developed for different stages of the malaria life cycle, but the assay used in this study has been developed for high-throughput screening for compounds that inhibit malarial growth in the human blood stage of the cycle. The lack of DNA and RNA in mature erythrocytes facilitates assaying malarial growth inside red blood cells by quantifying DNA with nucleic acid intercalating dyes. The assay (SYBR Gold) developed in the Gilberger laboratory is an adaptation of the malaria SYBR Green I-based fluorescence (MSF) assay reported by Johnson *et al*. (2007). The assay was modified to involve a 96-hour incubation period, rather than 48-72 hours, and uses SYBR Gold, rather than SYBR green to improve sensitivity. The *in vitro* antiplasmodial activity exhibited by both methanolic and ethylacetate extracts of *L. cylindrica* particularly with the methanolic extract which expressed the most efficacy maybe because it contain more metabolites than others. Anti-plaasmodial activities exhibited by plant extracts have been attributed to the presence of phenolics (Ravikumar *et al*., 2011), cardiac glycosides (Ene *et al*., 2009), alkaloids (Oliveira *et al*., 2009) terpenes (Moon *et al*., 2007), flavonoids and saponins (Ramazani *et al*., 2010) which are the secondary metabolites identified in this study. The mechanism of action might be by the inhibition of *P. falciparum* merozoites invasion into the red blood cells (Adams *et al*., 2005), inhibition of fatty acids biosynthesis (Tasdemir *et al*., 2007), inhibition of hemozoin biocrystallization by the alkaloids (Dubar *et al*., 2011), inhibition of proteins by terpenes (Kirby *et al*., 1989) and inhibition of β–haematin formation (Pabon *et al*., 2009). The significant reduction in *Plasmodium falciparum* growth observed in fractions F7-F10 of the methanolic leaves extract of *L. cylindrica* which also showed IC_{50} values close to the reference anti-malarial compound (WR-194,964) is an indication that these fractions (F7-F10) were more active in the red blood cell assay than other fractions (F1-F6). It is possible that the same class of the bioactive compounds is present in F7-F9 fractions since they show similar IC_{50} values. This therefore will warrant isolating and characterizing the active principle(s) in the F7-F10 fractions and conduct *in vitro* and *in vivo* anti-malarial studies in order to further substantiate anti-malarial claims of *L. cylindrica* as reported in folk medicine practice of Nigeria and as well to propose the possible mechanism of action. This is currently underway in our laboratory and will be provided in due course.

Conflict of interest

We declare there is no conflict of interest.

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References

Adams, Y., Smith, S.L., Schwartz-Albiez, R. & Andrews, K.T. (2005). Carrageenans inhibit the *in vitro* growth of *Plasmodium falciparum* and cytoadhesion to CD36, *Journal of Parasitology Research*, 97; 290-294.

Azeez, M.A., Bello, O.S. & Adewunmi, O.A. (2013). Traditional and medicinal uses of *Luffa cylindrica*: A review. *Journal of Medicinal Plants Studies*, 1(5), 102–111.

Dianne, J.T, Jeanne, M.C., Margarette, S.K., Oren, S.R., Aggrey, J.O., Piet A.K., Altaf, A.L., Bernard, L.N. & Fecko, O.K. (2003). Treatment history and treatment dose are important determinants of sulfadoxine-pyrimethamine efficiency in children with uncomplicated malaria in Western Kenya, *Journal of Infectious Diseases*, 187; 467-468.

Dubar, F., Egan, T.J., Predines, B., Kuter, D., Ncokazi, K.K., Forge, D., et al. (2011). The antimalarial ferroquine: role of the metal and intermolecular hydrogen bond in activity and resistance, *ACS Chemical Bioloy*, 6(3), 275-287

Ene, A.C., Atawodi, S.E., Ameh, D.A., Kwaneshie, H.O. & Agomo, P.U. (2009). *In vitro* antiplasmodial effect of chloroform extracts of *Artemisia macivarae* Linn and *Artemisia meritima* Linn. *African Journal of Biotechnology*, 8(23), 6612-6616.

Etim, E.A., Adebayo, Y.A. & Ifeanyi, O.E. (2018). Effect of *Luffa cylindrica* leaf extract on hematological parameters of Swiss albino mice, *Medicinal and Aromatic Plants* (Los Angelis), 7:318.

Inbaneson, S.J., Ravikumar, S. & Sugenti, P. (2012). *In vitro* anti-plasmodial effect of ethanolic extracts of traditional medicinal plant *Ocimum* species against *Plasmodium falciparum, Asian Pacific Journal of Tropical Medicine*, 103-106.

Johnson, J.D., Dennull, R.A., Gerena, L., Lopez-Sanchez, M., Roncal, N.E. & Waters, N.C. (2007). Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening, *Antimicrobial Agents and Chemotherapy*, 51:1926-1933.

Kirby, G.C., O'Neil, M.J., Philipson, J.D. & Warhurst., D.C. (1989). *In vivo* studies on the mode of action of quassinoids with activity against chloroquine resistant *Plasmodium falciparum*, *Journal of Biochemical Pharmacoloy*, 38, 367-4374.

Lambros, C. & Vanderberg, J.P. (1979). Synchronization of *Plasmodium falciparum* erythrocytic stages in culture, *Journal of Parasitology,* 65, 418-420.

Moon, Hi Jung J.C. & Lee, J. (2007). Anti-plasmodial activity of triterpenoids isolated from whole plants of *Viola* genus from South Korea, *Journal of Parasitology Research*, 100, 641-644.

Niharika, S., Naveen, K.K., Dinesh, M., Santosh, K.T. & Dinkar, S. (2015). Anti-plasmodial activity of medicinal plants from *Chhotanagpur plateau*, Jharkhand, India, *Journal of Ethnopharmacology*, 165, 152–162.

Oliveira, A.B., Dolabela, M.F., Braga, F.C., Jacome, R.L.R.P., Varotti, F.P. & Povoa, M.M. (2009). Plant derived antimalarial agents: new leads and efficient phythomedicines, *Journal of Brazilian Academy of Science*, 81(4), 715-740.

Pabon, A., Deharo, E., Zuluaga, L., Maya, J.D., Saez, J. & Blair, S. (2009). *Plasmodium falciparum*: effect on *Solanum nudum* steroids on thiol contents and beta-hematin formation in parasitized erythrocytes. *Journal of Experimental Parasitology*, 122, 273-279.

Partap, S., Kumar, A., Sharma, N.K & Jha, K.K. (2012) *Luffa cylindrica*: An important medicinal plant. *Journal of Natural Products and Plant Resources*, 2(1), 127-134.

Ramazani, A., Sardari, S., Zakeri, S. & Vaziri, B. (2010). *In vitro* anti-plasmodial and phytochemical study of five *Artemisia* species from Iran and *in vivo* activity of two species, *Journal of Parasitology Research*, 107:593-599.

Ravikumar, S., Jacob, I.S., Suganthi, P., Venkatesen, M. & Ramu, A. (2011). Mangrove plants as a source of lead compounds for the development of new anti-plasmodial drugs from South East coast of India, *Journal of Parasitology Research*, 108:1405-1410.

Saliu, O.A., Akanji, M.A., Idowu, O.A. & Saliu, N.B. (2019). Pharmacological evidence favouring the ethnomedicinal use of *Luffa cylindrica* leaf in the relief of pain and fever. *Direct Research Journal of Health and Pharmacology,* 7(4), 38-46.

Salman, A., Kanwal, W.K. & Syed, W.A. (2013). Analgesic activity of leaves, flowers and fruits peel of *Luffa cylindrica* (L.) Roem, *International Journal of Advanced Pharmaceutical Science,* 4(6), 1401-1408.

Sharma, N.K., Keshari, P., Jha, K., Singh, K. & Shrivastava, A.K. (2014). Hepatoprotective activity of *Luffa cylindrica* (L) M.J. Roem leaf extracts in Paracetamol intoxicated rats, *Indian Journal of Natural products & Resources,* 5(2), 43-148.

Tesdemir, D., Topaloglu, B., Perozzo, R., Brun, R., O'Neill, R., Carballeira, N.M. et al. (2007). Marine natural products from the Turkish sponge *Agelas oroides* that inhibit the enoyl reductase from *Plasmodium falciparum,Mycobacterium tuberculosis, Escherichia coli, Journal of Bioorganic and Medicinal Chemistry,* 15: 6834-6845.

World Health Organization (2013). World Malaria Report: 2010. WHO Press, Geneva, Switzerland, 2013.

Zofou, D., Tene, M., Ngemenya, M.N., Tane, P. & Titanji, VPK. (2011). *In vitro* anti-plasmodial activity and cytotoxicity of extracts of selected medicinal plants used by traditional healers of Western Cameroon, *Malaria Research and Treatments*, 561342, 1-6.