https://dx.doi.org/10.4314/ijs.v26i1.13

In vitro β-HEMATIN INHIBITORY ACTIVITY AND ANTIOXIDANT PROPERTIES OF FOUR SELECTED MEDICINAL PLANTS USED IN THE MANAGEMENT OF MALARIA IN SOUTHWESTERN NIGERIA

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(Received: 13th February, 2023; Accepted: 11th April, 2024)

ABSTRACT

This study extracted crude methanol-soluble phytochemicals from four selected medicinal plant leaves, and determined the antioxidant properties and inhibitory capacity of a malaria biomarker using *in vitro* β-hematin by the four medicinal plant leaves traditionally used for the treatment and management of malaria infection in the South-Western part of Nigeria. These were intended to establish scientific support for the acclaimed ethnomedicinal uses of the leaves of the selected plants for the treatment and management of malaria in South-Western Nigeria. The selected plants were Brimstone (*Morinda lucida,* ML (family Rubiaceae)), De Wild (*Alstonia boonei,* AB (family Apocynaceae)), Lemon grass (*Cymbopogon citratus,* CC (family Poaceae)) and Indian gooseberry (*Phyllanthus amarus,* PA(family Phyllanthaceae)). Each of the powdered plant materials (150 g) was suspended in 1000 ml of 80% methanol for 48 hours at room temperature with constant agitation. The crude extracts obtained were concentrated *in vacuo* at 40 °C. The *in vitro* antioxidant activities and anti-plasmodial activity of the plant leaf was performed using free radicals scavenging and β-hematin inhibitory assay. The order of the antioxidant activities results for the crude extracts showed PA>AB>CC>ML for DPPH radical scavenging activity, CC>ML>PA>AB for metal chelating capacity and CC>AB>ML>PA for hydroxyl radical scavenging property, while the results obtained for ascorbic acid equivalent (AEE) showed CC>PA>ML>AB and CC>AB>ML>PA for both TAC and FRAP, respectively. *Cymbopogon citratus* possessed better antioxidant activity and potency than other crude extracts. The IC_{50} obtained for the β-hematin inhibition for the extracts at 10 mg/mL were; 1.13±0.04, 0.43±0.08, 0.63±0.01, 0.16±0.01 ML, AB, CC and PA respectively while 7.59±0.04 μg/ml was obtained for chloroquine (CQ) standard drug at 1mg/mL. The study concluded that the crude ME obtained for each sample possesses antioxidant properties and inhibition of β-hematin formation which is an important malaria biomarker.

Keywords: Antiplasmodial, Antioxidant, β-hematin, Southwestern Nigeria.

INTRODUCTION

The disease of chordates caused by a protozoan of *the Plasmodium* genus is called malaria (Saleh *et al.*, 2019). It remains a major menace affecting public health, mostly in the tropical regions of the world (Elahe, *et al.*, 2021). About half of the global population suffers from malaria infection. World Health Organization (WHO) malaria report of 2019 indicated that 229 million suffered from malaria infection with an estimated 405,000 deaths (WHO, 2020). Among all the countries infected by malaria globally, sub-Saharan African region countries take 51% in which Nigeria alone accounted for 27% in the region. Malaria is among the deadly infections of the tropical and sub-tropical regions that have negatively impacted the poor (WHO 2019, Adebayo and Kretlli, 2011). Malaria is reported to be destructive and a fatal infectious disease affecting major countries under development around the Globe (Areola, *et al.*, 2016).

Africa continent suffers the greatest effects of this disease, putting children and pregnant women at high risk of death most especially, children under 5 years (WHO, 2020). Sub-Saharan Africa accounted for over 95% of the cases worldwide. Children and Pregnant women were said to be the most prone population for malaria, with over two-thirds of malaria mortality in children under the age of 5 years (WHO, 2019).

The resistance of the malaria parasite to many drugs used as antimalaria has put more challenges

to prevention and management of the illness. In the ethnomedicinal practices, many plants were involved in the management and treatment of malaria. The improvement in traditional botanical knowledge serves as an important promising tool of plant usefulness for human and animal medicine bio-prospecting instruments (Adekola *et al.*, 2021). Africa's continent has been endowed with an abundance of plant resources that have been used not only as dietetics but also as potent herbs used ethnobotanically for ages (Iwu, 1993). The use of plants in combating malaria is a common practice among rural dwellers and some in the urban settlements especially, in the Southwestern part of Nigeria (Figure 1). The study plants are *Alstonia boonei* (Apocycnaceae), *Morinda lucida* (Rubiaceae), *Cymbopogon citrates* (Poaceae), and *Phallanthus amarus* (Phyllanthacea)

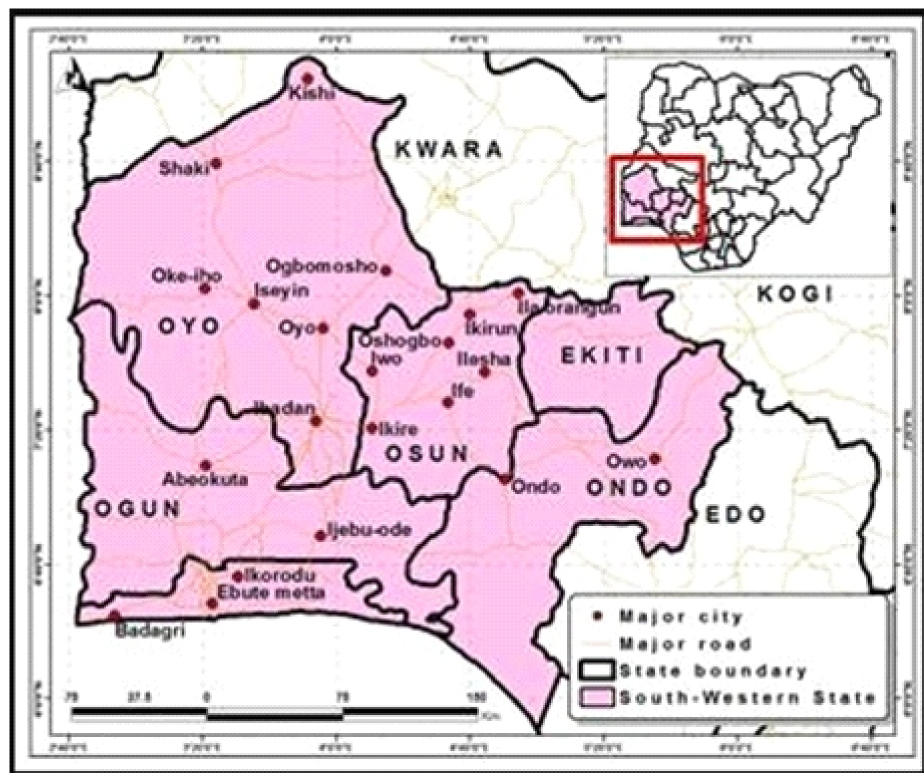

Figure 1: Map of Southwestern Nigeria (Researchgate)

Alstonia boonei (De – Wild)

Alstonia comprises about 40 species. *Alstonia boonei* De Wild (Figure 2), belongs to the family Apocynaceae. Two of the species are indigenous to Africa while others are around the world (Adotey, *et al.*, 2012). *Alstonia boonei* is known as Ahun in Yoruba, Egbu-ora in Igbo, Ukhu in Edo and Ukpukunu in Urhobo, it is abundantly present in the rain-forest and lowland areas of Nigeria (Adebayo and Krettli, 2011). In some other parts of the world, *Alstonia* is called Australian fever bush, Australian quinine, Devil tree, Dita bark, fever bark or palimara (Goose, *et al.*, 1999). West and Central African countries commonly use the root bark in combination with other herbs for arthritis management (Kweifio-Okai, 1991a&b; Obiagwu, *et al.*, 2014), the bark of the stem has also been reported to have the antioxidant property (Akinmoladun, *et al.*, 2007; Obiagwu, *et al.*, 2014). The bark of plant stems or leaves is taken orally as decoction or "teas" and also as a "steam therapy" ingredient for malaria (Adebayo and Krettli, 2011). Tablet of the stem bark has been produced as an antimalarial remedy (Majekodunmi, *et al.*, 2008; Chime, *et al.*, 2013). Previous phytochemical screenings reported that *Alstonia boonei* leaf having a very good promising antiplasmodial activity (Enemakwu, *et al.*, 2015), the stem bark phytochemical analysis exposed the presence of alkaloids, saponins, tannins, steroids, flavonoids and cardiac glycosides in substantial quantities (Chime, *et al.*, 2013). Several chemical compounds have been isolated from *A. boonei* amongst which include; alkaloids, tannins, iridoids

and triterpenoids (Goose, *et al.*, 1999; Adotey, *et al.*, 2012).

Figure 2: *Alstonia boonei* Plant

Morinda lucida (Brimstone)

Brimstone which is botanically called *Morinda lucida* Benth (Figure 3), is a plant of tropical rainforest belonging to the family Rubiaceae (Adeyemi, *et al.*, 2014), it is referred to as a nutrient factory due to its richness in vitamins A and E, the two prevailing antioxidants, that could be useful in degenerative diseases like atherosclerosis management (Adeleye *et al.*, 2018). In South-Western Nigeria, *Morinda lucida* is one of the medicinal plants used in the treatment of malaria. It's a tree of 9–18 m in height, with a dense crown of slender crooked branches (Adebayo and Krettli, 2011). It has moderately coarse wood, is medium weight and fairly hard. Parts of this plant like the stem bark, aerial parts or root bark are commonly used in most West African countries for antimalarial and other tropical diseases (Adebayo and Krettli, 2011). Its antimalarial activity has been reported to be affected by seasonal variation (Adebayo and Krettli, 2011). Different countries and tribes called it different names. It is known as Brimstone in English; Huka or Eze-ogu amid the Igbo tribe of Southeast Nigeria, Oruwo amongst Yoruba tribe in South-western Nigeria; Sangogo in Cote d' Ivoire; Twi, Kon kroma in Ghana and Ewe amake or atakake in Togo (Adeneye, 2013; Adeyemi, *et al.*, 2014). The filtrate of the macerated fresh leaves of the plant in fresh palm wine is used in South-West Nigeria orally for the control of blood sugar in diabetic suspected patients (Adeyemi *et al.*, 2014), the "oral teas" produced from the leaves usually taken orally has been reported to possess analgesic, laxative and anti-infections, anti-cancer, antispermatogenic and for malaria properties. The presence of steroids in *Morinda lucida* has been reported which makes it an important herb against cerebral malaria and an effective antiplasmodial agent. Up to 10 phytochemicals are reported to contain in the *Morinda lucida* among which include anthraquinones, alkaloids, tannins, flavonoids, triterpenoids, glucosides and saponins (Abu, *et al.*, 2016; Adeleye, *et al,* 2018).

Figure 3: *Morinda lucida* Plant

Cymbopogon citratus (Lemon Grass)

Cymbopogon citratus (figure 4), also known as Lemon grass and *Koko-oba* in most South-Western States of Nigeria is a tall perennial grass of India native, mostly planted in tropical and subtropical countries (Cheel, *et al.*, 2005; Figueirinha, *et al.*, 2008; Nambiar, *et al.*, 2012; Sangodele, *et al.*, 2014). *Cymbopogon citratus* is a monocotyledonous aromatic grass that belongs to the Poaceae family with rhizomes and densely tufted fibrous roots (Nambiar, *et al.*, 2012; Sangodele, *et al.*, 2014). It is made of short underground stems with ringed segments, coarse, slightly leathery slender sharp edges and pointed apex green leaves in dense clusters (Carlin, *et al.*, 1986; Ernst, 2008; Nambiar, *et al.*, 2012; Sangodele, *et al.*, 2014). Among other things, lemon grass (LG) is used in different parts of the world as folk medicine, as a food ingredient,

as folk medicines and cosmetic additive (Nambiar, *et al.*, 2012). In Asia LG is widely used in cuisines and sedatives; in India, it is used as febrifuge and immuno-stimulant while in Nigeria, it is used for stomach problems and typhoid (Brian, *et al.*, 2002; Aibinu, *et al.*, 2007; Sangodele, *et al.*, 2014).

Figure 4: *Cymbopogon citratus* Plant

Phyllanthus amarus (Indian gooseberry)

Phyllanthus amarus (Figure 5) Schum. Thonn. (Euphorbiaceae) is an annual herb grows between 6-15 inches in height. Its stem is angular with numerous distichous, elliptic-oblong leaves. Flowers are yellow and numerous. The shape of the fruits is capsule, globose, smooth and very small, it is indigenous to the Amazon Basin. 'Phyllanthus' as a name means "leaf and flower" (Patel *et al.*, 2011). Nearly 800 species of the genus Phallathus are distributed globally throughout the equatorial regions, the most notable species among them is *Phyllanthus amarus* due to its widely reported pharmacological activities (Joseph and Raj, 2011; Zubair *et al.* 2017). The therapeutic effects have been acknowledged as an anti-diabetic, and anti-cholesterol properties, anti-cancerous and cellular protective actions, liver protective and detoxification actions, antiviral actions, antispasmodic, pain relieving anti-inflammatory activity and normalising elevated urinary calcium levels in calcium stone forming patients (Ott *et al.*, 1997; Islam *et al.*, 2008; Bunalema *et al.*, 2014). Furthermore, extracts of PA possess antiparasitic, antibacterial and antimicrobial activity. It is also used for its wound healing properties (Islam*, et al.*, 2008). It possesses antiviral, anti-parasitic, antimalarial, antimicrobial, anti-cancer, anti-diabetic and anti-cholesterol agents (Thamlikitkul *et al.*, 1991; Ott *et al.*, 1997, Patel *et al.*, 2011). The protective and detoxification activities on organs such as the liver and kidney have been reported, it also possesses wound-healing and cellular protection properties (Islam, *et al.*, 2008, Patel *et al.*, 2011).

Figure 5: *Phyllanthus amarus* Plant

MATERIALS AND METHODOLOGY

MATERIALS

Chemicals: Chloroquine sulphate, Dimethyl sulfoxide (DMSO), $Fecl_3$, and Metahnol (Sigma-Aldrich,,Germany), Hemin Chloride (Sigma-Aldrich, USA), Acetic acid (PENTA Czech Republic), Sodium acetate, Sodium hydroxide (BDH, London), diphenyl-1-picrylhydrazil-hydrate (DPPH), Distilled water, etc. All chemicals used are of analytical grade.

All the plant leaves used were obtained within Obafemi Awolowo University, Ile-Ife Campus

METHODS

Pulverization and Extraction

Freshly obtained leaves of each plant sample were identified at the IFE-HERBARIUM of the Botany department, Obafemi Awolowo University. The plants were thoroughly rinsed with water and dried for three weeks under shade, then pulverized using kitchen blender.

The powdered sample was sieved, (150 g) was macerated in 1 liter of 80% methanol / 20% distilled water (v/v) for 48 hours, filtered using Whatman 1 filter paper and the filtrate was concentrated using a rotary evaporator at 40 °C *in*

vacuo under pressure to obtain the crude extracts. The crude extracts obtained were kept in the refrigerator at a low temperature (4 °C) for preservation toward preparation for the study.

EVALUATION OF ANTIOXIDANT PROPERTIES OF THE EXTRACTS

DPPH Radical Scavenging Activity

Procedure for DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) radical scavenging activity as reported by Baliyan, *et al* 2022, was adopted with slight modification. One millilitre of different concentrations of each extract was added to 1000 µl of 0.3 mM DPPH in methanol. The mixtures were mixed thoroughly and incubated at room temperature in the dark cupboard and the absorbance was read after 30 minutes at 517 nm against the control which contained 1 ml methanol in 1000 µl of 0.3 mM DPPH in methanol. The percentage of DPPH inhibition was calculated using the formula:

$$\% \text{ Scavenging activity} = \frac{\text{Abs (control)} - \text{Abs (Sample)}}{\text{Abs (control)}} \times 100$$

The concentration at 50 % inhibition (IC_{50}) was calculated from a linear regression plot of percentage inhibition against the concentration of extracts

Ferric Reducing Antioxidant Power (FRAP) Properties

The spectrophotometry procedure explained by Adekola *et al.,* 2022, was adopted for the FRAP assay. The basis of this assay is the reduction of a colourless ferric-tripyridyltriazine complex (10 m mM TPTZ, acetate buffer (300 mM pH 3.6) and 20mM $FeCl_3$) FRAP reagent, to its blue ferrous coloured form because of the effect of the donated electron from the antioxidant materials present.

An Aliquot of 1 mg/ml hydroxyl-methanolic extracts (HME) (50 µl) was separately added to FRAP reagent (1.5 ml), while distilled water was used instead of sample as control, mixed and stand for 10 minutes at room temperature without direct sunlight ray. Following the same procedure, different concentration of ascorbic acid was prepared and used to obtained standard linear calibration curve, the absorbance was measured at 593nm. The ferric reducing power of each plant sample was calculated and expressed as Ascorbic acid Equivalent (AAE) / mg

Evaluation of Total Antioxidant Capacity

The antioxidant capacity for each crude methanolic extract was performed adopting the method of Adekola *et al.,* (2022). The principle of the method is based on the capacity of the extract to reduce Molybdate (VI) to Molybdate (V) by production of a green phosphate / Molybdate (V) complex at an acidic pH. The assay mixture typically consists of 100 µl of extract aliquot, and 1000 µl of the TAC reagentz solution made up of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Each tube used for the assay was capped and incubated in a water bath at 95 °C for 90 minutes and then allowed to cool to room temperature. At 630 nm the absorbance was taken against a reagent blank which contained 1000 µl of reagent solution with 100 µl volume of methanol. The results obtained were recorded as Ascorbic acid equivalents (AAE) / mg.

Hydroxyl Radical Scavenging Activity

The assay was determined by adopting the procedure of Halliwell *et al.* (1987; 1989) as described by Rahman *et al.* (2015). The Fe^{3+}-ascorbate –EDTA-H_2O_2 system (Fenton reaction) generates the hydroxyl radicals (Rahman *et al.*, 2015). The principle is based on the quantification of the degradation deoxyribose product, which produces a pink colouration upon heating with Thiobarbituric Acid (TBA) at low pH. 1 ml of the iron-EDTA solution (0.1 mM EDTA, 3.0 mM deoxyribose, 0.1 mM $FeCl_3.6H_2O$, 2 mM H_2O_2, 0.1 mM Ascorbic acid in 10 mM phosphate buffer, pH 7.4) was added to the extracts at different concentrations making the the reaction mixture. The mixtures were incubated at 37 °C of human physiological temperature for 1 hour in the water bath, 1.0 ml of 1 % (w/v) TBA in 0.25 N HCl, and 1.0 ml of 10 % TCA was then added. The mixtures were heated for 20 minutes in boiling water at 100 °C and cooled with water. The absorbance at 532 nm of the molybdate-TBA pink colour formed was measured against the reagent blank. The percentage inhibition of 2-deoxy-D-ribose oxidation was determined to evaluate the hydroxyl radical scavenging capacity of the extracts. The percentage of hydroxyl radical scavenging activity

was calculated using the formula:

Percentage Hydroxyl Radical Scavenging activity $= A_0 - A_1 / A_0 X 100$
Where: A_0 = Absorbance of the control and A_1 = Absorbance of Sample
The percentage of inhibition was then plotted against the concentration of the extract from which the IC_{50} was calculated. Each concentration was repeated three times

Determination of Metal Chelating Capacity
Metal chelating capacity was determined by the method described by Singh and Rajini, (2004). Chelating Fe^{2+}- ferrozine complex reagent (2 mM $FeCl_2.4H_2O$ and 5 mM of ferrozine) was used.
$FeCl_2.4H_2O$ (1 ml) was mixed into an equal volume of various concentrations of the extracts. After incubation at room temperature for 5 minutes, 1 ml of ferrozine was added and vigorously mixed to initiate the reaction and further incubated for 10 minutes at room temperature after which the absorbance of the mixture was measured spectrophotometrically at 562 nm. EDTA was used as a positive control. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula:
% Inhibition $= (A_{control} - A_{sample}) / A_{control} \times 100$
(Where $A_{control}$ = absorbance of the control sample (contains $FeCl_2$ and ferrozine, complex formation molecule) and A_{sample} = absorbance of extracts)

IN VITRO INHIBITION OF Β-HEMATIN SYNTHESIS

The method of Rodrigues *et al*., (2011) to determine the ability of the methanolic crude extracts of the leaves of the selected medicinal plants to inhibit the β-hematin formation was carried out *in vitro* using chloroquine diphosphate as standard drug. Solution of varied concentrations of both standard and extracts were prepared separately. Chloroquine standard was dissolved in distilled water (1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/ml) and each was separately dissolved in 80% methanol v/v (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/ml). Each concentration was added to 50µl of DMSO in different sterilized Eppendorf tubes (in triplicate) where 50 µl of 0.5 mg/ml hemin chloride was added. Distilled water was used as a negative control. The synthesis of β-hematin was initiated by adding 250 µl of 0.2M acetate buffer pH 4.4 to the hemin chloride in the tube and the reaction mixture was incubated at 37 °C for 48 hours. The reaction mixtures were centrifuged at 4,000 rpm for 15 minutes to obtain the Hemozoin pellet while the supernatant was discarded. The pellet obtained was washed twice with 200 µl DMSO to remove unreacted hemin chloride. The final washed pellet was dissolved by adding 200 µl of 0.2N sodium Hydroxide (NaOH) and further diluted with 400 µl 0.1N NaOH. The absorbance was read at 405 nm and the results of the Mean value of the Standard deviation obtained was expressed as a percentage of inhibition of β-hematin synthesis thus;
$I (\%) = (A_o - A_i) / A_o \times 100$
Where I (%) = Percentage Inhibition, A_o = Absorbance of the Control (reaction mixture without extract or standard) and A_i = Absorbance of the extract or standard

RESULTS AND DISCUSSION

Extraction Yields of the Samples
The extraction of the plant leaves samples in 1000 ml of 80 % (v/v) Methanol/water yielded 10.41 g, 17.86 g, 9.17 g, and 6.96 g from 150g of the powdered leave samples of *Alstonia boonei, Morinda lucida, Phyllanthus amarus* and *Cymbopogon citratus* representing 6.94 %, 11.9 %, 6.11 % and 4.64 % respectively (Table 1). All data obtained was analyzed using Mean value and regression method.

Table 1: % Yields of samples crude extracts of *A. boonei, M. lucida, C. citratus* and *P. amarus*

Plant Name (Part Used)		Percentage Yield (%) (g)
Alstonia boonei	(Leaves)	6.94
Morinda lucida	(Leaves)	11.90
Cymbopogon citrates	(Leaves)	4.64
Phyllanthus amarus	(Leaves)	6.11

IN VITRO ANTIOXIDANT ASSAY

DPPH Radical Scavenging Activity

The reaction of DPPH with an antioxidant compound that can donate hydrogen is reduced according to the equation below;

$DPPH + RH \rightarrow DPPH_2 + R$

The DPPH assay gives information about the reactivity of the assay compound with stable free radicals. DPPH assay is used to investigate the ability of the extract to scavenge free radicals. The conversion of the deep violet colour to light yellow was measured spectrophotometrically at 517 nm. The rate at which the deep violet/purple DPPH solution decolorates was equivalent to the ability of the extract to donate electrons or protons to DPPH radicals. At varied concentrations, all the methanolic crude extracts exhibited DPPH radical scavenging activities. The IC_{50} for methanol extracts of *Alstonia boonei* (AB), *Morinda lucida* (ML), *Cymbopogon citratus* (CC) and *Phyllanthus amarus* (PA) values were calculated with that of Ascorbic acid (AA) as follows; 1.13 ± 0.04, 0.43 ± 0.008, 0.16 ± 0.01, 0.63 ± 0.01 and 7.59 ± 0.04 respectively (Table 2) indicating PA>AB>CC>ML. The IC_{50} of the activity was generated from the percentage of inhibition using the formula below;

$I(\%) = (A_o - A_i) / A_o \times 100$

Where, I (%) = Percentage Inhibition, A_o = Absorbance of the control (reaction mixture without extract) and A_i = Absorbance of the reaction mixture with extracts

Table 2: DPPH Scavenging property of Methanol-soluble crude Extract of the Extracts

Extracts					Ascorbic Acid	
Conc. (mg/ml)	ML	AB	PA	CC	Conc. (μg/ml)	AA
0.03125	14.24 ± 0.06	16.45 ± 1.49	45.77 ± 1.55	19.76 ± 0.06	0.625	5.31 ± 0.12
0.0625	15.35 ± 0.06	33.91 ± 3.05	54.59 ± 2.71	21.04 ±0.32	1.25	6.11 ± 0.12
0.125	17.83 ± 0.91	54.41 ± 1.03	60.11 ± 0.90	26.28 ± 2.20	2.5	14.45 ± 0.60
0.25	23.89 ± 0.12	56.80 ± 1.16	62.68 ± 0.25	33.73 ± 0.06	5	33.37 ± 0.48
0.5	28.58 ± 0.84	60.20 ± 0.19	63.51 ± 0.32	41.63 ± 0.45	10	66.11 ± 0.12
IC_{50} (mg/ml)	1.13 ± 0.04	0.43 ± 0.008	0.16 ± 0.01	0.63 ± 0.01	IC_{50} (μg/ml)	7.59 ± 0.04

Each of the values is the mean ± S.E.M of three readings (n=3),
(ML = *Morinda lucida*, AB = *Alstonia boonei*, CC = *Cymbopogon citratus*, PA = *Phyllanthus amarus* and AA = Ascorbic Acid)

Ferric Reducing Antioxidant Power (FRAP)

The antioxidant reduction power of the crude methanolic extract of the plant samples was measured and evaluated by the reduction of Fe^{3+} to Fe^{2+} in this order; ML, AB, PA and CC. The values obtained were 192.84 ± 0.56, 203.98 ± 7.69, 68.70 ± 6.56 and 237.93 ± 0.19 mg/g AAE (Ascorbic Acid Equivalent) respectively. The *Cymbopogon citratus* (CC) has the highest activity as follows; CC>AB>ML>PA (Table 3)

Total Antioxidant Capacity (TAC)

The change in the total antioxidant capacity assay of the methanolic crude extracts reveals a concentration-dependency result. The values obtained for *Morinda lucida* (ML), *Alstonia boonei* (AB), *Phyllanthus amarus* (PA) and *Cymbopogon*

citratus (CC) were 25.84 ± 1.27, 21.62± 2.68, 28.62 ± 3.67 and 30.62 ± 2.41 mg/g AAE (Ascorbic Acid Equivalent) respectively. Among the extracts, *Cymbopogon citratus* (CC) also displayed the highest total antioxidant value CC>PA>ML>AB (Table 3).

Table 3: Total Antioxidant Capacity (TAC) and Ferric Reducing Antioxidant Power (FRAP)

Plant Crude Methanolic Extracts	Total Antioxidant Capacity (TAC) (AAE mg/g) ± SEM	FRAP (AAE mg/g) ± SEM
Morinda lucida (ML)	25.84 ± 1.27	192.84 ± 0.56
Alstonia boonei (AB)	21.62 ± 2.68	203.98 ± 7.69
Phyllanthus amarus (PA)	28.62 ± 3.67	68.70 ± 6.56
Cymbopogon citratus (CC)	30.62 ± 2.41	237.93 ± 0.19

Each of the value represented the mean ± S.E.M of three readings (n=3)

Metal-Chelating Capacity

One of the antioxidant properties of medicinal plants is the metal-chelating capacity activity. Metal ion chelating property is the antioxidant reducing capacity of concentration of the catalyzing transition metal in Lipid peroxidation (LPO) (Meera and Sivakumar, 2019). All the methanolic crude extracts exhibit metal chelating activity at different concentrations (Table 4). Ferrous and ferrozine complex formation indicated the chelating activity. The IC_{50} for methanol extracts of *Alstonia boonei* (AB), *Morinda lucida* (ML), *Cymbopogon citratus* (CC), *Phyllanthus amarus* (PA) and EDTA values were calculated as follows; 1.36 ± 0.09, 0.55 ± 0.04, 0.25 ± 0.01, 1.07 ± 0.01 and 0.12 ± 0.003 µg/ml. *A. boonei* has the highest metal ion chelating capacity. EDTA>CC>ML>PA>AB (Table 4.4). The IC_{50} of the activity was generated from the percentage of inhibition using the formula below;

$I (\%) = (A_o - A_i) / A_o \times 100$

Where, I (%) = Percentage Inhibition, A_o = Absorbance of the control (reaction mixture without extract) and A_i = Absorbance of the reaction mixture with extracts

Table 4: Metal Chelating Capacity of Methanol-soluble crude Extract of the Extracts

Extracts					EDTA	
Conc. (mg/ml)	ML	AB	PA	CC	Control (µg/ml)	% EDTA
0.03125	21.38 ± 0.29	6.73 ± 0.54	13.65 ± 1.27	35.73 ± 0.64	0.02	8.30 ± 0.82
0.0625	25.59 ± 0.67	12.47 ± 1.13	16.67 ± 0.21	41.66 ± 0.78	0.04	22.50 ± 1.54
0.125	31.94 ± 0.08	19.24 ± 1.97	19.89 ± 0.97	44.22 ± 0.54	0.06	27.10 ± 4.52
0.25	35.11 ± 0.37	21.92 ± 0.40	23.45 ± 0.24	47.32 ± 0.73	0.08	33.90 ± 1.54
0.5	45.60 ± 2.70	25.55 ± 3.02	30.33 ± 0.35	65.79 ± 0.91	0.10	39.40 ± 0.82
IC_{50} (mg/ml)	0.55 ± 0.04	1.36 ± 0.09	1.07 ± 0.01	0.25 ± 0.01	IC_{50}	0.12 ± 0.003

Each of the values stands for the mean ± S.E.M of three readings (n=3)
(ML = *Morinda lucida*, AB = *Alstonia boonei*, CC = *Cymbopogon citratus*, PA = *Phyllanthus amarus* and EDTA = Ethyleneditetramin)

Hydroxyl Radical Scavenging Activities Determination

Another antioxidant property of a medicinal plant is its ability to scavenge hydroxyl radicals (Guchu *et al*., 2020). All the tested methanolic crude extracts demonstrated significant hydroxyl radical scavenging activities. The IC_{50} for methanol extracts of *Alstonia boonei* (AB), *Morinda lucida* (ML), *Cymbopogon citratus* (CC) and *Phyllanthus amarus* (PA) values obtained were as follows, 0.43±0.008, 1.13±0.04, 0.63±0.01 and 0.16±0.01 mg/ml with activity order of PA>AB>CC>ML (Table 5). PA demonstrated the highest hydroxyl radical scavenging power.

The IC_{50} of the activity was generated from the percentage of inhibition using the formula below;
$I (\%) = (A_o - A_i) / A_o \times 100$
Where, I (%) = Percentage Inhibition, A_o = Absorbance of the control (reaction mixture without extract) and A_i = Absorbance of the reaction mixture with extracts

Table 5: Hydroxyl Radical Scavenging Activity of Methanol-soluble crude Extract of the Extracts

Extracts					BHT	
Conc. (mg/ml)	ML	AB	PA	CC	Conc. (µg/ml)	BHT
0.03125	14.24 ± 0.06	16.45 ± 1.49	45.77 ± 1.55	19.76 ± 0.06	0.00625	5.31 ± 0.12
0.0625	15.35 ± 0.06	33.91 ± 3.05	54.59 ± 2.71	21.04 ±0.32	0.0125	6.11 ± o.12
0.125	17.83 ± 0.91	54.41 ± 1.03	60.11 ± 0.90	26.28 ± 2.20	0.025	14.45 ± 0.60
0.25	23.89 ± 0.12	56.80 ± 1.16	62.68 ± 0.25	33.73 ± 0.06	0.05	33.37 ± 0.48
0.5	28.58 ± 0.84	60.20 ± 0.19	63.51 ± 0.32	41.63 ± 0.45	0.1	66.11 ± 0.12
IC_{50} (mg/ml)	1.13 ± 0.04	0.43 ± 0.008	0.16 ± 0.01	0.63 ± 0.01	IC_{50} (µg/ml)	7.59 ± 0.04

Each of the values stands for the mean ± S.E.M of three readings (n=3)
(ML = *Morinda lucida*, AB = *Alstonia boonei*, CC = *Cymbopogon citratus*, PA = *Phyllanthus amarus* and BHT = Butylated Hydroxyl Toluene)

***In Vitro* Antiplasmodial Effect of the Selected Plant Extracts of *A. boonei, M. lucida, C. citratus* and *P. amarus* using β-hematin Inhibitory Formation**

β-hematin formation inhibitory properties results by the plant extracts were reported in Table 6, using Chloroquine (CQ) as a standard drug.

Table 6: Effect of the selected plant extracts on β-hematin formation

In vitro % Inhibition of β-hematin Results for the Plant Leave Crude Extracts					Chloroquine	
Conc (mg/ml)	ML	AB	CC	PA	Conc (mg/ml)	% Inhibition
10	41.75±2.92	34.06±3.97	21.17±5.64	50.23±3.97	1	73.10±0.12
5	29.95±3.23	24.84±4.60	16.98±6.75	46.53±5.01	0.5	69.69±0.25
2.5	9.30±3.23	18.15±3.76	14.63±3.83	32.35±3.83	0.25	66.78±0.12
1.25	4.35±2.70	13.83±3.07	10.33±4.42	25.82±4.42	0.125	61.11±1.26
0.625	3.87±2.91	10.35±8.65	8.77±3.83	16.66±3.83	0.0625	51.76±1.76
IC_{50}	11.27±2.91	15.64±3.12	27.55±8.65	8.43±3.83	IC_{50}	0.076±0.04

(ML = *Morinda lucida*, AB = *Alstonia booni*, CC = *Cymbopogon citratus*, PA = *Phyllanthus amarus* and CQ = Chloroquine)

DISCUSSION

This study aimed to determine the "Antiplasmodial and Antioxidant" activities of methanolic extracts of four plant leaves namely; *Morinda lucida* (ML)*, Alstonia boonei* (AB)*, Cymbopogon citratus* (CC) and *Phyllanthus amarus* (PA), using *in-vitro* method and also to establish relationship between antiplasmodial and antioxidant activities of each plant with scientific evidence for their ethnomedicinal usage in the treatment and management of malaria.

The results of the DPPH assay reveals the plant extracts scavenged DPPH free radicals in a dose-dependent manner as presented in Table 2, IC_{50} values of *Morinda lucida* (ML), *Alstonia boonei* (AB), *Cymbopogon citratus* (CC), *Phyllanthus amarus* (PA) and Ascorbic acid (AA) (standard drug) are 1.13±0.04, 0.43±0.008, 0.63±0.01, 0.16±0.01 mg/ml and 7.59±0.04µg/ml respectively. PA

extract demonstrated greater activity with IC_{50} of 0.16±0.01. the order of the DPPH radical scavenging activity is PA>AB>CC>ML which is in agreement with Timothy *et al.,* (2014)

The ability of a compound's antioxidant reduction against oxidative effects of reactive oxygen/nitrogen species is linked to the electron transfer activity of the compound which translates to its potential antioxidant power as demonstrated by the crude extracts in both the TAC and FRAP results (Table 3). (Dastmalchi *et al.*, 2007; Riaz *et al.*, 2011; Adekola *et al.*, 2021). The TAC values range from 30.62±2.41, 28.62±3.67, 25.84±1.27 to 21.62±2.68 AAE / mg respectively for CC>PA>ML>AB while FRAP values obtained were 237.93±0.19, 203.98±7.69, 192±0.56 and 68.70±6.59 for CC>AB>ML>PA. For both assays, CC possessed the highest antioxidant value of 30.62±2.41 and 237.93±0.19 for TAC and FRAP respectively.

The result of Metal Chelating Capacity (MCC) assay reveals *Cymbopogon citratus* possessing more inhibition of ferrozine-Fe^{2+} complex formation when compare with other extracts obtained as presented in Table 4 using EDTA as the standard. The order of the metal chelating activities for the extracts were CC>ML>PA>AB with IC_{50} values of 0.25±0.01, 0.55±0.04, 1.07±0.01 and 1.36±0.09 mg/ml respectively while that of EDTA is 0.12±0.003 mg/ml.

The hydroxyl radicals scavenging ability of the extracts were presented in Table 4.5 with IC_{50} values obtained as follows, 0.43±0.008, 1.13±0.04, 0.63±0.01 and 0.16±0.01 mg/ml (Table 5) for *Alstonia boonei* (AB)*, Morinda lucida* (ML)*, Cymbopogon citratus* (CC) and *Phyllanthus amarus* (PA) respectively. The activity order is PA>AB>CC>ML with PA possessing the highest hydroxyl radical scavenging power.

The antiplasmodial activities results obtained from the *in vitro* β-hematin inhibitory process showed that all the four methanol-soluble crude leaf extracts show appreciable inhibition of β-hematin formation in comparison with the chloroquine standard drug (Table 6). The results obtained also fall in line with the report of Abiodun *et al.,* (2018).

CONCLUSION

From the antioxidant activity for the crude extracts, *C. citratus* and *P. amarus* possessed greater free radical scavenging activity.

This study revealed that methanol-soluble crude extract of *Morinda lucida, Alstonia boonei, Cymbopogon citratus and Phyllanthus amarus* possess both antioxidant and antiplasmodial activity against *Plasmodium falciparum* parasite through *in vitro* results obtained for β-hematin inhibitory activity, which serve as support for their ethnomedicinal uses as antimalaria.

The results obtained demonstrated that *Cymbopogon citratus* and *Phyllanthus amarus* displayed better activities for both antioxidant activity and beta hematin inhibitory activity ahead of other extracts (*A. boonei* and *M. lucida*). The findings indicate that the plant samples can be used as promising therapeutic plants in drug discovery for the treatment and management of malaria. Further studies should be carried out to profile the phytochemical constituents, isolate active compounds and ascertain the drug-ability of the extracts.

ACKNOWLEDGEMENT

We sincerely acknowledge the role played by Prof. Kehinde T.O of the Zoology Department and laboratory Staff of both the Medical Biochemistry Department, Biochemistry and Molecular Biology Department, Obafemi Awolowo University, Ile-Ife for the success of this work.

CONFLICT OF INTEREST

We declared that there is no conflict of interest with any individual, group or party

REFERENCES

Abiodun, O. O. and Oladepo, O. M. (2018). Beta Hematin Inhibition: Evaluating the Mechanism of Action of Some Selected Antimalarial Plants. *Acta Pharmaceutica Sciencia,* 56(3): 61-69

Abu, N. E., Ezeomeke, S. I., Azegba, P., and Davidson, G. I. (2016). Phytochemical, nutritional and anti-nutritional properties of leaves, stem bark and root of trees used in popular medicine for the treatment of malaria in South-eastern Nigeria. *Journal of Medical Plant Research,* 10(38): 662-668.

Adebayo, J. O., and Krettli A. U. (2011). Potential antimalarials from Nigeria plants: A review. *Journal of Ethnopharmacology,* 133: 289-302.

Adekola, M. B., Areola, J. O., Fagbohun, O. F., Agaolu, F. T., Ogundepo. G. E, Fajobi, A. O., and Babalola, O. O. (2021). *In-vitro* antioxidant and anti-inflammatory activities of ethanol stem-bark extract of *Blighia saprida* K.D. koenig. *Journal of Pharmaceutical Analysis* doi.10.1016/j.jpha.2021.04.002.

Adeleye, O. O., Ayeni, O. J. Ajamu, M. A. (2018). Traditional and Medicinal uses of *Morinda lucida*. *Journal of Medicinal Plants studies*, 6(2): 249-254.

Adeneye, A. A. (2013). Profile of Morinda India leaf fractions on blood glucose and lipids in normal and alloxan-induced Hyperglycemic rats. *Journal of Pharmacological*, 4(5): 408-413.

Adeyemi, T. O. A., Ogboru, R. O., Idowu, M. D., Owoeye, E. A., and Isese, M. O. (2014). Phytochemical screening and health potentials of *Morinda lucida* Benth. *International Journal of Innovation and Scientific Research*, II(2): 515 - 519.

Adotey, J. P. K, Adukpo, G. E., Boahen, Y. O. and Armah, F. A. (2012). "A review of the ethnobotany and pharmacological importance of *Alstonia boonei* - De wild (Apocynaceae)". *International Scholar Research Network Pharmacology*, 1: 1-9

Aibinu, I., Adenipekun, T., Adelowotan, T., Ogunsanya, T., and Odugbemi, T. (2007). Evaluation of the antimicrobial properties of different parts of *Citrus anrantifolia* (Lime fruit) as used locally. *African Journal of Traditional Complementary and Alternative Medicine.* 2: 185 - 190.

Akinmoladun, A. C., Ibukun, E. O., Afor, E. et al; (2007). "Chemical Constituents and antioxidant activity of *Alstonia boonei*". *African Journal of Biotechnology*, 6(10): 1197-1201.

Akmar-Ishak, S., Fariza-Azimi, F., Syahnaz-Zahari, A. and Fredalina-Basri, D. (2021). Ex-vivo study of Antimalarial Activity of *Canarium Odontophyllum* Leaf Extracts Against Plasmodium Berghei NK65. *Journal of Microbes, Infection and Chemotherapy* doi:10.54034/mic.e1255

Amiri, O., Zekhnini, A., Bouhaimi, A., Tahrouch, S. and Hatimi, A. (2018). Anti-inflammatory Activity of Methanolic Extract from *Pistacia atlantica* Desf. Leaves. *Pharmacognosy Journal,* 10(1):71-76

Areola, J. O., Omisore, N. O., and Babalola, O. O. (2016). Antiplasmodial activity of stem-bark extract of *Milicia excels* (WELW) C.C. Berg against Rodent malaria parasites (*Plasmodium berghei*) in mice. *Ife Journal of Science,* 18(4): 905-911.

Atanu, F. O., Idih, F. M., Nwonuma, C. O., Hetta, H. F., Alamery, S. and Batiha, G. E. (2021). Evaluation of Antimalarial Potential of Extracts from *Alstonia boonei* and *Carica papaya* in *Plasmodium bergei*-Infected Mice. *Journal of Evidence Based Complement Alternative Medicine.* doi:10.1155/2021/2599191

Avwioro, G. (2010). Effectiveness of some medicinal plant decoction in the treatment of malaria in Nigeria. *Journal of Annual Report of Biological Research*, 1(2): 230 - 237.

Baliyan, S., Makherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P. and Chang, C. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitattive Phytochemical Analysis of *Fiscus religiosa. Molecules*, 27(4): 1326

Brian, T. S., and Ikhlas, A. K. (2002). Comparison of Extraction methods for marker compounds in the essential oil of lemongrass by GC. *Journal of Agricultural Food Chemistry*, 50: 1345 - 1349.

Bunalema, L., Obakiro, S., Tabuti, J. R. S., Waako, P. (2014). Knowledge on plants used traditionally in the treatment of tuberculosis in Uganda. *Journal of Ethnopharmacology*, 151: 999 - 1004

Carline, E., Constar, J., and Silva-Filho, D. P. (1986). Pharmacology of Lemon grass (*Cymbopogon citratus* Stapf). Effects of teas prepared from leaves on laboratory animals. *Journal of Ethnopharmacology*. 17(1): 37-64.

Cheel, J., Theoduloz, C., Rodrguez, J. and Schmeda, -H.G. (2005). Free radical scavengers and antioxidants from lemongrass (*Cymbpogon citratus* (CD.) Stapf.). *Journal of Agricultural and Food Chemistry*, 53: 2511-2517

Chime, S. A., Ugwaoke, E. C., Ouyishi, I. V., Brown, S. A. and Onunkwo, G. C. (2013). Formulation and Evaluation of Alstonia boonei Stem Bark Powder Tablets. *Indian Journal of Pharmaceutical Sciences*, 75(2): 226-230

Dastmalchi, K., Dorman, D., Oinonen, P. P. and Darwis, Y. (2008). Chemical composition and *in vitro* antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. *Lebensmittel-Wissenschaft und-Technologie,* 41(3): 391-400

Egan T. (2004). Heamozoin formation as a target for the National design of new antimalarials. *Journal of Drug Des. Rev.*; 1:93-110.

Egan, T., and Ncokazi, K. (2005). Quinoline antimalarial decreases the rate of beta-hematin formation. *Journal of Inorganic Biochemistry*, 99: 1532–1539.

Elahe, T., Tebit, E.K., Parya, K., Saeed, N., David, S. L., and Faham, K. (2021). Antiplasmodial, antimalaria / activities and toxicity of African Medicinal Plants: a systemic review of literature. *Malaria*; 20: 349.

Enemakwu, T. A., Adeyemi, M. M., and Salihu, L. (2015). *In vitro* antiplasmodial activity and phytochemical screening of *Alstonia boonei* De wild used in treating malaria-associated symptoms. *International Journal of Pharmacological* Research, 5(9): 207-213.

Ernst, E. (2008). "Chiropractic: a critical evaluation". *Journal on pain symptom Management*, 35(5): 544 - 6213.

Figueirinha, A., Paranhos, A., Perez-Alonso, J. J., Santos-Buelga, C., and Batisa, M. T. (2008). *Cymbopogon citratus* leaves. Characterization of flavonoids by HPLC-PDA-ESI/MS and a source of bioactive polyphenols. *Journal of Food Chemistry*, 110: 728-728

Goose, B. K., Bryson, T. A. and Gokou, T. (1999). Study of triterpenoids from *Alstonia boonei*. *Societé Quest-Africaine de Chimie*, 5(8): 123-128

Halliwell, B., and Gutteridge, J. M. C. (1989). Free radicals in biology and medicine. *Clarendon Press*, 3: 617 - 783.

Halliwell, B., Gutteridge, J. M. C., and Aruoma, O. I. (1987). The deoxyribose method: A simple test-tube assay for determination of rate constants for reactions of hydroxyl radicals. *Journal of Analytical Biochemistry*, 165: 215 - 219.

Islam, A., Mazumoler, U. K., Gupta, M., and Ghosal, S. (2008). Phyto-Pharmacology of *Phyllanthus amarus*, an overview. *Pharmacologyonline Journal*, 3: 202 - 209.

Iwu, M. M. (1993). A handbook of African Medicinal Plants by CRC Press Inc. Florida

Iwu, M. M. (1993). Handbook of African Medicinal Plants. *CRC. Press,* Boca Raton, FL, ISA, PP: 116 - 118.

Joseph, B. and Raj, S. J. (2011). Pharmacological Properties of *Phyllanthus amarus*. *International Journal of Pharmacology*, 7: 40–45.

Klein, E. Y. (2013). Antimalarial Drug Resistance: A review of the biology and Strategies to delay emergence and spread. *International Journal of Antimicrobial Agents*, 41(4): 311-317

Kweifo-Okai, G. (1991). "Anti-inflammatory activity of a Ghanian anti-arthritic herbal preparation: I". *Journal of Ethnopharmacology*, 33(3): 263 - 267.

Kweifo-Okai, G. (1991). "Anti-inflammatory activity of a Ghanian anti-arthritic herbal preparation: II". *Journal of Ethnopharmacology*, 33(3): 129 - 133.

Majekodunmi, S. O., Adegoke, O. A., and Odeku, O. A. (2008). Formulation of the extract of the stem bark of *Alstonia boonei* as tablet dosage form. *Tropical Journal of Pharmacology Research*, 7: 987 - 994.

Nambiar, V. S., and Matela, H. (2012). Potential Functions of Lemon Grass (*Cymbopogon citratus*) in Health and Disease. *International Journal of Pharmaceutical and Biological Archives*, 3 (5): 1035 – 1043.

Obiagwu, M. O., Ihekwereme, C. P., Ajaghaku, D. L., and Okoye, F. B. C. (2014). The useful medicinal properties of the Root-Bark extract of *Alstonia boonei* (Apocynaceae) May Be connected to Antioxidant Activity. *International Scholarly Research Network on Pharmacology*. Vol: 2014

Oduola, T., Bello, I., Adeosun, G., Ademosun, A., Raheem, G., and Avwioro, G. (2010). Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to *Morinda lucida* leaf extract. *North American Journal of Medical Science,* 2: 23–233.

Ott, M., Thyagarajan, S. P. and Gupta, S. (1997). Phyllanthus amarus supresses hepatitis B virus by interrupting interactions between HBV enhancer I and cellular transcription factors. *European Journal of Clinical Investigation,* 27: 908 – 915.

Patel, J. R., Tripathi, P., Sharma, V., Chauhan, N. S., and Dikit, V. K. (2011). Phyllanthus amarus. Ethnomedicinal uses, phytochemistry and Pharmacology: *A review. Journal of Ethnopharmacology,* 138: 286-313.

Plewes, K., Leopold, S. J., Kingston, H. W. F. and Dondrop, A. (2019). Malaria: What's New in the Management of Malaria? *Infectious disease clinics of North America,* 33(1): 39-60

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry*, 269: 337 - 341

Rahman, M. M., Islam, M. B, Biswas, M., and Alam, A. H. M. K. (2015). *In vitro* antioxidant and free radical scavenăăging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *Biomed Central Research Notes*, 8: 621.

Riaz, T., Shehzad, W., Viani, A., Pompanon, F, Taberlet, P. and Coissac, E. (2011). ecoPrimers: inference of new DNA barcode makers from whole genome sequence analysis. *Journal of Nucleic Acid Research,* 39(21): e145

Rodrigues, J. R., Lourenco, D., and Gamboa, N. (2011). Disturbance in Hemoglobin Metabolism and *in vitro* Antimalarial Activity of Azole Antimycotics. *Revista do Instituto de Medicina Tropical de Săo Paulo*, 53(1): 25-29.

Saleh, A. M. A., Elnakib, M. M., Malek, D. M. A., and Morsy, T. A. (2019). Mini Review on Malaria and Human Immunodeficiency Virus (HIV) in Sub-Sahara Africa. *Journal of the Egyptian Society of Parasitology*; 49(1): 61-72.

Sangodele, J. O., Okere, S. O., Ogunwole, E., Adams, M. D., and Shafe, M. O. (2014). Antiplasmodial activity of aqueous leaf extract of Cymbopogon citratus against *plasmodium falciparum* infected rats. *American Journal of Biomedical and Life Sciences*, 2(3): 60-64.

Singh, N. and Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, 85(4): 611 - 616

Thamlikitkul, V., Wasuwat, S. And Kanchanapee, P. (1991). Efficacy of Phyllanthus amarus for eradication of hepatitis B Virus in chronic carriers of Hepatitis B Virus. *Lancet*, 2: 764-766.

World Health Organization (2008). Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. Geneva.

World Health Organization, (2018). World Malaria Report 2018. *World Health Organization.* Retrieved December 3, 2021, from https://apps.who.int/iris/handle/10665/275867

World Health Organisation (2019). Annual World Malaria Report, *Geneva* (2020), ISBN 978-92-4-156572-1

World Health Organization. (2019). Malaria Vaccines. *World Health Organization,* Retrieved December 3, 2021, from, https://www.who.int/immunization/research/development/malaria

World Health Organization, (2020). Annual World Malaria matter. Retrieved October 4, 2021, from https://www.who.int/teams/global-malaria-programme

World Health Organization, World Malaria Report 2021. Available at http://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021. Retrieved on January 24, 2022

Zubair, M. F., Atolani, O., Ibrahim, S. O. Adebisi, O. O., Hamid, A. A. and Sowunmi, R. A. (2017). Chemical Constituents and Antimicrobial Properties of *Phyllanthus amarus* (Schum&Thoun). *Bayero Journal of Pure and Applied Sciences*, 10(1): 238-246