In vitro Antiplasmodial and Cytotoxicity Evaluation of Ethanol Leaf Extract of *Erythrina senegalensis* DC

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Authors' contributions

This work was carried out in collaboration among all authors. Author DAA wrote the first draft of the manuscript and did the experimental works of the study. Author AMOA generated the idea and designed the study. Author ASA managed the literature searches, edited the manuscript and finalized it. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

This study evaluates *in vitro* antiplasmodial and cytotoxicity activity of an ornamental plant commonly employed in Northern part of Nigeria for curing malaria infection. Fresh mature leaves of *Erythrina senegalensis* were collected, air dried, ground, percolated in ethanol for two weeks and evaporated to dryness at room temperature. The dried ethanol crude extract was fractionated with pet-ether, chloroform, ethyl acetate and methanol in order of increasing polarity. These were screened for phytochemicals, *in vitro* antiplasmodial and cytotoxicity test using standard procedures. The results of the phytoconstituents revealed the presence of alkaloids, tannins, flavonoids and terpenoids which could be responsible for the medicinal properties of the extract. Ethanol crude extract, pet-ether and ethyl acetate fractions were discovered to be non-cytotoxic exhibiting $LC_{50} > 1000$ µg/ml respectively. Only methanol fraction showed slight toxicity with LC_{50} value of 630 µg/ml. The highest activity of the extract and its fractions against *Artemia salina* larvae was recorded at 1000 µg/ml dose whereas lowest larvicidal activities and lethalities occurred at 10

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µg/ml dose. The ethanol extract and its fractions were significantly active against *Plasmodium falciparum* strain (K1) with percentage elimination of the parasites were in the range of 77% - 83% at 5000 µg/ml. Ethyl acetate (71%) and methanol fraction (71%) were found to be the most active fractions at the lowest concentration of 625 µg/ml. This finding justifies the usage of the plant in traditional medicine by traditional healers and local populace against malaria disease. The observed value for cytotoxicity assay of LC_{50}>1000 µg/ml recorded for the ethanolic extract suggested that oral intake of the extract could be deemed safe and would not be lethal at low/moderate dose as lethality is directly proportional to dosage and oral consumption of the extract as over dosage could lead to lethality and subsequently, mortality over a long period of time due to bioaccumulation. The isolation of antiplasmodial compounds from the leaves of the plant is necessary as it could be a potential candidate for novel antimalarial drugs. Continuous utilization of the leaves as antimalarial and the wide range of its safety at low and moderate dosages further supports its use among the less privileged and vulnerable people in malaria endemic areas in lieu of existing expensive modern antimalarial drugs.

1. INTRODUCTION

Malaria was once considered to originate from fetid marshes, thus the name malaria (bad air). However, scientists disclosed that the actual cause of malaria was a single-celled haemo(blood) parasite or protozoa known as *Plasmodium*. Malaria is widely distributed in south western part of Asia, tropical and sub-tropical Africa. The following Plasmodium species have been discovered to cause malaria in humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodimum Knowlesi* [1]. *Plasmodium falcipaium* is responsible for more than half (50%) of all malaria infections worldwide.

There is a concern on the increase in the resistance of malaria parasites to the commonly and cheapest antimalarial drug such as chloroquine for treatment of malaria in Nigeria. Artemisinin, a Chinese medicinal plant product, based on active principle of *Artemisia annie* has been introduced into the Nigerian market [2]. However, not much has been done to present indigenous medicinal plants possessing antimalarial properties.

Nigeria native medicinal plants employed in fighting malaria are still to be exhibited and projected in conferences as foreign plants in spite of our rich plant diversity. The quest for an interest in the scientific investigations of these common and local medicinal plants native to Nigeria was on the ground of their popular use to tackle several diseases which include malaria infection. Therefore, research into the impacts of these local plants of medicinal value is expected to validate and justify the utilization of these

plants against malaria [3]. However, majority of the plants employed in traditional medicine have not been sufficiently studied. *Erythrina senegalensis* DC is one of such local plants with medicinal value which belongs to the family Fabacea. The plant is a small tree or a thorny shrub which is commonly known as coral tree (English) and minjirya (Hausa, Nigeria). The leaves, root and stem bark are employed in traditional medicine by traditional healers to treat variety of illnesses ranging from malaria, fever, diarrhea, gastro-intestinal disorders, body pains, yellow fever to secondary sterility in female amongst others [4,5.6].

Previous empirical studies informed that aqueous extract of *E. senegalensis* stem bark indicated weak activity against *Plasmodium berghei* [7]. The roots ethanolic extract showed strong activity against *Plasmodium falciparum*, a multi-resistant strain K1 exhibiting IC50 of 1.82 µgmL-1 [8] while the methanol extract suppressed the growth of the same strain (IC50 = 99.7 µgmL-1). *Erythrina abyssinica* stem bark ethyl acetate extract disclosed activity against chloroquine-sensitive (D6) and resisitant (W2) *P. falciparum*, indicating IC50 values of 7.9±1.1 and 5.3±0.7 µgmL-1, respectively [9]. The stem bark ethyl acetate extract of *E. fusca* showed antiplasmodial activity against the multidrug resistant K1 strain of *Plasmodium falciparum* [10,11]. The methanol extract of the bark of *Erythrina variegata* revealed significant anti-malarial activity toward *Plasmodium falciparum in vitro* using the lactate dehydrogenase assay. The ethyl acetate fraction demonstrated the most activity, exhibiting equipotency against both strains of parasite with IC_{50} of 23.8 µg/mL against 3D7 and 9.3 µg/mL against K1. An

isoflavonoid, warangalone was isolated from the ethyl acetate fraction which disclosed anti-malarial activity against both strains of parasite used with IC50 of 3.7 µg/mL against K1 and 4.8 µg/mL against 3D7 [12]. Flavonoids of pterocarpane-type with antiplasmodial activities were isolated from *Erythrina fusca* stem bark [13]. Although, there are reasonable number of published scientific literatures on the antiplasmodial activities of various extracts obtained from the root and stem bark of the plant, *in vivo* cytotoxicity of its leaf extracts as well as *in vivo* antiplasmodial effects of *E. senegalensis* methanolic leaf extract has been reported but currently, there is a dearth of published scientific data/information on the *in vitro* antiplasmodial and *in vitro* cytotoxicity activities of ethanol leaf extract from *Erythrina senegalensis* and this current investigation was necessitated to further corroborate the aforementioned recent study. Hence, the present study was aimed at evaluating the *in vitro* antiplasmodial and *in vitro* cytotoxicity activity of ethanol leaf extract of *Erythrina senegalensis* DC.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

Fresh leaves of *Erythrina senegalensis* were collected on the 6th of April, 2017, from Mada Village, Gasau Local Government Area, Zamfara State, Nigeria and the plant material was authenticated by Mal. Baha'uddeen Said Adam at the Herbarium of Bayero University, Kano with the voucher number: 0310.

2.2 Preparation, Extraction of Plant Materials and Fractionation Procedure

The fresh leaves of *Erythrina senegalensis* were air dried at room temperature and ground into fine powder using pestle and mortar. Fine powder of the plant sample weighing 250 g was percolated in 1.3 L of ethanol (96%) in three separate sample bottles repeatedly at room temperature for two (2) weeks. The ethanol liquid crude extract was then decanted and filtered using No 1 Whatman filter paper and the ethanol liquid extract was concentrated with the aid of rotatory evaporator (R110) at 40°C and evaporated to dryness by exposure. The ethanol crude liquid extract was allowed to dry properly, weighed and labeled as ES-1. The ethanol crude extract (ES-1) was fractionated with petroleum-

ether, chloroform, ethyl acetate and methanol according to the standard method [14]. The order of fractionation was as follows: Petroleum ether < Chloroform < Ethyl acetate < Methanol. Each of the dried fractions of ethanol crude extract (petroleum-ether, chloroform, ethylacetate and methanol fraction) was coded as ES-1-01, ES-1-02, ES-1-03 and ES-1-04 respectively, weighed and stored until investigated for their phytochemicals, *in vitro* antiplasmodial and *in vitro* cytotoxicty activities.

2.3 Phytochemical Screening

2.3.1 Preparation of test solutions/samples for phytochemical screening

The test solutions/samples was subjected to phytochemical screening for the presence or absence of secondary metabolites using standard procedures [15,16,17]. The secondary metabolites investigated include: alkaloids, saponins, tannins, flavonoids, resins, steriods and terpeniods.

2.4 Brine Shrimp Lethality Test of Ethanol Crude Extract and Its Fractions

Eggs of Brine shrimp (*Artemia salina*-Leach) were hatched in a hatching chamber which contained salt water obtained from the ocean or sea. The chamber used for hatching was positioned under a natural light for forty eight (48) hours to enable the eggs to rapidly hatch into the larvae.

2.4.1 Preparation of test solutions/samples for brine shrimp lethality test

Each of the dried crude leaf extract and its fractions (20 mg) was dissolved separately in 2ml of methanol which constituted the stock solutions. Also, 20 mg of a positive control, potassium heptaoxodichromate (VI) was dissolved in 2 ml of distilled water. 500 µl, 50 µl and 5 µl were measured using micropipette from the stock solution of each test extract/fraction including the positive control which are equivalent to 1000, 100 and 10 µg/ml and were transferred into the vials respectively. A negative control contains sea/ocean salty water without any of the test samples. Each of the doses was tested in triplicates. In specific terms, each of the test solutions measured out from stock solutions of the crude extract and each of its fractions were put in 9 vials (9 vials per test solution) and 9 vials for positive control were air dried or evaporated

to dryness under a shade in about forty eight hours around 25°C. Subsequently, the dried test samples were tested for their activity against Brine Shrimp Larvae: *Artemia salina*. Also, 500 µl, 50 µl and 5 µl of negative control (salty sea water) were measured by using micropipette and transferred into 9 vials (3 vials for each dose in triplicates) [18].

2.4.2 Procedure for brine shrimp lethality test

To each dried test sample in a vial, 1-2 drops of Dimethyl sulphur (IV) oxide solvent was introduced in order to rapidly dissolve each of the test samples in the vials before transferring 10 *Artemia salina larvae* and salty sea water. A pasteur pipette was used to transfer ten(10) larvae of the brine shrimps into each of the vials containing the test solution/sample, after which natural salty sea water was added into the vials until the volume of salt water rose to 5 ml in each of the vials. Immediately after twenty four hours later, the surviving brine shrimp larvae were counted at each dosage and recorded. *Artemia salina* Leach larvae were considered or regarded as dead if they were lying immotile at the base of the vials. The total number of mortality and then percentage of mortality for each dose in triplicate for all test samples including positive and negative control were determined. Finally, LC50 values (µg/ml) at 95% confidence interval for each concentration for all test samples plus positive and negative control were calculated by employing a computer software application [19].

$$\% \text{ Mortality} = \frac{\text{Total No. of Dead } Artemia\ salina \text{ Larvae per Dosage}}{\text{Total Initial No. of Live } Artemia\ salina \text{ Larva per Dosage}} \text{ X } 100$$

2.5 Malaria Parasite Bioassay of the Extract and Fractions

2.5.1 Preparation of test solutions

A stock solution of 10,000 µg/ ml was prepared by dissolving 20 mg in 2 ml of Dimethyl sulphoxide (DMSO). Solution of 625µg/ml, 1250 µg/ ml, 2500 µg/ml, and 5000 µg/ml were prepared from the stock solution by serial dilution.

2.5.2 Source of malaria parasite for assay

Infected human blood samples containing parasitaemia of *Plasmodium falciparum* were collected from Bayero Univesity Clinic, Murtala Mohammed Specialist Hospital, Kano and Aminu Kano Teaching Hospital, Kano in K3-EDTA coated disposable plastic sample bottles tightly fitted with plastic corks [20].

Venous blood from patients recommended for malaria parasites test (MP Test) using 5 cm^3 disposable plastic syringes and needles (BD and 20 SWG) was collected and the samples were immediately transferred into K3-EDTA disposable plastic sample bottles with tightly fitted plastic corks and mixed thoroughly and then transported to the Microbiology laboratory at Bayero University in a thermo flask containing water maintained at 40°C [21].

2.5.3 Determination of *Plasmodium falciparum* (positive blood samples) using thin smear method)

Using a clean capillary tube, a small drop of each blood sample was placed at the centre of a clean glass slide at least 2 mm from one end. A cover slip was placed at angle 45°C in front of each drop and drawn backward to make contact with each drop. The drop was run along the full length of the edge of the cover slip. Smears were formed by moving the cover slip forward on glass slide. The thin smears were immersed in 30 ml of methanol contained in a Petri dish for 15 minutes. Geimsa's stain was dropped on each smear and allowed for about 10 minutes [21].

Excess stain was washed with clean tap water. The smears were air dried by hanging the glass slides inverted in a rack. Each dried smear was microscopically observed under a high power objective (x 100) using oil immersion after which an average parasitaemia was determined using the reading of 3 microscopic fields [22].

2.5.4 Separation of the erythrocytes (5% parasitaemia) from the serum of the blood samples

50% Dextrose solution (0.5 ml) was added to each of 5 m 1 defibrinated blood sample and then centrifuged at 2500 rpm for 15 minutes in a spectral merlin centrifugation machine. Supernatant layers were separated from the sediments. The later was diluted with 2-3 drugs normal saline solution [21] and further centrifuged at 2500 rpm for 10 minutes. The resulting supernatants were discarded. Samples with higher parasitaemia (above 5%) were diluted with fresh malaria parasite negative erythrocytes [22].

2.5.5 Preparation of *Plasmodium falciparum* culture medium

Venous blood (2 ml) from the main vein of white healthy rabbits pinnae was withdrawn using a disposable 5 ml syringe (BD 205 WG). This was defibrinated by allowing it to settle for at least one hour [21]. The defibrinated blood was centrifuged at 1500 rpm using spectre merlin centrifuge for 10 minutes and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500 rpm for five minutes, and the supernatant layer was added to the first test tube. The sediments were discarded and the serum collected was supplemented with the salt of RPMI 1600 medium (KCl 5.37 mM, NaCl 10.27 mM, $MgSO_4$ 0.4 mM, NaHPO4 17.73 mM, Ca $(NO_3)2$ 0.42 mM, NaHCO32.5 mM, and glucose 11.0 mM.(BDH Ltd, UK) as demonstrated by Devo et al. (1985). The medium was sterilized by 40µg/ml gentamicin sulphate [23].

2.5.6 *In-Vitro* bioassay of the activity of ethanol extract and its fractions on *Plasmodium falciparum* culture

A 0.1 ml of test solution and 0.2 ml of the culture medium were added into a tube containing 0.1 ml of 5% parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to the test fraction/samples was determined microscopically after incubation for 24, 48 and 72 hours at 320C. The incubation was undertaken in glass bell jar containing a lighted candle to ensure the supply of required quantity of CO_2 about 5% O_2 gas, 2% and 93% of nitrogen gas [24].

2.5.7 Determination of the activity

At the end of the incubation period of 72 hours (3 days), a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and stained by Giemsa's staining techniques. The mean number of erythrocytes appearing as blue discoid cells containing life rings of the parasite (that appeared red pink) was estimated and the percentage elimination by the samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasites after incubation period of 72 hours, using the formula below:

$$\% = N/Nx \times 100$$

Where,

%=Percentage Activity of the Extracts/Fractions
N = Total number of cleared RBC after 72 hrs
Nx = Total number of parasitized RBC
Note: RBC= Red Blood Cells [24].

3. RESULTS AND DISCUSSION

The screening results of phytochemicals indicated the presence of some secondary metabolites in the crude extract and its fractions. In Table 1, the ethanol crude extract revealed the presence of tannins, alkaloids, flavonoids, saponins, reducing sugars and terpenoids. Pet-ether fraction showed the presence of saponins, flavonoidsandtannins. Chloroform fraction indicated only the presence of tannins. The presence of saponins, tannins, alkaloids, terpenoid and flavonoids were detected in ethyl acetate fraction. Methanolic fraction showed the presence of saponins, reducing sugars (primary metabolite) and tannins (Table 2). Steriods and resins were not detected in the crude leaf extract and each of its fractions in the present study. The phytochemical investigation of this study revealed the presence of some secondary metabolites (alkaloids, tannins, saponins, terpenoids and flavonoids) in the leaf extract of the plants (Table 1). The findings are comparable to the results of other researchers that worked on the extracts obtained from various body parts of *E. senegalensisas* already published [25,26,27,28,7].

Table 1. Results of phytochemical investigation of ethanol crude extract and its fractions

Phytochemical constituent	ES-1	ES-1-01	ES-1-02	ES-1-03	ES-1-04
Tannins	+	+	+	+	+
Saponins	+	+	-	+	+
Alkaloids	+	-	-	+	-
Flavonoids	+	+	-	+	-
Resins	-	-	-	-	-
Steroids	-	-	-	-	-
Terpenoids	+	-	-	+	-

Key: ES-1: Ethanol Crude Extract; ES-1-01: Pet-ether Soluble fraction; ES-1-02: Chloroform Soluble fraction; ES-1-03: Ethyl acetate Soluble fraction; ES-1-04: Methanolic Soluble fraction. + = present; – = absent

Table 2. Results of cytotoxicity test for the ethanol extract and fractions

Extract/Fraction	Concentration (µg/ml)	LC$_{50}$ (µg/ml)
ES-1	1000 1000 10	>1000
ES-01-1	1000 1000 10	>1000
ES-01-2	1000 1000 10	>1000
ES-01-3	1000 1000 10	>1000
ES-01-4	1000 1000 10	630.7
Control (+) (K$_2$Cr$_2$O$_7$)	1000 1000 10	4
Control (-)	1000 1000 10	>1000

The availability of secondary metabolites in plants determines some biological activities in animals and man, hence, this is responsible for using them as herbs [29]. The presence of these phytochemicals in the plant has led to the justification of traditional uses of *Erythrina senegalensis* leaves in herbal medicinal practices in curing variety of diseases including malaria which have been reported on the plant.

The cytotoxicity test results obtained by using *Artemia salina* Leah larvae (Brine shrimp Test) whose values were expressed in LC$_{50}$ µg/ml at 95% confidence interval shows that the high toxicity level of Potassium heptaoxodichromate (VI) (4 µg/ml) was significantly lesser than the values obtained for toxicity levels of ethanol crude leaf extract (>1000), pet-ether soluble fraction (>1000), chloroform fraction (>1000), ethyl acetate fraction (>1000) and methanolic fraction having LC$_{50}$ µg/ml of 630.7 µg/ml (Table 2). From these test results of the samples, it was evident that only methanolic fraction (ES-1-04) with LC50 µg/ml of 630.7 was slightly toxic while ES-1 (LC50 µg/ml >1000), ES-1-01 (LC50 µg/ml >1000), ES-1-02 (LC50 µg/ml >1000) and ES-1-03 (LC50 µg/ml >1000) samples were non-toxic due to the fact that their LC50 µg/ml values were greater than 1000 whereas the LC50 µg/ml of methanolic fraction (630.7 µg/ml) was less than 1000 (Table 2). The consideration of LC50 µg/ml values less than 1000 suggest toxicity while

LC50µg/ml values which are greater than 1000 signify non toxicity [18]. The brine shrimp Assay of the leaf extract and each of its fractions evaluated on *Artemia salina* shows that only methanol soluble fraction demonstrated slight cytotoxicity (Table 2) which may be as a result of the presence of saponins/alkaoids in low content and this fraction could be a source of bioactive principle that may be useful as antiproliferative, antitumor, pesticidal and other bioactive agents. This study also indicated that activities of the leaf extract and its fractions against *Artemia salina* larvae and cytotoxicity of the methanol fraction are dose dependent. The low cytotoxicity of the methanol soluble fraction of the leaf crude extract reported in this study concurred with Wandji et al. [30] who isolated *Erysenegalein E*. from the stem bark of *Erythrina senegalensis* using methanol solvent. *Erysenegalein E*. which was isolated from the methanol extract obtained showed a certain degree of toxicity. Non toxicity of the ethanol crude extract in the current study was in agreement with the result obtained by the group of Atsamo and coworkers [31] who assessed the decoction of the stem bark of *Erythrina senegalensis* for possible toxicity and lethality since this part of the plant is employed in traditional medicine in Cameroon to treat liver disorders. Their finding showed that wide range of safety exists for the medicinal use of this decoction and this further supported the utilization of the stem bark extract of the plant as

a protective agent for the liver (hepatoprotective agent). The observed non-cytotoxic nature of the leaf extract of *E. senegalensis* in this study was in contrast with the findings of Wilson et al. [32] and Nnama et al. [33] who discovered that aqueous and ethanol leaf extracts of the plant exhibit low *in vivo* cytotoxicity in rats at relatively higher dose test concentrations of 100 mg/kg up to 1000 mg/kg per body weight and 4000 mg/kg down to 500 mg/kg per body weight respectively which could be due to relatively lower dose test concentrations of 10 µgml to 1000 µg/ml employed in this current *in vitro* cytotoxic study. Even though, methanol fraction of the plant leaf extract showed low toxicity (*in vitro*) at the same test doses. In the present study, methanolic fraction of the leaf extract of the plant was found to possess low larvicidal/cytotoxicity activity which justifies the traditional use of the leaves of the plant in question as a contraceptive agent by

traditional healers. If this fraction is harnessed, purified and assayed further, it may be an alternative source of agent(s) for family planning.

The *in vitro* antiplasmodial evaluation of the ethanol leaf crude extract and its fractions were carried out and the activities of the test solutions/samples were calculated as the percentage elimination of the malaria parasite after incubation periods of 24, 48 and 72 hours. The ethanol leaf crude extract and each of its fractions showed certain degrees of antiplasmodial activity (Table 3). At concentration of 5000 µg/ml, the ethanol leaf crude extract exhibited highest parasitic elimination of 85%, followed by pet-ether fraction (83%), ethyl acetate fraction (83%) and methanolic fraction (83%) indicated equipotency manifested in their percentages of malarial parasites elimination. The chloroform fraction also showed a

Table 3. *In-Vitro* antiplasmodial evaluation of ES-1 to ES-1-04

Extract/Fraction	Parasite initial count per dose	Conc. (µg/ml)	Parasitaemia final count per dose	Number of parasitaemia mortality after 72 hours per dose	% Elimination of parasites at the end of incubation per dose/ Conc
Artemeter and Lumenfantrine(+ control)	52	5000	2	50	96
	52	2500	4	48	92
	52	1250	5	47	90
	52	625	8	45	87
ES-1	52	5000	8	44	85
	52	2500	12	40	77
	52	1250	17	35	67
	52	625	23	29	56
ES-1-01	52	5000	9	43	83
	52	2500	12	40	77
	52	1250	16	36	69
	52	625	21	31	60
ES-1-02	52	5000	12	40	77
	52	2500	16	36	69
	52	1250	22	30	58
	52	625	24	28	54
ES-1-03	52	5000	9	43	83
	52	2500	10	42	81
	52	1250	13	39	79
	52	625	14	38	71
ES-1-04	52	5000	9	43	83
	52	2500	10	42	81
	52	1250	11	41	79
	52	625	15	37	71

Key: ES-1: Ethanol Crude Extract; ES-1-01: Pet-ether soluble fraction; ES-1-02: Chloroform soluble fraction; ES-1-03: Ethyl acetate soluble fraction; ES-1-04: Methanol soluble fraction

remarkable anti-plasmodial activity of 77% for 5000 µg/ml. At the dose of 2500 µg/ml, ethyl acetate and methanolic fractions showed maximum and equipotent effect of 81%, followed by pet-ether fraction and chloroform fraction and ethanol extract (77%) revealing significant activities against multidrug resistant *Plasmodium falciparum* strain K1 (*in vitro*) while positive control exhibited activity of 92% at the same dosage which is higher than the effects of each of test extract and its fractions. Furthermore, at the dosage of 1250 µg/ml, ethyl acetate and methanolic fractions demonstrated highestand equipotent activity of 79%, followed by pet-ether fraction (69%), ethanol extract (67%) and chloroform fraction (58%) with least percentage reduction of the parasitaemia. When compared to positive control (87%) at equal concentration, it was noticed that the positive control had much higher and significant antiplasmodial suppressive/elimination activity than each of the test extract and its fractions against multidrug resistant *Plasmodium falciparum* strain K1 (*in vitro*). At the lowest concentration of 625 µg/ml, ethyl acetate and methanolic fractions revealed the highest activity of 71%, followed by n-hexane fraction (60%). Ethanol extract (56%) and chloroform fraction (54%) indicated low/slight antiplasmodial activity at 625 µg/ml. The positive control (lumenfantrine/artemether) showed interesting antiplasmodial effects of 96% at 5000 µg/ml and 87% at 625 µg/ml. The percentage elimination of *Plasmodium falciparum* strain (K1) by positive control/reference standard (96%) was insignificantly higher than that of ethanol extract and its fractions at highest dose of 5000 µg/ml. However, at least concentration of 625 µg/ml, percentage reduction of *Plasmodium falciparum* strain (K1) by positive control/reference standard (87%) was found to be significantly greater than ethanol extract and each of its fractions. Treatment with increased doses of the crude ethanolic extract and its fractions resulted in relative reductions (*in vitro*) of *Plasmodium falciparum* strain (K1) as indicated by the parasitaemia (malaria parasites) final count when compared with the initial count of parasitaemia. This finding, actually confirms/validates the report that *E. senagalensis* leaves have been effectively used in folklore medicine for the treatment of malaria [34,4]. The antiplasmodial activities of ethanol extract and its fractions could be accrued to the existence of bioactive secondary metabolites such as tannins, terpenoids, flavonoids and alkaloids in the leaves of the plant [7,33,25,28,26,27]. Joanne et al. [35] discovered that flavonoids, alkaloids, terpenoids,

tannins and steroids possess anti-plasmodial activity. Bioland, [36]; Deloron et al. [37] and Quinghaosu, [38]; Sanjib, [39]; Francois and Nicholas, [40]; WHO, [41] also reported that quinine; quinidine; cinchonine which are alkaloids and artemisinin, artemotil, arteether, artemether and artesunate which are terpenes and their derivatives exhibited antiplasmodial activities respectively. Reported data based on laboratory investigations on *in vivo* and *in vitro* antiplasmodial assay of other parts (stem bark and root) of *Erythrina senegalensis* and other bioactive species in *Erythrina* genus revealed similar/comparative results with the findings of this study. For instance, the antiplasmodial activities (*in vitro*) of various extracts from stem bark and root of *E. senegalensis* as well as stem bark of *E. abyssinica*, *E. fusca* and *E. variegate* were reported [7,8,9,10,11,12,13]. The antiplasmodial activities of different extracts from specific parts of these plant species in Erythrina genus could be attributed to the presence of bioactive secondary metabolites such as flavonoids, terpenes/terpenoids, tannins, alkaloids, steroids and allied substances detected in certain part of the plants [28,27,25,26,33,41]. The results of this study is consistent with the study of Osuntokun et al. [42] who reported that methanolic leaf extract of *E. senegalensis* has significant *in vivo* antiplasmodial/antimalarial effects on *Plasmodium berghei* in rats although at relatively higher dose concentrations (200 mg/kg, 400 mg/kg and 600 mg/kg per body weight) when compared with dose test concentrations of 625 µg/ml, 1250 µg/ml, 2500 µg/ml and 5000 µg/ml used in the current study. Antiplasmodial activity of *E. senegalensis* leaves analysed in this present investigation has confirmed the traditional use of the leaves of *Erythrina senegalensis* in curing malaria infection and there is no hazard/lethality particularly when consumed orally at controlled low/moderate doses since it is safe at any of these doses due to the fact that extracts from the leaves of the plant have been scientifically investigated for their toxicity in rats and discovered to have low cytotoxicity (*in vivo*) at relatively regulated low and moderately high dose concentrations [32,33]. Antiplasmodial activity (*in vitro*) of the leaf extract of *Erythrina senegalensis* which has been further experimentally established from the results of this research properly explains and justifies the use of the leaves of plant to treat malaria disease in the Northern part of Nigeria and other countries in West Africa in the tropics.

4. CONCLUSION

The plant phytochemicals contributed to the efficacy of the extract and its fractions which has made it useful in the treatment of several ailments including malaria infection. The results obtained suggest that ethanol crude extract, pet-ether, chloroform and ethyl acetate soluble fractions shows no cytotoxicity whereas methanol fraction is slightly cytotoxic against *Artemia salina* Leah larvae. The antiplasmodial activities of the extract and its various fractions showed high activity at the highest concentration of 500 µg/ml. The leaves of *Erythrina senegalensis* can be employed as therapeutic agent in low to moderate doses, particularly among the rural populace where modern drugs are not affordable. Hence, its uses as anti-malarial herb in traditional medicine in Northern part of Nigeria has been scientifically proven based on the *in vitro* antiplasmodial data obtained from this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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