

IN-VITRO ANTIPLASMODIAL ACTIVITY OF AQUEOUS AND ETHANOL STEM AND LEAF EXTRACTS OF *SENNA OCCIDENTALIS* (COFFEE SENNA)

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ABSTRACT

Development of resistance against the frontline anti-malarial drugs has created an alarming situation, which requires intensive drug recovery to develop new, more effective, affordable and accessible anti-malarial agents. Plants as Senna occidentalis produce a wide variety of phytochemical constituents, which are secondary metabolites and are used either directly or indirectly in the pharmaceutical industry. Phytochemical screening and anti-plasmodial activity of the aqueous and ethanol extracts of S. occidentalis (L.) leaves and stems were studied in this work. The preliminary screening of the leaf extracts revealed the presence of alkaloids, saponins, cardiac glycosids, quinine, phenol, flavonoids, amino acids and carbohydrate and showed absence of tannin. Likewise stem extracts which shows absence of phenol in addition to tannin in ethanol extract. These extracts were assayed at various concentration using double serial dilution (20mg/ml, 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml) for anti-plasmodial effect after 24, 48 and 72 hours respectively, and the activity of the extracts were obtained as percentage activity of the extracts after 72 hours of incubation period. The result of anti-plasmodial activity revealed that both aqueous and ethanol stem and leaf extracts of the plant were effective against the malaria parasite. However, the aqueous stem extract showed greater activities than the ethanol extract. At extract concentration of 20mg/ml, both ethanol and aqueous extracts produced highest parasite clearance rate after 72 hours of incubation with percentage elimination of 77%. From these observations, S. occidentalis is likely to contain promising chemical compounds which can be utilized as an effective plant-based medicine for the treatment of malaria.

Keywords: Anti-plasmodium, in-vitro Malaria, parasite , phytochemicals.

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INTRODUCTION

Malaria is a severe disease caused by parasites that are transmitted to individuals through the bites of infected female anopheles mosquitoes. It's preventable and curable. In 2019, there were estimated 229 million cases of malaria worldwide. The estimated range of malaria deaths stood at 409 000 in the year 2019. Youngsters aged below 5 years are the prime vulnerable group affected by malaria. In 2019, they accounted for 67% (total=274 000) of all malaria deaths worldwide. The World Health Organization (WHO) African Region carries a disproportionately high share of the global malaria burden. In 2019, the region was home to 94% of malaria cases and deaths (WHO 2020). Traditional medicines have been used to treat protozoal infection for thousands of years and are the key sources of the two main groups (artemisinin and quinine derivatives) of recent antimalarial drugs. Medicinal plants contain active compounds with medicinal properties (Nkunya 1996) and herbal preparations account for 30-50% of the overall medicine consumption (Gelfand, 1965). Experts have described how British explorers were cured of malaria by rural people in Southern Africa using ancient medicines long before colonization. The WHO defines herbal medicine to include herbal materials, herbal preparations and finished herbal product that contain active ingredients components of the plants, or different plant materials, or combination thence (WHO 2002). In Africa, the use of indigenous plant plays a significant role in the traditional method of malaria treatment by providing good sources for the detection of phytochemicals in them, that are anti-plasmodial in nature. These phytochemicals include interalia, alkaloids, glycosides, phenols, saponins, terpenoids and flavonoids (Omoregie *et al.*, 2012). *Senna occidentalis* (Linn.) (formerly *Cassia occidentalis*) is a weed of the family Leguminosae, and is distributed throughout the tropical and sub-tropical regions of the world. Its common name is *coffee senna*. It can be found in open pastures and within the field of cultivated cereals like soybean, corn, sorghum and others; therefore, throughout the harvest there is little to prevent this plant from mixing with the cultivated crops (Lar, 1973; Barbosa-Ferreira *et al.*, 2005). *Coffee senna* is a very leafy, malodorous annual to woody plant that may grow up to 2 metres tall but is typically smaller. The plant is often annual. It is generally gathered from the wild for native medicinal use, while the seed is typically used as coffee (Barbosa-Ferreira *et al.*, 2005).

MATERIALS AND METHODS

Sampling area and Sample collection

Senna occidentalis sample was collected along Zaria road, Karfi, in Kura Local Government Area, Kano state, Nigeria during November 2020. The plant was identified by botanist at the herbarium section of the Department of Biological Sciences, Bayero University, Kano, where an Accession Number BUKHAN 0073 was assigned to it.

Processing of Plant materials

Stems of *S. occidentalis* were collected in bulk for preparation of extracts. The stem collected was air dried at room temperature in the Department of Biochemistry, Bayero University, Kano, Nigeria for 16 days for use in the determination of phytochemicals and

anti-malarial properties.

Extraction of the Phytochemicals

The extraction method used was maceration. The sample was ground into coarse powder using mortar and pestle and stored in an air tight bottle prior to use for analysis. After weighing, 150g of the ground sample of the stem bark was obtained. It was dissolved in 1000 ml of aqueous solution in an air tight bottle. The sample was shaken and kept for 48 hrs. The extract was filtered using a chess cloth and Whatman filter paper No. 1 (24 cm), to obtain filtrates of aqueous. It was allowed to evaporate for 16 days and the residue was weighed.

Phytochemical Screening

Qualitative phytochemical analysis was carried out to identify secondary metabolites such as alkaloids, carbohydrates, cardiac glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins and amino acids using standard procedures.

Detection of Alkaloid

Filtrates were treated with Wagner's reagent (Iodine in Potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloid (Tiwari *et al.*, 2011).

Detection of Carbohydrates

Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars (Tiwari *et al.*, 2011).

Detection of Cardiac Glycosides

To 2 ml of filtrates, 1ml of glacial acetic acid was added followed by the addition of 1ml ferric chloride. And then 1ml concentrated sulphuric acid was added. Formation of green-blue colouration or brown-green indicates the presence of cardiac glycosides (Parekh and Chanda, 2007).

Detection of Saponins

Exactly 0.5mg of extract was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins (Tiwari *et al.*, 2011).

Detection of Phenols

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols (Tiwari *et al.*, 2011).

Detection of Tannins

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins (Tiwari *et al.*, 2011).

Detection of Flavonoids

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids (Tiwari *et al.*, 2011).

Detection of Proteins and Amino Acids

The extracts were treated with few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins (Tiwari *et al.*, 2011).

Malaria Parasite Assay

Sourcing of Malaria Parasites for the Assay

Malaria parasites infected blood samples were obtained. Bayero University Old campus clinic at Kano provided clinical blood samples containing heavy parasitemia of *Plasmodium falciparum*. The samples were immediately transferred into K3- EDTA disposable plastic sample bottles with tightly fitted plastic corks, mixed thoroughly and then transferred to the Microbiology laboratory at Bayero University, Kano in a thermoflask containing water maintained at 4°C as demonstrated by Dacie (1968).

Confirmation of *Plasmodium falciparum* + Blood Samples using Thin Smear Method

After thorough mixing, a small drop of each blood sample was placed at the centre of a clean grease- free glass slide, at least 2cm from the edge using a clean capillary tube. A clean cover slip was placed in front of each drop at an angle about 45° and then drawn backward to be in contact with the drop of blood, the drop was then allowed to run along the full length of the edge of the cover slip. With a fast and smooth movement, the cover slip was pushed forward to form even thin smear on each glass slide. The smears were immersed in a petri-dish containing absolute methanol for 30 seconds, and then covered with several drops of Giemsa's stain for 10 minutes. Then excess stain was washed with a clean tap water, and the smears were air dried on a rack with the glass slides kept upside down (Cheesbrough, 2009). The air dried smears were observed under a microscope (CETI Belgium, BUK. BIO 99.01) using a high power objective (×100) under an oil immersion. The smears were traversed thoroughly for *P. falciparum* infected red blood cells. An average parasitemia was obtained from the reading of 3 microscopic fields (Hanne *et al.*, 2002). Blood samples with 5% parasitemia, were used for the assay. 5% parasitaemia was obtained by taking the average number of infected RBCs to total number of RBCs.

% = average number of infected RBCs

Total number of RBCs (Muktaret *et al.*, 2006).

Total RBCs = average number of infected blood + average number of non infected blood

Separation of the Erythrocytes

0.05ml of 5% dextrose solution was added to each 5ml of the blood samples and defibrinated, and then centrifuged at 2500 rpm for 15 minutes (in a spectra merlin centrifugation machine). The supernatant layers were discarded, and the sediments were diluted with normal saline (red blood cells diluting fluid) (Dacie, 1968), and centrifuged at 2500 rpm for 10 minutes. Similarly, the supernatants were discarded, and the samples with higher parasitemia (above 5%) were diluted with fresh malaria parasite negative erythrocytes (Hanne *et al.*, 2002).

Preparation of *Plasmodium falciparum* Culture Medium

The media was prepared by dissolving 10.4g of the powdered material into one litre

of sterile distilled water. Add 2 gram Sodium Bicarbonate (or 26.67 ml of 7.5% Sodium Bicarbonate Solution). Venous blood (2ml) from the main vein of white healthy rabbit's pinnae was withdrawn using a disposable 5ml syringe (BD 205 WG). This was defibrinated by allowing it to settle for at least one hour (Dacie and Lewis, 1968). The defibrinated blood was centrifuged at 1500 rpm using spectra merlin centrifuge for 10mins and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500 rpm for 5mins 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 (Trager, 1982). Adjust pH to 0.2-0.3 units below the required pH using 1N HCl or 1N NaOH.

Preparation of the Test Concentrations

An electronic digital balance was used to measure 40mg of each of the extracts and then dissolved in 1ml of dimethylsulphoxide (DMSO) in separate vials (stock solution). Using serial doubling dilution, five different concentrations (20mg/ml, 10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml) of each extract were prepared (Imam *et al.*, 2015).

An *in-vitro* Assay of the Activity of the Extracts on *Plasmodium falciparum* Culture

Exactly 0.1ml of test solution and 0.2ml of the culture medium were added into a tube containing 0.1ml of 5% parasitemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to the tested fractions was determined microscopically after incubation for 24, 48 and 72 hours at 37°C. The incubation was undertaken in a bell jar glass containing a lighted candle to ensure the supply of required quantity of 5% carbon dioxide, 2% oxygen gas, and about 93% nitrogen gas as demonstrated by Muktar *et al.*(2006).

Determination of Activity of Antimalarial

At the end of each incubation period, a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and stained by Geimsa'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 average percentage elimination by the samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasites after each incubation period, using the formula below;

$$\% = \frac{N}{N_x} \times 100$$

Where, % = percentage activity of the extracts

N = Total number of cleared RBC

N_x = Total number of parasitized RBC

[RBC = Red Blood Cells]

(Muktar *et al.*, 2006).

RESULTS

Percentage yields and physical properties of the four extract of *S. occidentalis* stem and leaf are presented in Table 3.1. The aqueous stem extract (ASE) and leaf extract (ALE) of the plant

was a reddish brown, which were gummy. The ethanol stem extract (ESE) and leaf extract (ELE) of the plant was greenish black and powdery. The result showed that the ASE and ESA yielded 6.00% and 3.55% respectively, while ALE and ELE yielded 5.34% and 5.20% respectively.

The result of phytochemical screening of aqueous and ethanol leaf extract showed (Table 3.2) the presence of alkaloids, cardiac glycosides, saponins, phenols, protein and amino acids, carbohydrates and flavonoid. However, tannin was found to be absent. While aqueous and ethanol stem extracts showed the presence of alkaloids, cardiac glycosides, saponins, protein and amino acids, carbohydrate and flavonoid. However, tannins and phenols were found to be absent.

The result of anti-malarial activity of aqueous and ethanol stem extracts is tabulated at Table 3.3. 20mg/ml of the aqueous stem extracts showed highest elimination of the parasites at the end of the incubation period, with percentage elimination of 77%, while 72% for aqueous leaf. The least activity was observed with stem ethanol extract at 1.25mg/ml which yielded a percentage elimination of only 35%.

Table 3.1: Percentage yield and Physical Properties of Stem Bark Extracts from *Senna occidentalis*

Property	ASE	ESE	ALE	ELE
Weight of plant sample (g)	140	150	120	100
Weight of plant extract (g)	8.40	5.34	6.44	5.20
Percentage yield (%)	6.00	3.55	5.34	5.20
Colour of extract	Reddish-brown	Greenish-black	Reddish brown	Greenish black
Texture of extract	Gummy	Powdery	Gummy	Powdery

Abbrv: ASE=Aqueous stem bark extract, EBE =Ethanol stem bark extract, ALE = Aqueous leaf extract and ELE= Ethanol leaf extract

Table 3.2: Phytochemical Constituents of Aqueous leaf Extract and Ethanol leaf Extract of *Senna occidentalis*

Phytochemicals	Leaf		Stem	
	Aqueous	Ethanol	Aqueous	Ethanol
Alkaloids	+	+	+	+
Carbohydrate	+	+	+	+
Cardiac glycosides	+	+	+	+
Flavonoids	+	+	+	+
Phenols	+	+	+	-
Proteins and amino acid	+	+	+	+
Quinone	+	+	+	+
Saponins	+	+	+	+
-Tannins	-	-	-	-

Key: + = present , - = absent

Table 3.3: *In-vitro* Anti-malarial Activity of *S. occidentalis* Aqueous and Ethanol Stem Extracts with percentage Elimination after 24, 48 and 72 hours of Incubation

Solvent	Concentration (mg/ml)	Average no. of parasite before incubation	Average no. of parasite during incubations			Average no. of parasite after incubation.	Percentage elimination at the end of incubation
			24hrs	48hrs	72hrs		
	PC (Arthem)	10	2.0±1.0	1.0±1.0	0.3±0.6	1.1±0.9	89
	NC	10	17.0±1.0	18.3±2.0	24±2.6	19.8±3.7	0
Aqueous	20	10	2.7±0.6	2.3±0.6	2.0±1.0	2.3±0.5	77
	10	10	2.7±0.6	2.3±0.6	2.3±0.6	2.4±0.2	76
	5	10	3.7±0.6	3.3±0.6	2.7±1.5	3.2±0.5	68
	2.5	10	3.7±0.6	4.0±1.0	3.3±0.6	3.7±0.4	63
	1.25	10	4.3±0.6	3.7±2.9	4.3±2.1	4.1±0.3	59
	Ethanol	20	10	3.7±0.6	2.7±0.6	2.7±0.6	2.9±0.6
	10	10	3.7±0.6	3.3±0.6	3.3±1.5	3.4±0.2	66
	5	10	4.3±1.5	4.7±0.6	4.3±1.5	4.4±0.2	56
	2.5	10	6.0±1.0	5.7±0.6	5.0±2.0	5.6±0.5	44
	1.25	10	6.7±1.5	6.7±1.2	6.0±1.0	6.5±0.4	35

Key: PC = positive control, NC = negative control

Table 3.4: *In vitro* Antimalarial Activity of *S. occidentalis* Aqueous and Ethanol Leaf Extracts with percentage Elimination after 24, 48 and 72hours of Incubation

Solvent	Concentration (mg/ml)	Average no. of parasite before incubation	Average no. of parasite during incubation				Average no. of parasite after incubation.	Percentage elimination at the end of incubation
			48hrs	24hrs	48hrs	72hrs		
Aqueous	20	10	3.3±0.6	2.7±0.6	2.3±0.6	2.8±0.5	72	
	10	10	4.3±0.6	3.6±0.6	2.7±0.6	3.6±0.8	64	
	5	10	4.3±0.6	4.0±2.0	4.0±1.0	4.1±0.2	59	
	2.5	10	4.7±0.6	4.0±0.6	3.7±2.1	4.2±0.5	58	
	1.25	10	5.3±0.6	4.7±0.7	3.3±0.6	4.4±0.6	56	
Ethanol	20	10	3.7±0.6	3.3±0.6	3.0±0.0	3.3±0.4	67	
	10	10	3.7±0.6	3.7±0.6	3.0±1.7	3.5±0.4	65	
	5	10	4.3±1.5	4.0±1.0	4.0±1.0	4.0±0.6	60	
	2.5	10	5.3±1.0	4.7±0.6	4.7±0.6	4.8±0.5	52	
	1.25	10	6.0±1.0	5.7±1.2	5.7±1.2	6.0±0.5	40	

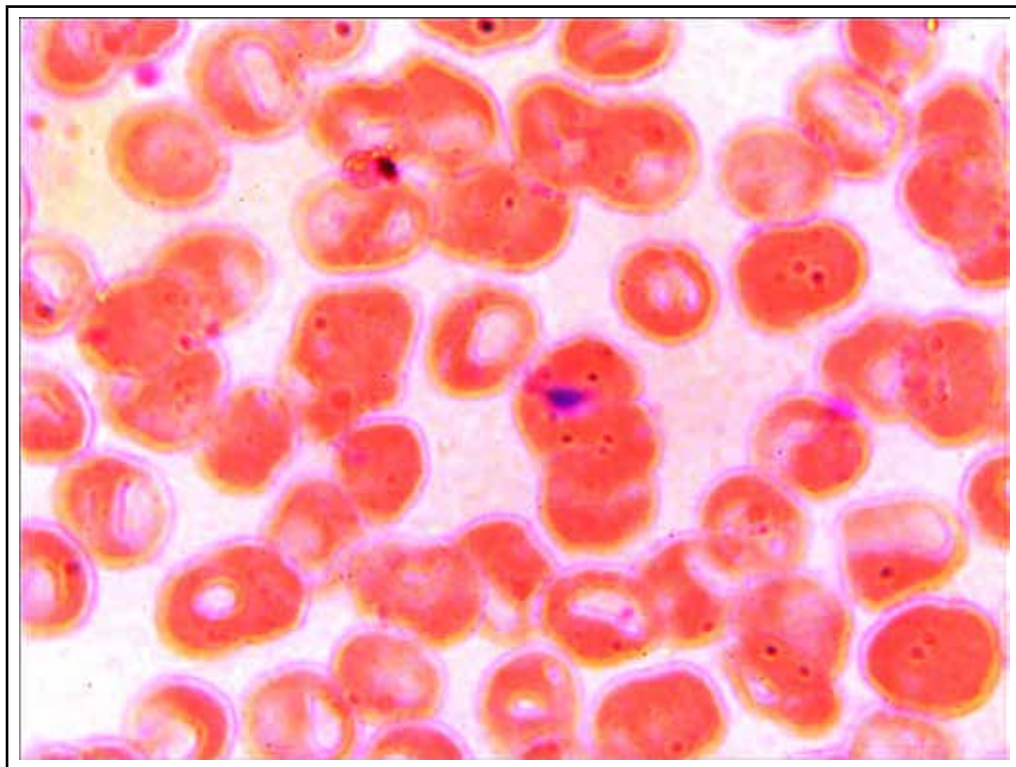


Image 1: Infected RBCs

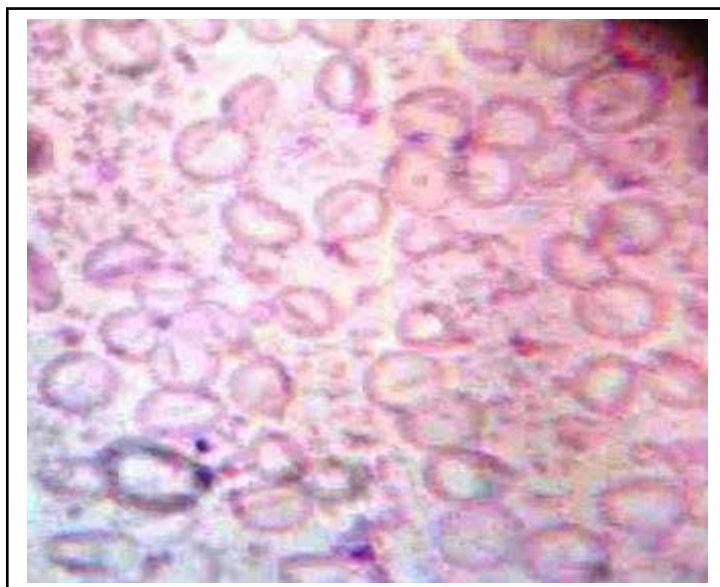


Image 2: Activity of Aqueous stem extract after 72 hours incubation which shows highest percentage elimination of 77%

DISCUSSION

Phytochemical analysis is very useful in the evaluation of some bioactive components of some plants (Anwar *et al.*, 2007). Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract which may also be used to search for bioactive agents that could be used in the synthesis of very useful drugs (Sibanda and Okoh, 2008). The result of phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, cardiac glycosides, tannins, steroids and saponin in leaf and stem of *Senna occidentalis* as shown in Table 4.1 and 4.2. The result of the study revealed that *Senna occidentalis* leaf and stem can be linked with the report of Aja *et al.* (2010) which showed that *Talinum triangulare* leaves are rich in phytochemicals. This result also supported the reports of Aja *et al.* (2015) that revealed high levels of phenols and other phytochemicals in *Dissotis rotundifolia* and *Cajanus cajan* leaves and seeds. Aja *et al.* (2011) have revealed that *Moringa oleifera* leaf is a good source of phytochemicals which is in line with the present result on *Senna occidentalis* leaves. This result does not correlate with the report of Nwali *et al.* (2012) which showed that *Bryophyllum pinnatum* leaf contained low levels of phytochemicals.

Phenolics and flavonoids act as cytoplasmic poisons with their inhibitory effect on cellular enzymes (Iwu, 1985). Flavonoids had revealed significant anti-parasitic activities against various strains of malaria parasite, trypanosome and leishmania (Al-Adhroeyb *et al.*, 2010). Flavonoids also are known for their anti-microbial, cytostatic and anti-inflammatory properties (Hodek *et al.*, 2002).

Phenols can be used in reduction of risk for infection in minor skin irritations and also kills germs. Phenols can improve effectiveness at relieving of itching and it can be added to lotion meant for the relief of insect bites and sunburn and other painful itching skin conditions (Burkill, 1995). Appreciable amount of flavonoids was found in *Senna occidentalis* leaf. This indicates that *Senna occidentalis* leaves could be a good source of flavonoids and as such is good for the management of cardiovascular diseases and oxidative stress, since flavonoids are biological antioxidants; flavonoids provide protection against these diseases by contributing along with antioxidants, vitamins and enzymes to the total antioxidant defense system to human body (Carson and Riley, 2003). Flavonoids possess substantial anti-mutagenic and anti-carcinogenic activities due to its antioxidant and anti-inflammatory properties (Gills, 1992). High level of alkaloids in *Senna occidentalis* plant obtained in the result showed that *Senna occidentalis* is a good source of alkaloids. Alkaloids have been implicated in inducing a stress response and apoptosis in human breast cancer cell (Harborne, 1973). Alkaloids which are nitrogen-containing naturally occurring compounds commonly found to have anti-microbial properties (Hou and Kumamoto, 2010). The alkaloids can be used as a central nervous system stimulant as well as powerful pain relievers (Jain *et al.*, 2008).

From Table 4.3 it can be seen that the average number of parasites after incubation increased as the concentration of the extract was reduced. This indicates that higher concentrations of the extracts were found to be more effective on the parasites. The elimination rate for both the extracts was seen to be almost rapid within the first 24hrs, which then goes somehow steady within the next incubation periods. Besides, previous studies on *J. curcas* leaves showed that it contained some phytochemicals which includes alkaloids, saponins, tannins, flavonoids,

phenols, glycosides, steroids and terpenoids. The aqueous and methanolic extracts showed a remarkable antiplasmodial activity with great percentage eliminations and the result revealed that both extracts of *J.curcas* exhibited some anti-malarial activity with a maximum of 95.4% Plasmodia elimination (Imam *et al.*, 2016).

However in this study, 20mg/ml of the aqueous stem extracts showed highest elimination of the parasites at the end of the incubation period, with percentage elimination of 77%, while 72% for aqueous leaf. The least activity was observed with stem ethanol extract at 1.25mg/ml which yielded a percentage elimination of 35%. This shows that the stem of *S occidentalis* could be more effective than the leaf of other plants in the treatment of malaria. Also the aqueous extract was observed to have higher percentage elimination at the various concentrations when compared with the ethanol extract. This is suggested to be due to the aqueous extract having more phytochemicals than in the ethanol extract. These observations suggest that the activity of the extracts may be cytotoxic for the malaria parasite, there by inhibiting their growth and development.

CONCLUSION

The study revealed that *Senna occidentalis* leaf is a good source of phytochemical and this may inform the use of this plant in ethno-medicine. These secondary metabolites may contribute to the medicinal important of the plant and its effective use in malarial management. We have found how the highest anti-malarial activity was found in aqueous stem with highest percentage elimination of 77% after incubation. Also the lowest activity of the extract was ethanol stem with lowest percentage elimination of 35% after 72 hours of incubation. Hence, stem of the *S. occidentalis* has higher activity against Plasmodium in comparison to the leaf.

Declarations: Not applicable

Funding: The present research paper does not involve any financial support.

Conflict of Interest/competition interests: Authors do not have any competitive interests in the research.

Availability of Data and Material: The data are original.

Authors Contribution: The data was conceptualized by AUY; analysis and investigation by AMSY.

Ethics Approval: There was no need of ethical clearance as no animal species were utilized for the study

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