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The Potency of African Locust Bean Tree as Antimalarial

[1]M.I. Builders, [2]F. Tarfa and [3]J.C. Aguiyi
[1]Department of Pharmacology and Therapeutics, College of Health Sciences, Bingham University, PMB 005, Karu, Nigeria
[2]Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development (NIPRD), PMB 21, Abuja, Nigeria
[3]Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, PMB 2084, Jos, Nigeria

Corresponding Author: Modupe Iretiola Builders, Department of Pharmacology and Therapeutics, College of Health Sciences, Bingham University, Karu, Nassarawa, Nigeria Tel: +2348054629053

ABSTRACT

This study was aimed to evaluate the antiplasmodial and antipyretic activities of the stem bark of African locust bean tree. The stem barks of African locust bean tree were extracted with methanol to obtain methanol extract. The antipyretic, acute toxicity, chemical constituents, antioxidant properties as well as the trace metallic content of this extract were determined. The extract was also subjected to column chromatography to obtain four fractions, these fractions were preliminary tested for antiplasmodial potency and methanol fraction which gave the most potent effect was fully evaluated. Flavonoids, tannins, terpenes, saponins, sterols, phenols and reducing sugars as well as Mg, Ni, Ca, Fe, Zn, Na, K and Cu were detected in the extract. It also showed a strong free radical scavenging activity on DPPH (2,2-diphenyl-2-picrylhydrazyl). An oral median lethal dose (LD_{50}) greater than 5 g kg^{-1} in mice was established for the crude extract and a significant dose dependent antipyretic and inhibition of parasitaemia in suppressive, curative and prophylactic tests. The antiplasmodial and antipyretic activities of the extract were tracked to the methanol fraction when evaluated with rodent malaria model *Plasmodium berghei berghei* and clinical isolates of *Plasmodium falciparum*. studies have established sufficient evidence collaborating the antimalarial activities of the stem bark of African locust bean tree, though the active principles are yet to be identified, further studies to elucidate these are ongoing.

Key words: African locust bean tree, antiplasmodial, antipyretic, antioxidant property, acute toxicity

INTRODUCTION

Malaria is a potentially deadly tropical disease characterized especially by cyclical bouts of fever, chills, shaking and sweating often accompanied by muscle aches and headache. It is a mosquito-borne disease of humans caused by a protozoan of the genus *Plasmodium*. Most of the lethal cases are caused by *Plasmodium falciparum*, the most virulent of the four *Plasmodia* species that infect humans. Infestation with *P. falciparum* is responsible for hundreds of millions of cases and more than one million deaths each year (White, 2004).

The increased attention on malaria is mainly due to the continuing high mortality and morbidity caused by this disease and has been related to both increased resistance of the vector to

most of the available insecticides and the causative parasites to the commonly used antimalarial drugs (Marsh, 1998). To stem the challenge of resistance to antimalarials there has been an intensified drive to develop new chemical entities with antimalarial potential and the plant kingdom remains an in exhaustive source of biochemical entities with useful pharmacological activities. A review of the medicinal plants used in the northern central and southwestern part of Nigeria for the treatment of malaria reveals the rich floral diversity of Nigeria (Idowu *et al*., 2010).

"African locust bean tree" popularly known as the *Parkia biglobosa* (Jacq.) R.Br. ex G. Don belongs in the family Fabaceae formerly Leguminosae and the subfamily Mimosoideae have been used traditionally as food and medicine and are of high commercial value in the south west and northern parts of Nigeria. Many traditional medicine practitioners in Nigeria have identified the stem bark and leaf of African locust bean tree as a condiment of great value in the treatment of malaria (Shao, 2002; Builders *et al*., 2011a). The stem bark is boiled and taken orally in form of decoction for malaria fever; there are also reports on the use of the bark of African locust bean tree, for the treatment of fever in Ghana (Asase *et al*., 2005; Igoli *et al*., 2005; Kayode *et al*., 2009). The establishment of its anti-inflammatory and analgesic activities only partially collaborate the claimed efficacy by the Traditional Medicinal Practitioners in Nigeria for the treatment of malaria (Gronhaug *et al*., 2008).

The aim of this study is to evaluate the antiplasmodial and antipyretic activities of the stem bark of African locust bean tree so as to authenticate its wide spread use by Traditional Medicinal Practitioners, especially in north central Nigeria for the treatment of malaria.

MATERIALS AND METHODS

Sample collection and identification: The stem barks of African locust bean tree were collected in February, 2009 in Chaza village in Niger state of Nigeria. The identification and authentication were done by (Ethno botanist) Mallam Muazam Wudil of department of Medicinal Plant Research and Traditional Medicine of National Institute for Pharmaceutical Research and Development, (NIPRD), Abuja, Nigeria where a voucher specimen (NIPRD/H/6228) was deposited at the herbarium for reference. The stem barks were cleaned, dried at 50°C in a hot air oven (Salvis ISG 160, Germany) for 72 h and milled into a coarse powder.

Chemicals and reagents: All chemicals were purchased from Sigma-Aldrich, USA.

Extraction of plant materials

Methanol extract: A 200 g quantity of the coarse powder was extracted with 2 L methanol for 48 h using a Soxhlet apparatus (Quicket, UK). The extract was filtered through Whatman No. 1 (Whatman International Ltd., Maidstone, UK) paper and evaporated under reduced pressure using a rotary evaporator to a yield of 20.17% w/w referred as crude extract. The dried extract was stored at 4°C until used.

Fractionation of the methanol extract: The methanol extract was fractionated successively with hexane, ethyl acetate and methanol. A total of 34 fractions (100 mL each) were collected and combined into four main groups (MF1 to MF4) on the basis of their Thin Layer Chromatography (TLC) profiles. These combined fractions (MF1 to MF4) were concentrated over water bath and allowed to evaporate to dryness at room temperature. The fractions were preliminary tested for antiplasmodial potency and MF4 (methanol fraction) which gave the most potent effect was fully evaluated (Adzu *et al*., 2007).

Identification of the chemical constituents: The methanol extract and the methanol fractions of the African locust bean stem bark were subjected to qualitative chemical screening for the identification of the various classes of chemical constituents using the method described by Builders *et al.* (2011b). Identification of tannins and flavonoids was further carried out by TLC using pre-coated silica gel 60G F_{254} Merck plates with different eluting systems (Wagner and Bladt, 2004).

Antioxidant potential: Ascorbic acid was used as the antioxidant standard and the methanol extract with methanol fraction were used for the antioxidant assessment. The extract, methanol fraction and ascorbic acid at equal concentrations of, 0.175, 0.25, 0.5, 1 and 2 m mL^{-1} in methanol were prepared. The radical scavenging activities of the extract and methanol fraction against 2, 2-Diphenyl-1-picrylhydrazyl radical were determined by UV spectrophotometry at 517 nm (Ayoola *et al.*, 2008), 1 mL of the extract or methanol fraction was placed in a test tube and 3 mL of methanol was added followed by 0.5 mL of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity presented as percentage inhibition was calculated using Eq. 1:

$$\text{Ihibition (\%)} = \frac{A_b - A_a}{A_b} \times 100 \quad (1)$$

where, A_b is the absorbance of the blank sample and A_a is the absorbance of the drug (methanol extract or methanol fraction or ascorbic acid).

Elemental analysis: The dried extracts 1.0 g was ashes in oven electric muffle furnace maintained at 400 and 450°C, for about 6-7 h to destroy all organic materials present in the sample. The crucible containing pure ash was dried in a desiccator. Thereafter the ash was digested with triple mixture acid: sulfuric acids: sulphuric: perchloric acid (11:6:3) to obtain a clear solution, 2.5 mL 6 M nitric added to ensure complete dissolution. The solution was then made to 25 mL with double distilled water and read up with a flame absorption spectrophotometer (Hitachi Model 80-80 polarize Zeeman Atomic, USA). Sodium and potassium was determined by flame fluorimeter (Miroslave, 1998).

In vivo antiplasmodial study

Animals: Swiss male albino mice (20-25 g body weight) were obtained from the animal facility centre of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and used for the study. The animals were fed *ad libitum* with standard feed and had free access to water (Abuja Municipal water Supply). They were also maintained under standard conditions of humidity, temperature and 12 h light/ darkness cycle. The animals were acclimatized for two weeks before the commencement of the study. A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulations of the ENV (ENV/MC/CHEM, 1998). The principle of laboratory animal care was also followed in this study (NIH Publication No. 85-23, 1985). The acute toxicity of the extract was determined by evaluating its median lethal dose (LD_{50}) using the Lorke method (Lorke, 1983).

Antipyretics studies: The effect of yeast-induced pyrexia on albino Wistar rats was evaluated by determining the body temperatures of the rats by measuring Rectal Temperature (RT) at

predetermined intervals (Al-Ghamdi, 2001). Fever was induced in the rats by injecting 15%w/v suspension of Brewer's yeast (*Saccharomyces cerevisiae*) at a dose of 1 mL kg^{-1} body weight subcutaneously. The rectal temperature of each rat was again determined after 24 h of yeast administration. Rats that did not show a minimum increase of 0.5°C in temperature 24 h after yeast infection were discarded. Thirty five selected rats were grouped into five and immediately treated as follows: group 1 received normal saline, group 2 received acetaminophen (150 mg kg^{-1} b.wt. p.o), while groups 3, 4 and 5 were received extract (25, 50 and 100 mg kg^{-1} b.wt. p.o), respectively. The rectal temperatures of all the rats were then determined by inserting a digital thermometer (Omron Digital fever Thermometer, Omron Health Care, China) into the rectum of each rat at 30 min intervals for 120 min, similar protocol was employed for methanol fraction at 12.50, 25 and 50 mg kg^{-1} doses.

Parasite inoculation: The malaria parasite used was a chloroquine-sensitive strain of *Plasmodium berghei berghei* (NK-65), obtained from the National Institute for Medical Research (NIMR), Lagos, Nigeria and kept at the Department of Pharmacology and Toxicology, NIPRD, Idu, Abuja, Nigeria. The parasites were maintained by serial blood passage in mice (Adzu *et al*., 2007). Parasitized erythrocytes were obtained from a donor- infected mouse by cardiac puncture in heparin and made up to 20 mL with normal saline. Animals were inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 10^7 parasitized erythrocytes on day zero. Infected mice with parasitaemia of 5-7% were allocated to five groups of six mice each (Ishih *et al*., 2004).

***In vivo* antimalarial assay:** A series of experiments were carried out to evaluate the *in vivo* anti-malarial activities of the methanol extract of African locust bean tree stem bark at 25, 50 and 100 mg kg^{-1} doses as compared to control groups treated with 0.2 mL of normal saline and reference groups treated with standard drugs (Chloroquine diphosphate 25 mg/kg/day). Malaria infection was first established in male mice by the intraperitoneal (i.p.) administration of donor male Swiss albino mouse blood containing about 1×10^7 parasites. The three different methods of treating malaria infections, i.e., 4-Day suppressive test, curative and prophylactic methods were applied according to Chung *et al*. (2009), Chandel and Bagai (2010) and Okokon *et al*. (2005), respectively. The laboratory tests were started with oral administrations of the compound in the 4-day suppressive tests (early malaria infection) and further screened for their curative (established malaria infection) and prophylactic (residual malaria infection) activities. Thick blood smears were prepared and blood films were fixed with methanol. The blood films were stained with Giemsa and then microscopically examined with 100-x magnification. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice. Similar protocol was employed for methanol fraction at 12.50, 25 and 50 mg kg^{-1} doses.

Antiplasmodial assay

Patients' selection: Three fresh blood specimens were collected from three patients suffering from fever and other malaria symptoms with confirmed infection by *P. falciparum*. Already prepared dried -in-acridine orange-stained thin smears were examined for *Plasmodium* species identification. The parasite density was determined by counting the number of infected erythrocytes among 20,000 erythrocytes from each patient, 4 mL of venous blood was collected in a tube coated with EDTA. Samples with monoinfection due to *Plasmodium falciparum* and a parasite density between 1 and 2% were used for the *in vitro* antimalarial tests (WHO, 2001).

***In vitro* test:** The assay was performed in duplicate in a 96-well microtiter plate, according to WHO method *in vitro* micro test (Mark III) that is based on assessing the inhibition of schizont maturation. RPMI 1640 (Sigma Company, USA) was the culture medium used for cultivation of *P. falciparum*. Dilutions were prepared from the methanol extract, MF4 and drug concentrations in the wells ranged from 100-3.125 μg mL^{-1} for the methanol extract, from 50 to 1.594 μg mL^{-1} for methanol fraction, from 0.1 to 0.03 μg mL^{-1} for chloroquine phosphate. Fifty micro liters from blood mixture media was added to each well in plate and incubated in a candle jar (with gas environment of about 3% O_2, 6% CO_2 and 91% N_2) (Karou *et al*., 2003; Ogunlana *et al*., 2009) at 37.0°C for 24-30. After incubation, contents of the wells were harvested and stained for 5 min in an already prepared dried -in-acridine orange reagent. The developed schizonts were counted in five fields of vision (>200 total cells) using a fluorescence microscope (Partec cyscope fluorescence microscope, Germany) at a magnification of 40.

Statistical analysis: Data were expressed as the mean±standard error of mean (SEM). Statistical analysis of data was carried out using one-way analysis of variance (ANOVA). The IC_{50} values were determined graphically on a log dose-response curve (log concentration versus percent inhibition curves) by interpolation while those values for the antioxidant activities were calculated from the linear regression of plots of concentration of the test compounds (mg mL^{-1}) against the percentage of inhibition of (2,2-Diphenyl-1-picrylhydrazyl).

RESULTS

Phytochemical tests: The result of the phytochemical screening of the crude methanol extract of African locust bean tree and methanol fraction is presented in Table 1. The analysis revealed the presence of saponins, tannins, terpenes, flavonoids and phenols, reducing sugars and sterols in the crude methanol extract while tannins, terpenes and flavonoids were found in the methanol fraction. However cardiac glycosides, resins, volatile oil and anthraquinones were absent.

Thin layer chromatography: The presence of tannins and flavonoids was further confirmed by thin layer chromatography and their R_f values have been presented (Table 2). Different screening systems were used to obtain better resolution of the components.

Table 1: Phytochemical analysis of the methanol extract and methanol fraction of African locust bean tree stem bark

Chemical compounds	Methanol extract	Methanol fraction
Tannins	+	+
Flavonoids	+	+
Terpenes	+	+
Alkaloids	-	-
Saponins	+	-
Phenols	+	-
Sterols	+	-
Resins	-	-
Volatile oil	-	-
Anthraquinones	-	-
Cardiac glycosides	-	-
Reducing sugars	+	-

-: Compound not detected, +: Compound detected

Table 2: TLC screening of methanol extract and methanol fraction of African locust bean tree stem bark

Drug	Tannins (R_f values)	Flavonoids (R_f values)	Detection reagent	Colour of spots
Methanol extract	0.92	0.51	Methanol ferric chloride	Blue, greenish blue
	0.97	069		
Methanol fraction	0.89	0.54		
	0.97	0.81		
Standard*	0.97	0.50		

*:Catechin (tannin) and Rutin (flavonoid)

Table 3: Elemental analysis of the methanol extract of African locust bean tree stem bark

Elements	Conc. (mg/100 g)
Sodium	-
Potassium	1.26±0.10
Calcium	60.4±0.23
Magnesium	300.8±0.64
Manganese	18.1±0.01
Iron	10.1±0.45
Copper	1.02±0.88
Zinc	9.55±0.37
Cadmium	-
Arsenium	0.11±0.94
Lead	0.03±0.72
Nickel	3.01±0.39

Value are Mean±SE

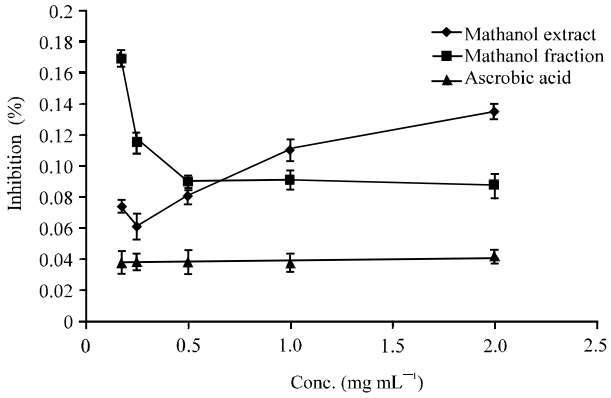

Fig. 1: Antioxidant properties of methanol extract and methanol fraction of African locust bean tree in relation to ascorbic aid

Antioxidant potential: Figure 1 shows the antioxidant potential of the crude extract of African locust bean tree and methanol fraction as determined by their inhibition of the free radical activities of DPPH. Ascorbic acid was used as the prototype antioxidant agent. The antioxidant activities of the methanol extract and methanol fraction were concentration dependent, increasing with concentration. The sensitivity of the antioxidant activity of the methanol fraction was higher than that of the crude un-fractionated methanol extract.

Elemental analysis: Table 3 shows the metallic elements detected in the methanol extract of African locust bean tree. Magnesium was the most abundant macro element (300.8 mg/100 g) closely followed by Calcium (60.40 mg/100 g) and the least was Nickel (3.01 mg/100 g). Amongst

the micro elements, manganese was most abundant (18.10 mg/100 g) while the contents of zinc and iron were (9.55 to 10.10 mg/100 g). The Arsenium and lead contents were very low, Cadmium was not found in the extract.

Acute toxicity tests: There was no mortality in animals at all doses of the extract up to 5000 mg mL^{-1}. The absence of death at doses up to 5 g extract/kg showed that LD_{50} of the methanol extract of African locust bean tree is greater than 5 g kg^{-1} p.o. Rubbing of nose and mouth on the floor of the cage and restlessness were the only behavioral signs toxicity shown by the animals, these disappeared within 24 h of extract administration.

Antipyretic studies: The methanol extract caused a reduction dependent decrease in rectal temperature at the highest dose of 100 mg kg^{-1}, the effect became significant at 30 to 90 min. The reduction was comparable with that of acetaminophen. Methanol extract caused inhibition of the pyrexia induced yeast after 30 min at variable doses (25-50 mg kg^{-1}, p.o). Methanol fraction exhibited a significant reduction in the yeast induced elevated temperature after treatment from 60-120 min, the inhibition was comparable with that of acetaminophen (Table 4).

***In vivo* antiplasmodial study:** Figure. 2 shows the antiplasmodial activities of the crude methanol extract and methanol fraction of the stem bark of African locust bean tree in relation to chloroquine. The crude extract and methanol fraction exhibited dose dependent reduction in parasitaemia at the different doses administered. Methanol fraction showed higher inhibition (93.29±1.2%, 96.28±1.4% and 100±1.0) of parasitaemia.

Table 4: Antipyretic properties of methanol extract and methanol fraction of African locust bean tree stem bark

Drug (mg kg^{-1})	BBT (°C)	0.0 h	0.5 h	1.0 h	1.5 h	2.0 h
Control	37.31±0.9	38.56±0.27	38.38±0.29	38.40±0.10	38.19±0.34	38.42±0.1
M. extract (25)	37.58±0.11	38.60±0.17	38.43±0.09	38.62±0.21	38.29±0.41	38.17±0.52
M. extract (50)	37.47±0.21	38.56±0.0	38.42±0.14	38.50±0.01	38.14±0.15	38.02±0.35
M. extract (100)	37.49±0.32	38.18±0.23	37.83±0.12*	37.88±0.30*	37.81±0.44*	38.00±0.38
M. fraction (12.5)	37.66±0.26	38.80±0.20	37.65±0.17	38.49±0.19	38.40±0.27	38.28±0.23
M. fraction (25)	37.60±0.11	38.63±0.16	38.52±0.10	38.41±0.24	37.91±0.17*	37.98±0.18*
M. fraction (50)	37.42±0.19	38.57±0.9	38.29±0.26	37.95±0.13*	37.92±0.19*	37.95±0.17*
PCM (150)	37.53±0.29	38.60±0.11	38.55±0.22	37.99±0.10*	37.81±0.20*	37.95±0.16*

M.: Methanol, PCM: Acetaminophen, *Significantly different from the control at $p<0.05$, BBT: Basal body temperature

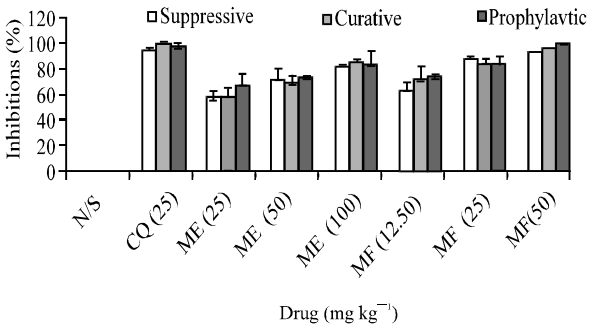

Fig. 2: *In vitro* suppressive, curative and prophylactic antiplasmodial activities of methanol extract (ME) and methanol fraction (MF) of African locust bean tree

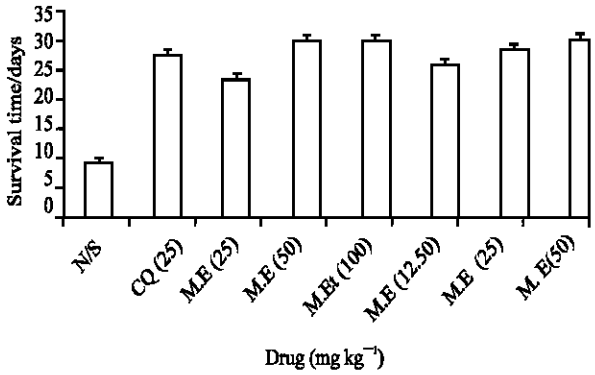

Fig. 3: Mean survival period of mice treated with methanol extract and (ME) methanol fraction (MF) of African locust mean tree

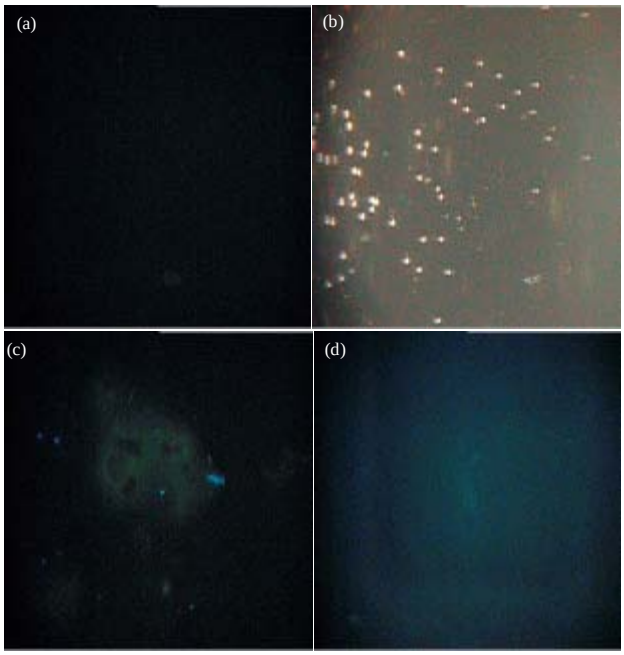

Fig. 4(a-d): Photomicrograph of the *in vitro* antiplasmodial activities of the methanol extract and methanol fraction, (a) Complete RPMI medium, (b) Untreated RPMI medium with *O. falciparum,* (c) RPMI medium treated with methanol extract (d) and RPMI medium treated with methanol fraction

Mean survival time: Figure 3 shows the mean survival time for the extract and fraction treated mice were dose dependent and ranged from 23.4±1.6-30.0±1.0 days. Mice treated with 50 and 100 mg kg^{-1} extract survived the total duration of the study, similar observation was observed with mice treated with 50 mg kg^{-1} of the fraction.

***In vitro* antiplasmodial activity:** The photomicrographs of the *in vitro* antiplasmodial activities of the methanol extract and methanol fraction are presented in Fig. 4. The crude methanol extract

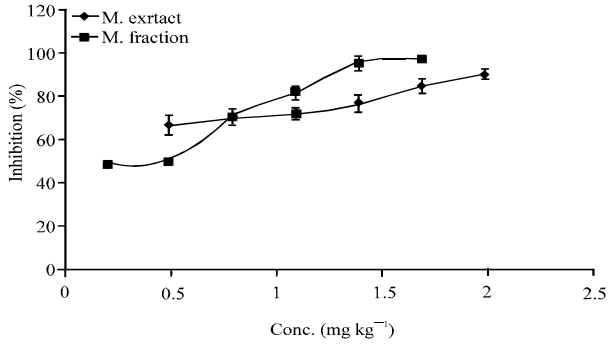

Fig. 5: *In vitro* antiplasmodial activities of methanol (M.) extract and methanol fraction of African locust bean tree

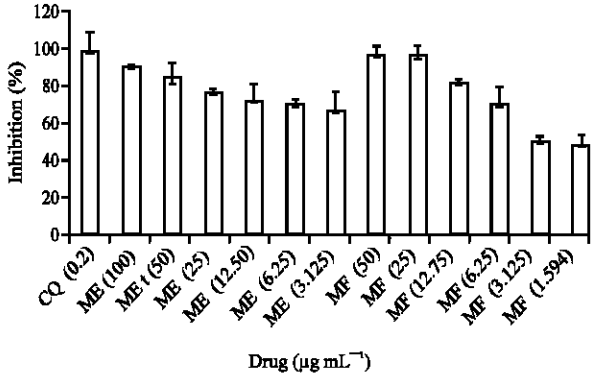

Fig. 6: *In vitro* antiplasmodial activities of methanol extract (ME) and methanol fraction (MF) of African locust bean tree versus chloroquine

and methanol fraction showed concentration dependent growth inhibition of *P. falciparum* (Fig. 5). The IC_{50} of the crude extract and methanol fraction were 12.92 and 0.12 µg mL^{-1}, respectively. The maximum plasmodia inhibitions were 90±1.2% and 96.7±1.0 for the crude extract and methanol fraction respectively and 98.5±1.0% for chloroquine phosphate (Fig. 6).

DISCUSSION

Extraction was carried out to remove impurities or recover a desired product by dissolving the plant materials in methanol which have certain selectivity for the extracted materials. (Scholz *et al*., 2000; Parekh *et al*., 2006). With sequential solvents extraction by fractionation, which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol), a wide polarity range of compound was extracted (Kuntal *et al*., 2010), it therefore possible to isolate the most active fraction (Methanol fraction).

Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor *et al*., 2001). Therefore, the traditional use of the stem bark of African locust bean tree for the treatment of malaria could be attributed to the presence of certain phytochemicals that constitute the bioactive

principles in the plant. Numerous plants containing a wide variety of phytochemicals as their bioactive principle have shown antiplasmodial activities (Francois *et al.*, 1996; Kim *et al.*, 2004; Monbrison *et al.*, 2006).

Thin layer chromatographic studies showed the confirmation of active principles like tannin and flavonoids on 0.97 and 0.54 Rf values for methanol fraction which were more close to standards 0.97 and 0.54 Rf with prominent blue and greenish blue coloration in both.

Many secondary plant substances had been assessed either for *in vitro* activity against *P. falciparum* or *in vivo* activity against *P. berghei* (Saxena *et al.*, 2003; Fidock *et al.*, 2004). Tannins, flavonoids and terpenes (Jimoh and Oladiji, 2005; Alshawsh *et al.*, 2007) are the classes of compounds possessing antimalarial activities.

The presence of flavonoids and tannins in the African locust bean is likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Ayoola *et al.*, 2008; Al-Adhroey *et al.*, 2010). The antioxidant effect of the African locust bean may represent another mechanism that contributes to its antimalarial activity.

Elemental analysis of the extract indicated the presence of pharmacologically useful trace metal elements with established usefulness various body functions. These elements are used extensively in chemotherapy and are essential in human and animal health. The mineral Mg detected in this plants are involved in over 300 enzymatic reactions of the body involving glycolysis, Krebs cycle, nucleic acid synthesis, amino acid activation, muscle regulations and protein synthesis. Thus due to high content of Mg, African locust bean tree may have the potential to cure malaria (Moses *et al.*, 2002; Koche, 2011).

WHO recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like arsenic, cadmium and lead, which amount to 1.0, 0.3 and 10 ppm, respectively (WHO, 1998), these values are within the range found in our sample. Quinine- Zinc complex had been confirmed to have 3 times antimalarial potency over Quinine sulphate (Ogunlana *et al.*, 2009) Iron, Manganese and copper are important trace elements in the human body, they play crucial roles in haemopoiesis, control of infection and cell mediated immunity (Anhwange *et al.*, 2004; Oluyemi *et al.*, 2006), therefore the presence of these minerals contributes to the antimalarial activity of African locust bean tree.

The acute toxicity of African locust bean tree has been investigated to determine any adverse effect that may arise as a result of a single contact or multiple exposures in a short time within 24 h period. Though African locust bean tree has been used by TMPs without any mortality due to toxicity, this claim has been authenticated by the lack of death at oral treatment of over 5000 mg kg^{-1} b.wt. of the extract. The results thus suggest that the methanol extract of the stem bark of African locust bean tree is has low toxicity (Subhan *et al.*, 2008; Builders *et al.*, 2012).

The ability of African locust bean tree to reduce the experimentally elevated body temperature shows that African locust bean tree possesses significant antipyretic effect on yeast induced pyrexia. The reduction in yeast induced fever by African locust bean tree might be due to its influence on the prostaglandin biosynthesis since it is involved in the regulation of body temperature. In general, it is believed that several mediators and multiple processes play a vital role in the pathogenesis of fever. Inhibition of any of these mediators is said to bring about antipyresis and as to how they interfere with PG synthesis is not clearly established (Aronoff and Neilson, 2010).

Methanol fraction exerted similar suppressive, prophylactic and curative antiplasmodial activities with chloroquine by the extent of inhibition of parasitemia, the African locust bean tree extract also indicated similar antiplasmodial activities however to a lower potency. The extract with its fraction also exhibited dose dependent chemo-suppressive and curative activities and also enhanced the mean survival time period of the treated mice particularly the group administered with the 100 mg/kg/day of the extract.

The IC_{50} of the methanol extract and methanol fraction of African locust bean tree were determined as 12.92 and 0.12 μg mL^{-1}. According to the norm that active extract has IC_{50}< 5 μg mL^{-1} and moderate active extract 5 μg mL^{-1}< IC_{50}<50 μg mL^{-1} (Rasoanaivo *et al*., 1992), the methanol fraction of African locust bean tree could be considered active while the methanol extract is moderately active when compared with that of the standard, chloroquine phosphate (0.050 μg mL^{-1}). The antiplasmodial activity of the plant was found to reside majorly in methanol fraction, this may be indicative of a significant potential for isolating purer compound.

CONCLUSION

The phytochemical assessments of the extract and purified fraction of the stem bark of African locust bean tree showed the presence of phytochemicals with established antimalarial activities. The high value of its LD_{50}, antipyretic as well as its effective *in vivo* and *in vitro* antiplasmodial activities explains its safety and effectiveness in its use for the treatment of malaria. Our studies have established sufficient evidence collaborating the antimalarial activities of the stem bark of African locust bean tree though the active principles are yet to be identified, further studies to elucidate these are in progress in our laboratories.

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