

**IN VITRO ANTIPLASMODIAL, ANTITRYPANOSOMAL, ANTILEISHMANIAL AND CYTOTOXIC
ACTIVITIES OF VARIOUS FRACTIONS OF *ABRUS PRECATORIUS* LEAF**

Formatted: Numbering: Restart each page

AUTHORS: DR. SAGANUWAN ALHAJI SAGANUWAN¹
PROF. PATRICK AZUBUIKE ONYEYILI¹
PROF. IGOCHE GEORGE AMEH²
DR. NGOZI JUSTINA NWODO³
PROF. RETO BRUN⁴

1. DEPARTMENT OF VETERINARY PHYSIOLOGY,
PHARMACOLOGY AND BIOCHEMISTRY,
COLLEGE OF VETERINARY MEDICINE,
UNIVERSITY OF AGRICULTURE,
P.M.B. 2373, MAKURDI,
BENUE STATE, NIGERIA.
2. DEPARTMENT OF PARASITOLOGY AND MEDICAL MICROBIOLOGY,
COLLEGE OF HEALTH SCIENCES,
USMANU DANFODIYO UNIVERSITY,
P.M.B. 2254, SOKOTO,
SOKOTO STATE, NIGERIA.
3. DEPARTMENT OF PHARMACEUTICAL AND MEDICINAL CHEMISTRY,
FACULTY OF PHARMACEUTICAL SCIENCES
UNIVERSITY OF NIGERIA NSUKKA,
ENUGU STATE, NIGERIA.
4. Swiss Tropical and Public Health Institute, Basel, Switzerland

CORRESPONDING AUTHOR: DR. SAGANUWAN ALHAJI SAGANUWAN

E-mail: pharm_saga2006@yahoo.com

Tel: +2347039309400

+2348027444269

ABSTRACT

Background

Infectious diseases are the worst problem in tropical and subtropical regions of the world. The Nupe ethnic group from Nigeria has been using the leaves of *Abrus precatorius* for treatment of malaria and various forms of cancers. However And studies have shown that the plant has antimicrobial and anti-cancer activities. In this study the crude methanolic extract, methanolic, ethyl acetate, chloroform and n-hexane fractions of *Abrus precatorius* leaf were tested *In vitro* against chloroquine and pyrimethamine resistant *Plasmodium falciparum* K1, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovoni* and rat skeletal myoblasts (L-6 cells).

Formatted: Font: Italic

Methods

The leaf ingredients were extracted and separated via methanol, ethyl acetate, chloroform and n-hexane solvents using column chromatography. In vitro activity against erythrocytic stages of *P. falciparum* was determined by a modified [3H]-hypoxanthine incorporation assay. In vitro activities against *T. brucei rhodesiense*, *T. cruzi* and *L. donovoni* and cytotoxicity against L6 cells line were assayed. Regression analysis was adopted for computation of the 50% inhibitory concentrations. The antiprotozoan activity of the extracts was qualified as active when IC₅₀ value was less than 50µg/ml. The extract that showed selectivity index higher than 3.29 was considered to have potential for safer therapy.

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Results

n-hexane fraction showed the best antiplasmodial activity with inhibitory concentration (IC₅₀) value of 12.1 µg/ml followed by chloroform fraction (23.0 µg/ml), crude methanolic extract (30.4 µg/ml) and ethyl acetate fraction (45.9 µg/ml) with selectivity index of 3.66, 1.90, 2.18 and 3.29 respectively. Chloroform fraction showed the best activity against *T. brucei rhodesiense* with IC₅₀ value of 17.9 µg/ml and selectivity index of 2.44 and followed by n-hexane fraction with IC₅₀ (34.5 µg/ml) and selectivity index of 1.28.

Formatted: Font: Italic

Conclusions

Since leaf extract has significant antiplasmodial, antitrypanosomal and antileishmanial activities in vitro, bioassay guided isolation of the active principles can be done with a view to discovering novel drugs for the treatment of malaria, trypanosomiasis and leishmaniasis.

Keywords: *Abrus precatorius*, cytotoxicity, *Plasmodium falciparum*, Leishmania donovoni, *Trypanosoma species*, fractionation.

Formatted: Font: Italic

Background

Infectious diseases are the world's leading cause of deaths, killing almost 50,000 human every day. Malaria occurs in 100 countries with about 40% of the world's population at risk^[1]. Urban and Periurban malaria are now substantial problems in certain areas of Africa and Asia^[2]. Nigeria accounts for a quarter of all malarial cases in the African region, transmission in the Southern part of the country occurs all year round and is more seasonal in the northern part. Despite the increased funding for malaria control in Nigeria which rose from US \$17 million in 2005 to US \$60 million in 2007, provided by the Federal Government, the Global Fund and the World Bank^[3] it is still the cause of the death before the age of 5 years in $\frac{1}{5}$ and $\frac{1}{3}$ of children in Urban and rural areas of Nigeria respectively^[4]. Saganuwan and Adelaiye reported that those between the age range of 0 and 39 years are mostly affected by malaria in the middle belt of Nigeria^[5].

African trypanosomiasis caused by *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* in East, West and Central Africa have put 3.5 million people under surveillance in endemic countries and 55 million people are at risk of infection^[6]. But American trypanosomiasis (Chagas' disease) is caused by *Trypanosoma cruzi* and affects man, opossums, rodents, armadillos, dogs, cats and domestic animals^[7]. *T. cruzi* affects 16 to 18 million people in Latin America and is responsible for death of more than 45,000 patients per year^[8]. The chronic phase typically occurs 10 – 20 years after the parasite infection and affects 10 to 30% of those infected. It is transmitted to human by triatomine bugs or through blood transfusion^[9].

Formatted: Font: Italic

Visceral leishmaniasis is a chronic, severe, protozoan disease of humans, dogs and rodents characterized by cutaneous, mucocutaneous lesions, lymphadenopathy, weight loss, anaemia, lameness, renal failure and occasionally epistaxis or ocular lesions. It is caused by leishmania species including *leishmania donovani*, a causative agent in the Mediterranean area and the Middle East^[7].

Formatted: Font: Italic

Abrus precatorius is a member of *papilionaceae* used to treat microbial infections caused by *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumonia* ^[10-13] and malaria infection caused by *Plasmodium berghei*^[14]. The plant has *In vitro* antiplasmodial activity against *P. falciparum*,^[15] having abruquinone B as the antiplasmodial and cytotoxic principle.^[16]

The constituents of *Abrus* leaf are tannins, saponins, cardiac glycoside, steroid, flavonoid, alkaloid, total glycoside, saponin glycoside and 19.21% oil^[17]. *Abrus precatorius* is being used by Nupes in Bida Emirate of Niger State, Nigeria and proven to have antiplasmodial activity against *P. berghei* in mice needs to be tested *in vitro* against other protozoan parasites including *P. falciparum* with a view to providing effective antiprotozoal drugs.

Formatted: Font: Italic

Methods

Plant materials

Abrus precatorius leaves used for the studies were collected from Ajule in Ankpa local government area of Benue State, Nigeria and identified by Mallam Umaru Denge in Herbarium of Biological Science Department, Usmanu Danfodiyo University Sokoto, Nigeria where a voucher specimen with voucher number V/No. 2005029 has been deposited. The leaves collected were air dried to constant weight under an open shade and ground into fine powder using grinding machine.

Extraction of leaves

Five hundred grammes (500g) of *Abrus precatorius* leaf powder was dissolved in 1200ml of absolute methanol stirred for one hour and filtered with Whatman filter paper no. 1 into measuring cylinder. The filtrate was therefore concentrated at 60 °C in a desiccator. A total of 35g of filtrate was obtained. The crude methanolic extract was

fractionated with methanol, ethylacetate, chloroform and n-hexane solvents using column chromatography.

Column chromatographic separation of the crude methanolic extract

Ten gramme (10.0g) of the dried crude methanolic extract of *Abrus precatorius* leaf was separated using column chromatography. About 200.0g slurry of activated silica gel (70 – 230 mesh) was packed to two-third the length of the glass column (150 cm x 1.5cm, 1D). The methanol extract was dissolved in 60 ml of methanol-water mixture (1:2) v/v translating to 20ml of methanol and 40 ml of water and the mixture was introduced into the column. The column was eluted with 1.2L of n-hexane, 1.0L of chloroform, 1.2L of ethylacetate and finally washed with 1.0L of methanol using the flow rate of 5cm³/sec [18, 19]. This afforded four solvent fractions, which were concentrated, dried and tested against chloroquine-pyrimethamine resistance *Plasmodium falciparum* strain (K₁), *Trypanosoma brucei*, *rhodesiense*, *Trypanosoma cruzi*, and *Leishmania donovani*. The study got prior approval from the University of Agriculture Makurdi Bioethic Committee and issued the permit (P/No. 2011001).

Determination of *In vitro* antiprotozoan activity and cytotoxicity

Trypomastigotes of *Trypanosoma brucei rhodesiense*, strain STIB900; amastigotes of *Trypanosoma cruzi*, Strain Tulahuen C₄ and *Leishmania donovani* axer, Strain MHOM-ET-67/L82; IEF of *Plasmodium falciparum*, strain K1 and L6 cells line of rat skeletal myoblasts were used for *In vitro* assay of various fractions of *Abrus precatorius* leaf using melarsoprol, benzimidazole, mitefosine, chloroquine and podophyllotoxin as standards respectively.

Activity against *Plasmodium falciparum*

In vitro activity against erythrocytic stages of *P. falciparum* was determined by a modified [3H]-hypoxanthine incorporation assay^[20], using the chloroquine- and pyrimethamine-resistant K1 strain and the standard drug chloroquine. The parasite cultures were incubated in RPMI 1640 medium with 5% Albumax (without hypoxanthine) and exposed to serial drug dilutions in micro-titer plates. After 48 h of incubation at 37°C, in a

reduced oxygen atmosphere, 0.5 μ Ci 3H-hypoxanthine was added to each well. The cultures were incubated for a further 24 h before they were harvested onto glass-fiber filters and washed with distilled water. The radioactivity was counted using a BetaplateTM liquid scintillation counter (Wallac, Zurich). The results were recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. IC₅₀ values were calculated from the sigmoidal inhibition curves using Microsoft Excel. Chloroquine was the reference drug used^[21,22].

Activity against *Trypanosoma brucei rhodesiense*

Trypanosoma brucei rhodesiense, STIB 900 strain, and the standard drug, melarsoprol, were used for the assay. This stock was isolated in 1982 from a human patient in Tanzania and after several mouse passages cloned and adapted to axenic culture conditions. Minimum Essential Medium (50 μ L) supplemented with 25 mM HEPES, 1g/L additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate and 15% heat inactivated horse serum was added to each well of a 96-well micro-titer plate^[23, 24]. Serial drug dilutions of seven 3-fold dilution steps covering a range from 100 to 0.123 μ g/mL were prepared. Then 10⁴ bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 μ L was added to each well and the plate was incubated at 37 °C under a 5% CO₂ atmosphere for 72 h, 10 μ L Alamar Blue (resazurin, 12.5 mg in 100 mL double-distilled water) was then added to each well and incubation continued for a further 2–4 h. The plates were then read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analysed using the micro plate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA)^[25].

Activity against *Trypanosoma cruzi*

Rat skeletal myoblasts (L6 cells) were seeded in 96-well microtitre plates at 2000 cells/well in 100 μ L RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. The medium was removed after 24 h and replaced by 100 μ L per well containing 5000 trypomastigotes of *T. cruzi* Tulahuen strain C2C4 containing the β -galactosidase (Lac Z) gene. After 48 h, the medium was removed from the wells and replaced by 100 μ L fresh medium with or without a serial drug dilution of seven 3-fold dilution steps covering a range from 100 to 0.123

µg/mL. After 96 h of incubation, the plates were inspected under an inverted microscope to confirm growth and sterility of the controls. The substrate CPRG/Nonidet (50 µL) was added to all wells. A color reaction developed within 2–6 h was read photometrically at 540 nm. Data were transferred into the graphic programme SoftmaxPro (Molecular Devices), which calculated IC₅₀ values. Benzimidazole was the reference drug used^[26].

Activity against *Leishmania donovani*

Amastigotes of *L. donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37 °C in SM medium at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% CO₂ in air. One hundred microlitre of culture medium with 10⁵ amastigotes from axenic culture with or without a serial drug dilution was seeded in each of the 96-well microtitre plates. Serial drug dilutions covering a range from 100 to 0.123 µg/mL were prepared. After 72 h of incubation the plates were inspected under an inverted microscope to confirm growth and sterility of the controls. Ten microliter Alamar Blue (12.5 mg resazurin dissolved in 100 mL distilled water) was then added to each well and all the plates were incubated for another 2 h. The plates were then read with a Spectramax Gemini XS microplate fluorometer using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Decrease of fluorescence (=inhibition) was expressed as percentage of the fluorescence of control cultures and plotted against the drug concentrations. From the sigmoidal inhibition curves the IC₅₀ values were calculated. Miltefosine was used as the reference drug.^[27]

Cytotoxicity against L6 cells

Assays were performed in 96-well microtiter plates, each well containing 100 µL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4 × 10⁴ L6 cells (a primary cell line derived from rat skeletal myoblasts). Serial drug dilutions of seven 3-fold dilution steps covering a range from 100 to 0.123 µg/mL were prepared. After 72 h of incubation, the plates were inspected under an inverted microscope to confirm growth and sterility of the controls. Ten microliter of Alamar Blue solution was then added to each well and the plates incubated for another 2 h. The plates were then read

with a Spectramax Gemini XS microplate fluorometer using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analysed using the microplate reader software Softmax Pro. Podophyllotoxin was the reference drug used^[21, 22, 28].

Statistical analysis

Quantitative values obtained per treatments were converted to percentage inhibitory concentrations. Regression analysis was adopted for computation of the 50% inhibitory concentrations.

Results and Discussion

Antiplasmodial activity

In vitro activities of crude methanol extract, methanol, ethyl acetate, chloroform and n-hexane fractions of *Abrus precatorius* leaf against chloroquine- and pyrimethamine resistant strain of *Plasmodium falciparum* (K₁) are summarized in table 1.

Table 1. *In vitro* antiplasmodial activity of various fractions of *Abrus precatorius* leaf on *Plasmodium falciparum* K1

Fraction	Inhibitory concentration (IC ₅₀) (µg/mL)	Selectivity index
Chloroquine (standard)	0.073	0.070
Crude methanolic extract	30.4	3.29
Methanolic fraction	>50	>2.00
Ethyl acetate fraction	45.9	2.18
Chloroform fraction	23.0	1.90
n-Hexane fraction	12.1	3.66

Formatted Table

All the extracts showed significant antiplasmodial activity at the concentration ranging between 12.1 and > 50 µg/mL. The *In vitro* antiplasmodial activity was exhibited by crude methanol extract (30.4 µg/mL), methanol fraction (>50 µg/mL), ethyl acetate fraction (45.9 µg/mL), chloroform fraction (23.0 µg/mL) and n-hexane fraction (12.1 µg/mL).

respectively. Their values of selectivity index are crude methanolic extract (>3.29), methanolic fraction (2.00), ethylacetate fraction (>2.18), chloroform fraction (1.90) and n-hexane fraction (3.66) respectively. But the IC_{50} value for chloroquine was 0.073 $\mu\text{g/ml}$ with selectivity index of 0.070.

The antiplasmodial activities of the extracts were qualified as “active” when IC_{50} is $\leq 50 \mu\text{g/ml}$. Any extract having activity beyond this range was considered inactive. The extracts that showed selectivity index greater than 3.29 was considered to have the potential for safer therapy. The exhibition of antiplasmodial activity by all the fractions of *Abrus precatorius* leaf agrees with the report of Bagavan *et al.* indicating that *In vitro* antiplasmodial activity of *Abrus precatorius* is at concentrations range between 30 and 70 $\mu\text{g/ml}$. However, in the present study n-hexane showed the best activity with IC_{50} of 12.1 $\mu\text{g/ml}$ indicating that n-hexane fraction may have antiplasmodial principle.

Abruquinone B isolated from the aerial parts of *Abrus precatorius* showed antiplasmodial and cytotoxic activities [16]. Saganuwan *et al.* had earlier reported *in vivo* antiplasmodial activity of the plant aqueous extract in mice [1429]. Ramazani *et al.* reported that some plants have promising anti-plasmodial activity with IC_{50} values $\leq 50 \mu\text{g/ml}$. The plants are *Prosopis Juliflora*, *Boerhavia elegans* and *Solanum surattense*, [29] *Surattense*, [30] *Phyllanthus emblica*, *Syzygium aromaticum*, *Gloriosa superba*, *Annona squamosa* and *Musa paradisiaca* [15]. *Acetogenins* from members of *Annonaceae*, [3034] exhibited *In vitro* antiplasmodial activities [3132]. Drug resistance strains of *P. falciparum* have been found in many endemic areas of the world and many conventional anti-malarial drugs have been associated with treatment failure [3233], hence the antiplasmodial activity of *Abrus precatorius* leaf is an indication that *P. falciparum* K₁ is susceptible to the plant fractions and the extract perhaps due to presence of abruquinone B in the plant [16]. Our findings also agree with the report of Mesia *et al.* indicating that plant phytochemicals can display antiplasmodial activity *In vitro* as proven by stem barks of *Croton mubango*, *Nauclea pubegoni* and the leaves of *Pyrenacantha staudtii* with IC_{50} values of 3.2, 5.3 and 15.2 $\mu\text{g/ml}$ respectively [3334].

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Antitrypanosomal activity

Chloroform fraction showed the best anti-trypanosomal activity against *Trypanosoma bruceirhodesiense* with the IC₅₀ value of 17.9 µg/mL followed by n-hexane fraction (IC₅₀ of 34.5 µg/mL), crude methanolic extract (IC₅₀ of 53 µg/mL), methanolic fraction (IC₅₀ value of 99.5 µg/mL) and lastly ethyl acetate fraction with IC₅₀ value of >100µg/mL respectively. But the IC₅₀ value for melarsoprol was 0.005µg/mL with selectivity index of 1.00. The selectivity index for crude methanolic extract (>1.89), methanolic fraction (>1.01), ethyl acetate fraction (>1.00), chloroform fraction (2.44) and n-hexane fraction (1.28) were respectively recorded (table 2).

Formatted: Font: Italic

Formatted: Font: Italic

Table 2. *In vitro* activity of various fractions of *Abrus precatorius* leaf on *Trypanosoma brucei* subspecies *rhodesiense*

Fraction	Inhibitory concentration (IC ₅₀) (µg/mL)	Selectivity index
Melarsoprol (standard)	0.005	1.00
Crude methanolic extract	53	>1.89
Methanolic fraction	99.5	>1.01
Ethyl acetate fraction	>100	>1.00
Chloroform fraction	17.9	2.44
n-Hexane fraction	34.5	1.28

Table 3 shows *In vitro* activity of various fractions of *Abrus precatorius* leaf on *Trypanosoma cruzi*. Chloroform fraction exhibited best anti-trypanosomal activity against *T. cruzi* with IC₅₀ value of 53.6 µg/mL whereas crude methanolic extract showed the least anti-trypanosomal activity with IC₅₀ value of 71.4 µg/mL. Whereas methanolic, ethylacetate, and n-hexane fractions exhibited IC₅₀ values of 70.5, 62.1 and 53.6 µg/mL respectively. The SI values for Benzimidazole, crude methanolic, methanolic and ethylacetate fractions are 0.02, >1.40, >1.42 and >1.61 respectively. Chloroform and n-hexane fractions have the least selectivity index of 0.82.

The exhibition of antitrypanosomal activity by chloroform and n-hexane fractions of *Abrus precatorius* leaf at concentration ranging between 17.9 and 34.5 µg/mL (table 2) is corroborated by the report of Sowadogo *et al.* indicating that the methanolic extracts of *Lantana ukambensis*, *Xeoderris sthulmanii*, *Pavinaria curatelifolia*, *Ozorra insignis* and *Ficus platyphylla* showed significant antitrypanosomal activity with minimum lethal concentrations between 1.5 and 25 µg/mL^[3435]. Methylene chloride, methanol and aqueous extracts of *Cassia sieberiana*, *Hymenacardia acta*, *Pericopsis laxifolara*, *Strychnos spinosa* and *Trichilia emetica* showed antitrypanosomal activity against *T. brucei* with IC₅₀ values ranging from 1.5 to 39 µg/mL^[3537].

Formatted: Font: Italic

Formatted Table

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Table 3. *In vitro* activity of various fractions of *Abrus precatorius* leaf on *Trypanosoma cruzi*

Fraction	Inhibitory concentration (IC ₅₀) (µg/mL)	Selectivity index
Benzimidazole (standard)	0.315	0.02
Crude methanolic extract	71.4	>1.40
Methanolic fraction	70.5	>1.42
Ethyl acetate fraction	62.1	>1.61
Chloroform fraction	53.6	0.82
n-Hexane fraction	54.1	0.82

But the fact that all the extracts showed antiplasmodial activity against *Trypanosoma cruzi* with IC₅₀ value greater than 50µg/ml may indicate that the plant is less potent against *T. cruzi*.

Antileishmania activity

The *In vitro* assay of various fractions of *Abrus precatorius* leaf on *Leishmania donovoni* showed that n-hexane fraction has the best activity having IC₅₀ value of 15.7 µg/mL in comparison with the rest of fractions. Chloroform, methanolic and ethyl acetate fractions and methanolic extract showed IC₅₀ values of 24.1 µg/mL, 42.3 µg/mL, 47.4 µg/mL and >100 µg/mL respectively.

The fact that all the test fractions except methanolic fraction showed antileishmanial activity against *L. donovoni* at concentrations range between 12.1 and >50µg/ml agree with the report of Sowadogo *et al.* indicating that plants have antileishmanial activity. *L. ukambensis* showed significant antileishmanial activity with an inhibitory concentration (IC₅₀) of 6.9 µg/ml [3435]. The bark of *Chondendion tomentosum* and *Cedrela odorata* are active against *Leishmania* [36]. Lenta *et al.* reported *In vitro* antileishmanial activity of *Allanblackia manticola* and *Symptonia globulifera*. Benzophenone, guttiferone A and F isolated from *A. manticola* and *S. globulifera* were responsible for the antileishmanial activity of the plants.

Formatted Table

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Table 4. *In vitro* activity of various fractions of *Abrus precatorius* leaf on *Leishmania donovani*

Fraction	Inhibitory concentration (IC ₅₀) (µg/mL)	Selectivity index
Mitefosine (standard)	0.234	0.02
Crude methanolic extract	47.4	>2.12
Methanolic fraction	>100	>1.00
Ethyl acetate fraction	42.3	>2.36
Chloroform fraction	24.1	1.81
n-Hexane fraction	15.7	2.82

But mitefosine (standard) showed non-comparable IC₅₀ value of 0.234 µg/mL in comparison with the tested fractions. Their SI values are; mitefosine (0.02), crude methanolic extract (>2.12), methanolic fraction (>1.00), ethylacetate fraction (>2.36), chloroform fraction (1.81) and n-hexane fraction (2.82) respectively (table 4).

Formatted: Font: Italic

Formatted Table

Table 5. *In vitro* cytotoxic activity of various fractions of *Abrus precatorius* leaf on rat skeletal myoblasts (L-6 cells) line

Fraction	Inhibitory concentration (IC ₅₀) (µg/mL)
Podophyllotoxin (standard)	0.005
Crude methanolic extract	>100
Methanolic fraction	>100
Ethyl acetate fraction	>100
Chloroform fraction	43.7
n-Hexane fraction	44.3

Formatted Table

Cytotoxic activity

Chloroform fraction showed highest cytotoxic activity having IC₅₀ value of 43.7 µg/ml. Methanolic extract, ethylacetate and methanolic fractions showed IC₅₀ values of >100 µg/ml. But n-hexane fraction showed inhibitory concentration of 44.3 µg/ml (table 5).

The n-hexane solvent can be used to extract esters from medicinal plants^[3738]. *Terpenoids* and *furanoterpenes* are responsible for *In vitro* activity against *P. falciparum*^[21]. Tasdemir *et al.* also reported antitrypanosomal and antileishmanial activities of flavonoids against *T. brucei rhodesiense*, *T. cruzi* and *L. donovani*^[3839]. Aerucyclamides A – D are heterocyclic peptides and nostocarboline, an alkaloid respectively produced by *Microcystis aeruginosa* and *Nostoc* displayed activity against *P. falciparum* with a pronounced selectivity towards rat myoblasts^[3940]. The correlation between antiprotozoan activity and cytotoxicity of the test fractions and crude methanolic extract agrees with the report of Schmidt *et al.* that antiprotozoal activities are significantly correlated with cytotoxicity and the major determinants for activities are α and β-unsaturated structural elements, also known to be essential for other biological activities of sesquiterpene lactones. However, certain compounds are more toxic against protozoa than against mammalian cells while others are more cytotoxic than active against the protozoa^[4041]. The selectivity index (SI) is defined as the ratio of the L₆ toxicity to the anti-protozoan activity and is determined by dividing the IC₅₀ values for L₆ by the IC₅₀ value for

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

protozoan parasites. The extract with higher selectivity value indicate potentially safer therapy. Therefore, n-hexane was safest having the SI of 3.67 followed by crude methanol extract (3.29). But the rest of fractions have low SI of 1.9-2.18.

Conclusion

n-hexane and chloroform fractions of *Abrus precatorius* leaf showed the best inhibitory activities against chloroquine and pyrimethamine resistant *Plasmodium falciparum* with IC₅₀ value of 12.1 µg/ml and *Leishmania donovoni* (IC₅₀; 15.7 µg/ml) respectively. Chloroform fraction showed the best cytotoxic activity against rat myoblasts (L6) cell lines. Flavonoid, saponin, glycoside, quinone and alkaloids have been suspected to be responsible for anti-protozoan activities of *Abrus precatorius* leaf.

Formatted: Font: Italic

Formatted: Font: Italic

Competing interest

The authors declare that they don't have competing interests.

ACKNOWLEDGEMENT

I wish to acknowledge the contributions of Mr Emmanuel Mbaoji, Chief Technologist of Pure and Applied Chemistry Laboratory and Mr Agbo, A.N., Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka for their assistance in Chromatographic separations. I appreciate immensely the contributions of Mr Kehinde Ola Emmanuel, the man who typed the work and Mr Abuto Monday, a corp member with National Youth Services for collection of *Abrus precatorius* leaves.

Formatted: Font: Bold

Formatted: Left

References

1. Cheesebrough M. Examination of blood for malaria parasite. District Laboratory Practice in Tropical Countries Part 1, Low price edition. Cambridge University Press, Cambridge, UK; 2005: 434.
2. Dellacollete C. Malaria epidemic response: What clinician needs to know? Mera July, 2004: 3.
3. WHO. World malaria report. WHO Bulletin 2008: 99.
4. Akubue PI. Textbook of Pharmacology. Africana First Publishers Limited, Onitsha Nigeria; 2006: 603.
5. Saganuwan AS, Adelaiye PO. **The epidemiology of malaria in University of Agriculture Makurdi Health Centre**. Afr J Clin Exper Microbiol 2007, **8**(3): 122 – 128.
6. WHO. World Health Report 2004, Statistical Annex, 2004.
7. Kahn CM, Lorie S. The Merck Veterinary Manual 9th ed., Merck and Co., Inc., Whitehouse Station in Partnership with Merial Ltd.; 2005: 2712.
8. WHO. Chagas' disease. Thirteen Programme Report UNDP/WB/TDR, Geneva; 1997: 112 – 123.
9. Viotti R, Vigliano C, Armenti H, Segura E. Treatment of chronic chagas' disease with benznidazole: clinical and serological evolution of patients with long-term follow-up. Am Heart J 1994, **127**: 151 – 162.
10. Saganuwan AS, Gulumbe ML. In vitro antimicrobial activities testing of *Abrus precatorius* cold-water leaf extract on *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Proceedrugs of 2nd Annual Conference, Nigerian Society for Indigenous Knowledge and Development CRUTECH, Obubra, 9th – 12th Nov. 2005: 93 – 97.
11. Saganuwan AS, Gulumbe ML. **In vitro antimicrobial activities testing of *Abrus precatorius* cold-water leaf extract on *Salmonella typhimurium*, *Escherichia coli* and *Klebsiella pneumoniae***. J Sci Technol Res 2005, **4**(3): 70 – 73.
12. Adelowotan O, et al. **The in vitro antimicrobial activity of *Abrus precatorius* (L) fabaceae extract on some clinical pathogens**. Nigerian Postgrad Med J 2008, **15**(1): 32 – 37.

Formatted: Justified, Line spacing: 1.5 lines

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

13. Bobbarala V, Vadlapudi V. ***Abrus precatorius* L. seed extracts antimicrobial properties against clinically important bacteria.** Int J pharm Technol Res 2009,1(4): 1115-1118.
14. Saganuwan AS, et al. **In vivo antiplasmodial activity by aqueous extract of *Abrus precatorius* in mice.** Revista Latinoamericana de Quimica 2011,39(1 – 2): 32 – 44.
15. Bagavan A, et al. **In vitro antimalarial activity of Medicinal plant extracts against *Plasmodium falciparum*.** Parasitol Res 2011,108: 15 – 22.
16. Limmatvapirat C, Sirisopanapon S, Kittakoop P. **Antitubercular and antiplasmodial constituents of *Abrus precatorius*.** Planta Medica 2004,70(3): 276 – 278.
17. Saganuwan AS, Onyeyili PA, Suleiman AO. **Comparative toxicological effects of orally and intraperitoneally administered aqueous extract of *Abrus precatorius* leaf in Swiss albino mice.** Herba Polonica 2011,57 (3): 32 – 44.
18. Azogu CI: Laboratory Organic Chemistry. Techniques, Qualitative Analysis, Organic Preparations and Spectroscopy. Second edition, Maybinson Book Publishers, New Jersey, USA; 2010: 269.
19. Beckett AH, Stenlake JB. Practical pharmaceutical Chemistry. Fourth edition – Part two, CBS Publishers and Distributors, New Delhi; India; 2008: 602.
20. Martile H, Pink JRL. *Plasmodium falciparum* malaria parasite cultures and their use in immunology. In: Iefkovites, I. and Perines, B. (eds.) Immunological Methods, Academic Press, San Diego: 1990: 221 – 234.
21. Orhan I, et al. **Inhibitory activity of marine sponge-derived natural products against parasitic protozoa.** Marine Drugs 2010,8: 47-58.
22. Scala F, et al. **Bromopyrrole alkaloids as lead compounds against protozoan parasites.** Marine Drugs 2010,8: 2162 – 2174.
23. Baltz T, et al. **Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evans*, *T. rhodesiense* and *T. gambiense*.** The EMBO J 1985,4: 1273 – 1277.
24. Thuita JK, et al. **Efficacy of the diamidine DB75 and its prodrug DB289, against murine models of human African trypanosomiasis.** Acta Tropica 2008,108: 6-10.
25. Raz B, et al. **The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T.b. rhodesiense* and *T.b. gambiense*) in vitro.** Acta Tropica 1997,68: 139 – 147.

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

26. Buckner FS, et al. **Efficient technique for screening drugs for activity against *Trypanosoma cruzi* using parasites expressing beta-galactosidase.** Antimicrob Agent Chemother 1996;**40**: 2592 – 2597.
27. Mikus J, Steverdrug D. **A simple colourimetric method to screen drug cytotoxicity against *Leishmania* using the dye Alamar Blue.** Parasitol Int 2008;**48**: 265 – 269.
28. Nwodo NJ, Brun R, Osadebe PO. **In vitro and in vivo evaluation of the antitrypanosomal activity of fractions of *Holarrhena africana*.** J Ethnopharmacol 2007;**113**: 556 – 559.
2930. Ramazani A, et al. **In vitro and in vivo antiplasmodial activity of *Boerhavia elegans* and *Solanum surattense*.** Malaria J 2010;**9**: 124.
3031. Bermejo A, et al. **Acetogenins from Azimonaceae: recent progress in isolation, synthesis and mechanism of action.** Nat Prod 2005;**22**: 269 – 303.
3132. Rakotomanga M, et al. **Antiplasmodial activity of acetogenins and inhibitory effects on *Plasmodium falciparum* adenylate translocase.** J Chemother 2004;**16**: 350 – 356.
3233. Ridley PG. **Medical need, scientific opportunity and the drive for antimalarial drugs.** Nature 2002;**415**: 686 – 693.
3334. Mesia GK, et al. **Antimalarial activities and toxicities of three plants used as traditional remedies for malaria in the Democratic Republic of Congo: *Croton mubango*, *Nauclea pubegoni* and *Pyrenacantha standtii*.** Ann Trop Med Parasitol 2005;**99** (4): 345 – 357.
3435. Sawadago WR, et al. **In vitro antileishmanial and antitrypanosomal activities of five medicinal plants from Burkina Faso.** Parasitol Res, 2011, PMID: 22037827, p1.
3536. Gonzalez-Coloma A, et al. **Antileishmanial antitrypanosomal, and cytotoxic screening of ethnopharmacologically selected plants.** Parasitol Res 2012;**4**: 1381 – 1392.
3637. Hoet S, et al. **In vitro antitrypanosomal activity of ethnopharmacologically selected Beninese, plants.** J Ethnopharmacol 2004;**91**: 37 – 42.
3738. Dwivedi MK, et al. **Homocysteine and cardiovascular disease.** Biotechnol Molecul Biol Rev 2011;**6**(5): 101-107.
3839. Tasdemir D, et al. **Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo structure-activity relationship, and quantitative**

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

structure-activity relationship studies. Antimicrob Agent Chemother 2006,**50**(4): 1352 – 1364.

3940. Portmann C, et al. **New antiplasmodial natural products from cyanobacteria: Linking their ecological role in to their therapeutic potential.** Planta Medica 2009,**75**: DOI:10.105513-0029-1234265.

4041. Schmidt TJ, et al. **Quantitative structure-antiprotozoal activity relationships of sesquiterpene lactones.** Molecules 2009,**14**: 2062-2076.