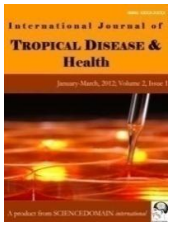

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In vitro Antiplasmodial, Antitrypanosomal, Antileishmanial and Cytotoxic Activities of Various Fractions of *Abrus precatorius* Leaf

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Authors' contributions

This work was carried out in collaboration between all the authors. Author SAS managed the literature searches, performed the statistical analysis, wrote the protocol, and the first draft of the manuscript. Author PAO designed the study. Authors IGA, NJN and RB managed the analyses of the study. All authors read and approved the final manuscript.

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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 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*

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rhodesiense, *Trypanosoma cruzi*, *Leishmania donovoni* and rat skeletal myoblasts (L-6 cells).
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 IC_{50} value was less than 50 μg/ml. The extract that showed selectivity index higher than 3.29 was considered to have potential for safer therapy.
Results: n-hexane fraction showed the best antiplasmodial activity with inhibitory concentration (IC_{50}) 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 IC_{50} value of 17.9 μg/ml and selectivity index of 2.44 and followed by n-hexane fraction with IC_{50} (34.5 μg/ml) and selectivity index of 1.28.
Conclusion: Since leaf extract has significant antiplasmodial, antitrypanosomal and antileshmanial 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.

1. 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 millionin 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 $^1/_5$ and $^1/_3$ 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 triatonine bugs or through blood transfusion [9].

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

Abrus precatorius is a member of *papillionaceae* 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.

2. METHODS

2.1 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.

2.2 Extraction of Leaves

Five hundred grammes (500g) of *Abrus precatorius* leaf powder was dissolved in 1200 ml 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.

2.3 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 20 ml 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 $5cm^3$/sec [18,19]. This afforded four solvent fractions, which were concentrated, dried and tested against chloroquine-pyrimethamine resistance *Plasmodium falciparum* strain (K_1), *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Leishmania donovoni.* The study got prior approval from the University of Agriculture Makurdi Bioethic Committee and issued the permit (P/No. 2011001).

2.4 Determination of *In vitro* Anti-protozoan Activity and Cytotoxicity

Trypamastigotes of *Trypanosoma brucei rhodesiense*, strain STIB900; amastigotes of *Trypanosoma cruzi*, Strain Tulahuen C_4 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.

2.5 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 µCi 3H-hypoxanthine was addedto each well. The cultures were incubated for a further 24 h before they were harvested onto glass-fibber filters and washed with distilled water. The radioactivity was counted using a Betaplate^{TM} 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. IC_{50} values were calculated from the sigmoidal inhibition curves using Microsoft Excel. Chloroquine was the reference drug used [21,22].

2.6 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 µL) 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 dilutionsteps covering a range from 100 to 0.123 µg/mL were prepared. Then 10^4 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 µL was added to each well and the plate was incubated at 37°C under a 5% CO_2 atmosphere for 72 h, 10 µL 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 588nm. Data were analysed using the micro plate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA) [25].

2.7 Activity against *Trypanosoma cruzi*

Rat skeletal myoblasts (L6 cells) were seeded in 96-well microtitre plates at 2000 cells/well in 100 µL RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. The medium was removed after 24h and replaced by 100 µL 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 µL fresh medium with or without a serial drug dilution of seven3-fold dilution steps covering a range from 100 to 0.123 µg/mL. After 96 h of incubation, the plates *Mar. Drugs* 2010, *8* 55 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 Softmax Pro (Molecular Devices), which calculated IC_{50} values. Benzmidazole was the reference drug used [26].

2.8 Activity against *Leishmania donovani*

Amastigotes of *L. donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37°C in SMmedium at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% CO_2 in air. One hundred microlitre of culture medium with 10^5 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 IC_{50} values were calculated. Miltefosine was used as the reference drug [27].

2.9 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 4×10^4 L6cells (a primary cell line derived from rat skeletal myoblasts). Serial drug dilutions of seven 3-folddilution 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].

2.10 Statistical Analysis

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

3. RESULTS AND DISCUSSION

3.1 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* (K_1) are summarized in Table 1.

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), ethylacetate 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. Butthe IC_{50} value for chloroquine was 0.073 μg/ml with selectivity index of 0.070.

The antiplasmodial activities of the extracts were qualified as "active" when IC_{50} is ≤ 50 μg/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. [15] indicating that *in vitro* antiplasmodial activity of *Abrus precatorius* is at concentrations range between 30 and 70 μg/ml. However, in the present study n-hexane showed the best activity with IC_{50} of 12.1 μg/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. [14] had earlier reported *in vivo* antiplasmodial activity of the plant aqueous extract in mice. Ramazani et al. [29] reported that some plants have promising anti-plasmodial activity with IC_{50} values ≤ 50 μg/ml. The plants are *Prosopis juliflora, Boerhavia elegans* and *Solanum surattense*, *Phyllanthus emblica, Syzygium aromaticum, Gloriosa superba, Annona squamosa* and *Musa paradisiacal* [15]. *Acetogenris* from members of *Annonaceae* [30], exhibited *in vitro* antiplasmodial activities [31]. 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 [32], hence the antiplasmodial activity of *Abrus precatorius* leaf is an indication that *P. falciparum* K_1 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. [33] 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 μg/ml respectively [33].

3.2 Antitrypanosomal Activity

Chloroform fraction showed the best anti-trypanosomal activity against *Trypanosoma brucei rhodesiense* with the IC_{50} value of 17.9 μg/mL followed by n-hexane fraction (IC_{50} of 34.5 μg/mL), crude methanolic extract (IC_{50} of 53 μg/mL), methanolic fraction (IC_{50} value of 99.5 μg/mL) and lastly ethyl acetate fraction with IC_{50} value of >100μg/mL respectively. But the IC_{50} value for melarsoprol was 0.005 μg/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).

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

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 IC_{50} value of 53.6 μg/mL whereas crude methanolic extract showed the least anti-trypanosomal activity with IC_{50} value of 71.4 μg/mL. Whereas methanolic, ethylacetate, and n-hexane fractions exhibited IC_{50} 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. [34] 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 [34]. 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 IC_{50} values ranging from 1.5 to 39 μg/ml [35].

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

3.3 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 IC_{50} value of 15.7 μg/mL in comparison with the rest of fractions. Chloroform, methanolic and ethyl acetate fractions and methanolic extract showed IC_{50} 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 >50 μg/ml agree with the report of Sowadogo et al. [34] indicating that plants have antileishmanial activity. *L. ukambensis* showed significant antileishmanial activity with an inhibitory concentration (IC_{50}) of 6.9 μg/ml. 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.

But mitefosine (standard) showed non-comparable IC_{50} 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).

3.4 Cytotoxic Activity

Chloroform fraction showed highest cytotoxic activity having IC_{50} value of 43.7 μg/ml. Methanolic extract, ethylacetate and methanolic fractions showed IC_{50} values of >100 μg/ml. But n-hexane fraction showed inhibitory concentration of 44.3 μg/ml (Table 5).

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

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

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

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

The n-hexane solvent can be used to extract esters from medicinal plants [37]. *Terpenoids* and *furanoterpenes* are responsible for *In vitro* activity against *P. falciparum* [21]. Tasdemir et al. [38] also reported antitrypanosomal and antileishmanial activities of flavonoids against *T. brucei rhodesiense*, *T. cruzi* and *L. donovoni*. 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 [39]. The correlation between antiprotozoan activity and cytotoxicity of the test fractions and crude methanolic extract agrees with the report of Schmidt et al. [40] 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. The selectivity index (SI) is defined as the ratio of the L_6 toxicity to the anti-protozoan activity and is determined by dividing the IC_{50} values for L_6 by the IC_{50} value for 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.

4. CONCLUSION

n-hexane and chloroform fractions of *Abrus precatorius* leaf showed the best inhibitory activities against chloroquine and pyrimethamine resistant *Plasmodium falciparum* with IC_{50} value of 12.1 μg/ml and *Leishmania donovoni* (IC_{50}; 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.

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COMPETING INTEREST

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

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