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Research Article

Antiplasmodial and Cytotoxic Activities of Extracts from *Jacaranda mimosifolia* and *Pseudospondias microcarpa*

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ABSTRACT

Jacaranda mimosifolia and *Pseudospondias microcarpa* two medicinal plants used in Cameroon to treat various diseases such as malaria, typhoid fever, hyperglycemia, gonococci, anemia, diarrhea, filaria, angulillulose, rheumatisms were screen against K1 strain of *P. falciparum in vitro*. Out of the 15 extracts tested, two showed good (IC507.74–8.6 µg/mL), eleven exhibited moderate (IC50 from 10.50–36.55 µg/mL), while only one displayed mild (IC50 >50 µg/mL) antiplasmodial activity. Methylene cholride and ethyl acetate extracts from stem bark of *J. mimosifolia* were the most active with IC50 of 7.74 µg/mL and 8.6 µg/mL respectively. The cytotoxicity of these highly active extracts on HEpG2 were 20 µg/mL and 17.38 µg/mL with the good selectivity indices of 2.5839 and 2.0209 respectively. This study have revealed for the first time the antiplasmodial activity of solvent extracts of *J. mimosifolia*and *P. microcarpa* and justify the traditional use of these plants. These plants are good candidates for isolation of anti-protozoal compounds which could serve as new lead structures for drug development.

Keywords: *Jacaranda mimosifolia*, *Pseudospondias microcarpa*, antiplasmodial activity, cytotoxicity.

INTRODUCTION

Malaria is a major parasitic disease in the world, especially in Africa. Annually, about 300-500 million of clinical cases and 1.2–2.8 million of deaths are register[1,2,3]. Moreover, *P. falciparum* the most widespread etiological agent for human malaria has become increasingly resistant to standard antimalarials e.g. chloroquine, sulphadoxinepyrimethamine and artemisinin[4]. Consequently, new drugs or drug combinations are urgently needed today for the treatment of malaria. These drugs should have novel modes of action or be chemically different from the drugs in current use[5].

Plants have always been considered to be a possible alternative and rich source of new drugs[6]. and most of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates[7,8]. Realizing that many drugs originated from medicinal plants, it is quite likely that several other plants described in thetraditional medicine literature may lead to other plant derived novel antimalarials[9]. In fact, due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the populations depend on traditional medical remedies mainly from plants[10,11].

In fact, *Jacaranda mimosifolia* is used in traditional medicine in different countries to cure wounds, ulcers, and as an astringent in diarrhoea and dysentery[12,13].Whereas, *Pseudospondias microcarpa* is extensively used in herbal medicine inCentre, East, South and Littoral regions of Cameroon inthe treatment of various diseases like: Hyperglycemia, gonococci, anemia, diarrhea, filaria, angulillulose, rheumatisms, ankylostomia, ascaridiose, cestodose, malaria, typhoid fever, haemorrhoid, oedemas, rheumatisms, stomach ache, icterus, pediculoses, diabetes, trypanosomiasis, leishmaniasis and elephantiasis[14,15,16,17,18,19]. In the best of our knowledge no investigation on antimalarial activity on these two plants species is reported. Therefore, to fill this gap in, the present study was designed to evaluate the antiplasmodial and

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Table 1: Antiplasmodial activity of crude extracts *J. mimosifolia* against *P. falciparum* and cytotoxicity study on HEpG2 cells

Part of Plant	Extracts	IC_{50} K1 strain (µg/mL)	IC_{50} HEpG2 Cells(µg/mL)	SI
Root back	Hexane	> 25	> 25	NA
	Methylenechloride	10.50	7.99	0.7609
	Ethylacetate	11.16	53.65	4.8073
	Methanol-CH_2Cl_2(1:1)	15.98	> 50	>3.1289
Stem back	Hexane	21.45	> 25	>1.1655
	Methylenechloride	7.74	20	2.5839
	Ethylacetate	8.60	17.38	2.0209
	Methanol CH_2Cl_2 (1:1)	26.55	> 50	>1.8832
References drugs				
Chloroquine		0.034	/	/
Doxydine		0.00418	/	/
Artesunate		0.00119	/	/
Atovaquone		0.00081	/	/

A not applicable; SI selectivity index; SI Plasmodium=IC_{50} HEpG2 cells/ IC_{50}*P. falciparum* K1

cytotoxic activity of solvent extracts of *Pseudospondias microcarpa* and *Jacaranda mimosifolia*.

MATERIALS AND METHODS

Plant materials

The roots bark and stem bark of *P. microcarpa*(41437/HNC) and *J. mimosifolia* (50081/HNC) were collected in Yaoundé (February 2012) and identified at the Cameroon National Herbarium (HNC) where voucher specimens are deposited.

Preparation of extracts

The air-dried root backs and stem bark of *J. mimosifolia* was pulverized and 500g was exhaustively macerated successively with *n*-Hexane (n-Hex, 3 L), Methylene chloride (DCM, 3 L), ethyl acetate (EA, 3 L), of Methanol-Methylene chloride (MeOH-DCM, 5 L) (1:1v/v) at room temperature for 72 h. The macerate was filtered and evaporated under reduced pressure to obtain MeOH-DCM crude extract (50 g) and (45g) respectively. The macerate was filtered and evaporated under reduced pressure to obtain eight extract (2 g), (2.6 g), (5 g), (3 g), (3.5 g) (3 g), (20 g) and (25 g) respectively

The air-dried and powdered root (500 g) and stem bark (500 g) of *P. microcarpa* were exhaustively macerated successively with *n*-Hexane (n-Hex, 3 L), Methylene chloride (DCM, 3 L), ethyl acetate (EA, 3 L), Methanol (MeOH, 2 L) respectively at room temperature for 72 h. The macerate was filtered and evaporated under reduced pressure to obtain eight extract (2 g), (3 g), (2 g), (40 g), (4 g), (6 g), (40 g) and (70 g) respectively.

In vitro culture of *Plasmodium falciparum* and antimalarial activity

The evaluation of antiplasmodial activity of extracts was conducted *in vitro* on one culture-adapted *P. falciparum*K1 strain. *P. falciparum* strains was maintained in fresh A+ human erythrocytes at 2.5 % haematocrit in complete medium (RPMI 1640 with 25 mM HEPES, 25 mM $NaHCO_3$, 10 % of A+ human serum) at 37 ° C under reduced O_2 atmosphere (gas mixture 6 % CO_2, 10 % O_2, 84 % N_2). Parasitaemia was maintained daily between 1% and 6 %. The *P. falciparum* drug susceptibility test was carried out by comparing quantities of DNA in treated and control cultures of parasite in human erythrocytes according to an SYBR Green I fluorescence-based method using 96-well fluorescence plate reader. Parasite culture was synchronized at ring stage with 5 % sorbitol. Extracts dissolved in appropriate solvent (final concentration less than 0.5 % v/v) were incubated in a total assay volume of 200 µL (RPMI, 2 % haematocrit and 1 % parasitaemia) for 72 h in a humidified atmosphere (6 % CO_2, 14 % O_2, 80 % N_2) at 37 ° C, in 96-well flat-bottom plates. Duplicate assays were performed for each sample. After incubation, 125 µL supernatant was discarded and cells were washed twice with 125 µL 1X PBS. 15 µL re-suspended cells were transferred to 96-well flat bottom non-sterile black plates (Greiner Bio-one). 15 µL of the SYBR Green lysis buffer (2XSYBR Green, 20 mMTris base pH 7.5, 20 mM EDTA, 0.008 % w/v saponin, 0.08 % w/v Triton X-100) was added to each well. Negative control (treated by solvent) and positive control (chloroquine) were added to each set of experiments. Plates were incubated for 15 min at 37° C and then read on a TECAN Infinite F-200 spectrophotometer with excitation and emission wavelengths at 485 and 535 nm respectively. The concentrations of extracts required to induce a 50 % decrease in parasite growth (IC_{50}) were calculated from three independent experiments[20].

Cytotoxic activity on HepG2 cells using MTT assay

HepG2 cell line used for cytotoxicity was maintained at 37 °C, 6 % CO_2, with 90 % humidity in RPMI supplemented with 10 % fœtal bovine serum, 1 % l-glutamine (200 mM) and penicillin (100 U/mL)/streptomycin (100 µg/mL) (complete RPMI medium). The Cytotoxic properties of plant extracts on HEp-2 cells was performed in 96-well flat-bottom tissue-culture plates according to method described by Mosmann[21]with slight modifications. Briefly, 5.10^3 cells in 100 µL of culture medium (RPMI + 10 % CO_2) were inoculated into each well of 96-well plates and incubated at 37 °C in a humidified 6 % CO_2 atmosphere. After 24 h incubation, 100 µL of medium with various product concentrations (final concentration less than 0.5 % v/v)

Table 2: Antiplasmodial activity of crude extracts *P. microcarpa* against *P. falciparum* and cytotoxicity study on HEpG2 cells

Part of Plant	Extracts	IC_{50} K1 strain (µg/ml)	IC_{50} HEpG2 Cells(µg/ml)	SI
	Hexane	> 50	NA	NA
	Methylenechloride	36.55	> 50	>1.3679
	Ethylacetate	27.59	20	0.7249
Root back	Methanol	13.46	> 25	>1.8573
	Hexane	> 25	> 25	NA
	Methylenechloride	> 12.5	> 12.5	NA
Stem back	Ethylacetate	18.25	> 25	>1.3698
	Methanol	*	*	*
References drugs				
Chloroquine		0.034	/	/
Doxydine		0.00418	/	/
Artesunate		0.00119	/	/
Atovaquone		0.00081	/	/

NA not applicable; SI selectivity index;* Not determined; SI Plasmodium=IC_{50} HEpG2 cells/ IC_{50} *P. falciparum* K1

were added and the plates were incubated 72 h. Duplicate assays were performed for each sample. At the end of the treatment and incubation, each plate-well was microscope-examined for detecting possible precipitate formation before the medium was aspirated from the wells. Then, 10 µL yellow MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) solution (5 mg MTT/mL in PBS) was added to each well with 100 µL of medium without fœtal bovine serum. Cells were incubated for 2 h at 37 °C to allow MTT oxidation by mitochondrial dehydrogenase in the viable cells. After 2 h, the MTT solution was aspirated and DMSO (100 µL) was added to each well to dissolve the resulting blue formazan crystals. Plates were then shaken vigorously (300 rpm) for a few minutes. The absorbance was measured at 570 nm using a BIO-TEK ELx808 Absorbance Microplate Reader. Doxorubicin was used as positive control. Cell viability was calculated as percentage of control (cells incubated without compound). The 50% cytotoxic concentration (HepG2 CC_{50}) was determined by non-linear regression analysis processed on dose–response curves, using the Table Curve software 2D v.5.0. CC_{50} values represent the mean value calculated from three independent experiments.

Selectivity index

The selectivity index was determined as the ratio between the cytotoxic (HEpG2 cells) and antiparasitic (K1 strain) activity.

RESULTS

In the present study, 15 crude extracts from two different plants were evaluated for their antimalarial potencies. The results shown that activities were plants, plant parts and solvent dependant. The IC50 of extracts from *Jacaranda mimosifolia* were ranged from 7.74 to 26.55 µg/ml whereas IC50 of *Pseudospondias microcarpa* were from 13.46 to >50 µg/mL. The thresholds for the *in vitro* antimalarial activity of the plant extracts were based on the classification according to Gessler *et al.*[22]where: extract with IC50 less than 10 µg/mL is considered very good; 10 to 50 µg/mL considered moderate and over 50 µg/ml considered to have low activity. Based on this classification, two extracts have showed good (IC50 7.74–8.6 µg/mL), eleven exhibited moderate (IC50 from 10.50–36.55µg/mL), and one displayed mild (IC50 >50 µg/mL) antiplasmodial activity (Table 1 and 2). Good antiplasmodial potencies were displayed by methylene chloride extract (IC50=7.74 µg/mL) and ethyl acetate extract (IC50=8.6 µg/mL) of stem bark of *Jacaranda mimosifolia.* The antiplasmodial activity exhibit by these plant extracts was less than activity of reference antiplasmodial compounds (0.00081 to 0.034µg/mL). The cytotoxicity of these highly active extracts on HEpG2 were 20 µg/mL and 17.38 µg/mL with the selectivity indices of 2.5839 and 2.0209 respectivelymethylene chloride and ethyl acetate were the good extraction solvent for root bark and stem bark of *Jacaranda mimosifolia* whereas methanol was the best solvent for root bark and ethyl acetate the best for stem bark of *Pseudospondias microcarpa*. In general, extracts from *Jacaranda mimosifolia* were more potents than extracts from *Pseudospondias microcarpa*.

DISCUSSION

Indigenous healthcare systems have always played a vitalrole in the management of community health and discovery of novel chemotherapeutic agents. Thus, the analysis of this traditional medicine led to numerous new active molecules, and in the fight against malaria, a better knowledge of traditional medicine will help in the discovery of new active molecules[23]. In the present study, two medicinal plants *Jacaranda mimosifolia* and *Pseudospondias microcarpa* known for their traditional medicinal usage and pharmacological activities were evaluated for their antiplasmodial activity against *P. falciparum* K1 and their toxicity against HEpG2 cell line. Four solvent were used including methanol, methylene chloride, ethyl acetate and hexane. Methylene-chloride and ethyl acetate extracts from stem bark of *Jacaranda mimosifolia* were found to be the promisisng whereas methanol extract from root bark was for *Pseudospondias microcarpa*. This indicates the importance of choice of good solvents and plant parts towards enriching promising

metabolites[24].As stated by Rasoanaivo et al.[25]extracts that presented an IC_{50} under 20 µg/mL could be considered as potential sources for antimalarials while those that presented an IC50 < 10 µg/mL have to be included in further investigations (mostly research of new active principles). In the present study, methylene chloride and ethyl acetate extracts from stem bark of *Jacaranda mimosifolia*among the 15 tested reached this value (IC < 10 µg/ml). In accordance with our funding, Beourouet al.[26]reported that the dichloromethane extract from stems of *Acokanthera oppositifolia* displayed a moderate activity on both strains of *P. falciparum* with IC values of 9.82 µg/mL (FcB1) and 8.95 µg/ml (F32). Also, the dichloromethane extract of areal part of*Cadaba farinosa* showed good to moderate antiplasmodial activities on both strains (FcB1 IC50 = 3.05 µg/mL and F32 IC50 = 6.25 µg/mL). Moreover, Lekana-Doukiet al.[27] reported that dichloromethane extracts of *Tetrapleura tertaptera* and *Copaifera religiosa* showed promising activity against *P. falciparum* strains FCB (chloroquine-resistant) and 3D7 (chloroquine-sensitive), indicating that they contained the main active compounds. Several authors have pursued the search for natural products with antimalarial effect in plants in the past and in recent years[28,29,30]. The rationale for such studies is based on the resistance of theparasites to conventional treatment as observed in the case of malaria[31].In Africa and Asia there has been an intense and enthusiastic search for active compounds against *P. falciparum* especially[32,33,34]. The present study represents an important contribution to this field since the data obtained, together with that already reported, has shown to be inedited. Studies in progress are being made for identifying the chemical component(s) responsible for the antimalarial effect of the plant extracts.

CONCLUSION

The results of this study have shown that the extracts of *Jacaranda mimosifolia* nd *Pseudospondias microcarpa* exhibited antimalarial activity. To our knowledge, this is the first antiplasmodial evaluation described for plants used in this study. These results justify the traditional use of the plants in the treatment of infectious disease including malaria. Further work is suggested to isolate, identify and characterize the active principles from these plants.

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