Trop J Nat Prod Res, April 2021; 5(4):609-612

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Short Communication

Evaluation of *In Vitro* Antiprotozoal and Cytotoxic Activities of Selected Medicinal Plants used in Nigerian Folk Medicine

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ARTICLE INFO

Article history:
Received 02 November 2020
Revised 16 March 2021
Accepted 07 April 2021
Published online 03 May 2021

ABSTRACT

The global burdens of trypanosomiasis, malaria and leishmaniasis have continued to impoverish the developing countries. These protozoan parasitic diseases are still endemic in sub-Saharan Africa; and drug resistance and toxicities have further aggravated this situation. The aim of the study is to validate some folkloric claims on the uses of the selected plants in ethnomedicine in Nigeria. The study selected eight plants based on their use in the management of parasitic protozoan diseases and evaluated their antiprotozoal as well as cytotoxic activities. The methanol extracts of the plants were tested for *in-vitro* activities against *Trypanosoma brucei rhodesiense* (Tbr), *Trypanosoma cruzi* (Tcr), *Leishmania donovani* (Ldon) and *Plasmodium falciparium* as well as mammalian skeletal L6 myoblast for cytotoxicity. The results showed moderate to low *in-vitro* antiprotozoal and cytotoxic activities. *Aspilia africana* (IC_{50} 8.15 µg/mL) and *Caesalpinia pulcherrima* (IC_{50}, 3.98 µg/mL) showed significant *in-vitro* anti-Tbr activity with selectivity indices of 6 and 9.7 respectively. *C. pulcherrima* (IC_{50}: 12.14 µg/mL, Ldon; 14.0, Pfc), *Dissotis rontundifolia* (IC_{50}: 18.45 µg/mL, Tbr), *Ficus glumosa* flower (IC_{50}: 16.05 µg/mL, Tbr), *Morinda morindiodes* (IC_{50}: 13.46 µg/mL, Tbr), *Senna alata* (IC_{50}: 10.51 µg/mL, Tbr; 18.07 µg/mL, Ldon) and *Sphenocentrum jollyannum* (IC_{50}: 11.53 µgmL, Tbr; 18.30 µg/mL, Ldon; 13.26 µg/mL, Pfc) showed moderate activities. Further separation of *C. pulcherrima* extract resulted in improved antileishmanial (IC_{50}, SI: 4.57 µg/mL, 4.6) and antiplasmodial (IC_{50}, SI: 3.80 µg/mL, 5.6) activities. This study has shown that some plants used in folk medicine in Nigeria could be potential sources of lead compounds for parasitic infection.

***Keywords*:** Antitrypanosomal, Antiplasmodial, Antileishmanial, Cytotoxicity, Folk medicine.

Introduction

The importance of plants as a source of new chemical entities in drug discovery can never be overemphasized. To date, plant-based natural products are still an important lead to the discovery and design of new biologically active molecules.[1] Over 51 % of new small molecule drugs (molecular weight < 500) are natural compounds; natural products derived or inspired by structures of active natural compounds and have yet to be minded from many plants, marine environments, microbial world, toxins, venoms and animals.[1]

Rural dwellers still rely on folk medicines for the treatment of infectious diseases. The medicines are usually prepared as decoctions, infusions, powders, ashes, teas or as poultices.[2] The herbal preparations are administered by traditional healers whose expertise in diagnosis, preparations, treatments and follow-up is usually not documented.[3] The practitioners also claim that their procedures are cheaper, safer and more effective than orthodox medicines. The claimed affordability is usually attributed to the availability of these plants within their community. In most cases, however, the claimed safety and efficacy need to be validated. It is, therefore, necessary to scientifically validate the potential use of folk medicines for the treatment of parasitic infections, which plants present a good alternative as a source of new antiparasitic agents. The validation is significant for two reasons. Available data relied on the *in-vivo* model, which is not as specific as the *in-vitro* method. The commonly used *P. berghei* in the *in-vivo* models is not as pathogenic as the *P. falciparum* in sub-Saharan Africa; thus, this study.

We selected eight different plants used in folk medicine to determine their antiparasitic and cytotoxic activities: *Aspilia africana* (Pers.) C.D. Adams (Asteraceae), *Bombax buonopozense* P. Beauv. (Bombacaceae), *Caesapinia pulcherrima* (L) Sw. (Leguminosae), *Dissotis rotundifolia* (Sm.) Triana (Melastomataceae), *Ficus glumosa* Del. (Moraceae), *Morinda morindiodes* Bak. (Rubiaceae), *Senna alata* (L.) Roxb. (Leguminosae) and *Sphenocentrum jollyannum* Pierre (Menispermaceae). These plants are used in folk medicine for various purposes such as antiprotozoal agents.[4-20]

In African traditional medicine, *Aspilia africana* is reported to possess many activities such as wound healing, anti-inflammatory, antimalarial *(P. berghei*) and antiulcer.[4,5] The leaves, roots and stem bark of *B. buonopozense* have antidiarrheal, antimicrobial, antimalarial (*P. Berghei*), antiulcer, analgesic and antipyretic activities.[6,7] The antimicrobial, antiviral, antiulcer and anti-inflammatory activities of *C. pulcherrima* have been documented.[8,9] *D. rotundifolia* is characterized by its vast activities including antidiarrheal, antitrypanosomal (*T. brucei brucei*), antioxidant and antimicrobial.[10,11] *F. glumosa* is known in folk medicine for its antioxidant, antidiabetic, anti-atherogenic, hypolipidemic and gastroprotective activities.[12,13] *M. morindiodes* possesses several pharmacological activities such as antiplasmodial (*P. berghei*), antitrypanosomal (*T. b. brucei*), hypoglycemic and hypolipidemic activities.[14-16] The antioxidant phenolics and the antifungal anthraquinone derivatives of *S. alata* have also been reported.[17,18] The anti-inflammatory of furanoditerpene, antihelmintic, antimalarial, hypolipidemic and hypoglycemic activities of *S. jollyanuum* have been reported.[19,20]

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Citation: Nnadi CO, Okorie NH, Nwodo NJ. Evaluation of *In Vitro* Antiprotozoal and Cytotoxic Activities of Selected Medicinal Plants used in Nigerian Folk Medicine. Trop J Nat Prod Res. 2021; 5(4):609-612. doi.org/10.26538/tjnpr/v5i4.2

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

To the best of our knowledge, there is no sufficient scientific evidence that supports the antiprotozoal properties of these medicinal plants against *T.b. rhodesiense*, *T. cruzi*, *L. donovani* and *P. falciparum*. This study, therefore, examines the *in-vitro* antiprotozoal and cytotoxic activities of these plants against the four protozoan parasites that cause diseases of impoverished rural dwellers.

Materials and Methods

Plant collection

All the plant materials were collected by a plant taxonomist, Mr. Alfred Ozioko of the International centre for ethnomedicine and drug development (InterCEDD). The collection was done in 2017 and 2018 in Nsukka Nigeria. The plants were properly identified and the voucher specimens of the plants were kept at the herbarium (Table 1). The plant names and other relevant pharmacognostic profiles were further confirmed at http://www.theplantlist.org.

Preparation of extracts

The collected plant materials were cleaned, dried under shade for 2 weeks and pulverized into 1 mm coarse powder. A 100 g each of the pulverized plant material was separately macerated in 500 mL of methanol (MeOH) for 48 h on a magnetic stirrer. The mixtures were filtered and concentrated *in vacuo* at 40 ºC. The dried extracts were preserved at 4 ºC before further investigation.

Based on the *in-vitro* activity data of the methanol extract (Table 2), the dried crude extracts (1.1 g) of *C. pulcherrima* was separately dissolved in 100 mL of 10 %v/v MeOH-water and the resulting mixtures successively partitioned with 200 mL (2 x 100 mL) each of *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and *n*-butanol in the increasing order of polarity using a separatory funnel. All the fractions were also concentrated *in vacuo* at 40 ºC.

In-vitro antiparasitic and cytotoxicity assays

The *in-vitro* screening for the antiparasitic activity of the extracts against *Trypanosoma brucei rhodesiense* (bloodstream trypomastigotes, STIB 900 strain), *Trypanosoma cruzi* (amastigotes Tulahuen C4 strain), *Leishmania donovani* (amastigotes MHOM-ET-67/L82 strain), *Plasmodium falciparum* (intraerythrocytic form, NF54 IEF strain) and cytotoxicity test against mammalian L6 cell line from rat skeletal myoblasts were performed according to the established standard protocol using melarsoprol (Tbr), benznidazole (Tcr), miltefosine (Ldon), chloroquine (Pfc) and podophylotoxin (L6) as controls.[21,22] The serial dilutions of the extract/fractions were prepared in the 100 µL growth media from the top concentration of 200 µg/mL concentration and diluted accordingly. The culture and test samples were incubated for 48 h at 37 ºC in a 5 % CO_2 atmosphere It is imperative to note that only the activity data of the dichloromethane soluble of *C. pulcherrima* against parasites are reported due to the inactivity of the other fractions (n-hexane, ethyl acetate and n-butanol) against the parasites at the concentration tested.

Table 1: Profiles of studied plant materials

Plants name	Voucher No.	Parts collected	Yield (%w/w)
A. africana	InterCEDDA2aa1	Flower	0.80
B.buonopozense	InterCEDDB8bb7	Stem bark	3.24
C. pulcherrima	InterCEDDL7cc2	Leaves	1.16
D. rotundifolia	InterCEDDM0dr1	Leaves	4.85
F. glumosa (l)	InterCEDDM1fg9	Leaves	3.02
F. glumosa (fr)	InterCEDDM1fg8	Fruits	0.72
M. morindiodes	InterCEDDR2mm5	Leaves	4.82
S. alata	InterCEDDL9sa1	Leaves	3.90
S. jollyannum	InterCEDDM6sj6	Leaves	5.10

Statistical Analysis

The IC_{50} values were calculated by non-linear regression using an equation for a sigmoid dose-response curve with variable slope (Prism 5.0, GraphPad Software, CA). The IC_{50} data were expressed as mean IC_{50} ± SEM, (n = 3).

Results and Discussion

All the plant extracts showed moderate to low *in-vitro* antiprotozoal activities with regards to the four parasitic protozoa tested (Table 2). With an IC_{50} < 10 µg/mL, extracts of *A. africana*, *C. pulcherrima* and *S. alata* showed moderate activity against Tbr while *C. pulcherrima* elicited moderate activity against Pfc. Moderate to low antiprotozoal activities were observed in extracts with IC_{50} < 20 µg/mL which included *C. pulcherrima* (Ldon and Pfc), *D. rotundifolia* (Tbr), *F. glumosa* fruits (Tbr), *M. morindiodes* (Tbr), *S. alata* (Tbr and Ldon) and *S. jollyannum* (Tbr, Ldon and Pfc). The extracts *B. buonopozense* and *F. glumosa* leaves showed low *in-vitro* activities against all the tested parasites.

All the extracts were also tested for cytotoxic activity against mammalian L6 myoblast. The results in Table 2 showed that all the extracts have cytotoxic IC_{50} > 40 µg/mL, aside *C. pulcherrima* with an IC_{50} of 38.77 µg/mL.

The extract of *C. pulcherrima* was subjected to a solvent-solvent fractionation in the solvent of increasing polarity as follows: n-hexane, dichloromethane, ethyl acetate and n-butanol. The partitioning yielded n-hexane (0.125 g), DCM (0.410 g), EtOAc (0.105 g) and n-butanol (0.095 g) fractions. The fractions were also tested for *in-vitro* activities against the parasites. The DCM soluble of *C. pulcherrima* showed significant improvement in activities against Ldon (IC_{50} 4.6 µg/mL) and Pfc (IC_{50} 3.8 µg/mL) with a slight reduction in the activity against Tbr (IC_{50} 7.8 µg/mL) when compared with the methanol extract, IC_{50} 12.1, 14.0 and 3.98 µg/mL respectively. There was also a slight increase in the cytotoxicity of the fraction (IC_{50} 21.25 µg/mL) compared with the crude extract (IC_{50} 38.77 µg/mL).

The study was carried out to identify plants with the potential to generate lead compounds with activities against the protozoan parasitic infections. The plants were identified based on the ethnopharmacological data obtained from the traditional herbal medicine users. They are primarily used in the treatment of parasitic protozoan infections in folk medicines in sub-Saharan Africa. The ethnomedicinal information we gathered indicated that rural dwellers relied on the aqueous and/or alcoholic extracts for the treatment of malaria and malaria-related symptoms in Eastern Nigeria. A literature survey revealed that some plants tested in this study had also shown some antiplasmodial and/or antitrypanosomal activities in the previous reports.[5,7,10,14-16,19] Our study showed that *A. aspilia*, *S. alata*, *F. glumosa* and *B. buonopozense* elicited low *in-vitro* antiplasmodial activity (IC_{50} > 20); *M. morindiodes* was inactive (IC_{50} > 50) while *S. jollyannum* showed moderate antiplasmodial activity (10 < IC_{50} < 20). On the antitrypanosomal activity (*T,b, rhodesiense*), both *D. rotundifolia* and *M. morindiodes* showed moderate activity (10 < IC_{50} < 20). Both *A. africana* and *C. pulcherrima* were exceptionally active (IC_{50} < 10). A comparison of the present study with available data is somewhat challenging due to two reasons. The *in-vivo* model was adopted in all the previous studies available. More so, *T. b. brucei*, instead of *T. b. rhodesiense* and/or *T. cruzi*, was used as an antitrypanosomal target while *P. berghei* was used for antiplasmodial studies. However, *in-vivo* studies have shown that *S. alata*, *B. buonopozense*, *M. morindiodes* and *S. jollyannum* possess antiplasmodial activity against *P. berghei*.[5,7,14,16,19] Similarly, extracts of *D. rotundifolia* and *M. morindiodes* were reported with antitrypanosomal activity against *T. b. brucei*. Both antiplasmodial and antitrypanosomal activities have not been reported for *C. pulcherrima*, however, diterpene alkaloids possessing a tetracyclic cassane-type furanoditerpenoid skeleton with γ-lactam ring isolated from a closely related species (*C. minax*) showed *in-vitro* antiplasmodial activity (IC_{50} 0.42 and 0.79 µM) against *P. falciparum*.[23] The screening for anti-*T. cruzi* activity was done for

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

only three plants- *C. pulcherrima*, *S. alata* and *S. jollyannum* and the study showed that the plants have low anti-*T. cruzi* activity (IC_{50} > 40) suggesting that these plants may not be a source of anti-*T. cruzi* molecules.

Three plants- *C. pulcherrima*, *S. alata* and *S. jollyannum*- were found with potential for antileishmanial activity with IC_{50}, SI of 12.1 µg/mL, 3.2; 18.1 µg/mL, 2.6 and 18.3 µg/mL, 2.7 respectively. Interestingly, this is not the first time *C. pulcherrima* is reported to possess antileishmanial activity. Some furanocassane diterpenoids, isolated from the root of *C. pulcherrima* were found to possess strong antileishmanial activity.[24] With the improved activity (IC_{50}, SI 4.8 µg/mL, 4.6) of the dichloromethane fraction of the leaves, *C. pulcherrima* as well as *S. alata* and *S. jollyannum* could be potential sources of leishmanicidal agents.

Table 2: *In-vitro* antiprotozoal activities of selected plants

Sample	*Tbr* (STIB900)	*T. cruzi*	*Ldon* (ax. am)	*Pfc* (NF54)	Cytotox (L6)
A. africana (fl)	8.15 ± 0.11	n.d	32.65 ± 3.23	33.25 ± 2.97	49.35 ± 2.93
B.buonopozense (b)	25.75 ± 0.21	n.d	33.70 ± 2.20	21.25 ± 6.20	49.60 ± 6.44
C. pulcherrima (l)	3.98 ± 0.22	42.65 ± 0.15	12.14 ± 1.01	14.00 ± 1.05	38.77 ± 0.65
C. pulcherrima (d)	7.84 ± 0.39	n.d	4.57 ± 0.04	3.80 ± 0.81	21.25 ± 0.29
D. rotundifolia (l)	18.45 ± 0.52	n.d	31.10 ± 3.71	22.75 ± 2.11	51.60 ± 0.75
F. glumosa (l)	27.40 ± 0.30	n.d	55.95 ± 0.87	33.75 ± 4.21	49.50 ± 1.27
F. glumosa (fr)	16.05 ± 0.06	n.d	33.90 ± 0.38	42.30 ± 8.10	45.65 ± 0.92
M. morindiodes (l)	13.46 ± 1.20	n.d	>100	>50	40.35 ± 1.19
S. alata (l)	10.51 ± 1.00	56.25 ± 0.92	18.07 ± 0.98	33.70 ± 5.23	46.87 ± 2.40
S. jollyanuum (l)	11.53 ± 0.05	48.90 ± 1.25	18.30 ± 1.11	13.26 ± 0.33	48.60 ± 1.55
Melarsoprol	0.002 ± 0.001	-	-	-	-
Benznidazole	-	0.509 ± 0.001	-	-	-
Miltefosine	-	-	0.09 ± 0.001	-	-
Chloroquine	-	-	-	0.002 ± 0.001	-
Podophyllotoxin	-	-	-	-	0.009 ± 0.001

Data expressed as mean IC_{50} ± SEM, n = 3, b = bark, fl =flower, fr = fruits, l = leaves, d = dichloromethane soluble, Tbr (*Trypanosoma brucei rhodesiense*), Ldon (*Leishmania donovani*), Pfc (*Plasmodium falciparium*). n.d = not done

Conclusion

The study has provided scientific evidence for the use of some of these plants in the management of parasitic protozoan infections in folk medicine. More importantly, potential sources of anti-*T. b. rhodesiense*, anti-*P. falciparum* and anti-*L. donovani* has also been identified. Isolation of the bioactive principles from *C. pulcherrima* is currently ongoing.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

Charles Nnadi and Ngozi Nwodo are grateful to the Swiss Tropical and Public Health Institute (STPHI), Basel Switzerland for the *in-vitro* assays.

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