In vitro antiprotozoal and cytotoxic activity of ethnopharmacologically selected Guinean plants

IN VITRO ANTIPROTOZOAL AND CYTOTOXIC ACTIVITY OF ETHNOPHARMACOLOGICALLY SELECTED GUINEAN PLANTS

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ABSTRACT

Based on an ethnobotanical survey, 41 Guinean plant species widely used in the traditional treatment of fever and/or malaria were collected. From these, 74 polar and apolar extracts were prepared and tested for their *in vitro* antiprotozoal activity along with their cytotoxicity on MRC-5 cells. A potent activity (IC_{50} <5 µg/mL) was observed for *Terminalia albida, Vismia guineensis, Spondias mombin* and *Pavetta crassipes* against *Plasmodium falciparum*; for *Pavetta crassipes, Vismia guineensis, Guiera senegalensis, Spondias mombin, Terminalia macroptera* and *Combretum glutinosum* against *Trypanosoma brucei brucei;* for *Bridelia ferruginea, G. senegalensis, V. guineensis, P. crassipes* and *C. glutinosum* against *T.cruzi.* Only the extract of *Tetracera alnifolia* showed a good activity (IC_{50} 8.1 µg/mL) against *Leishmania infantum.*The selectivity index of the active samples varied from 0.08 to >100. These results may validate at least in part the traditional use of some of the plant species.

Keywords: Guinea; antiprotozoal activity; *Plasmodium; Trypanosoma*; *Leishmania*; cytotoxicity

INTRODUCTION

Only a limited number of drugs are available for the control of protozoal diseases. Most trypanocidal and leishmanicidal therapeutics have been in use for more than 40 years leading to the occurrence of drug-resistance, a problem which is particularly well-known for malaria. The increasing risk of drug resistance to most of the available and affordable antimalarial, antitrypanosomal and antileishmanial drugs has become a major concern [1]. History shows that plants have been important sources and templates of new medicines against malaria, namely quinine and more recently artemisinin. Traditional medicine may be a potential rich source of new drugs, given the remarkable contribution it has made to the development of novel drugs over the past centuries and it is clearly an approach that should be continued. In Africa, 48 of its 52 countries (92%) are endemic for malaria and access to conventional antimalarials is inadequate for at least 80% of the population. Hence, traditional medicine remains popular mainly in rural areas for treating various illnesses including protozoan diseases [2, 3]. Although the use of traditional medicines that lack scientific validation will undoubtedly continue, there is an urgent need to differentiate between efficacious and safe products *vs.* ineffective and/or unsafe products. Considering the great potential of the Guinean resources in terms of plant biodiversity and traditional knowledge and practice, an ethnopharmacological investigation on 113 Guinean medicinal plants species was undertaken, 109 belonging to 84 genera across 46 families [4]. In the present investigation, 41 plant species were collected and further evaluated for their *in vitro* antiprotozoal activity and selectivity. The present investigation is the first report on the antiprotozoal activities of Guinean plant species widely used in traditional medicine.

RESULTS

A total of 74 extracts from 41 plant species were evaluated for their antiprotozoal and cytotoxic activities. The extracts were prepared from 23 leaf parts, 25 stem bark, 8 root bark and 2 whole plants to give 35, 25, 10 and 4 extracts respectively. From these, 53 extracts were polar (8 aqueous, 29 methanol, 16 ethanol) and 21 were apolar (14 chloroform, 7 hexane). All 74 extracts were screened *in vitro* for their antiprotozoal activity and for their cytotoxic activity against MRC-5 cells. The IC_{50} and CC_{50} values, as well as the selectivity index are presented in Table 1. The level of antiprotozoal activity was ranked according to the following criteria: strong ($IC_{50} \leq 5$µg/mL); good (5 µg/mL < $IC_{50} \leq 10$ µg/mL); moderate (10 µg/mL < $IC_{50} \leq 20$ µg/mL); weak (20 µg/mL < $IC_{50} \leq 40$ µg/mL); low (40 µg/mL < IC_{50} <64 µg/mL) and inactive ($IC_{50} \geq 64$ µg/mL). An extract is considered as non-cytotoxic when CC_{50} >32 µg/mL.

The extracts of *Vismia guineensis* showed the broadest antiprotozoal effect. The extracts of *Combretum glutinosum*, *Guiera senegalensis* and *Pavetta crassipes* showed the strongest activity against both *Trypanosoma* species (IC_{50} = 2.0 to 2.9 µg/mL) while *Lantana camara, Hymenocardia acida, Carica papaya* and *Alchornea cordifolia* showed good antitrypanosomal activity with an IC_{50} of 7.9 to 9.6 µg/mL.

Seven extracts from various plant parts of *Terminalia albida* (stem bark), *V. guineensis* (stem bark chloroform and methanol; root bark chloroform and methanol), *Spondias mombin* (leaves) and *P. crassipes* (leaves, alkaloid extract) exhibited strong antiplasmodial activity. Only *V. guineensis* was cytotoxic (CC_{50} = 5.2 and 11.9 µg/mL for respectively the root and stem bark). A good selectivity index (SI) was observed for *T. albida* (SI >100) and *S. mombin* (SI >23). Six extracts from 4 plant species showed a good antiplasmodial activity: *Mezoneuron benthamianum*, *Terminalia macroptera*, *Newbouldia laevis*, *V. guineensis* and *Alchornea cordifolia* (leaf and stem bark). Only the root bark of *N. laevis* and the leaves of *V. guineensis* were cytotoxic. Ten extracts from ten plant species were moderately active against *P. falciparum*: *A. cordifolia* (stem bark), *Hymenocardia acida* (stem bark), *Carica papaya* (leaves), *Trichilia emetica* (stem bark), *Ficus vallis-choudae* (stem bark), *Cochlospermum tinctorium* (root), *Paullinia pinnata* (leaves), *Morinda geminata* (root bark), *Albizia zygia* (stem bark) and *Azadirachta indica.* All these extracts were not cytotoxic, except *F. vallis-choudae* and *M. geminata*. Other extracts only showed weak, low or no activity.

Twelve extracts from nine plant species were strongly active against *T. b. brucei* including the crude alkaloid extract of a *Pavetta* sp., the extract of the whole plant of *Phyllanthus niruri* (chloroform and methanol extracts), *P. crassipes* (leaves), *Margaritaria discoidea* (leaves), *V. guineensis* (leaves and root bark), *G. senegalensis* (leaves), *S. mombin* (stem bark), *T. macroptera* (root bark), *C. glutinosum* (leaves). Most of these extracts showed a high cytotoxicity (CC_{50} <5 µg/mL) except *Pavetta* sp., *P. niruri, M. discoidea, T. macroptera* and *S. mombin*. The strongest activity against *T. cruzi* was recorded for 8 extracts from 5 plant species which were all cytotoxic (CC_{50} ≤21.2 µg/ml). These included *Bridelia ferruginea, G. senegalensis, P. crassipes, V. guineensis* (chloroform and methanol extracts of the root bark and the stam bark) and *C. glutinosum.* Various extracts exhibited a good inhibitory activity against *T. b. brucei* with an IC_{50} varying from 6.7 (stem bark of *V. guineensis*) to 8.6 µg/mL (leaves of *A. cordifolia*). A good leishmanicidal effect along with the best SI was recorded for the leaf extracts of *Tetracera alnuifolia*.

DISCUSSION

The present results support at least in part the traditional antimalarial use of most of the tested plant species since their extracts showed an IC_{50} <64 µg/mL. As all the tested plant species are widely used in Guinean traditional medicine, the other weakly or inactive species could possibly act on symptoms of malaria such as febrile illnesses and/or enhance immunological responses. In view of their high selectivity (SI >100 and 23) *T. albida* and *S. mombin* should offer the potential of safer therapy: *T. albida* is widespread and *S. mombin* is moderately distributed in the Guinean flora. The wide use of the most frequently cited plant species *V. guineensis, T. macroptera,* and *T. albida* is clearly supported by their strong to moderate *in vitro* antiplasmodial activity: *V. guineensis* (leaves, stem bark and root bark), *T. macroptera* (root bark), *T. albida* (leaves and stem bark) are intensively used by the herbalists and traditional practioners [4]. Although these species are moderately distributed in Guinea, the intense harvesting of stem bark or root bark could lead ultimately to their extinction. In order to preserve the ecology and sustainability, the use of leaves is recommended instead of stem bark or root bark. In this respect, it is interesting to note a good antiprotozoal property (IC_{50} ≤10 µg/mL) of the leaves *S. mombin, M. benthamianum, N. laevis* and *A. cordifolia* as antiplasmodials; *M. discoidea, V. guineensis, G. senegalensis, C. glutinosum, H. acida, A. indica, C. papaya, L. camara, A. cordifolia,* and *L. inermis* as antitrypanosomals (*T. b.*

brucei)*; B. ferruginea, G. senegalensis, C. glutinosum, V. guineensis, L. camara, C. papaya, H. acida* and *A. cordifolia* as antitrypanosomals (*T. cruzi*)*;* and *T. alnifolia* as antileishmanial. In general, the polar extracts (methanol, and/or water) were more active compared to the apolar ones (hexane, chloroform), which supports the wide use of solvents such as water in traditional medicine. However, the water extracts were sometimes significantly less active than the organic ones.

The antiplasmodial activity found here for some of the Guinean plant species was in accordance with previous reports on *V. guineensis* [5], *T. macroptera* [6] and *A. cordifolia* [7]. The *in vitro* antiplasmodial activity of *T. emetica* was in agreement with that of other *Trichilia* species, such as *T. glabra* (Costa Rica), *T. hirta* (Costa Rica) and *T. trifolia* (Puerto Rico) [8]. The effect against *T. b. brucei* was similar to previous reports on *H. acida, C. sieberiana* and *T. emetica* [9]. In contrast to the strong antiplasmodial activity of Guinean *V. guineensis*, the apolar and polar extracts of the stem bark of *V. guineensis* from Cameroon exhibited weak activities against both chloroquine-sensitive and multiresistant strains of *P. falciparum* [10]. Moreover, the methanolic extracts of the leaves and stem bark of *V. orientalis* from Tanzania were reported to be inactive against multidrug resistant *P. falciparum* strain K1 [11]. Also with regard to *C. papaya, C. tinctorium*, *G. senegalensis*, *E. senegalensis* and *A. indica* conflicting results have been reported before. Such discrepancies are most likely due to the use of plant samples from different geographical origins, their location and their period of collection [12-17].

Antiplasmodial activities of *Terminalia* species have been reported previously. However, this is the first report on the strong antiplasmodial effect of *T. albida* with IC_{50} = 0.6 µg/mL. Antiplasmodial activity has been recorded for the aqueous extract of the root bark of *T. macroptera* from Burkina Faso (IC_{50} = 1 µg/mL; K1 strain) [6]; the ethanol extract of the young leaves of *T. schimperiana* (IC_{50} = 2.4 µg/mL; K1 strain) [15], the ethanol extract of the stem or leaf of *T. glaucescens* (IC_{50} values ranging from 0.3 to 0.6 µg/mL on FcM29 and FeB1-Colombia chloroquine-resistant and Nigerian chloroquine-sensitive strains) [7], the methanol extract of the stem bark of *T. avicennioides* (IC_{50} = 14.1 µg/mL, K1 strain or 12.3 µg/mL, 3D7 strain) [18], the methanolic and aqueous root bark extracts of *T. mollis* active against chloroquine-sensitive strain 3D7 with IC_{50} values of 11.7 and 33.5 µg/mL, respectively [19], the stem bark and stem wood aqueous extracts of *T. spinosa* against chloroquine-sensitive K67 and chloroquine-resistant ENT36 strains of *P. falciparum* (IC_{50} <10 µg/ml) [20].

To fully evaluate the antioplasmodial potential of the active plant species, complementary *in vivo* investigations are needed. Indeed, while many extracts appear very promising *in vitro*, subsequent confirmation *in vivo* in mice infected with *Plasmodium* sp. have mostly been disappointing. For example, the curative and the suppressive activities of *E. senegalensis* were not significant [21]; the extract of *A. indica* was found to require very high doses (e.g. 800 mg/kg body weight) to exhibit significant *in vivo* activity while such high doses are clearly not therapeutically meaningful [22]; the aqueous extract of *C. papaya* and *A. indica* were inactive on chicken infected with *P. gallinaceum* [8].

Some of the species discussed above have already phytochemically been investigated for active compounds. Ellagic acid from *A. cordifolia* exhibited an IC_{50} value of 0.1 µg/mL against *P. falciparum* FcM29 [23]. Ellagic acid also showed *in vivo* activity in mice [24], and may also be responsible for the antimalarial activity of *Phyllanthus amarus* [25]. Vismione H from *V. guineensis* showd an IC_{50} of 0.09 µg/mL against *P. falciparum* NF54 [26]. Vismione D, emodine and other anthranoids from *V. orientalis* showed IC_{50} values <10 to 50 µg/mL against *T. b. rhodesiense* and *T. cruzi*, IC_{50} = 0.4 - 2.0 µg/mL against *L. donovani*, and IC_{50} = 1 - 50 µg/mL against *P. falciparum* K1 [27]. Gedunin from *A. indica* exhibited an IC_{50} of 0.02 - 0.04 µg/mL against *P. falciparum* [22], but did not suppress *Plasmodium berghei* in mice after intraperitoneal dosing at 90 mg/kg/day [16]. 3-*O*-*E*-*p*-Coumaroyl-alphitolic acid from *C. tinctorium* showed an IC_{50} value of 2.3 µM against *P. falciparum* [13].

To the best of our knowledge, this is the first evidence of the *in vitro* antiplasmodial activity of *S. mombin*. Due to the strong antiplasmodial activity (IC_{50} = 2.8 µg/mL) without any cytotoxicity (CC_{50} >64.0 µg/mL) of the methanol extract of the leaves, this plant could be a good antimalarial candidate, however, attention must be paid since toxicity along with an abortifacient effect of the leaf aqueous extract has been described [28, 29].

In conclusion, the present results support at least in part the traditional antimalarial use of most of the investigated plant species. Future perspectives include the identification of active antiprotozoal constituents present in *T. albida, S. mombin* and *T. alnifolia* through bioassay-guided fractionation.

MATERIAL AND METHODS

Ethnobotanical investigation

The selected plants were collected during an ethnobotanical survey conducted in the 4 Guinean regions from May 2008 to September 2010. Botanical identification was first conducted in the field, and confirmed by Dr. S.M. Keita (CERE, University of Conakry), M.S. Barry and N. Camara (CRVPM – Dubreka). Voucher specimens were deposited at the Herbarium of the CRVPM. Plant families and registration numbers of voucher specimens have been published before [4].

Preparation of plant extracts

Plant extracts were prepared by macerating 10 g of powdered dried plant material with 50 mL solvent (hexane, chloroform, methanol, or ethanol 70%) for 24 h. With regard to the aqueous crude extract, a decoction of 10 g dried plant powder was prepared in 150 mL distilled water for 30 min in order to approximate the traditional preparation method. Total alkaloid extracts were prepared from 50 g of plant material by chloroform to which ten drops of ammonia were added. The extracts were then filtered and each filtrate was evaporated *in vacuo* to dryness. Five mg were weighed and submited for antiprotozoal and cytotoxicity testing.

Biological evaluation

Antiprotozoal activity and cytotoxicity (MRC-5 cells) evaluation was carried out as previously described [30, 31]. The selective index (SI) is calculated as the ratio of the cytotoxicity on MRC-5 (CC_{50}) to the antiprotozoal activity (IC_{50}). For the different tests, appropriate reference drugs were used as positive control: tamoxifen for MRC-5, chloroquine for *P. falciparum*, miltefosine for *L. infantum*, benznidazole for *T. cruzi* and suramin for *T. brucei*. All reference drugs had pharmaceutical quality and were either obtained from Sigma-Aldrich (tamoxifen, suramin) or from WHO-TDR (Geneva, Switzerland; chloroquine, miltefosine, benznidazole).

Conflicts of interest

The authors have no conflicts of interest

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