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Antileishmanial and Trypanocidal Activities of Extracts and Aporphine Alkaloids Isolated from *Monodora* Genus (*Annonaceae*)

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Abstract

Leishmaniasis and trypanosomiasis are protozoan diseases caused respectively by the kinetoplastid protozoan *Leishmania* parasites transmitted by the female phlebotomine sandflies and *Trypanosoma* parasites transmitted by the tsetse fly. In the search for agents from tropical medicinal plants to treat these two neglected tropical diseases, serially extracted petroleum ether, dichloromethane and methanol extracts of the leaves of *Monodora crispata* and *Monodora brevipes*, and eleven aporphines alkaloids isolated from the dried powdered leaves of the two plants were evaluated against *Leishmania donovani promastigotes* and *Trypanosoma brucei brucei trypomastigotes*. The extracts of both plants and the isolated compounds displayed varied levels of antiprotozoal activities. The oxoaporphine compounds, (+)-anolobine (7) and (+)-listeferine (8), exerted the most significant activity against *L. donovani* (IC_{50}: 14.59 µM) and *T. brucei brucei* (LC_{100}: 50.02 µM) respectively. This is the first report on the antiprotozoal activity of the isolated compounds. The results offer potential for further studies of the oxoaporphines for enhanced antiprotozoal activity.

Keywords: Antileishmanial; Trypanocidal; Monodora; *Leishmania donovani*; *Trypanosoma brucei brucei*; Aporphine alkaloids

Introduction

Leishmaniasis and trypanosomiasis are separate groups of arthropod-borne diseases of humans and other animals caused by infection with protozoan hemoflagellates of the genus *Leishmania* and *Trypanosoma* respectively. Both genera belong to the *Trypanosomatidae* family of the order Kinetoplastida [1]. These kinetoplastid diseases also referred to as Neglected Tropical Diseases (NTDs), are a diverse group of communicable diseases that prevail in tropical and subtropical conditions in 149 countries and affect more than one billion people, costing developing economies billions of dollars every year [2].

The genus *Leishmania* has more than 20 species. These are transmitted to humans by the bites of infected female phlebotomine sandflies which, breed in forest areas, caves, or the burrows of small rodents. There are three main forms of the disease: Cutaneous leishmaniasis which is the most common form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body, leaving life-long scars and serious disability; visceral leishmaniasis or kala-azar, which is fatal if left untreated in over 95% of cases and is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia; and mucocutaneous leishmaniasis which leads to partial or total destruction of mucous membranes of the nose, mouth and throat [2]. According to WHO [2] an estimated 700,000 to 1 million new cases and 20,000 to 30,000 deaths occur annually.

Human African trypanosomiasis (HAT), also known as sleeping sickness is often fatal if left untreated [3]. The disease takes 2 forms, depending on the parasite involved: *Trypanosoma brucei gambiense* found in 24 countries in west and central Africa currently account for 97% of reported cases of sleeping sickness and causes chronic infection. The other, *Trypanosoma brucei rhodesiense* found in 13 countries in eastern and southern Africa represents fewer than 3% of reported cases. This causes acute infection. A third form known as American trypanosomiasis or Chagas disease occurs mainly in Latin America. The causal organism belongs to a different Trypanosoma subgenus and is transmitted by a different vector [4].

The drugs that are currently used for the treatment of these neglected tropical diseases suffer the limitations of toxicity, variable efficacy, requirements for parenteral administration and/or lengthy treatment regimen [3]. As a result, the search for new drug prototypes is an important requirement that needs to be pursued until the burden of these diseases can be reduced or eliminated. In the past few years, the interest in natural products as a potential source for the treatment of parasitic diseases has increased [5-8]. Traditionally, plants have been used for the treatment of protozoan diseases and phytotherapy has over the years received considerable attention in the search for alternative compounds with antiparasitic activity [9,10]. In our search for chemical agents from tropical medicinal plants for the treatment of Leishmaniasis and *Trypanosomiasis*, we evaluated eleven compounds, isolated from *Monodora crispata* and *Monodora brevipes* together with the serially extracted organic solvent extracts of the two plants against *Leishmania donovani promastigotes* and *Trypanosoma brucei brucei trypomastigotes*.

Materials and Methods

Plant materials

Leaves of *M. crispata* and *M. brevipes* were collected in August 2010 in Diapodoumé (South of Côte d'Ivoire). They were authenticated by Aké Assi from the Centre National de Floristique (Université de Cocody-Abidjan). Herbarium specimens were deposited at the Herbarium of the Laboratoire de Botanique (Université de Cocody-Abidjan), with the voucher numbers: MC-DADE-Diapodoumé2010-1 and MB-DADEDiapodoumé2010-1 respectively.

Extraction of plant materials

Dried powdered leaves of *M. crispata* and *M. brevipes* were each serially cold-extracted with petroleum ether, dichloromethane and methanol. The extracts were each concentrated under reduced pressure using the rotavapor at 40ºC into semisolid mass. The semisolid mass was further dried under vacuum to give a hardened amorphous mass, which was kept in the fridge until needed for use.

Isolated compounds

Eleven aporphines alkaloids (Figure 1) identified as, (-)-mocrispatine (1); (-)-pallidine (2); (-) N-méthylarmépavine (3); (+)-magnoflorine (4); (+)-menispermine (5); (-)-anolobine (6); (+)-anolobine (7); (+)-listeferine (8); (+)-réoméroline (9); (+)-laurotetanine (10) and (+)-corydine (11) isolated from the dried powdered leaves of M. crispata and brevipes [11,12] were used in this study.

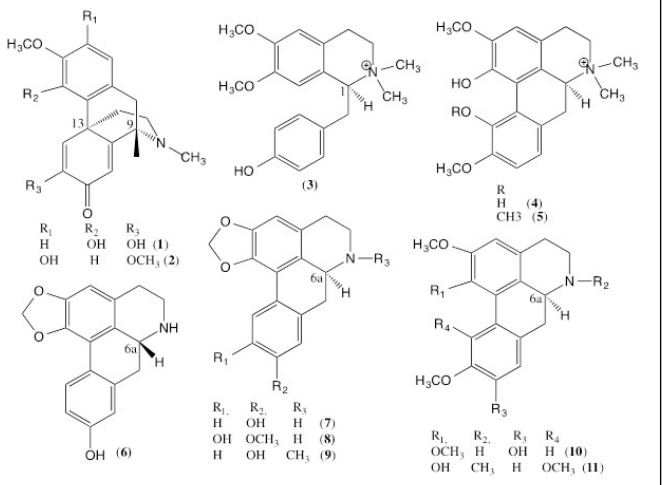

Figure 1: Aporphine alkaloids isolated from *M. crispata* and *M. brevipes*.

Determination of antileishmanial activity of test samples

The antileishmanial activity of the isolated compounds was tested *in vitro* against *L. donovani* (WHO designation: MHOM/ET/1967/L82), according to a method previously described [13]. This method is based on a dye, tetrazolium-dye, specific for dead parasites, thus allowing the measurement of an EC_{50} value. Briefly, promastigotes were cultivated in HEPES (25 mm)-buffered RPMI 1640 medium enriched with 10% fetal calf serum and 50 µg/mL gentamicin at 27°C in a dark environment. The screening was performed in flat bottomed 96-well plastic tissue-culture plates maintained at 27°C. Promastigotes forms from a logarithmic phase culture were suspended to yield about 106 cells/mL after haemocytometer counting. Each well was filled with 100 µl of the parasite suspension, and the plates were incubated at 27°C for 1 h before the addition of the samples, dissolved in Dimethyl Sulfoxide (DMSO) at different concentrations up to 100 µg/ml for both extracts and compounds. The viability of promastigotes was assessed by the Tetrazolium-dye (MTT) colorimetric method. The results are expressed as the effective concentrations (IC_{50}) inhibiting parasite growth by 50% after a 3-day incubation period. Pentamidine and was used as reference compound. Experiments were performed in triplicate. A maximum DMSO concentration of 0.1%, which did not show toxicity, was used in the final well volume of 200 ml.

Determination of trypanocidal activity of test samples

Compounds were tested for their activity against bloodstream forms of *T. brucei brucei* (CMP fast strain) as described elsewhere [14,15]. This method was based on the observation of the motility of cultivated parasites, followed by the inoculation to naive mice of the solution of immobile parasites with the LC_{100} of the tested compound. No infection confirms the trypanocidal effect for LC_{100}. Briefly, the bloodstream parasites were maintained *in vitro* without loss of infectivity for 24 h in the dark at 37°C in a 5% CO_2 atmosphere. Screening was performed in 96-well tissue-culture plate in a final well volume of 200 ml containing 2×10^5 parasites/ml, in supplemented minimum essential medium (Gibco, BRL). Samples were tested at varied concentrations up to 100 µg/ml for both extracts and compounds (diluted in DMSO) and the minimum lethal concentration (LC_{100}: defined as the minimum concentration at which no motile parasites were observed microscopically) determined. Confirmation of the LC_{100} was obtained by injecting naive mice intraperitoneally with 150 µl of the treated trypanosome suspension withdrawn from the well after a 24 h incubation period. The animals were aparasitaemic 30 days post-infection. Melarsoprol and pentamidine were used as reference compounds. Experiments were performed in triplicate. A maximum DMSO concentration of 0.1% which did not show toxicity was used in the final well volume.

Results and Discussion

Methanol, dichloromethane and petroleum ether extracts of *M. brevipes* and *M. crispata* showed various levels of antiprotozoal activity against *L. donovani promastigotes* and *T. brucei brucei* trypomastigotes in Table 1. The extracts were more active against *L. donovani* than *T. brucei brucei*; while all were active against *L. donovani* (EC_{50}<100 µg/mL), only three including the methanol extract of *M. brevipes*, dichloromethane and petroleum extracts of *M. crispata*, showed activity against *T. brucei brucei* (CL_{100}<100 µg/mL). *M. crispata* extracts were generally the most active; the dichloromethane and methanol extracts were respectively the most active against *L. donovani* and *T. brucei brucei*.

The eleven aporphine alkaloids; two promorphinanes: [(-)-mocrispatine (1), (-)-pallidine (2)], one benzylisoquinoline: [(-)-N-methylarmepavine (3)], four aporphines: [(+)-magnoflorine (4), (+)-ménispermine (5), (+)-laurotetanine (10) and (+)-corydine (11)], and four oxoaporphines [(-)-anolobine (6); (+)-anolobine (7); (+)-listeferine (8); (+)-roemeroline (9)] have shown varied levels of antiprotozoal activity against *L. donovani promastigotes* and *T. brucei brucei* trypomastigotes in Table 2.

Extracts	*Leishmania donovani*	*Trypanosoma brucei brucei*
Concentration	IC_{50} (µg/mL)	CL_{100} (µg/mL)
Monodora brevipes methanol extract	64.2	50.0
Monodora brevipes dichloromethane extract	56.5	>100
Monodora brevipes petroleum ether extract	60.7	>100
Monodora crispata methanol extract	33.9	12.5
Monodora crispata dichloromethane extract	10.8	31.3
Monodora crispata petroleum ether extract	16.6	62.5
Pentamidine	2.6	-
Mélarsoprol (Mel W)	-	0.2

Table 1: Antiprotozoal activity of extracts of *Monodora genus* (leaves).

Compound	*Leishmania donovani*	*Trypanosoma brucei brucei*
Concentration	EC_{50} (µm)	LC_{100} (µm)
(-)-mocrispatine (1)	193.93	199.68
(-)-pallidine (2)	53.82	191.13
(-) N-méthylarmépavine (3)	>200	>200
(+)-magnoflorine (4)	>200	>200
(+)-ménispérmine (5)	>200	>200
(-)-anolobine (6)	19.21	114.95
(+)-anolobine (7)	14.59	>200
(+)-listeferine (8)	26.69	50.02
(+)-réoméroline (9)	52.20	>200
(+)-laurotetanine (10)	23.24	191.13
(+)-corydine (11)	174.78	183.28
Pentamidine	7.7	-
Mélarsoprol (Mel W)	-	0.4

Table 2: Antiprotozoal activity of isolated compounds.

The compounds in a manner similar to the extracts were largely more active against *L. donovani promastigotes*. The oxoaporphines were generally the most active especially against *L. donovani* ($14.59 \leq IC_{50} \geq 52.20$ µm). The aporphine, (+)-laurotetanine (10), also showed good activity against *L. donovani* (IC_{50}=23.24 µm). The oxoaporphine alkaloid, (+)-listeferine (8) was the most active compound against *T. brucei brucei* with an LC_{100} value of 50.02 µm. Two such alkaloides, (+)-reomeroline (9) and (+)-anolobine (7) nevertheless did not show activity against *T. brucei brucei*. Interestingly however, (+)-anolobine (7) was the most active against *L. donovani* (IC_{50}=14.59 µm) and has IC_{50} value of about twice that of the standard drug, Pentamidine (IC_{50}=7.7 µm). Its IC_{50} value also falls within the range given for the putative antileishmania drug, sitamaquine ($2.9 \geq IC_{50} \leq 19.0$ µm) [8]. 7 is therefore a potential candidate for further studies as an antileishmanial compound. (+)-Listeferine (8), the most active compound against *T. brucei brucei*, on the other hand, was 125 fold less active than the reference drug, Mélarsoprol. Three compounds exhibited no activity against either parasite.

The generally low antiprotozoal activity of the aporphines does not make them good candidates for either leishmaniasis or trypanosomiasis therapy. However, the activity demonstrated by the oxoaporphines tells of their potential as important class of phytochemicals that could be a subject of activity optimization through structural modification. Indeed, some of the oxoaporphines which differ slightly in chemical structure showed clear differences in activity in this study. For example, (+)-Listeferine (8; Figure 1) which is a demethylated derivative of (+)-reomeroline (9), is about twice more active than the latter against *L. donovani*; an observation that demethylation at R_3 led to increased antiprotozoal activity of the latter.

Members of the *Annonaceae* family and their isolated compounds have demonstrated antiprotozoal activities including antiplasmodial, leishmanicidal and antitrypanosomal activities [6,7]. Literature data also revealed similar activity of the related aporphines. Oxoaporphines closely related to those evaluated in this study particularly possess promising antiprotozoal activity especially antitrypanosomal activities [5]. It is therefore not surprising that the oxoaporphines are the most active molecules in this study. This is the first report of the antiprotozoan activity of *M. crispat*, *M. brevipes* and the isolated compounds, and it portrays the oxoaporphine as potential candidates for further structural modification-activity study.

Conclusion

The organic solvent extracts of *M. crispat*, *M. brevipes* and their isolated aporphines showed varied levels of activity against *L. donovani promastigotes* than *T. brucei brucei* trypomastigotes. The oxoaporphines were particularly active especially against *L. donovani* and therefore are potential candidates for further studies for enhancement of their antileishmanaial activity.

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Ethical Approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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