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Leishmanicidal activity of fractions rich in aporphine alkaloids from Amazonian *Unonopsis* species

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Abstract: *In vitro* evaluation of alkaloidal fractions of twigs, barks and leaves from two *Unonopsis* species, *Unonopsis guatterioides* R.E. Fr. and *Unonopsis duckei* R.E. Fr., Annonaceae, against promastigote forms of *Leishmania amazonensis* revealed these species as sources of substances with promising leishmanicidal potential. All alkaloidal fractions from twigs, barks and leaves of *U. guatterioides* were classified as highly active, with IC50 1.07, 1.90, and 2.79 mg/mL, respectively. Only the alkaloidal fraction from the twigs of *U. duckei* was classified as inactive.

Introduction

Leishmaniasis is a tropical disease, considered by the World Health Organization (WHO) as the second most important protozoal public disease, even to infect two million people annually worldwide. In Brazil, leishmaniasis affects nineteen states, with approximately 90% of human cases related to the Northeast and there are major outbreaks in the Midwest, Southeast and North (Rath et al., 2003; Rocha et al., 2005; Gil et al., 2008). The treatment of leishmaniasis is based on complexes of trivalent or pentavalent antimony and the patients treated with this drug present some cardiotoxicity and gastrointestinal intolerances. Other problems associated with these types of medications are the pain at the injection site and in some cases kidney and liver failure (Bacab & Rodriguéz, 2001; Gil et al., 2008). Considering the difficulties of the treatment and the absence of vaccines, there is urgency in the search for new therapeutic drugs, including those of herbal medicines. According to the WHO, the plants are the best and largest source of drugs for mankind. Besides alkaloids, the literature describes other classes of natural substances with proven leishmanicidal activity *in vitro* assays on promastigote and/or amastigotes forms of *Leishmania*, such as: quinones, terpenes, phenolic derivatives, acetogenins, among others (Bacab & Rodriguéz, 2001). It is estimated that in the Amazon region more than 1200 species are marketed as medicinal plants (Maciel et al., 2002). The use of extracts from plants or potions for developing countries is a reality (Montanari & Bolzani, 2001) and ethnobotanical studies have shown the popular use of plants in the treatment of leishmaniasis (Bacab & Rodriguéz, 2001).

The Neotropical genus *Unonopsis* (Annonaceae) comprises about fifty species and presents a wide distribution through the Amazon region where there are species with restricted distribution as is the case of *U. duckei* (Maas et al., 2007). This genus is rich in aporphine alkaloids and its derivatives, being oxaporphine, azafluorenone, phenanthrene and bisaporphine the most common types (Laprévote et al., 1987; Arango et al., 1988; Guinaudeau et al., 1988; Siqueira et al., 1998; Waechter et al., 1999). Several biological activities are known for the aporphine alkaloids and their derivatives (Silva et al., 2007; Zanin & Lordello, 2007) where antiprotozoal activity is increasingly being studied and presenting good results, especially leishmanicidal activity (Waechter et al., 1999; Mishra et al., 2008). Other phytochemical reports brings data about the isolation of the triterpene polycarpol from the follow species: *Unonopsis guatterioides* (Touché et al., 1981; Silva et al., 2012b), *Unonopsis spectabilis* (Laprévote et al., 1987), *Unonopsis pacifica* (Arango et

al., 1988) and *Unonopsis glaucopetala* (Jayasuriya et al., 2005), and data of the composition of the essential oils of some species, such as *Unonopsis guatterioides* (Fournier et al., 1997) and *Unonopsis costaricencis* (Palazzo et al., 2009). In this short communication we report the evaluation of the *in vitro* leishmanicidal activity from the alkaloid fractions from the twigs, barks and leaves of *Unonopsis guatterioides* and *Unonopsis duckei.*

Materials and Methods

Plant material

The botanical material (twigs, barks and leaves) of *Unonopsis guatterioides* R.E. Fr., Annonaceae, was collected in the Campus of Federal University of Amazonas (UFAM) in January 2010. The specimen was identified by Prof. Dr. Antonio Carlos Webber from the Biology Department from UFAM, a voucher specimen was deposited in the herbarium of UFAM under registration number 8249. The botanical material (twigs, barks and leaves) of *Unonopsis duckei* R.E. Fr. was collected from Adolpho Ducke Forest Reserve (km 26 of AM-010 highway) from individual specimen previously marked (number 3289) and identified during the execution of the Ducke Reserve Flora Project. A voucher specimen under registration number 191265 is deposited in the herbarium of the Instituto Nacional de Pesquisas da Amazônia. After drying in an oven with air circulation at 50 °C for a period of two days, the material was powdered.

Preparation of the alkaloid fractions

The powdered material was extracted successively with *n*-hexane and methanol, during six days for each solvent which were renewed every three days. After evaporation of the solvent at reduced pressure, a part of methanol extracts of the barks of the trunk (4.1 g), twigs (4.4 g), and leaves (4.2 g) of *U. guatterioides* and barks of the trunk (4.2 g), twigs (4.3 g), and leaves (4.7 g) of *U. duckei* were subjected to acid-base treatment, according to the methodology to obtain the conventionally alkaloid fractions (Costa et al., 2006), where the methanol extracts were solubilized in CH_2Cl_2 (50 mL) and partitioned with a solution of 3% aqueous HCl (3 x 250 mL). In sequence the aqueous solutions were combined and adjusted with NH_4OH to pH 10, followed by the extraction with CH_2Cl_2 (3 x 200 mL). The CH_2Cl_2 fractions were combined, and the solvent evaporated under vacuum, providing, respectively, 53.6, 30.9, and 71.9 mg of alkaloid fractions of *U. guatterioides* and 25.6, 13.3, and 47.4 mg of alkaloid fractions of *U. duckei.*

In vitro *evaluation of the leishmanicidal activity*

Promastigote forms of *Leishmania amazonensis* (IFLA/BR/67/PH8) were maintained at 24 ºC in RPMI 1640 culture medium containing 50 µg/mL of gentamicin sulfate and supplemented with 10% of fetal bovine serum. To the evaluation of the leishmanicidal activity of the alkaloid fractions, $5x10^5$ promastigotes/mL were treated with 100, 50, 25, 12, and 6 µg/mL of the fractions, by six days at 24 °C. Every 24 h was performed the differential counting of the promastigotes using the erythrosin B vital exclusion dye at 0.04% in a Neubauer hemocytometer chamber under an optical microscope with a magnification of 400x. The drug pentamidine (50 mg/mL) was used as reference. Cultures containing dimethyl sulfoxide (DMSO) at a concentration of 0.7% were made to control the solvent fractions. Assays were performed in duplicate and repeated twice. The results were expressed as parasite growth inhibitory concentration (IC50) (Schmeda-Hirschmann et al., 1996; Camacho et al., 2003). The IC50 calculations were performed using the regression model by nonlinear dose-response curve of inhibition (log [inhibitor] x response), describing the relationship between various concentrations of the samples and the number of *Leishmania* in the growth curves obtained. The leishmanicidal activity of each sample was classified as highly active (IC50 <10µg/mL), active (10 mg/mL <IC50 <50 µg/mL), moderately active (50 µg/mL <IC50 <100µg/mL) and non-active (IC50> 100 µg/mL) (Osório et al., 2007).

Results and Discussion

Table 1 presents the results of the leishmanicidal activity of six alkaloid fractions. The fractions from *U. guatterioides* were classified as highly active, with the IC50 of alkaloid fractions of the twigs, leaves and barks, respectively, 1.07, 1.90, and 2.79 µg/mL. On the other hand, from the evaluated alkaloid fractions of *U. duckei*, only the portion derived from the leaves showed high activity, with IC50 of 4.00 µg/mL, with the fraction derived from the barks classified as active (IC50 32.16 µg/mL) and the fraction from the twigs classified as inactive. The pronounced activity of alkaloid fraction of the twigs (IC50 1.07 µg/mL) may be a consequence of the presence of the majority aporphine alkaloid anonaine (**1**) (Silva et al., 2012a). Previous studies revealed that this alkaloid has significant leishmanicidal activity against promastigotes of *L. braziliensis* and *L. amazonensis* (Queiroz et al., 1996). The observation of the high activity of the alkaloid fraction of the leaves (IC50 1.90 µg/mL) may also be related to the presence of a majority aporphine alkaloid asimilobine (**2**) (Silva et al., 2012a), which recently was related as an anti-leishmanial compound (Mollataghi et al., 2012).

The alkaloid fraction from the barks of the trunk of *U. guatterioides*, (IC50 2.79 µg/mL) also showed significant activity, that can be attributed to the oxaporphine alkaloids liriodenina (**3**) and lisycamine (**4**) (Silva et al., 2012a), which showed good leishmanicidal activity (Waechter et al., 1999) when evaluated against promastigotes form of *L. major* and *L. donovani.* Liriodenine also presented significant activity against *L. guyanensis* and *L. braziliensis* (Costa et al., 2006). Another alkaloid in the genus Unonopsis described with leishmanicidal potential against promastigote forms of *Leishmania in vitro* assays is the bisaporphine alkaloid unonopsine (**5**) isolated from *U. buchtienii* (Waechter et al., 1999) and from the species synonymy of *U. guatterioides, U. lindmanii* (Siqueira et al., 1998).

Table 1. Leishmanicidal activity *in vitro* of alkaloidal fractions of Amazonian *Unonopsis* species.

Botanical name	Extracts	Code	Plant part	IC50 (µg/mL)[a]
U. duckei	Alkaloid fraction	FADC	barks	32,16
		FADG	twigs	155,61
		FADF	leaves	4,00
U. guatterioides	Alkaloid fraction	FAGC	barks	2,79
		FAGG	twigs	1,07
		FAGF	leaves	1,90
Pentamidine[b]				9,8

[a]*Leishmania* (*Leishmania*) *amazonensis* strain PH8 (IFLA/BR/67/PH8); IC50, 50% inhibitory concentration; [b]Reference drug.

The present results demonstrate that both *U. duckei* and the *U. guatterioides* have a significant leishmanicidal activity. Prior knowledge of the chemistry of the species *U. guatterioides* reinforces the idea of potentiality of aporphine alkaloids in the fight against leishmaniasis. *U. guatterioides* can be considered a strong candidate to source of metabolites with leishmanicidal character, since in addition to all the fractions were highly actives, is a species that is widely distributed in the Amazon Region.

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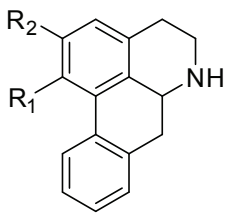
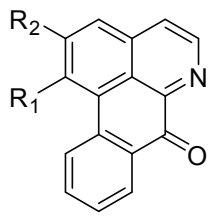
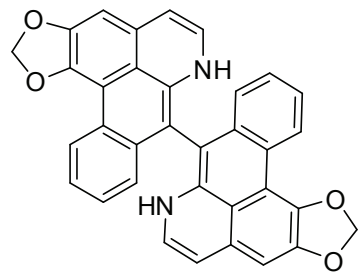


1 $R_1/R_2=OCH_2O$
2 $R_1=OCH_3$; $R_2=OH$



3 $R_1/R_2=OCH_2O$
4 $R_1=R_2=OCH_3$



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