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Antiprotozoal Activity of Ethanol Extracts of Some *Bomarea* Species

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Abstract

The antiprotozoal activity of 26 ethanol extracts derived from 13 species of the genus *Bomarea* (Alstroemeriaceae) were evaluated against the promastigote forms of three *Leishmania* species (*L. amazonensis* L., *L. braziliensis* Vianna, and *L. donovani* Laveran & Mesnil) and the epimastigote form of *Trypanosoma cruzi* Chagas. IC_{50} values for leishmanicidal activities were between 4.9 and 98.6 μg/mL, *B. setacea* extracts being the most active against the three species of *Leishmania*. Amphotericin B ($IC_{50} = 0.2$ μg/mL) and pentamidine ($IC_{50} = 10$ μg/mL) were used as control in this assay. IC_{50} values for antitrypanosomal activities were between 65.2 and 92.7 μg/mL, whereas the control benznidazole had an IC_{50} value of 2 μg/mL.

Keywords: Alstroemeriaceae, antitrypanosomal activity, biodiversity, *Bomarea*, leishmanicidal activity.

Introduction

According to the World Health Organization (WHO), infectious diseases were the second greatest cause of mortality in the world in 2005, accounting for approximately 25% of all deaths. Within tropical areas, parasites are responsible for major infectious diseases such as malaria, leishmaniasis, and Chagas' disease. The high incidence of this type of disease has prompted the scientific community to dedicate great effort in the search for solutions to these public health problems by exploring plant metabolites as potential therapeutic agents (Cunha et al., 2003).

Chagas' disease, a common tropical parasitosis in Latin America, is caused by *Trypanosoma cruzi* Chagas and affects, according to WHO, 16 million to18 million of the 90 million people exposed to triatomine vectors of the parasite. In Colombia, for example, it is estimated that 5% of the population is infected, and 3.5 million more are at high risk of infection. Chemotherapy for this disease (benznidazole and nifurtimox) has low success rates and produces severe side effects.

Leishmaniasis, a tropical and subtropical disease caused by trypanosomatide parasites of the genus *Leishmania*, infects 12 million people in the world and, in the case of cutaneous leishmaniasis, presents a morbility rate close to 2 million worldwide (Soares et al., 2007). This constitutes a group of diseases characterized by cutaneous or mucosal ulcers or, in its visceral form, fever, anemia, and substantial swelling of the liver and spleen. In the case of Colombia, the estimated annual incidence rate of leishmaniasis in its diverse clinical forms comprises between 6500 and 8500 cases (Agudelo et al., 2002). Chemotherapy using pentavalent antimonials or pentamidine produces undesirable side effects (Berman, 1997), and it seems that the parasites are becoming increasingly resistant to the existing antiparasite drugs (Soares et al., 2007).

A broad number of angiosperms have been explored for antiprotozoal activity, with recent research including species from the Fabaceae and Asteraceae (Camacho et al., 2003), Melastomataceae (Cunha et al., 2003), Rutaceae (Pepe et al., 2004), Annonaceae (Osorio et al., 2007), and

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Apocynaceae (Soares et al., 2007), but, to our knowledge, no information about antiprotozoal activity of species from the genus *Bomarea* Mirb. (Alstroemeriaceae) is found in the literature. This genus is composed of 100 to 110 species distributed from Chile to Mexico, with the highest diversity in the Northern Andes of South America (Alzate, 2005). The initial description of this genus included ethnobotanical information compiled in Chile stating that the plant had antivenereal and febrifuge uses (Mirbel, 1804). Several species of *Bomarea* have been traditionally used locally as contraceptives by indigenous Amazonian communities. Little ethnobotanical information is known about this genus and less about its biological potentialities.

Hostettman (1998) postulated that in order to evaluate a large number of plant species for their potentially medicinally active metabolites, it is necessary to perform rapid chemical and biological screening to determine the primary potential of the extracts. From this initial screening, one can decide which plants should be studied with an exhaustive analysis. Consequently, we evaluated the antiprotozoal activity of 26 ethanol extracts derived from 13 species of *Bomarea,* using established bioassays.

Materials and Methods

Plant material

The plant material of the 13 species of *Bomarea* was collected in diverse zones of Colombia and Ecuador (Table 1). Herbarium samples were determined by Dr. Fernando Alzate and deposited at the Herbarium of the Universidad de Antioquia (HUA), Colombia. Plant material was dried at 40°C in an oven with circulating air for a period of 24 h.

Preparation of extracts and fractions

Stems and leaves of the plants were dried at 40°C, powdered, then extracted by maceration in stoppered flasks containing ethanol at room temperature for 4 to 5 days, then filtered. Each filtered extract (500 mg) was diluted in 10 mL of methanol; then it was applied to a chromatographic column with Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO) and eluted by gravity with 95% ethanol. Two fractions were collected for each extract, and they were reconstituted in an appropriate form. For antiprotozoal activity, dimethyl sulfoxide (DMSO) was added to the vegetal fraction and then diluted as required in culture medium. Control drugs pentamidine, amphotericin B, and benznidazole were dissolved in water and then diluted as required in culture medium.

Parasites

Promastigote forms of *Leishmania amazonensis* L. (IFLA/BR/75/PH8), *L. braziliensis* Vianna (MHOM/BR/75/M2903), and *L. donovani* Laveran & Mesnil (MHOM/74/PP75) were cultured at 26°C in Scheneider's *Drosophila* medium containing 10% fetal bovine serum (FBS). *Trypanosoma cruzi* Chagas in its epimastigote form (Tulahuen strain) was cultured at 26°C in Liver Infusion Tryptose (LIT) medium, supplemented with 5% FBS.

Antiparasite activity

Promastigote forms of *L. amazonensis, L. donovani,* and *L. braziliensis* and the epimastigote form of *Trypanosoma cruzi* were used under concentrations of 5×10^4parasites/mL. Parasites were exposed to two of the fractions obtained for each of the extracts of the 13 species of *Bomarea* at concentrations ranging from 10 μg/mL to 100 μg/mL for 72 h.

The activity was evaluated by comparing the cultures exposed to the fractions with the control cell cultures and with those treated with the reference drug (benznidazole for *T. cruzi* and amphotericin B and pentamidine for *Leishmania* species). The protozoa were counted using light-transmitted microscopy. All assays were carried out in triplicate. IC_{50} values were established by a graphical method using the program Cricket Graph 1.3. Fractions considered to be active were those presenting IC_{50}values <10 μg/mL.

Cytotoxicity assay

The capacity of extracts of six species of *Bomarea* (*B. patinii, B. setacea, B. vestita, B. diffracta, B. bredemeyerana,* and *B. hieronymi*) to kill U-937 was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] method, following the previously reported methodology of Weniger et al. (2001). After 96 h of incubation in the presence of the compounds, the viability of cells was determined according to the amount of formazan produced after the addition of MTT. Cells cultured in the absence of the extracts, but maintained under the same conditions, were used as controls. A DMSO control DMSO was also included. Results are expressed as 50% lethal concentrations (LC_{50}), calculated by the probit method (Finney, 1971). All assays were carried out in triplicate .

Results

Leishmanicidal activity

The leishmanicidal activity (IC_{50}values) of the 26 ethanol extracts belonging to 13 species of *Bomarea* ranged from 4.9 to 98.6 μg/mL (Table 1), with the two fractions obtained from *B. setacea* (*B. setacea*-1 and *B. setacea*-2) being the most active ones. IC_{50} values for *B. setacea*-1 and *B. setacea*-2, respectively, are the following: 29.3–5.1 μg/mL for *L. amazonensis*, 5.1–5.4 μg/mL for *L. braziliensis,* and 4.9–5.3 μg/mL for *L. donovani*. *B. euryantha*-2, *B. edulis*-2, and *B. diffracta*-2 showed activity against *L. braziliensis*, with IC_{50} values of 6.2, 11.3, and 10.3 μg/mL, respectively.

Table 1. Evaluation of the antiprotozoal activity against promastigote forms of *Leishmania* spp. and the epimastigote form of *Trypanosoma cruzi* of fractions obtained from ethanol extracts of 13 species of *Bomarea*.

		Leishmania			
Voucher	Fraction	PH8 *(L. amazonensis)* IC_{50} (μg/mL)	M2903 *(L. braziliensis)* IC_{50} (μg/mL)	PP75 *(L. donovani)* IC_{50} (μg/mL)	Trypanosoma Epimastigote (Tulahuen) IC_{50} (μg/mL)
Alzate 2897	*B. bredemeyerana* (Willd.) Mirb.(1)	23.4	45.0	90.0	71.3
	B. bredemeyerana (Willd.) Mirb.(2)	45.0	28.8	45.0	83.3
Alzate 2714	*B. diffracta* Baker (1)	20.8	30.6	50.0	>100
	B. diffracta Baker (2)	19.8	**10.2**	24.9	79.2
Alzate 2951	*B. edulis* (Tussac) Herb. (1)	**11.3**	26.2	90.6	65.2
	B. edulis (Tussac) Herb. (2)	16.2	15.4	43.0	71.3
Alzate 2909	*B. euryantha* Alzate (1)	33.4	48.3	84.5	83.7
	B. euryantha Alzate (2)	17.3	**6.2**	96.5	67.2
Alzate 2923	*B. glaberrima* Pax (1)	35.8	35.8	48.7	65.2
	B. glaberrima Pax (2)	82.5	36.8	82.5	69.3
Alzate 2930	*B. glaucescens* (Kunth) Baker (1)	49.3	51.0	82.5	74.4
	B. glaucescens (Kunth) Baker (2)	28.7	36.8	96.5	71.3
Alzate 2890	*B. hieronymi* Pax (1)	42.9	47.0	98.6	83.7
	B. hieronymi Pax (2)	39.5	45.3	48.3	71.3
Alzate 2927	*B. hirsuta* (Kunt) Herb. (1)	96.5	90.6	96.6	71.3
	B. hirsuta (Kunth) Herb. (2)	32.6	41.2	41.2	75.0
Alzate 2931	*B. linifolia* (Kunth) Baker (1)	34.5	50.0	48.3	65.2
	B. linifolia (Kunth) Baker (2)	41.2	45.3	>100	92.7
Alzate 2922	*B. pardina* Herb. (1)	37.5	41.2	36.8	73.2
	B. pardina Herb. (2)	90.0	73.6	80.6	76.8
Alzate 2810	*B. patinii* Baker (1)	32.3	27.1	81.9	77.5
	B. patinii Baker (2)	82.5	82.5	97.5	71.3
Alzate 2713	*B. setacea* (Ruiz & Pav.) Herb. (1)	29.3	**5.4**	**5.3**	65.2
	B. setacea (Ruiz & Pav. Herb. (2)	**5.1**	**5.1**	**4.9**	69.7
Alzate 2801	*B. vestita* Baker (1)	33.4	24.7	69.0	65.2
	B. vestita Baker (2)	49.3	37.5	97.5	83.7

Fractions were applied in concentrations of 10 to 100 μg/mL. Highest antiprotozoal values are highlighted in **bold** font.
Pentamidine $IC_{50} = 10$ μg/mL.
Amphotericin B $IC_{50} = 0.2$ μg/mL.
Benznidazole $IC_{50} = 2.0$ μg/mL.

IC_{50} values for the controls were the following: 0.2 μg/mL for amphotericin B and 10 μg/mL for pentamidine.

Trypanocidal activity

No significant activity of the different fractions of *Bomarea* could be found against epimastigotes of *T. cruzi* Tulahuen strain, all measured IC_{50} values being >60 μg/mL (Table 1). IC_{50} value of benznidazole in these assays was 2.0 μg/mL.

Discussion

From the tested fractions obtained from ethanol extract of several *Bomarea* species, several were active against *Leishmania promastigotes*, but all were inactive against *T. cruzi* epimastigotes. According to Osorio et al. (2007), extracts with IC_{50} values <10 μg/mL might be considered active. According to this rule, eight fractions evaluated fulfill the criterion. The highest antiparasite activity was found with *B. diffracta, B. euryantha, B. edulis,* and*B. setacea,* the latter showing the strongest activity.

Globally, the best activity was found with *L. braziliensis* (MHOM/BR/75/M2903), a strain specifically associated with cutaneous leishmaniasis, which is characterized by its aggressiveness (Agudelo et al., 2002). Considering the two fractions obtained from each of the ethanol extracts, no significant differences could be found between the biological activity exhibited by fraction number 1 (highest molecular weight) compared with fraction number 2, although it is observed that from seven fractions with moderate leishmanicidal activity (IC_{50} values between 4.9 and 11.3 μg/mL), five belong to the second fraction.

Phytochemical studies of *Bomarea* species showed the presence of tuliposides (Bjorkner, 1982). Tuliposide A is known to be synthesized from tulipaline after the plant has

been wounded (Slob, 1973). This is a lactone that produces contact dermatitis in people exposed to *Bomarea* tissues. These compounds are also found in species of *Alstroemeria*, another genus of Alstroemeriaceae, for which Bjorkner (1982) states that all the species of this family should be considered allergenic. Yu et al. (1994) and Akendengue et al. (2002) report inhibitory activity of some lactones against *Leishmania* and *Trypanosoma* spp.

Steroidal saponines with hemolytic properties have also been reported in some species of *Bomarea* (Hegnauer, 1963). Saponines are a group of chemical compounds that have been documented to have effective antiprotozoal activity (Plock et al., 2001). Benzofuranones were isolated in this work from ethanol extracts of *Bomarea setacea* using GC-MS. Some benzofuranones showed antiparasite activity in mice (Kinnamon et al., 1998) and have been demonstrated to be active againts *L. donovani, T. brucei rhodesiense*, *T. cruzi*, and *Plasmodium* spp. (Mukhlesur & Gray, 2005).

Through a molecular systematics analysis, Alzate et al. (2008) proposed three evolutionary lineages (clades A, B, and C) within the genus. Three of the four species that present activity antiparasite activity belong to clade A. Species of clade A are restricted to lowlands and midlands of South and Central America and appear to constitute the most interesting infrageneric taxon to include in programs of bioprospection.

Finally, the secondary compounds of *Bomarea* appear to have low cytotoxicity, which adds to their potential utility in the treatment of human protozoal diseases.

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