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BRIEF COMMUNICATION

ANTI-TRYPANOSOMAL ACTIVITY OF PENTACYCLIC TRITERPENES ISOLATED FROM *Austroplenckia populnea* (CELASTRACEAE)

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SUMMARY

Four pentacyclic triterpenes isolated from *Austroplenckia populnea* and four compounds of known anti *T. cruzi* or anti-malarial activity were tested. Of those triterpenes tested 20α-hydroxy-tingenone showed high activity, epikatonic acid was less active, while populnilic and populninic acids were inactive against the trypanosome of the subgenus *Schizotrypanum* tested. Benzonidazole, nifurtimox, ketoconazole and primaquine presented a remarkable dose-dependent inhibitory effect reaching practically to a total growth inhibition of the parasite at the end of incubation time. The trypanosome tested appear to be a suitable model for preliminary screen for anti *T. (S.) cruzi* compounds.

KEYWORDS: *Austroplenckia populnea;* Pentacyclic triterpenes; Anti-trypanosomal activity; Growth inhibition.

Austroplenckia populnea Reiss (Celastraceae) is a tropical tree commonly found in the Minas Gerais State, Brazil. "Mangabarana, "Mangabeira-Brava", and "Marmelinho do Campo" are its popular names. The tea of its leaves is used as antidysenteric[2] and anti-rheumatic[6] in the popular medicine. Extracts and several constituents obtained from *A. populnea* have been investigated for biological activity including antitumor activity[8], as well as, antibacterial activity[11,16].

On the other hand, Chagas' disease remains a major public health problem in Latin America where it has been estimated that 16-18 millions people are chronically infected by *Trypanosoma cruzi*, it's etiological agent[17].

Since reduviid bugs (mainly the domiciate species *Triatoma infestans)*, are intermediate hosts for *T. cruzi*, control is largely carried out by use of residual insecticides in endemic areas. However, efficacy of this activity is limited by economic-social factors[1]. The chronicity combined with the involvement of the host's immune response in the pathogenesis of Chagas' disease makes the development of a effective vaccine particularly difficult. On the other hand, chemotherapy using the nitrofuran derivative nifurtimox or the nitroimidazole derivative benzonidazole because of their clinical effects continue inexpressive and controversial[14], claiming for new drugs.

In order to avoid handling the pathogenic *T. cruzi* for preliminary screening of anti-*T. cruzi* organic compounds GABORAK *et al.*[3], had earlier suggested the use of other members of the *Schizotrypanum* genus instead of, since a number of compounds of known anti-*T. (S.) cruzi* activity had showed similar activity when tested *in vitro* against non-pathogenic trypanosomes.

In the present study we used a trypanosome of the genus *Schizotrypanum [T. (S) cruzi]* isolated from a *Phyllostomus hastatus* bat collected in Minas Gerais State, Brazil[9], to test pentacyclic triterpenes isolated from *A. populnea*. This isolate was unable in produce detectable parasitemia in mice[9], and distinct from *T. cruzi* in their isoenzymatic and lectin agglutination profiles[10,15]. In addiction, trials *in vitro* are of permanent interest because the identification of active compounds may be useful for the development of new agents for the treatment of Chagas' disease.

A. populnea Reiss (Celastraceae) was collected in Nova Lima region, Minas Gerais State, Brazil. After botanical identification by Dr. Luiz Pedersoli, Departament of Botany, Universidade Federal de Minas Gerais, a voucher specimen (No. 10473) representing this collection has been deposited at the Herbarium of the Natural History Museum of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.

The cleaned bark wood (3.0 kg) and roots (270 g) of *A. populnea* was dried at room temperature and than milled in powder. The bark wood was submitted to extraction using methanol. This extract (656 g)

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was further fractionated with hexane, benzene and ethyl acetate. The roots were extracted with petroleum ether, dichloromethane and methanol. The phytochemical methods were described previously[12,13]. From the petroleum ether extract of the roots was isolated 48.0 mg of the 20α-hydroxy-tingenone[12], and from the bark wood extracts were isolated 209.4 mg of populninic acid 361.0 mg of epikatonic acid and 46.0 mg of populnilic acid[13].

The trypanosome strain *[T.(S) cruzi*] used in the experiments was isolated from a *P. hastatus* bat collected in Serrania, Minas Gerais State, Brazil. Isolation was performed by hemoculture in brain-hearth-infusion (BHI) culture medium supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS), 2% of 10% rabbit haemoglobin solution. Clone was obtained by successive plating technique in the same culture medium added with 0.75 agar. Flagellate was maintained by serial passage every 10 days and also by criopreservation in liquid nitrogen after addition of 10% glycerol (v/v) to culture.

Four pentacyclic triterpenes 20α-hydroxy-tingenone, epikatonic acid, populnilic acid and populninic acid were assayed (Table 2). Benzonidazole, nifurtimox, ketoconazole and primaquine drugs of known anti-trypanosomal, anti-fungical and anti-malarial activity were tested for comparison (Table 1).

T. (S) cruzi growth inhibition experiments were carried out in 16 x 150 mm screw-capped tubes containing of BHI medium added with 10% of foetal bovine serum (FBS) and 2% of a 10 mg/mL hemine (Sigma Co. US) solution, pH 7.2. The compounds (10 and 50 µg/mL) tested were previously solubilized in DMSO (Dimethyl sulfoxide), filtered in Millepore (0.2 µm) membrane and aseptically added to the tubes (0.1 mL/tube). In no-added control test tubes 0.1 mL of the solvent was added. Final volumes of *T.(S) cruzi* culture medium/tube was always 2.5 mL. Inocula consisted of 0.1 mL of a exponential growth phase culture which correspond to about 4.8 x 10^2 cells/mL. Cultures were incubated at 28 °C for 2-12 days. Growth was estimated with heamocytometer (Improved Double Neubauer). Cell motility and cellular integrity was observed by microscopical observation in fresh-smears.

The results obtained in these experiments were given in tables 1 and 2. Table 1 presents the effects of the well-known *T. (S.) cruzi* growth inhibitors drugs nifurtimox, benzonidazole, ketoconazole and primaquine[1], on the epimastigote forms of the *T. (S.) cruzi*-like trypanosome used. It can be seen that, for all assayed drugs occurred a remarkable dose-dependent inhibitory effect reaching practically to a total parasite growth inhibition, at the end of the incubation time. Those results are agreements with the GABORAK *et al.*[3] observation, and we can conclude that the trypanosome used appear to be a suitable model for the preliminary screen of anti-*T. cruzi* drugs, mainly in less-equipped laboratory in developing countries.

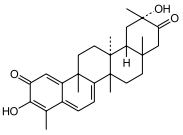
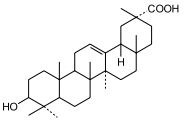
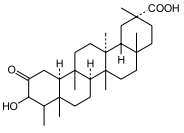
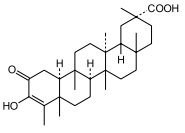
The activity of those pentacyclic triterpenes from *A. populnea* tested may be classified as follows; a) highly active: 20α-hydroxy-tingenone, b) less active: epikatonic acid; and c) inactive: populnilic acid and populninic acid (Table 2). Tingenone (Maitenine) other natural quinonoid[5], that differs from 20α-hydroxy-tingenone only by the presence of a methyl instead of the hydroxyl group at 20-position, inhibited *T. (S.) cruzi* growth mainly by DNA double-strand intercalation mechanism[17]. Otherwise, the presence of a carboxylic group at the 20-position, such as in epikatonic acid, populnilic acid or populninic acid rises loss of activity, possible by difficulties in cross cell cytoplasmatic membrane. Frequently changes in critical radicals affect markedly the drug activity, such as in primaquine in which the 6-methoxy group was found to be responsible for the toxic effect, since analogues which have different substituents at this position are inactive against *T. (S.) cruzi*[4].

Table 1
Inhibition growth* of *Trypanosoma (Schizotrypanum)* sp, isolated from bat *Phyllostomus hastatus*, by drugs with known anti-*T. cruzi* activity

Drug	Conc.	Culture time (Hours after inoculation)					
		48		96		192	
	µg/mL	Cell count	Inhibition (%)	Cell count	Inhibition (%)	Cell count	Inhibition (%)
Benzonidazole	00	$1.0x10^6$	-	$1.4x10^6$	-	$7.3x10^6$	-
	10	$4.9x10^5$	51.0	$6.9x10^5$	50.7	$8.2x10^5$	88.8
	50	$4.5x10^5$	55.0	$3.6x10^5$	74.3	$3.0x10^5$	95.9
Ketoconazole	00	$1.1x10^6$	-	$1.3x10^6$	-	$7.0x10^6$	-
	10	$6.3x10^5$	42.7	$7.2x10^5$	44.6	$8.0x10^5$	88.6
	50	$3.0x10^5$	72.7	$1.0x10^5$	92.8	0.0	100.0
Nifurtimox	00	$1.0x10^6$	-	$1.4x10^6$	-	$7.3x10^6$	-
	10	$2.5x10^5$	75.0	$4.5x10^5$	96.8	0.0	100.0
	50	$5.0x10^4$	95.0	0.0	100.0	0.00	100.0
Primaquine	00	$1.1x10^6$	-	$1.3x10^6$	-	$7.0x10^6$	-
	10	$2.7x10^5$	75.5	$4.0x10^4$	96.9	0.0	100.0
	50	$7.5x10^4$	93.2	0.0	100.0	0.0	100.0

*The results represent three replicates of each experiment with mean values expressed as cell number/mL and % inhibition of growth.

Table 2
Inhibition growth* of *Trypanosoma (Schizotrypanum)* sp, isolated from bat *Phillostomus hastatus*, by four pentacyclic triterpenes isolated from *Austroplenckia populnea*

Substance	Conc.	Culture time (Hours after inoculation) 48		96		192	
	μg/mL	Cell count	Inhibition (%)	Cell count	Inhibition (%)	Cell count	Inhibition (%)
	00	$5.7x10^5$	-	$6.0x10^5$	-	$1.1x10^6$	-
	10	$2.0x10^5$	64.9	$2.0x10^5$	66.7	$2.1x10^5$	80.0
20α-hydroxy-tingenone	50	$2.6x10^5$	54.4	$2.0x10^5$	66.7	$2.0x10^5$	81.8
	00	$7.0x10^5$	-	$1.0x10^6$	-	$2.0x10^6$	-
	10	$6.9x10^5$	1.4	$1.0x10^6$	0.0	$1.9x10^6$	5.0
Epikatonic acid	50	$6.8x10^5$	2.9	$6.0x10^5$	40.0	$3.2x10^5$	84.0
	00	$7.0x10^5$	-	$1.0x10^6$	-	$2.0x10^6$	-
	10	$6.7x10^5$	4.3	$1.1x10^6$	0.0	$1.9x10^6$	5.0
Populnilic acid	50	$6.5x10^5$	7.1	$1.0x10^6$	0.0	$1.9x10^6$	5.0
	00	$7.0x10^5$	-	$1.0x10^6$	-	$2.0x10^6$	-
	10	$7.0x10^5$	0.0	$9.7x10^5$	3.0	$2.0x10^6$	0.0
Populninic acid	50	$6.8x10^5$	2.9	$9.8x10^5$	2.0	$1.9x10^6$	5.0

*The results represent three replicates of each experiment with mean values expressed as cell number/mL and % inhibition of growth.

Of those pentacyclic triterpenes obtained from *A. populnea* tested, 20α-hydroxytingenone show high activity; epikatonic acid less active and populninic acid and populnilic acid were inactive against the trypanosome used. In addiction the *T. (S.) cruzi* trypanosome from *P. hastatus* showed to be a good biological approach for *T. cruzi* screening of organic compounds.

RESUMO

Atividade anti-tripanosomicida de triterpenes pentacíclicos isolados de *Austroplenckia populnea (*Celastraceae)

Foram testados quatro triterpenos pentacíclicos isolados de *Austroplenckia populnea* e quatro compostos de conhecida atividade anti-*T. cruzi* ou anti-malárica. Dos triterpenos testados 20α-hidroxi-tingenona mostrou atividade elevada, ácido epicatônico foi menos ativo, enquanto ácido populnílico e populnínico foram inativos contra o tripanossoma do subgênero *Schizotrypanum* testado. Benzonidazole, nifurtimox, cetoconazole e primaquina apresentaram efeito inibitório dose-dependente atingindo praticamente a inibição total do crescimento do parasita no final do tempo de incubação. O tripanossoma testado mostrou ser um modelo adequado para uma seleção preliminar de compostos anti. *T. (S.) cruzi*.

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