Received 16 April 2009; Accepted 29 October 2009

Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
20(1): 1-6, Jan./Mar. 2010

Artigo

Trypanocidal activity of flavonoids and limonoids isolated from Myrsinaceae and Meliaceae active plant extracts

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RESUMO: "Atividade tripanocida de flavonoides e limonoides isolados de extratos ativos de plantas de Myrsinaceae e Meliaceae". A atividade de extratos brutos de três espécies de *Rapanea* (Myrsinaceae) e de *Cipadessa fruticosa* (Meliaceae) foi avaliada *in vitro* contra formas tripomastigotas de *Trypanosoma cruzi*. Foram obtidos 33 extratos de diferentes órgãos das espécies estudadas, sendo que onze deles apresentaram atividades significantes (% de lise > 50) nos ensaios realizados. O fracionamento de um extrato ativo dos galhos de *R. lancifolia* (99,5%) resultou no isolamento de dois flavonoides (quercetina e taxifolina), que apresentaram baixa atividade tripanocida. De um extrato ativo dos frutos de *C. fruticosa* (97,7%) foram isolados os limonoides mexicanolídeos cipadesina, mexicanolídeo, febrifugina e cipadesina A, que foram moderadamente ativos sobre *T. cruzi*. Além disso, outros dois flavonoides (flavona e 7-metoxiflavona), previamente ensaiados contra *T. cruzi*, foram isolados do extrato hexânico dos galhos de *C. fruticosa* (100%). Os resultados obtidos aqui sugerem que as plantas avaliadas podem constituir fontes de novas substâncias ativas sobre o *T. cruzi.*

Unitermos: Myrsinaceae, Meliaceae, atividade tripanocida, flavonoides, limonoides.

ABSTRACT: The activity of crude extracts of three *Rapanea* species (Myrsinaceae) and *Cipadessa fruticosa* (Meliaceae) was evaluated *in vitro* against the trypomastigote forms of *Trypanosoma cruzi.* Thirty-three extracts from different organs of these species were assayed and eleven of them showed significant activity (lysis % >50). The fractionation of an active extract from branches of *R. lancifolia* (99.5%) led to the isolation of two flavonoids: quercetin and taxifolin, which have weak trypanocidal activity. Additionally, one active extract from fruits of *C. fruticosa* (97.7%) afforded mexicanolide limonoids: cipadesin, mexicanolide, febrifugin and cipadesin A, that were slightly active on *T. cruzi*. Moreover, other two flavonoids (flavone and 7-methoxyflavone), previously assayed against *T. cruzi*, were isolated from the hexane extract from branches of *C. fruticosa* (100%). The results presented here suggest that the plants evaluated could be a source of new active compounds against *T. cruzi.*

Keywords: Myrsinaceae, Meliaceae, trypanocidal activity, flavonoids, limonoids.

INTRODUCTION

Chagas' disease (American trypanosomiasis) affects 16-18 million people, mostly from Central and South America, where 25% of the total population is at risk of infection (WHO, 2004). It is caused by a flagellate protozoan, *Trypanosoma cruzi*, and transmitted to humans by blood-sucking triatomine insects and by blood transfusion (WHO, 1997). With the control of the insect vector (*Triatoma infestans*), infected blood transfusion is becoming the major cause for the spread of the disease (Dias, 1993). The only trypanocidal substance currently used to prevent the infection by this route is gentian violet, but its use is limited due to undesirable effects on the patients (Dias, 1993). The treatment of Chagas' disease is still a challenge, since the only current available drug (benznidazole) has strong side effects (de Castro, 1993). Thus, the search for more efficient and less toxic drugs is needed to control this disease.

In the past years, the study of compounds isolated from plants has received considerable attention in the search for alternative chemotherapy of parasitic diseases (Serrano et al., 2000) and many natural compounds have been identified as trypanocides (Duschak & Couto, 2007;

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Guimarães & Faria, 2007). Several extracts from plants of Meliaceae and Rutaceae families have been showed *in vitro* activity against *T. cruzi* (Mafezoli et al., 2000; Vieira et al., 2001; Ambrozin et al., 2004; de Mesquita et al., 2005). Therefore, natural products constitute a promising source of new active compounds for treatment of Chagas' disease.

In this paper, we report the results of the evaluation of *in vitro* trypanocidal activity of 33 crude extracts from different organs of *Rapanea lancifolia, R. guianensis, R. umbelata* Myrsinaceae, and *Cipadessa fruticosa* Meliaceae. Moreover, it is showed the trypanocidal activity of flavonoids isolated from hexane extracts from branches of *R. lancifolia* and *C. fruticosa*, as well the activity of four limonoids isolated from dichloromethane extract from fruits of *C. fruticosa.*

MATERIAL AND METHODS

Plant material

Plants were collected in different states of Brazil and identified by Dr. Maria Inês Salgueiro Lima from Department of Botany, UFSCar, SP and Dr. José Rubens Pirani from Department of Botany, USP, SP (Table 1).

Table 1. Botanical identification of plants assayed.

Plant	Herbarium/collected in	Voucher number
Rapanea lancifolia (Mart.) Mez.	HUFSCar - Department of Botany, São Carlos, SP/03/08/00	6700
Rapanea guianensis Aubl.	HUFSCar - Department of Botany, São Carlos, SP/03/08/00	6699
Rapanea umbellata (Mart.) Mez.	HUFSCar - Department of Botany, São Carlos, SP/03/08/00	6698
Cipadessa fruticosa Bl.	SPF – Department of Botany, USP, São Paulo, SP/01/20/01	110.664

Extraction and isolation of compounds

The powdered air-dried plant material was extracted three times (72 h) by maceration with hexane, CH_2Cl_2 and MeOH at room temperature. The solvent was removed under reduced pressure by rotary evaporation and the extracts obtained were assayed against *T. cruzi*.

The hexane extract (9.3 g) from branches of *R. lancifolia* (fresh weight: 1870 g) was fractionated through column chromatography on silica gel affording hexane, dichloromethane, ethyl acetate and methanol fractions. The methanol fraction (1.5 g) was chromatographed on silica gel using hexane-CH_2Cl_2-EtOAc-MeOH gradient, giving four fractions. Fraction 4 was fractionated in the same conditions to yield compounds **1** (3.2 mg) and **2** (3.7 mg).

The hexane extract (12.2 g) from branches of *C. fruticosa* (fresh weight: 1524 g) was submitted to vacuum liquid chromatography over silica gel using a hexane-CH_2Cl_2-EtOAc-MeOH gradient. The ethyl acetate fraction (6.8 g) was chromatographed on silica gel, eluting with hexane-CH_2Cl_2-acetone gradient to give nine fractions. Fraction 3 was fractionated as above, using hexane-CH_2Cl_2-acetone (6:3:1), affording nine fractions. Fraction 5 was three times chromatographed through column chromatography on silica gel, eluting with hexane-CH_2Cl_2-MeOH (7:2.5:0.5) to afford compounds **7** (16.7 mg) and **8** (8.2 mg).

The dichloromethane extract from fruits (15.0 g) of *C. fruticosa* (fresh weight: 180 g), was submitted to vacuum liquid chromatography over silica gel using a hexane-CH_2Cl_2-EtOAc-MeOH gradient, resulting in the fractions: hexane-CH_2Cl_2 (1:1); CH_2Cl_2; CH_2Cl_2/AcOEt (1:1); EtOAc and EtOAc/MeOH (1:1). Fractions CH_2Cl_2/AcOEt (1:1) and EtOAc/MeOH (1:1) were studied, as following described: the fraction CH_2Cl_2/AcOEt (1:1) (5.0 g) was again submitted to vacuum liquid chromatography on silica gel using a gradient elution (hexane, CH_2Cl_2, EtOAc, and MeOH), resulting in the four corresponding soluble fractions. The ethyl acetate fraction (2.3 g) obtained from the last fractionation was several times chromatographed on silica gel and finally submitted to preparative HPLC to afford for limonoids **3** (19.4 mg), **5** (8.1 mg), and **6** (8.5 mg). Limonoid **4** (48.9 mg) was isolated from fraction EtOAc/MeOH (1:1) (4.6 g) of extract through several chromatographies on silica gel.

Compounds **1-6** were identified through ^1H and ^{13}C NMR spectra, ^1H - ^1H COSY, HSQC and HMBC experiments and MS. The spectral data of **1** and **2** were in agreement with those published in the literature (Kuo et al., 1998; Moon et al., 2001) as well as the data of limonoids **3** (Luo et al., 2000), **4** (Paula, 1996), **5** (Govindachari & Kumari, 1998) and **6** (Leite et al. 2005a). Additionally, compounds **7** and **8** were characterized by comparison of ^{13}C NMR data with the literature (Kingsburry & Looker, 1975). The *in vitro* activity on *T. cruzi* of compounds **3 - 6** is described herein. On the other hand, the activities of compounds **1, 2, 7** and **8** were previously reported (Ambrozin et al., 2004; Tasdemir et al., 2006).

Bioassay

The bioassays were carried out according to the procedures described by Ambrozin et al. (2008). The activity of crude extracts was evaluated in triplicate at 4 mg/mL and pure substances at 250, 100, and 50 µg/mL. It is expressed as percent reduction of the parasite number (lysis) and IC_{50} (µmol/L) for compounds **3-6** were calculated using the program GraphPad Prims v.3.0.

RESULTS AND DISCUSSION

In this work, we assessed the trypanocidal activity of 33 extracts of three Myrsinaceae and one Meliaceae species (Table 2). Eleven of them (33%) showed significant activity (lysis % > 50) at 4 mg/mL against the trypomastigote forms of *T. cruzi*. The majority of the extracts of *C. fruticosa* presented relevant activity. The best result was obtained from the hexane extract from branches of *C. fruticosa* (CFBH) that reduced 100% of the parasite number. The majority of the extracts of the three *Rapanea* species assayed did not present significant results, since several extracts led to the total lysis of the parasites and the red blood cells, as well. However, some extracts of these species showed high trypanocidal activity without affecting the red blood cells, such as the hexane extract from branches of *R. lancifolia* (RLBH).

Similar studies involving the trypanocidal activity of Meliaceae and Rutaceae species have been reported by our group (Mafezoli et al., 2000; Vieira et al., 2001; Ambrozin et al., 2004, 2005a, 2008) showing that these plants can be considered a promising source of active compounds against trypomastigote forms of *T. cruzi*. Thus, the significant results obtained from the extracts of *C. fruticosa* are consistent with those previously reported for this family.

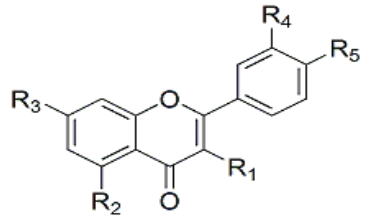
The investigation of the methanol fraction of the active hexane extract from branches of *R. lancifolia* (RLBH) led to the isolation of the flavonol quercetin (**1**)

Table 2. *In vitro* activity of the crude extracts (4 mg/mL) of *Rapanea* species and *Cipadessa fruticosa* on trypomastigote forms of *Trypanosoma cruzi.*

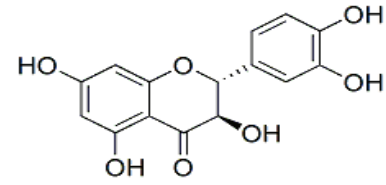
Plant	Plant part	Solvent extraction	Crude extract	Lysis %
Rapanea lancifolia (Myrsinaceae)	flowers	hexane	RLFLH	76.8
		dichloromethane	RLFLD	72.2
		methanol	RLFLM	TL[a]
	leaves	hexane	RLLH	TL[a]
		dichloromethane	RLLD	TL[a]
		methanol	RLLM	TL[a]
	branches	hexane	RLBH	99.5
		dichloromethane	RLBD	7.0
		methanol	RLBM	47.69
Rapanea guianensis (Myrsinaceae)	flowers	hexane	RGFLH	TL[a]
		dichloromethane	RGFLD	TL[a]
		methanol	RGFLM	TL[a]
	leaves	hexane	RGLH	TL[a]
		dichloromethane	RGLD	TL[a]
		methanol	RGLM	68.5
	branches	hexane	RGBH	TL[a]
		dichloromethane	RGBD	TL[a]
		methanol	RGBM	6.94
Rapanea umbellata (Myrsinaceae)	fruits	Hexane	RUFRH	TL[a]
		dichloromethane	RUFRD	69.4
		methanol	RUFRM	TL[a]
	roots	hexane	RURH	TL[a]
		dichloromethane	RURD	TL[a]
		methanol	RURM	TL[a]
Cipadessa fruticosa (Meliaceae)	fruits	hexane	CFFRH	95.5
		dichloromethane	CFFRD	97.7
		methanol	CFFRM	60.6
	leaves	Hexane	CFLH	33.7
		dichloromethane	CFLD	92.8
		methanol	CFLM	40.2
	branches	hexane	CFBH	100
		dichloromethane	CFBD	76.1
		methanol	CFBM	42.0

[a] total lysis

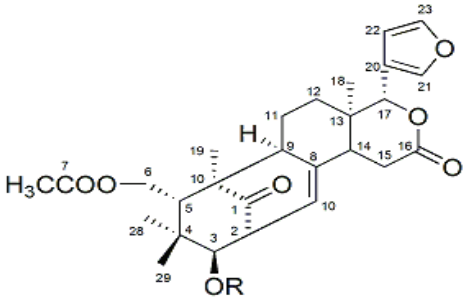
and the dihydroflavonol taxifolin (**2**). Several biological activities have been reported for these flavonoids. Quercetin (**1**), for example, presented antiprotozoal effect against *Plasmodium falciparum* (IC_{50} = 14 µM), *Trypanosoma brucei brucei* (IC_{50} = 13.2 µM), *Leishmania donovani* (IC_{50} = 63.8 µM) (Camacho et al., 2002), *T. brucei* and *T. rhodesiense* (Williamson & Finnigan, 1978). In addition, this compound was described as a constituent of an aqueous extract from *Lychnophora pinaster,* Asteraceae which showed trypanocidal activity (Silveira et al., 2005). Recently, high leishmanicidal activity was described for such compound (Tasdemir et al., 2006). Compounds **1** and **2** were previously assayed on trypomastigote forms of *T. cruzi*, but showed low activity (Tasdemir et al., 2006). The relevant activity of the original extract (RLBH) is probably associated with the presence of other compounds or mixture of them.



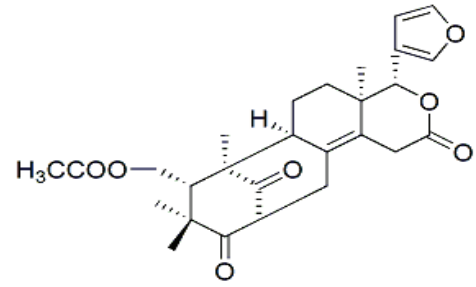
1: R_1=R_2=R_3=R_4=R_5=OH
7: R_1=R_2=R_3=R_4=R_5=H
8: R_1=R_2=R_4=R_5=H; R_3=OCH_3



2



3: R=$COCH(CH_3)CH_2CH_3$
4: R=Tiglate



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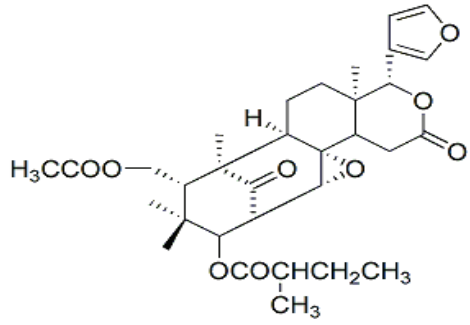

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The mexicanolide limonoids **3-6**, isolated from fruits of *C. fruticosa*, showed significant trypanocidal activity (Table 3). They were obtained from the very active dichloromethane extract of fruits (Table 2, CFFRD, 97.7 %). This extract also afforded other limonoids (Leite et al., 2005a,b), which were not assayed herein. Recently, we reported the inhibitory activity of crude extracts of Meliaceae and Rutaceae plants on glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) enzyme from *T. cruzi*. The results showed that crude extracts from *C. fruticosa* showed high ability to inhibit the enzyme activity (Leite et al., 2009). Thus, this specie could be considered as a promising source of lead compounds against Chagas' disease. In addition, the extracts from *C. fruticosa* showed high inhibition of adenine phosphoribosyltransferase (APRT), a key enzyme from *Leishmania* that has been proposed as a target for the rational search of new leishmanicidal drugs (Ambrozin et al., 2005b).

The trypanocidal activity of flavone (**7**) and 7-methoxyflavone (**8**), isolated from the active hexane extract from branches of *C. fruticosa* (CFBH), was previously published by Ambrozin et al. (2004). They showed that these flavonoids, isolated from an active fraction of *Conchocarpus heterophyllus* (Rutaceae), presented low activity against trypomastigote forms of *T. cruzi* (IC_{50} = 2116 and 787 µg/mL or 9531 and 1084 µmol/L, respectively) when compared with other flavonoids (Ribeiro et al., 1997; Ambrozin et al., 2004). Probably, the relevant activity obtained from the extract CFBH can be related to other characteristic metabolites of *C. fruticosa*. As limonoids in the present work showed trypanocidal activity, we believe that the activity of CFBH extract may be associated with these compounds.

Table 3. *In vitro* activity of cipadesin (**3**), febrifugin (**4**), mexicanolide (**5**), and cipadesin A (**6**) on trypomastigote forms of *Trypanosoma cruzi.*

Compound	Concentration (µg/mL) x Lysis % ± EPM[a]			IC_{50} (µmol/L)
	50	100	250	
3	6.39 ± 3.2	50.58 ± 2.9	80.81 ± 4.1	189.0
4	16.86 ± 3.4	69.18 ± 4.6	65.69 ± 2.9	168.0
5	11.39 ± 2.5	28.48 ± 5.7	77.21 ± 2.5	326.3
6	36.04 ± 5.8	55.81 ± 2.3	88.95 ± 2.9	136.1
gentian violet[b]	35.7 ± 1.6	100.0 ± 0.0	100.0 ± 0.0	76.0

[a] medium standard error
[b] positive control

In conclusion, the present study showed that the Myrsinaceae and Meliaceae plants constitute a potential source of active compounds for treatment of Chagas' disease. The phytochemical study of three active extracts of *R. lancifolia* and *C. fruticosa* led to the isolation of quercetin (**1**), taxifolin (**2**), flavone (**7**) and 7-methoxyflavone (**8**), which had showed weak activity against the trypomatigote forms of *T. cruzi,* and cipadesin (**3**), febrigufin (**4**), mexicanolide (**5**), and cipadesin A (**6**) that were appreciably actives. In addition, the trypanocidal activity of plants of Myrsinaceae family and limonoids **3-6** are been disclosed for the first time.

ACKNOWLEDGEMENTS

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) and Financiadora de Estudos e Projetos (FINEP) for the financial support. Also, authors thanks to Dr. Maria Inês Salgueiro Lima, and Dr José Rubens Pirani for identificating the investigated plants.

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