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Evaluation of the in vitro antischistosomal activity of Bidens sulphurea (Cav.) Sch. Bip. (Asteraceae)

Avaliação da atividade antiesquistossomal in vitro de Bidens sulphurea (Cav.) Sch. Bip. (Asteráceas)

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ABSTRACT

In this study, the in vitro schistosomicidal properties of the n-hexane (BS-1), dichlorometane (BS-2) and methanol (BS-3) extracts from the leaves of Bidens sulphurea against Schistosoma mansoni were investigated. The extract BS-3 at 200 µg/mL caused the death of 100% of the male and 75% of the female adult worms and promoted the separation of 75% of the coupled pairs into individual males and females within 120h. This extract was partitioned in n-hexane, dichloromethane, ethyl acetate, and methanol, furnishing the fractions BS-3.1, BS-3.2, BS-3.3, and BS-3.4, respectively. The fraction BS-3.2 was the most active, causing the death of all the male and female adult worms after 120 h, and the separation of all the coupled pairs of worms in the same period. The results of this preliminary study demonstrated for the first time the in vitro antischistosomal activity of B. sulphurea extracts.

Keywords: antischistosomal agents, Bidens sulphurea, natural products, plant extracts, Schistosoma mansoni.

RESUMO

Neste estudo, foram investigadas as propriedades esquistossomicidas in vitro dos extratos n-hexano (BS-1), diclorometano (BS-2) e metanol (BS-3) das folhas de Bidens sulphurea contra o Schistosoma mansoni. O extrato BS-3 a 200 µg/mL causou a morte de 100% dos vermes machos e 75% das fêmeas adultas e promoveu a separação de 75% dos pares acoplados em machos e fêmeas individuais dentro de 120h. Este extrato foi particionado

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em n-hexano, diclorometano, acetato de etila e metanol, fornecendo as frações BS-3.1, BS-3.2, BS-3.3 e BS-3.4, respectivamente. A fração BS-3.2 foi a mais ativa, causando a morte de todos os vermes adultos machos e fêmeas após 120 horas, e a separação de todos os pares de vermes acoplados no mesmo período. Os resultados deste estudo preliminar demonstraram pela primeira vez a atividade antiesquistossomal in vitro de extratos de B. sulphurea.
Palavras-chave: antischistosomal agents, Bidens sulphurea, produtos naturais, extratos de plantas, Schistosoma mansoni

1 INTRODUCTION

Human schistosomiasis is one the most significant and neglected tropical diseases in the world, second to malaria only.[1] This parasitosis affects more than 200 million people worldwide, and it is estimated that approximately 800 million are at risk of contracting it.[1] Praziquantel (PZQ) is the only medication that is currently effective against all the species of schistosome; however, it does not prevent re-infections and is inactive against juvenile schistosomes, besides having limited effect on already developed liver and spleen lesions.[2, 3] These limitations, in combination with considerable concern about the development of PZQ resistance, have motivated the scientific community to call for research and development of novel and inexpensive drugs against schistosomiasis.[3, 4] In this scenario, several essential oils, extracts, and isolated compounds obtained from plants have been investigated for their schistosomicidal potential.[5-7]

Bidens sulphurea (Cav.) Sch. Bip. (Asteraceae) is an annual erect herbaceous species native to Mexico,[8] popularly known as “cosmo-amarelo”, “picão-grande”, and “aster do México” in Portuguese, which is traditionally used to treat malaria in Brazil.[9] As part of our ongoing project on the antiparasitic activities of natural products,[6, 10-13] and considering previous reports on the schistosomicidal activity of the essential oil from *B. sulphurea* flowers,[14] this study aimed to evaluate the *in vitro* antischistosomal effects of the extracts from the leaves of *Bidens sulphurea* (Cav.) Sch. Bip. (Asteraceae) against *Schistosoma mansoni.*

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

Bidens sulphurea (Cav.) Sch. Bip. (Asteraceae) was collected at "Sítio 13 de maio" near Franca city (20º26'S 47º27'W 977m, State of São Paulo, Brazil) in May 2014. A voucher specimen (SPFR12020) was deposited at the Herbarium of Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Brazil (Herbarium SPFR).

2.2 EXTRACTION AND FRACTIONATION

Fresh leaves of *B. sulphurea* (400 g) were dehydrated, powdered, and sequentially extracted with *n*-hexane, dichlorometane, and MeOH (15 mL of solvent/g of powder) under sonication for 15 min, according to a previously reported methodology.[15] The phases were concentrated under reduced pressure to yield the *n*-hexane (**BS-1**), dichloro-methane (**BS-2**), and methanol (**BS-3**) extracts. The extract **BS-3** was resuspended in methanol and partitioned with *n*-hexane, dichloromethane, and ethyl acetate (3 x 50 mL). The solvent was removed under reduced pressure and the resulting fractions **BS-3.1**, **BS-3.2**, **BS-3.3**, and **BS-4** (the methanol fraction) were stored in a refrigerator at -4 ºC for further antischistosomal assays.

2.3 IN VITRO ANTISCHISTOSOMAL ASSAYS

The LE strain of *S. mansoni* was maintained in *Biomphalaria glabrata* snails and Balb/c mice and recovered after 8 weeks, according to a previously reported methodol-ogy.[16] The worms were washed in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen), kept at pH 7.5 with HEPES 20 mM, and supplemented with penicillin (100 UI/mL), streptomycin (100 µg/mL), and 10% bovine fetal serum (Gibco). For the *in vitro* test with *S. mansoni*, the sample (crude extracts or partition fractions) was dissolved in 1% DMSO and used at concentrations of 200 µg/mL, which were added to the medium containing one adult worm pair after a period of 24 h of adaptation to the culture medium. The parasites were kept for 120 h and monitored every 24 h, to evaluate their general

condition concerning motor activity and mortality rate.[17] Also, changes in pairing, egg production, and egg development were examined with the aid of an inverted microscope (Leitz).[18] RPMI 1640 medium and RPMI 1640 with 1% DMSO were used as negative control groups, and praziquantel (PZQ) was employed as the positive control group at a concentration of 3.1 µg/mL The experiment was carried out in quadruplicate and repeated at least 5 times.

Pairs of adult worms were incubated for 120 h with the sample (crude extracts or partition fractions) at 200 µg/mL), and the viability assays were conducted using the MTT assay, as previously described.[12] The experiment was accomplished in quadruplicate and repeated at least 5 times. Results are expressed as mean ± SEM. Data were statistically analyzed by one-way analysis of variance, followed by Tukey´s multiple comparison test.

3 RESULTS

The *in vitro* antischistosomal effects of the extracts from the leaves of *B. sulphurea* in *n*-hexane (**BS-1**), dichloromethane (**BS-2**), and methanol (**BS-3**), and the fractions of **BS-3** in *n*-hexane (**BS-3.1**), dichloromethane (**BS-3.2**), ethyl acetate (**BS-3.3**), and methanol (**BS-3.4**) are summarized in Table 1. All the extracts and fractions were tested at 200 µg/mL. It was observed that **BS-3** is more effective than **BS-2** and **BS-1**, causing the death of 100% of the male worms after a period of 120 h of incubation and promoting the separation of most of the worms into individual males and females in 120 h. On the other hand, these extracts caused no significant decrease in motor activities, even after 120 h. The adult worms remained coupled in the two negative control groups (RPMI 1640 medium and DMSO 1% plus RPMI 1640 medium), and no death of these parasites occurred. In contrast, the positive control (PZQ, 10 µg/mL) caused a total reduction in the motor activity, and death of parasites within 24 h.

The extract **BS-3**, the most effective against *S. mansoni* worms, was fractioned by partition to furnish the fractions **BS-3.1**, **BS-3.2**, **BS-3.3,** and **BS-3.4**. As shown in Table 1, all these fractions promoted the separation of all the coupled pairs of worms into individual males and females after 120 h of incubation, except for **BS-3.4**. However, only **BS-3.2** promoted the coupled pairs separation in 24 h. Moreover, **BS-3.2** also caused the death of 100% of male and female worms after 120 h, whereas incubation with **BS-3.1**

and **BS-3.3** resulted in the selective death of 100% of males. None of the fractions caused a significant reduction in the worm's motor activity.

4 DISCUSSION

The results obtained in the study indicate that the **BS-3** extract derived from the leaves of the *B. sulphurea*, displayed interesting *in vitro* schistosomicidal effects against *S. mansoni* at a concentration of 200 µg/mL after 120 h, even though no significant effect on the motor activity was observed. When compared to **BS-1** and **BS-2** extracts, the **BS-3** extract proved to be more effective in separating worm couples and causing the death of male worms. These results not only showed that **BS-3** exhibited *in vitro* antischistosomal activity, but also revealed that *S. mansoni* worms are more susceptible to **BS-3** than female ones. The differences between *S. mansoni* male and female worms in terms of susceptibility have also been reported in the literature for other extracts and essential oils [3, 11, 19] and are likely associated with the fact male worms are more exposed to drugs than female worms (female worms are enclosed in the male worm gynecophoral groove).[3]

The fractionation of **BS-3** into their *n*-hexane (**BS-3.1**), dichloromethane (**BS-3.2**), ethyl acetate (**BS-3.3**), and methanol (**BS-3.3**) phases revealed that **BS-3** was the most active fraction. Moreover, the higher activity of **BS-3.2** as compared to **BS-3** and fractions **BS-3.1**, **BS-3.3**, and **BS-3.4** may indicate that most of the active compounds of **BS-3** are likely in **BS-3.2**. On the other hand, none of the fractions were significantly more effective against *S. mansoni* than **BS-3** to reduce the worm's motor activity.

In the literature, methanol extracts from different plants have been reported for their antischistosomal effects, such as those obtained from *Eucalyptus globulus*,[20] *Furcraea selloa*,[21] *Jatropha curcas*,[22] *Momordica balsamina*,[23] *Carica papaya*.[24] *Actinopyga echinites*, and *Holothuria polii*.[25] Many compounds isolated from methanol extracts have also been reported for their antischistosomal activities, such as the steroidal glycoside (saponin),[21] 3-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-xylopyranoside gloriogenin from *Fuircraea selloa*, which led to 100% mortality of the *S. mansoni* (EC_{50} = 2.25 and 1.91 µg/mL for female and male worm respectively) at a concentration of 5 µg/mL.[21] Mallophenol B, 2,2,8-trimethyl-6-formyl-chrom-3-ene-7-O-β-D-glucopyranoside, and benzyl alcohol 7-O-(30,40,6'-tri-O-galloyl)-β-D-glucopyranoside isolated from *Eucalyptus globulus*, at a concentration of 20 ppm, caused almost 100% toxicity to

S. mansoni miracidia.[20] Balsaminol F (LC_{50} = 14.7 ± 1.5 µM after 24 h) and karavilagenin C (LC_{50} = 28.9 ± 1.8 µM after 24 h) isolated from the ethyl acetate fraction of the methanol extract of *Momordina balsamina.* At concentrations of 10-50 µM, these compounds induced significant reductions in the motor activity of the worms and significantly decreased the egg production. Furthermore, they were able to separate the adult worm pairs into male and female after 24 h.[23] Echinosides A and B isolated from *Actinopyga echinites* and *Holothuria polii* were highly active against *S. mansoni* adult worms, with LC_{50} of 0.19 µM and 0.27 µM, respectively.[25]

The antischistosomal effects of **BS-3** and its fraction **BS-3.2** can be attributed to the presence of specific bioactive compounds, which may be acting alone or synergistically with other compounds to produce an antischistosomal effect.[3] Previous studies have reported the occurrence of some classes of compounds in different *Bidens* species that can contribute to antischistosomal activity, such as alkaloids, tannins, fatty acids, phenolic acids, triterpenes, phytosterols, chalcones, aurones, and flavonoids (e.g., rutin and quercetin).[26-28] However, the composition of *Bidens* extracts may vary according to the species, extraction method, and growing conditions of the plant,[26] so the compounds responsible for the antischistosomal activity of **BS-3** and **BS-3.3** cannot be identified from the preliminary data reported here.

5 CONCLUSIONS

The methanol extract from *B. sulphurea* leaves and its dichloromethane phase displayed *in vitro* antischistosomal activity against *S. mansoni* adult worms at a concentration of 200 µg/mL, causing mortality and separation of coupled worms similar to PZQ after 120 h of treatment. The preliminary results reported here for the first time demonstrate the *in vitro* antischistosomal potential of *B. sulphurea* extracts. This is a noteworthy result because *B. sulphurea* occurs in many countries worldwide and is easily cultivated. Further phytochemical studies must be carried out to isolate and identify the compounds responsible for the antischistosomal activity of this extract.

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APPENDIX

Table 1: *In vitro* schistosomicidal effects of *n*-hexane (BS-1), dichloromethane (BS-2), and methanol (BS-3) extracts from the leaves of *B. sulphurea* and fractions of BS-3 obtained by partition in *n*-hexane (BS-3.1), dichloromethane (BS-3.2), ethyl acetate (BS-3.3), and methanol (BS-3.4) .

Samples	Period of incubation (h)	Separated couples (%)	Dead worms (%)		Reduction in the motor activity			
					Slight (%)		Significant (%)	
			M	F	M	F	M	F
Control[a]	24	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0
DMSO[b] 1%[b]	24	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0
PZQ[c]	24	0	100	100	0	0	100	100
	120	0	100	100	0	0	100	100
BS-1	24	0	0	0	25	0	0	0
	120	0	25	0	50	0	0	0
BS-2 [d]	24	25	0	0	0	0	0	0
	120	25	100	25	100	25	0	0
BS-3[d]	24	75	0	50	0	0	0	0
	120	75	100	75	0	0	0	0
BS-3.1 [d]	24	50	0	0	75	0	0	0
	120	100	100	0	100	0	0	0
BS-3.2	24	100	50	0	25	25	25	50
	120	100	100	100	0	0	0	0
BS-3.3	24	100	0	0	75	75	25	25
	120	100	0	0	100	100	0	0
BS-3.4	24	0	0	0	75	0	0	0
	120	0	0	0	100	0	0	0

[a] RPMI 1640 medium; [b] DMSO + RPMI medium; [c] Tested at (3.1µg/mL); [d] Tested at a concentration of 200 µg/mL. M: males; F: females.

Source: The Author