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Research Paper

Annona Species Activity Against *Schistosoma Mansoni* at Different Life Cycle Stages

Environmental Science

KEYWORDS : Annona species, Schistosoma mansoni, Schistosomicidal activity, Miracidicidal activity, Cercaricidal activity

Aldenir Feitosa Santos	Laboratório de Pesquisa - Centro Universitário Cesmac & Maceió –AL, Brasil
Kelly Barbosa da Silva	Laboratório de Pesquisa em Recursos Naturais, Instituto de Química e Biotecnologia- Universidade Federal de Alagoas & Maceió-AL, Brazil
Joao Gomes da Costa	Laboratório de Pesquisa - Centro Universitário Cesmac & Maceió –AL, Brasil
Fernanda Andrade de Cesar	Laboratório de Imunologia e Esquistossomose Experimental, Laboratório de Imunopatologia Keizo Asami - Universidade Federal de Pernambuco & Recife-PE, Brazil
Monica Camelo Pessoa de Azevedo Albuquerque	Laboratório de Imunologia e Esquistossomose Experimental, Laboratório de Imunopatologia Keizo Asami - Universidade Federal de Pernambuco & Recife-PE, Brazil
Antonio Euzebio Goulart Santana	Laboratório de Pesquisa em Recursos Naturais, Instituto de Química e Biotecnologia - Universidade Federal de Alagoas, Maceió-AL, Brazil

ABSTRACT

In this study, 22 ethanol extracts derived from 6 species of Annona were subjected to biological assays in order to evaluate their schistosomicidal, miracicidal and cercaricidal activity in vitro. The plant extracts A. crassiflora (root wood),A. muricata (leaf) and A. salzmannii (leaf) showed schistosomicidal activity at concentrations of 100-10 µgmL-1. It was possible to observe that 15 extracts were active, with emphasis on A. crassiflora (stem, root wood and root bark), A. glabra (seeds), A. muricata (leaf), A. pisonis (stem), A. salzmannii (leaf) and A. squamosa (seeds and roots) for showing cercaricidal activity at < 10 gmL-1. Regarding miracicida activity 11 extracts were active and among the plant extracts evaluated, the species A. crassiflora (pulp + seeds) and A. glabra (leaf) stood out for demonstrating miracicidal activity at 1 gmL-1. These results suggest that plant extracts might be promising for the development of new schistosomicidal, cercaricidal e miracicidal agents.

INTRODUCTION

Schistosomiasis is a parasitic disease with great social impact, being regarded as a relevant public health issue in 76 countries in Africa, Asia, and South and Central Americas. This parasite, characteristic of tropical regions, has been considered for more than 10 years to be the 2nd most prevalent tropical disease, second only to malaria. It affects more than 207 million people and 600 million others located in at risk areas, in more than 70 tropical countries [1-3]. Brazil is the country with the highest endemic area for schistosomiasis in the Americas. It is estimated that the disease affects 2.5 to 6 million people in Brazil [4-6]. Some factors contribute to both development and maintenance of Schistosomiasis mansoni breeding sites, such as subsistence cultivation; perennial cultivation; flooded areas; ponds or watercourses used for sport fishing, washing of utensils, and bathing; sand deposits on river banks with no vegetation and large debris.

Control of the disease can be carried out directly, with the use of toxic agents to the worm (schistosomicides) or their larval forms - miracidium (miracicides) and cercariae (cercaricides), or indirectly with the aid of molluscicides, health education and basic sanitation programs. A combination of the forms of control is the most effective way to combat the disease [7-9]. A strategy based on vaccines against schistosomiasis is still in the experimental stage.

Currently, control of schistosomiasis has been based on the use of the schistosomicide praziquantel, coupled in some cases, with the use of the molluscicide niclosamide. Despite the efficiency of the method, the prevalency rates for schistosomiasis have remained the same for decades, certainly influenced by socio-economic factors, the emergence of praziquantel-resistant strains of Schistosoma [3], the result of constant drug use in clinical and prophylactic treatments and, due to the financial inviability for the use of these toxic agents to the worms and their invertebrate hosts on a large-scale.

In this context, research with medicinal plants becomes a viable alternative, especially in countries with large biodiversity and rich cultural and ethnic diversity, like Brazil. Several research groups have invested in the search for an alternative way for controlling this disease [10]. Various natural compounds have been shown to be toxic to miracidia and cercariae [11, 12] and some plants were reported to possess components that can inhibit the penetration of cercariae through human skin [13]. Crude aqueous extract of Zingiber officinale [14] and pure compounds as artemether [15] and dihydroartemisinin [16] have showed in vivo schistosomicidal effects, but so far, no new drug has been marketed for the treatment of schistosomiasis [17].

The Annonaceae is a family of plants consisting of about 2300 to 2500 species and more than 130 genera. Although the Annonaceae are generally consumed as fresh fruits, they are also widely used in folk medicine. Several reports have characterized the pharmacological activity of these plants because of their bioactive compounds (mainly acetogenins, flavonoids and alkaloids) found in the roots, leaves, bark, seeds and fruit. The members of the Annonaceae family are mostly distributed in the Americas and Asia. Previous chemical and pharmacological investigations on some species of this family have indicated the presence of important bioactive compounds, exhibiting various pharmacological activities including antimicrobial, insecticidal and antiparasitic properties, in particular against Leishmania sp., Plasmodium falciparum and Trypanosoma cruzi. However, the Annonaceae family, considering its size (130 genera and 2300 species), is one of the least studied phytochemically.

Aiming to contribute to the search for plant species useful in the control of Schistosoma mansoni, in this research, 22 ethanol extracts derived from 6 species of Annona were subjected to biological assays in order to evaluate their schistosomicidal, miracicidal and cercaricidal activity in vitro.

MATERIALS AND METHODS

- **Plant material**

The different parts of the plant species were collected and a voucher of each plant deposited in the herbarium of the University of Brasilia (Brasilia, DF.) and at the herbarium of IMA-AL (Instituto do Meio Ambiente do Estado de Alagoas, Brazil), and were identified respectively by Prof. José Elias de Paula and Rosangela P. de Lira Lemos. The amount of vegetal material used for the preparation of extracts was dependent on their availability for collecting. The minimum amount was established as 500 g of fresh material [20].

- **Ethics Statement**

The study protocol was approved by Ethical Committee on the Use of Animals in the Center of Biological Sciences of the Federal University of Pernambuco (CEUA-UFPE, protocol 23076.047005/2014-21).

- **Schistosomes**

The BH strain (Belo Horizonte - MG, Brazil) of S. mansoniwas routinely maintained at the Laboratório de Imunopatologia Keiko Asami (UFPE) by passage through B. glabrata and mice [21-23].

- **Preparation of working solutions for the bioassays**

Adult schistosome bioassay: A stock solution (400 g/mL) was prepared for each sample by dissolving an aliquot of material (8 mg) in dimethylsulphoxide (DMSO; 200 L) and adding RPMI-1640 medium to a final volume of 20 mL. Appropriate aliquots of the stock solution were diluted with the parasite culture medium to yield concentrations of 200, 100, 50 and 10 g/mL. Six replicates of each bioassay were carried out for each sample concentration [21].

Miracidia bioassay: A stock solution (1 mg/mL) was prepared for each sample by dissolving an aliquot of material (15 mg) in DMSO (150 L) and adding de-chlorinated water to a final volume of 15 mL. Samples (150 L) were typically assayed in duplicate at concentrations of 100, 10 and 1 gmL-1.

Cercariae bioassay: A stock solution (200 g/mL) was prepared for each sample by dissolving an aliquot of material (3 mg) in DMSO (150 L) and adding de-chlorinated water to a final volume of 15 mL, as previously described [24]. The extracts were initially tested at initial concentrations of 100, 10 and 1 gmL^{-1}, to a final volume of 10 ml per concentration. Depending on the results obtained intermediate concentrations were evaluated.

- **Protocols for the bioassays**

Schistosomicidal activity: Strict aseptic techniques were employed throughout the experiments, Mercer and Chapell [25]. Two adult schistosomes, paired or unpaired, in parasite culture medium were placed into one well of a multi-well plastic tissue culture plate, an appropriate aliquot of sample solution was added, and the plates were incubated at 37 ± 1°C in a humid atmosphere containing 5% CO_2. The viability of the worms was observed under an inverted microscope each day until the 5th day after sample addition. Schistosomes were considered dead when no movement could be detected over a 3 min observation period. Positive controls (10 g/mL praziquantel and negative controls (RPMI-1640 medium containing 1% v/v DMSO) were included in every experiment, and a minimum of 30 schistosomes were employed in the assay of each treatment and control group.

Miracidicidal activity: Twenty miracidia were exposed to each concentration of a sample in multi-well plates as described previously [26], together with positive (0.1 g/mL Niclosamide) and negative controls (de-chlorinated water containing 1% v/v DMSO).

Cercaricidal activity: Approximately 500 freshly released cercariae suspended in de-chlorinated water were placed into a 25 mL beaker and an appropriate aliquot of sample solution was added as previously described [24].

- **Statistical methods**

The experiment was developed in a completely randomized arrangement in a factorial design (Annona plant extracts x concentrations) with five replications. Data were subject to analysis of variance and means were compared by the Tukey test at 5% probability. Estimates of the Pearson correlation coefficient between variables were also determined. All analyzes were performed using the computer program SAEG (Statistical and Genetic Analysis System).

RESULTS AND DISCUSSION

- **Schistosomicidal activity**

The chemotherapy of many helminth infections has been complicated by the emergence of resistant strains and/or tolerance to anthelmintic drugs in use. Recent studies in the laboratory and in the field have detected a reduction in effectiveness of praziquantel against schistosomes. In this context, many researchers have directed their work toward the search for new substances useful in the control of schistosomiasis, with plants as alternative sources.

In the bioassays to evaluate susceptibility of adult worms in vitro, a significant effect was observed against extract concentration ($p < 0.01$), exposure time ($p < 0.01$) and the interaction of the plant extract concentration x exposure time ($p < 0.01$), according to the analysis of variance (Table 1).

Table 1 –Analysis of variance for the activity of different Annonaceae extract concentrations with respect to exposure time.

Source of variation	Mean square	F
Extract concentration	44981.57	219.54**
Time	48505.13	236.74**
Concentration x time	3969.62	19.37**
Residue	204.89	

**Significant to 1% probability by the F test.

The results obtained were sorted into groups:

•Group I – plant extracts that showed schistosomicidal activity at concentrations of 750-500 μgmL^{-1}: Annona pisonis (stem) and A. squamosa (seeds) (Table 2).

•Group II – plant extracts that showed schistosomicidal activity at concentrations of 500-50 μgmL^{-1} :A. crassiflora (stem, root bark and fruit rind), A. squamosa (roots) andA. glabra (seed) (Table 3).

•Grupo III –plant extracts that showed schistosomicidal activity at concentrations of 100-10 μgmL^{-1}: A. crassiflora (root wood), A. muricata (leaf) and A. salzmannii (leaf) (Table 4).

In group I, the species A. squamosa (seeds) especially stands out, for producing 100% mortality in 24 hours at 750 $\mu gmL^{-1}$and in 72 hours at 500 μgmL^{-1}(Table 2). This extract also produced unpairing of the worms in the first 24 hours of contact and inhibition of oviposition. Just like praziquantel [27], the ethanol extract of the seeds of A. squamosa caused severe alterations in the tegu-

ment of the worm, which evolves through the formation of blisters until the "peeling" process and vesiculation. The deleterious action on the tegument of the worm is extremely important because it protects the schistosome from the host immune system acting on it, is involved in the absorption of nutrients and has a secretory function [27]. A. pisonis (stem) produced 100% mortality at 750 gmL^{-1} between the 2nd and the 5th day of exposure, producing unpairing of the worms in this same period, but without causing significant alteration in their tegument. These extracts were more active than the stem bark of Anthostema senegalenseis, Piliostigma thonningii, Sclerocarya birrea and that of the roots of Lannea barteri which showed schistosomicidal activity at 2000 μgmL^{-1}, and that of the extract of the stem bark of Khaya senegalensis which was active at 1000 μgmL^{-1}[10].

Table 2. Schistosomicidal action in vitro of ethanol extracts from species of Annonas from group I

Group I	Conc. (µg/mL)	Incubation period (h)	Dead worms (%)	Motor activity reduction (%)		Worms with Tegumental alterations (%)	
				Slight	Significant	Parcial	Extensive
Control[a]		24-120	0	0	0	0	0
Praziquantel	10	24	100	0	100	0	0
		48-120	100	0	100	0	100
A. pisonis - stem	750	24	0	0	0	0	0
		48-72	0	81	0	0	0
		96	95	100	0	0	0
		120	100	0	100	50	0
	500	24	0	0	0	0	0
		48-96	0	100	0	0	0
		120	25	75	25	0	0
A. squamosa - seeds	750	24-120	100	0	100	0	0
	500	24	0	100	0	0	0
		48	47.5	100	0	100	0
		72-120	100	0	100	100	0

a RPMI 1640 + 1% DMSO

In group II, the species A.squamosa (root) stood out, for producing 100% mortality at 250 gmL^{-1} in just 24 hours of exposure, with unpairing of worms and blistering in their tegument (Table 3). The A. crassiflora (stem and root bark) extracts produced 100% mortality in 48 hours of exposure at 100 $\mu gmL^{-1.}$ The extract of the stem produced unpairing of worms and the formation of blisters in the tegument within the first 24 hours of exposure. The extract of the root bark only induced unpairing after 48 hours of exposure. The results obtained with these extracts were superior to those displayed by the essential oil of Baccharis trimem, the seeds of Millettia thonningii and by the essential oil of Ageratum conyzoides. The essential oil of B. trimera at a concentration of 130 μgmL^{-1} produced a significant decline in motility and a worm mortality rate of 100% after 24 hours of exposure [28]. The seeds of M. thonningii only caused 100% mortality at 100 μgmL^{-1} after 72 hours of exposure [29]. The essential oil of Ageratum conyzoides produced a 75% mortality of females and 100% of males at 100 μgmL^{-1} in 120 hours of exposure [30]. The extract of A. glabra (seeds) showed results similar to M. thonningii (seeds) [29] producing 100% mortality within 72 hours of exposure and unpairing of worms.

Table 3. Schistosomicidal action in vitro of ethanol extracts from species of Annonas from group II

Group II	Conc. (µg/mL)	Incubation period (h)	Dead worms %	Motor activity reduction (%)		Worms with Tegumental alterations (%)	
				Slight	Significant	Parcial	Extensive
Control[a]		24-120	0	0	0	0	0
Praziquantel	10	24	100	0	100	0	0
		48-120	100	0	100	0	100
A. squamosa Roots	250µg/mL	24-120	100	0	100	0	0
	150µg/mL	24	90	0	100	0	0
		48-120	100	0	100	0	0
	80µg/mL	24-72	0	100	0	0	0
		96-120	100	0	100	0	0
A. crassiflora Stem	100	24	23	100	0	0	0
		48-120	100	0	100	100	0
	50	24	0	100	0	0	0
		48-72	0	100	0	100	0
		96-120	100	0	100	100	0
A. crassiflora Root bark	100	24	17.4	100	0	0	0
		48-120	100	0	100	0	0
	50	24-96	0	100	0	0	0
		120	0	0	100	0	0
A. glabra Seeds	250	24	84	0	100	0	0
		48-120	100	0	100	0	0
	100	24	65	100	0	0	0
		48	88	0	100	0	0
		72-120	100	0	100	0	0
	50	24-72	0	100	0	0	0
		96	78.5	0	100	0	0
		120	100	0	100	0	0
	30	24-120	0	0	0	0	0

a RPMI 1640 + 1% DMSO

The plant species of group III (Table 4) were considered as the most promising with respect to schistosomicidal activity, in that they caused deleterious effects on the worms up to 10 $\mu gmL^{-1.}$ manifesting as changes in their morphology, induction of the formation of blisters and the process of "peeling" in the tegument and induction of unpairing of the worms.

Table 4. Schistosomicidal action in vitro of ethanol extracts from species of Annonas from group III

Group III	Concentration (µg/mL)	Incubation period (h)	Dead worms (%)	Motor activity reduction (%)		Worms with Tegumental alterations (%)	
				Slight	Significant	Parcial	Extensive
Control[a]		24-120	0	0	0	0	0
Praziquantel	10	24	100	0	100	0	0
		48-120	100	0	100	0	100
A. crassiflora Root wood	70	24	100	100	0	0	100
		48-120	100	0	100	0	100
	50	24	50	0	100	0	100
		48	90	0	100	0	100
		72-120	100	0	100	0	100
	30	24	0	100	0	0	0
		48	0	100	0	100	0
		72	33	0	100	0	100
		96-120	100	0	100	0	100
	10	24	0	0	0	0	0
		48	0	100	0	0	0
		72-120	0	100	0	0	0
A. muricata Leaf	100	24	42.8	100	0	100	0
		48-120	100	0	100	100	0
	60	24	0	100	0	0	0
		48	5	100	0	70	0
		72	23.3	100	0	30	0
		96	52.6	50	50	0	0
		120	73.6	10	90	0	0
	30	24	0	0	0	0	0
		48-72	0	50	0	0	0
		120	0	100	0	30	0
A. zalzmannii Leaf	100	24	0	0	0	0	0
		48-120	100	0	100	0	100
	60	24-72	0	100	0	0	0
		96	7	100	0	0	0
		120	25	100	0	0	0
	30	24	0	0	0	0	0
		48-120	0	100	0	0	0

a RPMI 1640 + 1% DMSO

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The best results were seen by A. crassiflora (root wood) which induced alterations in morphology and behavior of the worm on the 1st day of exposure to 50 $\mu gmL^{-1,}$ and 100% mortality on the 3rd day of exposure, surpassing the results seen by the roots of Securidaca longepedunculata that showed activity only at 80 μgmL^{-1}[10]. At 30 gmL^{-1} this extract of Annona produced 100% mortality on the 4th day of exposure, with an affect similar to that seen by Euphorbia royleana [31] and inferior to that shown by Grateloupia livida (dried seaweed) that caused the death of 40.95% of S. japonicum adult worms at 25 µg/mL after 24 h of incubation 1 [32], and by Eriosema griseum (leaf) which showed activity at 40 gmL^{-1} [10]. The species A. salzmannii (leaf) and A. muricata (leaf) caused a maximum deleterious effect on schistosomes at 100 gmL^{-1} with only 24 hours of exposure. The schistosomicidal action of these extracts continued until 10 μgmL^{-1}(Table 4).

The species A. crassiflora (fruit rind), A. glabra (husk), A. muricata (stem), A. pisonis (stem bark), A. salzmannii (stem wood) and A. squamosa (leaf) were inactive against the worms at the concentrations evaluated and for the maximum exposure time.

It is important to emphasize that the adult worms of S. mansoni subjected only to the vehicle used in the solubilization of the different plant extracts remained viable throughout the observation period (10 days), showing vigorous activity without changes in their tegument.

- **Miracicidal activity**

An alternative way to combat Schistosomiasis is through the use of miracicidal substances. The miracidium of S. mansoni survives up to 24 hours in an aqueous medium, if the temperature conditions are suitable [33]. The use of miracicidal agents prevents infection of the snails and, thus the production of the infectious form of the disease, the cercariae.Among the plant extracts evaluated against the miracidium of S. mansoni, the species A. crassiflora (pulp + seeds) and A. glabra (leaf) mainly stood out for demonstrating miracicidal activity at 1 gmL^{-1}, followed by extracts of A. crassiflora (root wood), A. muricata (root) and A. salzmannii (leaf) active at 10 $gmL^{-1.}$ The extracts of A. crassiflora (stem), A. glabra (seeds), A. muricata (leaf), A. salzmannii (stem wood) and A. squamosa (seeds and root) produced 100% mortality of miracidia at 100 gmL^{-1}(Table 5). These results were better than those reported for the plants: Euphorbia milli (latex), that in the range of 100 -50 gmL^{-1} produced only 43% mortality after 2 hours of exposure and with the increase of exposure time to 4 hours, produced 80% mortality at 100 gmL^{-1} and 73% at 50 gmL^{-1} [34]; Tetrapleura tetraptera (fruits) and Lagenaria brevifolia (seeds and pulp) which produced 100% mortality after 60 min of exposure at concentrations of 1000 gmL^{-1} , 250 gmL^{-1} and 100 gmL^{-1}, respectively [35]. However, they were inferior to that shown by Nigella sativa (seeds), which produced 100% mortality at 5 gmL^{-1} after 1 min. of exposure [36].

Table 5. Miracicidal activity of plant species against the miracidia of Schistosoma mansoni Sabom

Species of Annona	Part Tested	Concentration (gmL^{-1})	Behavior of miracidia post evaluation			
			15min.	30 min.	1h	2h
A. crassiflora	Pulp and seeds	100	+ +	+ +	+ +	+ +
		10	+ +	+ +	+ +	+ +
		1	+	+	+	+ +
	Stem	100			+	+
	Root wood	100			+	+
		10			+	+
	Fruit rind	100				
A. glabra	Seeds	100			+	+
		10				
		1				
	Leaf	100	+ +	+ +	+ +	+ +
		10	+ +	+ +	+ +	+ +
		1			+	+ +
A. muricata	Leaf	100				+
	Roots	100	+	+	+	+ +
		10			+	+
		1				
	Stem	100				
A. pisonis	Leaf	100				
	Stem bark	100				
	Stem	100				
A. salzmannii	Leaf	100				
	Stem wood	100				+
A. squamosa	Seeds	100			+	+
		10				
	Roots	100			+	+
		10				
	Leaf	100				

(++) 100% miracidia mortality– total miracicidal activity; (+) about 90% miracidia mortality, () about 50% miracidia mortality, (-) 100% miracidia motility – absence of miracicidal activity.

- **Cercaricidal activity**

In Infection by S. mansoni occurs when the cercariae penetrates the body of a human through intact skin. Therefore, preventing such penetration is also a potential form of controlling infection. In recent years many topical agents have been evaluated for their ability to block the penetration of cercariae into the skin and some of these are highly effective, but have not been used clinically due to their potential toxicity, difficulty in manufacturing and/or difficulty in applying under field conditions [36]. These, among other factors, have stimulated the search for new chemical agents capable of providing such protection, and in this context, one of the first aspects to be analyzed was the cercaricidal activity of the samples, with respect to immobilization and mortality of the larvae.

In analyzing the results obtained against cercariae of S. mansoni, it was possible to observe that the extracts from A. crassiflora (stem, root wood and root bark), A. glabra (seeds) A. muricata (leaf), A. pisonis (stem), A. salzmannii (leaf) and A. squamosa (seeds and roots) showed activity at concentrations below 10 $gmL^{-1,}$ extracts from A. crassiflora (fruit rind), and A. squamosa (leaf) were active between 14-20 gmL^{-1}, extracts from A. muricata (stem), A. pisonis (husk) and A. salzmannii (stem wood) were active between 21-50 gmL^{-1}, and the extract of A. muricata (stem bark) produced 100% mortality between 51-100 gmL^{-1}(Table 6).

Table 6. Cercaricidal activity of plant species against the cercariae of Schistosoma mansoni Sabom

Species of Annona	Part tested	Concentration (gmL^{-1})	Behavior of cercariae post evaluation			
			15 min.	30 min.	1h	2h
A.crassiflora	Stem	1		+	+	+ +
		0,5		+	+	+
	Root wood	15		+	+ +	+ +
		10		+	+ +	+ +
		50				+
		1				
	Root bark	2			+	+
		1			+	+
	Fruit rind	40	+	+	+	+ +
		20				+
A. glabra	Seeds	4	+	+	+ +	+ +
		3,5				
		3				
A. muricata	Leaf	20			+	+
		10			+	+
		9,5				
	Stem bark	100				+
		95				+
		90				
	Stem	50				+
		45				+
		40				
A. pisonis	Stem bark	55		+	+	+
		50			+	+
		45				+
		40				
	Stem	8	+	+	+	+
		7			+	+
		6,5			+	+
	Stem wood	60		+	+	+ +
		55				+
		50				+
A. squamosa	Seeds	6	+	+	+	+
		4	+	+	+	+
		3,5				+
		3				
	Roots	6		+	+	+
		4		+	+	+
		3,5				
	Leaf	20				+ +
		16				+
		14				+
		10				

(+ +) 100% mortality –precipitated and immobile cercariae – total cercaricidal activity; (+) 100% of the cercariae were precipitated and with abnormal movements, loss of infection capacity - cercaricidal activity; () about 50% of cercariae were precipitated and with abnormal movements – partial cercaricidal activity, (-) 100% motility – absence of cercaricidal activity.

These results were lower than seen by Nigella sativa (seeds) which produced 100% mortality at 5 gmL^{-1} after 5min of exposure [26], but still warrant further investigation of these extracts as topical agents against schistosomiasis, primarily in situations where contact with the contaminated water is inevitable or there is a high risk of accidental exposure.

All of these plant species were more toxic to cercariae of S. mansoni that the latex of Euphorbia milli which proved slightly toxic to these larvae, producing only 14.4% mortality after 4 hours of exposure at a concentration of 100 gmL^{-1}[34]. The results were also higher than those reported by Ajaye et al. [35] for the species Tetrapleura tetraptera (fruits) and Lagenaria brevifolia (seeds) which produced 100% mortality after 60 min of exposure at concentrations of 1000 gmL^{-1} and 250 gmL^{-1}, respectively.

The cercaricidal activity shown by plant extracts was higher or equivalent to schistosomicidal activity. These results indicate that the cercariae are more susceptible than the schistosomes and that this feature is inherent in the morphology of these target organisms. The cercariae are covered by a membrane of three layers that protects the inside from an external agent acting on it [37] immediately after penetration into the vertebrate host, such that the inside of the resulting schistosomulum is protected by a seven-layer membrane, making it less susceptible than the cercariae [38].

Another aspect to be noted is that the species A. crassiflora (stem), A. glabra (seeds),A. muricata (leaf), A. salzmannii (leaf) and A. squamosa (seeds and roots) were active against adult worms and their larval forms in addition to already showing molluscicidal activity [39]. This result indicates the possibility of investing in alternative methodologies for schistosomiasis control, and for when the plant species can be used at different phases of the life cycle of the worm. Additionally A. salzmannii (stem wood) shows a bioselectivity that enables direct attack on the worm and its larval stages without affecting the snail [39].

CONCLUSIONS

In this study 06 Annonas species were evaluated. The activity displayed by these plants ranged from selective attack on miracidium and cercariae to the adult worm, characteristics which give evidence for the possibility of their use for control of these stages in situations where the disease is highly prevalent. In addition, the option to combat the larval forms of the worm, in the aquatic environment, without the necessity to make use of molluscicidal agents, which reduces environmental impact, is an action that is indicated as a prophylactic method in areas at high risk of contamination or near these areas.

Similarly to praziquantel, the species were active against adult worms causing mortality, decreased motor activity and tegument disorders, demonstrating beyond this the advantage of also acting on the larval forms of the worm, and thus being considered as a potential source for new schistosomicidal agents.

Considering the results seen it is worth to emphasize the importance of conducting in vivo tests on mice infected with S. mansoni and to determine the mechanism of action of anti-schistosomal plant species.

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