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Original Article

The schistosomicidal activity of ethanolic extracts from branches, leaves, flowers and fruits of *Handroanthus impetiginosus* (Mart. ex DC.) Mattos (Bignoniaceae) plant and metabolic profile characterization by UPLC-ESI-QTOF analysis

Atividade schistosomicida do extrato etanólico dos galhos, folhas, flores e frutos de plantas de *Handroanthus impetiginosus* (Mart. ex DC.) Mattos (Bignoniaceae) e caracterização do perfil metabólico por análise de UPLC-ESI-QTOF

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Abstract

Schistosomiasis, caused by *Schistosoma mansoni* Sambon, 1907, is a severe and widely distributed parasitic disease, affecting about 200 million people worldwide. The disease is recognized by elevated mortality rates, especially among those living in areas of poor sanitation. Currently, the chemotherapeutic treatment is solely based on using the praziquantel drug. Therefore, there is a need for the discovery of new medicines for the treatment of this parasitosis. Thus, this work aimed to evaluate the schistosomicidal activity of ethanolic crude extracts from the branches, leaves, flowers, and fruits of *Handroanthus impetiginosus* (Mart ex DC.) Masttos and characterize its metabolic profile by UPLC-ESI-QTOF analysis. Evaluation of plant extract on *S. mansoni* was carried out in adult worms *in vitro*, in which the mortality rate was quantified, and the damages in the tegument of the worms were monitored. All extracts induced changes in the viability of adult males of *S. mansoni*, causing the death of the parasites, which was directly dependent of the concentration.

Keywords: Schistosomiasis, chromatography, mass spectrometer, neglected diseases.

Resumo

Schistosomíase, causada pelo *Schistosoma mansoni* Sambon, 1907, é uma doença parasitária severa e amplamente distribuída, afetando cerca de 200 milhões de pessoas pelo mundo. A doença é reconhecida pelo alto índice de mortalidade, especialmente dentre as populações que vivem em ambientes de pouca vigilância sanitária. Geralmente, o tratamento é apenas baseado no uso da droga praziquantel. Entretanto, há uma necessidade da descoberta de novos medicamentos para o tratamento dessa parasitose. Por isso, o presente trabalho visa avaliar a atividade schistosomicida dos extratos etanólicos cru dos galhos, folhas, flores e frutos de *Handroanthus impetiginosus* (Mart ex DC.) Masttos e caracterizar seu perfil metabólico por análise de UPLC-ESI-QTOF. Avaliações dos extratos vegetais em *S. mansoni* foram conduzidos em vermes adultos *in vitro*, pelos quais a taxa de mortalidade foi quantificada, e danos no tegumento dos vermes foram monitorados. Todos os extratos induziram mudanças na viabilidade de vermes adultos de *S. mansoni*, causando a morte desses parasitas, o que foi diretamente dependente da concentração.

Palavras-chave: Esquistossomose, cromatografia, espectrômetro de massa, doenças negligenciadas.

1. Introduction

Schistosomiasis is a parasitic disease caused by trematodes of the genus Schistosoma. The infection is directly related to poor sanitation. According to the World Health Organization (Savioli et al., 2017), this parasite belongs to neglected tropical diseases (NTDs), which are endemic among the poorest and most vulnerable

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populations. These diseases are considered neglected, as they receive reduced investment in research, development and production of new drugs, vaccines, and the low effectiveness of control programs (Fonseca et al., 2020).

Based on the questions presented, one of the intervention alternatives to deal with the problem is the use of natural products for the treatment of this disease. As a millennial practice, medicinal plants have contributed to medicine been gaining more space as an effective and complementary alternative throughout history (Thomford et al., 2018). In addition to preventing, curing, or minimizing disease symptoms, products of natural origin are more affordable to the population and public health services than chemically synthesized products (Dutta et al., 2019).

In this context, *Handroanthus impetiginosus* is a tree species native from Brazil that can reach 20 to 35 m high, mainly known as ipê-roxo, pau-d'arco-roxo, and ipê-roxo-da-mata (Pimenta et al., 2022). It is known for its medicinal characteristics as analgesic, anti-inflammatory, antineoplastic, antibiotic, diuretic and antiseptic action (Ahmad et al. 2020). The known active compounds of this species are coumarins, flavonoids, tannins, saponins, resins, naphthoquinones, anthraquinones such as lapachol, α-lapachol, β-lapachol, chloro-hydro-lapachol as well as minerals: silicon, calcium, iron, cobalt and vitamins. They are responsible for several ecological issues and reported biological activities, including antineoplastic, antitumor, anticoagulant, antimalarial, and analgesic (Silva Júnior et al., 2019).

The search for anthelmintic compounds, mainly from natural sources, has intensified recently (Jiao et al., 2020; Paula et al., 2020, 2022; Oliveira et al., 2020a; Lima et al., 2021, 2022). All this search is summarized in a recently published work, which shows the studies carried out for the treatment of schistosomiasis from the mid-1910s to the year 2016, where more than 100 compounds studied were gathered with information about *in vitro* and *in vivo* therapeutic action, toxicity, and pharmacokinetic profiles (Lago et al., 2018). All these efforts may represent hope in controlling schistosomiasis and show the need to search for new compounds to provide effective alternatives to treating the disease. Thus, this research aimed to evaluate the antiparasitic properties of crude ethanolic extracts from different parts of *H. impetiginosus* (branches, leaves, flowers, and fruits) against *S. mansoni*. In addition to the investigation of the metabolic profile by UPLC-ESI-QTOF approaches.

2. Material and Methods

2.1. Plant material collection

The branches, leaves, flowers and fruits of *H. impetiginosus* were collected in the urban area at coordinates (20º31'19.19" S, 47º24'36" W). Dr. Milton Groppo performed the botanical identification, and the core material was deposited in the Herbarium of the Department of Biology at FFCLRP-USP with the registration number SPFR 16658.

2.2. Extracts obtainment

For the crude ethanolic extracts preparation, the branches, leaves, flowers and fruits of *H. impetiginosus* were left for 15 days to dry separately at room temperature. Afterward, they were desiccated for 48 hours in a circulating air oven at 45ºC, then ground in a vertical rotor mill with fixed knives. – MARCONI – MA680. The powder mass was obtained as follows: 1.945g for branches, 4.570g for leaves, 1.400g for flowers, and 1.925g for fruits. The material was pulverized, macerated, and filtered, and the liquid extract was concentrated in a rotary evaporator. The ethanol extract masses were obtained 500 mg branches (EtOH-Br), 4.570g leaves (EtOH-Le), 1.400g flowers (EtOH-Fl) and 1.925g fruits (EtOH-Fr).

2.3. Maintenance of S. mansoni's lifecycle

The biological cycle of *S. mansoni*, strain LE (Luis Evangelista), is routinely maintained in the Parasitology Research Laboratory of the Research Center in Exact and Technological Sciences, University of Franca, using Balb/c mice as a vertebrate host and snails of the species *Biomphalaria blabrata* Say, 1818, as an invertebrate host (Hackett, 1993). Adult parasites were obtained by perfusion of the hepatic portal system of mice 54±2 days after infection, with approximately 200 cercariae, according to conditions previously described by Smithes and Terry (1965). After 49 days of the disease, adult worms were recovered through perfusion of the mesenteric and portal veins, according to Smithes and Terry (1965). After collection, the parasites were maintained in RPMI 1640 medium (Inlab Diagnóstica, Diadema, Brazil) buffered with HEPES 20 µM, pH 7.5, supplemented with penicillin (100 UmL) (Cultilab, Campinas, Brazil) and maintained until the use in RPMI-1640 culture medium (Gibco), containing 10% Fetal Bovine Serum (SFB) (Gibco).

2.4. In vitro evaluation of schistosomicidal activity

The branches, leaves, flowers, and fruits ethanol extracts were evaluated for changes in the tegument and schistosomicidal activity *in vitro* against adult male worms of *S. mansoni*. Adult male worms were recovered from Balb/c mice as described above. Subsequently, an adult worm was transferred per well into a 24-well culture plate containing 2 mL of RPMI 1640 culture medium (Inlab) buffered with 20 µM HEPES, pH 7.5, supplemented with penicillin (100U/mL), streptomycin (100µg/mL) (Cultilab, Campinas, BR) and 10% fetal bovine serum (Cultilab) and incubated in a humidifying atmosphere at 37ºC in the presence of 5% CO_2.

After 24 hours of incubation, the crude extracts of branches, leaves, flowers and fruits separately previously dissolved in (DMSO) (Sigma-Aldrich) and added to RPMI 1640 (Inlab) medium and then tested at concentrations of 6.25 µg/ mL; 12.5 µg/ml; 50.0 µg/ml; 100.0 µg/ml and 200.0 µg/mL, based on concentrations determined in previous experiments by our research group (Magalhães et al., 2009). The parasites were incubated under the same conditions described above. Each well was observed after 24, 48, and 72 hours of contact with the samples, comparing them with the controls. Mortality and

integument detachment of male parasites was evaluated using an inverted microscope (Carl Zeiss, Göttingen, Germany). Mortality was determined according to a scale from 0 to 3 where: (3= worms with normal movement. 2= decreased motor activity, 1= minimal motor activity with occasional movements, and 0= death of the worms without movement for more than 2 minutes of observation) (Ramirez et al., 2007).

The parasites without movement for more than 2 minutes of observation were washed with RPMI 1640 medium, transferred to culture plates with the same medium without the substances, and monitored as described above. As a negative control, young male worms maintained in RPMI 1640 medium with 0.1% DMSO (Sigma-Aldrich) were used. In the experiment, PZQ at a concentration of 1.56µM was used as a positive control. Three independent experiments were carried out, with five young male worms being evaluated per concentration in each experiment (a total of 25 parasites). Results were expressed as the mean ± standard deviation (SD) of adult male viability. The LC_{50} (50% lethal concentration of parasites) was determined by the non-linear regression curve using the dose-response inhibition equation. The test was performed using GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, California, USA).

2.5. Statistical analysis

Values were expressed as mean ± SD of the samples. A one-way analysis of variance (one-way ANOVA) was used, followed by determining the significance of differences between the control and treated groups (Dunnett post-test). Tests were performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, California, USA).

2.6. Mass spectrometer instrument system and analysis

A C18 ACQUITY ®HSS T3 ultra-analytical reversed-phase column (1.8 µm, 100 x 2.1 mm, 40 °C) was used for the development of the chromatographic runs in a Xevo-qTOF/MS (Waters Corp., Milford, USA) ultra-performance liquid chromatography coupled to quadrupole time of flight mass spectrometer (MS) instrument (UPLC-QTOF/MS). The mobile phases contained (A) 1% acidified H_2O with formic acid and (B) acetonitrile at a flow rate of 0.5 mL/min. Blank samples were a mixture of ACN: water (1:1 v/v). The injection volume consisted of 5 µL of each extracted sample (branches, leaves, flowers and fruits from *H. impetiginosus*). The resulting chromatographic method gradient was developed as follows: 1% of B and 99% of A (0.1 min), 80% of A and 20% of B (until 7.5 min), 20% of A and 80% of B (until 8.5 min), 1% of A and 99% of B (until 8.6 min) and 99% of A and 1% of B (until 10 min).

Mass data were acquired as MS^E independent data acquisition (DIA) using the Masslynxs™ MS Software (Waters Corp., Milford, USA). The ESI (electrospray) ionization source operated in negative and positive modes, and MS data were collected in the centroid mode. The lock spray was calibrated using a leucine-encephalin standard solution at the frequency of 10s with mass ratio (*m/z*) at *m/z* 554.2622 ESI- in the negative mode and at *m/z* 556.2768 ESI^+ in the positive ionization mode. The collision energy parameters were performed with alternative high and low energy scans, 10 eV and 40 eV, respectively. The source temperature operating parameters were cone voltage (40V), capillary voltage (3.0 kV), desolvation temperature (300°C), desolvation gas flow (600 L/h) and cone gas flow (30 L/h and 120°C).

2.7. MS Data treatment and chemical annotation

The MS Fragment ion spectra (MS^E) raw data-independent centroided acquisition of the RT-*m/z* pair and peak area information was processed for each ionization mode using the MS DIAL software 4.80 version. The raw data ions were collected for mass data processing steps, which included the application of peak corrections, data filtering, baseline correction, isotope exclusion ([isotope (M+1)+H]$^+$, [isotope (M+2) + H]$^+$, [isotope (M+1)–H]$^-$ and [isotope (M+2)–H]$^-$), deconvolution, chromatogram building and peak alignment algorithms. Adduct search set up for mass detection in the ESI+ including [M+H]$^+$, [M+Na]$^+$, [M+K]$^+$ and to the negative [M+H]$^-$, [M+Cl]$^-$ and [M+Br]$^-$. For dereplication, the data matrix with *m/z*-Rt pair and peak area were exported to MS FINDER tool 3.52 after peak blank exclusion and peaks without eligible MS^2 data. The elemental composition was implemented for molecular formula determination. The dereplication was also supported by manually checking the online Dictionary of Natural Products (DNP®) chemical library. An excel format document (.csv) was exported with metabolite annotation.

3. Results and Discussions

3.1. Metabolic profile and dereplication

Adequate separation of the peaks could be observed in the chromatograms of all *H. impetiginosus* crude extract samples (Figure 1). The metabolic profile obtained from the UPLC-ESI-QTOF chromatograms of leaves, fruits, flowers, and branches indicated widespread chemical variance in the different vegetal parts of *H. impetiginosus* extracts. The MS data treatment of both ESI+/ESI- modes allowed the putative identification of the majority known metabolites in the samples. The most shared metabolite classes presented in the extract samples were unveiled using MS finder and DNP libraries matching exact mass and molecular formula, which indicated the presence of most naphthoquinones and flavonoids (Tables 1 and 2).

Studies indicated that naphthoquinones potassium salts of isolapachol and lapachol can exhibit molluscicidal activity in the life cycle of *Schistosoma mansoni* (Lima et al., 2002). The lapachol, isolapachol or α/β lapachone metabolite could not be differentiated only by mass spectra since they share the same molecular and main fragment ions (*m/z* 243.1006 and respective *m/z* 225.0907, *m/z* 201.0540 and *m/z* 187.0391) (Figure 2). Although, they were dereplicated in all the samples, especially in the branches and leaves samples. Further studies involving validated MS experiments with standard compounds can implement energy and fragmentation studies aiming univocally differentiation of these quinones by MS technics,

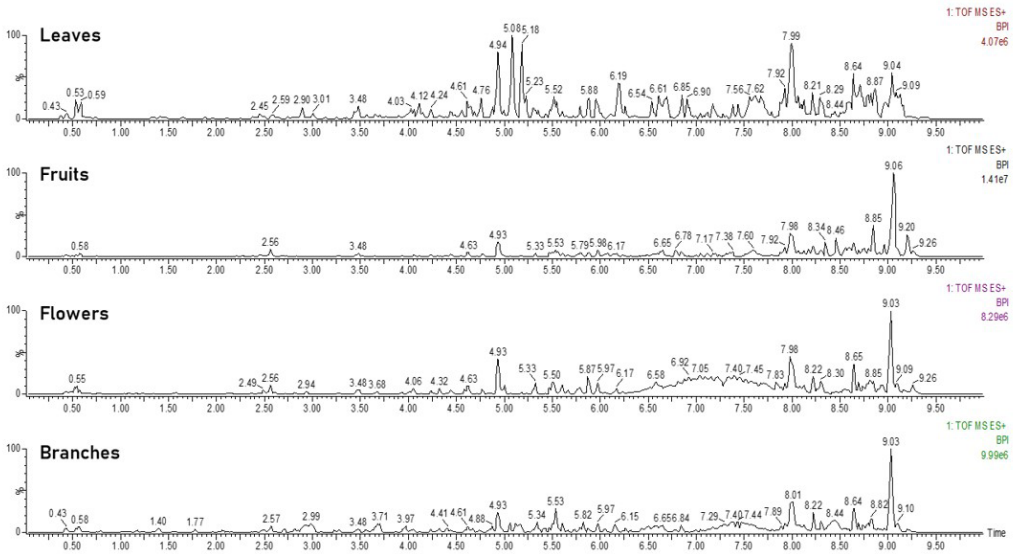

Figure 1. BPI chromatograms for each type of *H. impetiginosus* aerial parts extract for the positive ionization mode (ESI+).

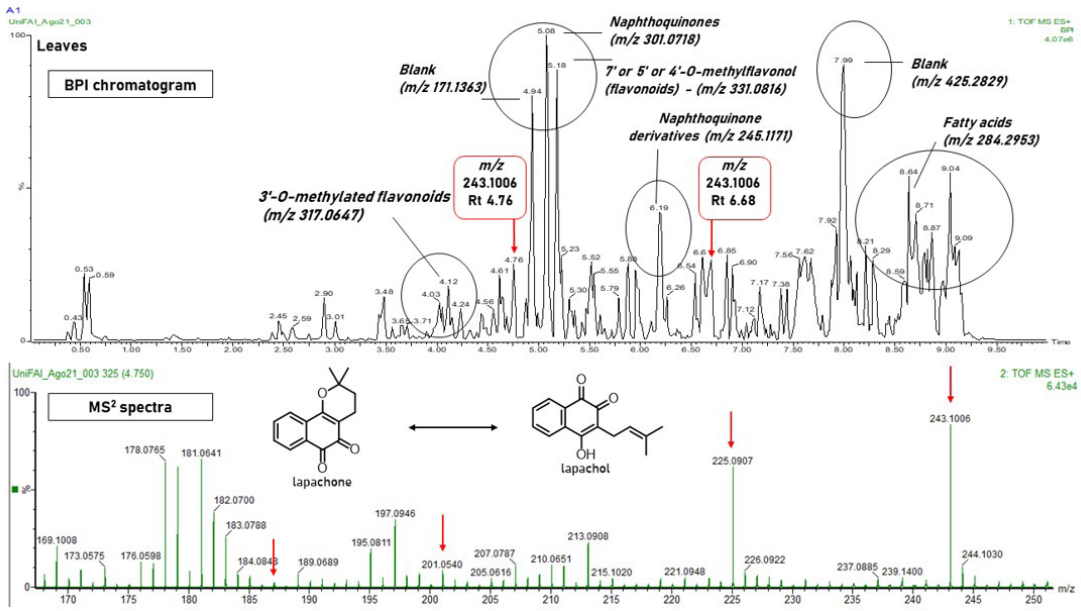

Figure 2. BPI chromatograms of *H. impetiginosus* EtOH- leaves extract evidencing high and low energy channels (10 and 40 eV) selected at Rt 4.76 min and *m/z* 243 in the positive ionization mode (ESI+). EtOH-Le – leaves.

which in our case were evidenced at 2 different retention times (Rt 4.76 and 6.68 min) (Figure 1 and 2).

Furthermore, the chemical analyses indicated that naphthoquinones are present in all metabolic content of the analyzed samples. Thus, our study demonstrated that this metabolite class could be encountered in the whole vegetal material of *H. impetiginosus*, corroborating the literature that the *H. impetiginosus* specie is rich in the quinone class content (El-Hawary et al., 2021). Besides the quinones, several other metabolite classes reported in the literature were annotated in this dereplication, including the iridoid glycosides, benzoic acid derivatives, furanonaphthoquinones, hydroxyanthraquinones, long-chain fatty acids, triterpenoids and phenolic compounds, such as phenylpropanoid glycosides (Table 1 and 2).

3.2. Percentage of death in adult male S. mansoni worms

The evaluated extracts reduced motility and caused parasite death depending on concentration and incubation time (Figures 3 and 4). Monitoring parasites kept in culture

Table 1. List of highest peak areas and respective samples analyzed in the ESI⁺. EtOH-Le - leaves, EtOH-Fr - fruits., EtOH-Fl - flowers and EtOH-Br - branches.

m/z	Rt (min)	FM	Hits	Adduct type	EtOH-Le	EtOH-Fr	EtOH-Fl	EtOH-Br
189.0548	6.20	$C_{11}H_8O_3$	Plumbagine (Naphthoquinones)	[M+H]+	1735491	169317	238738	167153
215.1051	6.64	$C_{10}H_{10}N_6$	Aminoimidazoles derivatives	[M+H]+	1963992	151734	127684	149121
243.1006	6.68	$C_{15}H_{14}O_3$	α/β-lapachone or lapachol	[M+H]+	6513792	145918	144620	138948
243.1006	4.76	$C_{15}H_{14}O_3$	α/β-lapachone or lapachol	[M+H]+	2549406	173449	182827	171144
245.1171	6.20	$C_{15}H_{16}O_3$	Naphthoquinone derivatives	[M+H]+	1815944	49326	47052	81290
271.0611	2.99	$C_{15}H_{10}O_5$	Genistein or *n*-methoxy-2-acetylnaphtho[2,3-b] furan-4,9-dione	[M+H]+	78179	598920	160083	25540854
284.2947	8.64	$C_{18}H_{37}NO$	Octadecanamide (fatty acids)	[M+H]+	18314056	18682856	23915892	22563552
287.0569	2.70	$C_{15}H_{10}O_6$	2-Acetylnaphtho[2,3-b] furan-4,9-dione; 7-methoxy, 8-hydroxy or kaempferol/luteolin	[M+H]+	312468	2904474	203347	8703838
295.1319	5.06	$C_{19}H_{18}O_3$	2-arylbenzofuran derivatives (neolignans)	[M+H]+	3203028	115739	114731	116892
301.0753	5.09	$C_{16}H_{12}O_6$	2-Acetylnaphtho[2,3-b] furan-4,9-dione; 7,8-dimethoxy	[M+H]+	21326266	461049	479681	594847
303.0874	4.02	$C_{16}H_{14}O_6$	7' or 5' or 4'-*O*-methylflavonol (flavonoids) or *n, n*-methoxy-2-Acetylnaphtho[2,3-b] furan-4,9-dione	[M+H]+	2658854	218089	145109	171877
317.0647	4.10	$C_{16}H_{12}O_7$	Isorhamnetin and other 3'-*O*-methylated flavonols	[M+H]+	2290106	244523	79607	137442
329.1009	5.53	$C_{18}H_{16}O_6$	7 or 5-*O*-methylated flavonoids	[M+H]+	1000473	9306508	749920	22707646
419.1340	5.17	$C_{21}H_{22}O_9$	Anthraquinone glycosides	[M+H]+	56187	1890034	517874	14429116
447.1187	8.96	$C_{21}H_{18}O_{11}$	Flavonoid-7-*O*-glucuronides	[M+H]+	16851	450221	93338	16816662
449.1061	2.28	$C_{21}H_{20}O_{11}$	Flavonoid-7-*O*-glycosides (ex. Luteolin 7-glucoside)	[M+H]+	19213	1496370	63015	68107
465.1019	2.6	$C_{21}H_{20}O_{12}$	Flavonoid-3-*O*-glycosides (ex.Quercetin 3-galactoside)	[M+H]+	149513	1623354	80245	189372
471.3467	6.8	$C_{30}H_{46}O_4$	Triterpenoids	[M+H]+	104215	5855020	434702	466070
507.1494	3.65	$C_{24}H_{26}O_{12}$	Flavonoid-7-*O*-glycosides	[M+H]+	15423	952940	80707	115487
540.2094	3.05	$C_{25}H_{33}NO_{12}$	Iridoid glycosides (ex. Lonijaposide B)	[M+H]+	5493	854701	297184	211136
559.2764	8.84	$C_{27}H_{42}O_{12}$	Iridoids or sesquiterpenoids glycosylated	[M+H]+	205912	8782545	1110272	284546
595.1447	8.68	$C_{30}H_{26}O_{13}$	Flavonoid 3-*O*-*p*-coumaroyl glycosides	[M+H]+	2217613	605423	645151	976514
611.1635	2.47	$C_{27}H_{30}O_{16}$	Flavonoid-3-*O*-glycosides (ex. Rutin)	[M+H]+	24413	845894	132031	847781

Table 2. List of the highest peak areas and respective samples analyzed in the ESI⁻. EtOH-Le - leaves, EtOH-Fr - fruits., EtOH-Fl - flowers and EtOH-Br - branches.

m/z	Rt (min)	FM	Hits	Adduct type	EtOH-Le	EtOH-Fr	EtOH-Fl	EtOH-Br
137.02255	1.7	$C_7H_6O_3$	4-hydroxybenzoic acid	[M-H]-	450	81851	143150	252226
149.05991	5.5	$C_9H_{10}O_2$	Cinnamyl alcohols (ex. 4-Coumaryl alcohol)	[M-H]-	11938	0	0	0
163.03891	2.5	$C_9H_8O_3$	4-hydroxycinnamic acid (*E*-p-coumaric acid)	[M-H]-	248109	298	121	8399
179.03358	3.0	$C_9H_8O_4$	Caffeic acid or 4-Hydroxyphenylpyruvic acid	[M-H]-	33511	1445	2351	827
247.0972	4.3	$C_{14}H_{16}O_4$	Coumaric acids and derivatives (ex. Prenyl cis-caffeate)	[M-H]-	263274	40	54	0
269.04431	2.9	$C_{15}H_{10}O_5$	Hydroxyanthraquinones (ex. Emodin)	[M-H]-	217	1729	109	200254
269.04431	5.1	$C_{15}H_{10}O_5$	Flavones or isoflavones (ex. Apigenin)	[M-H]-	103316	174	295	1459
271.06003	4.0	$C_{15}H_{12}O_5$	Flavanones (ex. Naringenin)	[M-H]-	40509	36	14	2104
285.03851	4.0	$C_{15}H_{10}O_6$	Flavones (ex. Luteolin)	[M-H]-	68630	772	30	2958
285.03851	3.5	$C_{15}H_{10}O_6$	Flavonols (ex. Kaempferol)	[M-H]-	344	45015	2891	13944
285.07654	4.9	$C_{16}H_{14}O_5$	*O*-methylated flavonoids (ex. Sakuranetin)	[M-H]-	294111	151	266	327
287.22076	4.4	$C_{15}H_{12}O_6$	Flavanonols (ex. Aromadendrin)	[M-H]-	560	12086	1036	138932
293.21051	6.1	$C_{18}H_{30}O_3$	Lineolic acids and derivatives	[M-H]-	4648	122823	41639	38664
295.22672	6.5	$C_{18}H_{32}O_3$	Long-chain fatty acids	[M-H]-	32576	101889	127722	121846
299.05667	5.1	$C_{16}H_{12}O_6$	*O*-methylated flavonoids (ex. Chrysoeriol)	[M-H]-	646447	7820	12312	9322
299.07791	1.0	$C_{13}H_{16}O_8$	Phenolic glycosides	[M-H]-	0	4401	25174	187678
301.0715	4.0	$C_{16}H_{14}O_6$	*O*-methylated flavonoids (ex. Hesperetin)	[M-H]-	800928	1954	2518	2177
305.10358	2.9	$C_{16}H_{18}O_6$	Naphthopyrans or pyranocoumarins	[M-H]-	75	412	5346	161094
329.06757	5.2	$C_{17}H_{14}O_7$	7-*O*-methylated flavonoids (ex. 3,7-dimethylquercetin)	[M-H]-	411714	2196	2079	2157
329.23105	4.3	$C_{18}H_{34}O_5$	Long-chain fatty acids	[M-H]-	2960	63026	411557	347901
419.09583	2.8	$C_{20}H_{20}O_{10}$	Flavonoid *O*-glycosides	[M-H]-	122	6336	243059	563569
445.07559	2.9	$C_{21}H_{18}O_{11}$	Flavonoid *O*-glucuronides	[M-H]-	202	8656	197	387048
453.33487	8.0	$C_{30}H_{46}O_3$	Triterpenoids (ex. Ganoderic acid Y/ Ganoderiol F)	[M-H]-	322	193061	20502	24215
455.35144	8.2	$C_{30}H_{48}O_3$	Ursolic acid (or other triterpenoids ex. oleanolic acid / betulinic acid)	[M-H]-	28152	168792	54931	93709
499.37875	8.0	$C_{32}H_{52}O_4$	Triterpenoids (ex. dysolenticin F)	[M-H]-	0	232917	20456	30646
515.11926	3.0	$C_{25}H_{24}O_{12}$	Quinic acids and derivatives (ex. Dicaffeoylquinic acid)	[M-H]-	189848	143	46	37

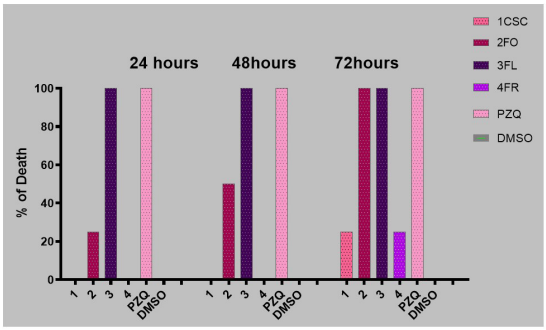

Figure 3. Effect *of H. impetiginosus* extracts on the survival of adult male *S. mansoni* worms. Adult male worms were incubated in an RPMI medium containing a concentration of 200 µg/mL of 1-branches, 2-leaves, 3-flowers, and 4-fruits.

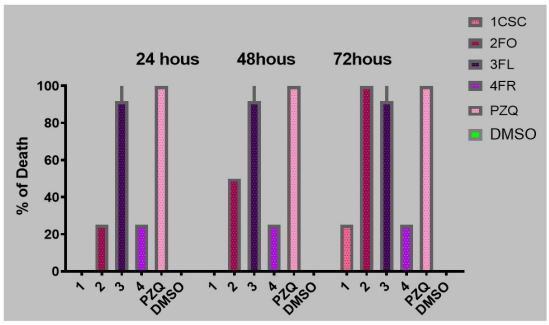

Figure 4. Effect of *H. impetiginosus* extracts on the survival of adult male *S. mansoni* worms. Adult male worms were incubated in an RPMI medium containing a concentration of 100 µg/mL of 1-branches, 2-leaves, 3-flowers, and 4-fruits

for 24h, 48h, and 72h indicated a schistosomicidal effect on adult male *S. mansoni* worms. For this incubation period, 100% mortality was achieved with extracts at a concentration of 200 µg/mL from flowers for 24h; and with the crude ethanolic extracts of the leaves for 72h. At a 100 µg/mL concentration, the crude extracts that reached 100% mortality in 48h and 72h were from flowers and leaves.

3.3. In vitro evaluation of H. impetiginous extracts in the induction of integument changes of adult worms of S. mansoni

The external morphology of the male *Schistosoma mansoni* presents the gynophore canal (GC), a longitudinal fold in the posterior portion that shelters the female and helps the mating and reproduction. The anterior portion is characterized by a smooth, cylindrical integument, an oral sucker, and a ventral sucker with numerous cylindrical papillae (Figure 5).

Changes promoted by the ethanolic extracts EtOH-Br, EtOH-Le, EtOH-Fl, and EtOH-Fr doses on adult male worms at 24, 48, and 72h are shown in Figures 5a-e. The extract promoted an apparent effect on the musculature of the worms with the evolution of dorsoventral curvature in a time-dependent manner, assuming the form of a corkscrew after 24h of incubation. After 48h of incubation at 12.5 µg/mL of the EtOH-Le, opening, and relaxation in the gynophore canal were observed. After 24h of incubation at 25 µg/mL of the EtOH-Fr, bubbles were observed in the anterior region around the sucker of the worms (Figures 5b and 5c). Moreover, severe changes in the integument were characterized by edema, displacement, wrinkled surface, and erosion, which were observed when incubated at 50 µg/mL of EtOH-Br, and 50 µg/mL of EtOH-Fl for 72h, respectively, throughout the incubation period (Figure 5d and 5e), an evident longitudinal contraction of the muscles occurred, and the worms presented in the form of a corkscrew (Figure 5e).

Furthermore, populations use plants containing naphthoquinones from different locations to treat diseases, from parasitic diseases to different types of cancer. For example, countries with plant species that produce naphthoquinones, such as Brazil, China, and Japan, have been contributing intensively with chemical and pharmacological studies of these species (Rani et al., 2022). Several therapeutic applications are attributed to β-lapachone; in this sense, studies indicate antibacterial, antifungal (Guimaraes et al., 2021), antiviral (Mokarizadeh et al., 2020), antiarthritic (Gong et al., 2021), anti-inflammatory (Oliveira et al., 2020b), trypanocidal (González et al., 2020) and schistosomicidal. Edingl, Tersan, and Waite, in 1947, stated that naphthoquinone derivatives could inhibit aerobic glycolysis in *S. mansoni* adult worms. Other studies indicate molluscicidal activity on *B. glabrata* (intermediate host of *S. mansoni*) and its eggs and activity on the cercariae of *S. mansoni* (Jali et al., 2018).

Ortho-naphthoquinone has moderate activity against *S.mansoni* compared to the reference drug (PZQ). In addition, it exhibits activity in other phases of the parasite's biological cycle, a feat not achieved with praziquantel, which only fights the adult form of the worm (Silva Júnior et al., 2019). The activity of a topical preparation of β-lapachone was tested against *S. Mansoni* cercariae in rat tails. The preparation showed total blockage of cercariae penetration when applied to the tail 24 hours before infection. Infection depends on the complete penetration of cercariae (Silva Júnior et al., 2019).

Aires et al. (2014) evaluated the schistosomicidal activity of β-lapachone 50mg/kg/day, intraperitoneally, on *S. mansoni* worms in mice and observed moderate activity. There was a decrease in the number of worms by 29.78%, 37.2%, 24.2%, and 40.22% when administered during the skin schistosomulus, pulmonary schistosomulus, juvenile worms, and adult worms phases, respectively. In addition, in all groups, there was a decrease in the number and diameter of hepatic granulomas in the control group.

Subsequently, the same research group evaluated the activity of β-lapachone on the integument of *S. mansoni* worms. They observed that after 24 hours of incubation, exposure to different concentrations of β-lapachone led to the death of 67% to 100% of the worms. Changes in worm motility were also observed, besides scaling, blistering, and tegument rupture (Silva Júnior et al., 2019). However, the pharmacological potential of this naphthoquinone is not indicated for the systemic treatment of these parasites

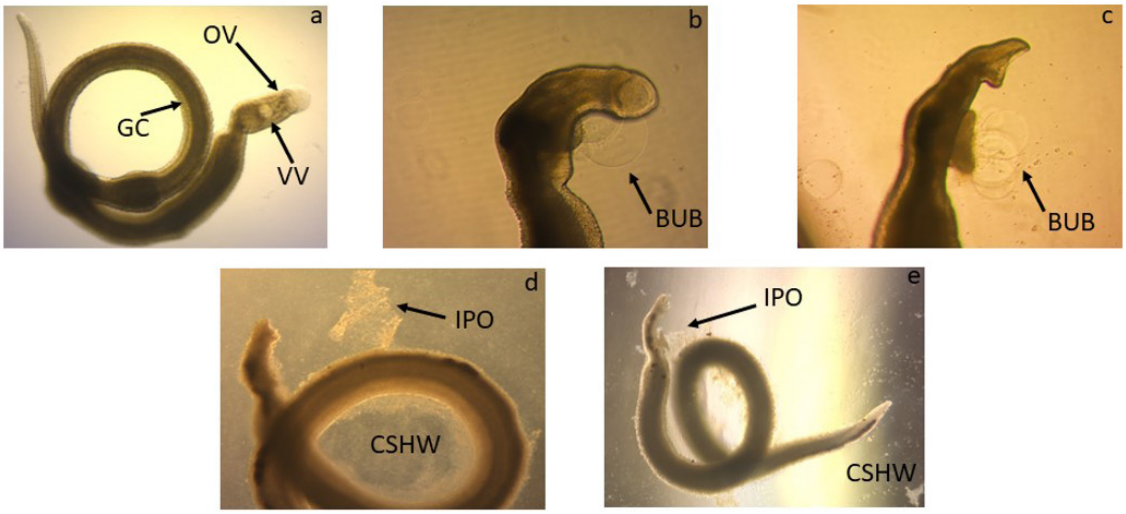

Figure 5. a-e Images obtained through an inverted microscope at 40x magnification, of the integument of adult male *S. mansoni* worms from the negative control group (a), group treated with leaves (b), fruits (c), branches (d) and flowers (e) extracts. Captions: gynophore canal (GC), oral sucker (OV), ventral sucker (VV), bubbles (BUB) , integument peeling off (IPO) and corkscrew-shaped worm (CSHW).

due to the adverse effects caused by its high toxicity in mammalian cells. Although, that opens a range of new possibilities for the research and synthesis of β-lapachone derivatives, as well as association with phytotherapeutic medicines aiming at increased effectiveness and decreased toxicity in humans (Silva Júnior et al., 2019).

4. Conclusion

All extracts caused changes in the viability of adult males of *S.mansoni* at 49 days. The extracts caused the death of the parasites directly dependent on the concentration and incubation time. The extracts caused contractions in the adult male worms before the schistosomicidal effect. At lethal concentrations, the extracts caused changes in the integument of adult male worms, revealing that the damage caused occurs in a dose-dependent manner. These results open new possibilities for researching herbal medicines to treat neglected tropical diseases, such as schistosomiasis.

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