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Evaluation of the Schistosomicidal Potential of Guttiferone - A Obtained from *Garcinia brasiliensis´*s Seed

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Abstract: Schistosomiasis is a serious public health issue in developing countries mainly from the African, Asian and South American continents. Praziquantel is the only medication used in the treatment of schistosomiasis. Such fact justifies the search of therapeutic alternatives for the disease, presenting shorter course of treatment, lower occurrence of side effects and lack of resistance of the parasite to the compound. In the present study, it was evaluated the schistosomicidal activity of ethanolic extract of the *Garcinia brasiliensis´*s seed (EES); hexane (FHS), ethyl acetate (FAES) and aqueous (FAS) fractions, obtained by partition; and a pure constituent isolated from this species: the benzophenone guttiferone-A (gut-A), on adult worms of *Schistosoma mansoni*, using *in vitro* tests. Mortality (ED90) and damage on the membrane surface and excretory system activity were noted at concentrations of 98.0; 97.0; 89.0 and 18.0 µg/mL for the ESS; FHS; FAES and gut-A, respectively. Whereas for gut-A, these data were confirmed by Hoechst 33258 and Resorufin fluorescent probes. The results obtained indicate the gut-A as a promising natural compound, as *in vitro* assays provide sustainability to carry out *in vivo* tests, in order to search new drugs for the treatment of this disease.

Keywords: Benzophenones, *Garcinia brasiliensis*, Guttiferone-A, Organic compounds, *Schistosoma mansoni*, Schistosomiasis.

1. Introduction

Schistosomiasis, caused by the helminth *Schistosoma mansoni*, is the parasitic disease with the second greater socioeconomic impact worldwide, malaria being the first. It affects varied geographic areas; approximately 209 million people infected in 76 countries by one of the different species of *Schistosoma*, and over 700 million people living under the risk of infection, mainly in African, Asian and American countries (Steinmann et al., 2006). Due the lack of a

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schistosomiasis vaccine, the control of this disease relies on reducing morbidity in individual and communities levels, with safe and effective drugs (Gryseels et al., 2006).

The strategy for controlling schistosomiasis is grounded primarily in the treatment of infected individuals, with the use of medications such as praziquantel (Doenhoff et al., 2009), which hasn´t prevented reinfections, as well as and appears ineffective against young forms of the parasite (Xiao et al., 2011). In addition, there is a considerable concern over the emergence of strains resistant to this drug (Liang-Xian et al., 2013), and reports in which praziquantel induced hemorrhage in lung tissue of infected individuals (Flisser and McLaren, 1989; Noya et al., 2002). Therefore, the development of new effective drugs is necessary for schistosomiasis control.

The search of antiparasitic compounds from natural sources has increased over the past decade (Boissier et al., 2009; Botros et al., 2009; de Araújo et al., 2007; Magalhães et al., 2012, 2009; Mahran et al., 2007). In this context, plants are an important source of biologically active compounds that can provide structures for the development of new drugs (Tonuci et al., 2012). Therefore, examples such as *Zingiber officinale* (Matos-Rocha et al., 2013), *Piper cubeta* (Esperandim et al., 2013), *Curcuma longa* (Morais et al., 2013) and *Solanum lycocarpum* (Miranda et al., 2012) have been studied as natural products with schistosomicidal activity.

Garcinia brasiliensis is a plant popularly known as 'bacupari'. In folk medicine, *G. brasiliensis´*s leaves are used in the treatment of tumors, urinary tract inflammation, arthritis and pain relief (Pereira et al., 2010). Some compounds isolated from this plant presented promising effects, such as 7-epiclusianone, a benzophenone tetraisoprenilada that has been showing anti-inflammatory and analgesic activity (Santa-Cecília et al., 2012, 2011), antimicrobial (Naldoni et al., 2009a), antitumoral (Martins et al., 2009), effective in the treatment of asthma (Coelho et al., 2008). Also isolated from this plant, fukugetina is a biflavonoid with anti-inflammatory activities (Lim et al., 2006), inhibition of HIV-1 reverse (LIN et al., 1997), inhibition of tumor angiogenesis (Pang et al., 2009) and proteases of *L. (L.) amazonensis* (Pereira et al., 2010).

The guttiferona-A (gut-A) (Fig. 1) is the most abundant natural benzophenone isolated from the seed of *G. brasiliensis*. Many pharmacological activities have been reported for this metabolite, including activities such as anti-HIV (Gustafson et al., 1992), cytotoxicity (Chaturvedula et al., 2002), trypanocidal, antiplasmodial, antioxidant (Ngouela et al., 2006), inhibition of serine and cysteine proteases (Martins et al., 2009) and antimicrobial (Monzote et al., 2011; Naldoni et al., 2009b). For possessing broad spectrum of biological activities, the objectives of the present study were the evaluation of gut-A schistosomicidal potential, isolated from the seeds of *G. brasiliensis*.

2. Materials and Methods

2.1. Plant Material

*G. brasiliensis´*s fruits were collected on February of 2011, in Viçosa region, Minas Gerais, Brazil (latitude 20° 45'14" south and longitude 42° 52'55" west). The botanical identification was performed by Dr. João Augusto Alves Meira Neto, at the botanical garden of the Federal University of Viçosa. The species is well cataloged under the specimen number IC2604, deposited in the herbarium of the Federal University of Viçosa.

2.2. Extraction and Isolation Procedures

*G. brasiliensis´*s seeds were ground and submitted to extraction by maceration with ethyl acetate, at ambient temperature. After 24 hours, the crude extract was obtained by filtration and evaporation of the solvent under reduced pressure, followed by renewal of the extraction solvent. This process was repeated until the material was exhaustively extracted. The isolation of the gut-A compound was performed by column chromatography (silica gel) of the crude extract (Santos et al., 1999). The substances were eluted with a mixture of hexane/ethyl acetate, with a gradual increase

of polarity, and the collected fractions were monitored by thin layer chromatography. The product of interest was obtained pure, as a yellow solid. The identity of the substance was confirmed by comparison with literature data, obtained by spectroscopic techniques: IR, UV, MS, ^{1}H NMR and ^{13}C (Martins et al., 2009).

2.3. Chromatographic Analysis and Quantification

The extract, fractions and pure substance chromatograms were obtained in a Liquid Chromatograph (Shimadzu UFLC 20), using a NST column (Nano Separation Technologies) C18-154605 (150 x 4.6 mm column, particle size of 5.0 μm), according to Almeida et al. (2008).

The moveable phase consisted of a mixture of 5 mmol/L acetic acid solution (eluent A) and 0.1% v/v methanol/acetic acid solution (eluent B). The injection volume was 20.0 μL on 1.0 ml/min flow rate. In the initial 10 minutes, the analysis was performed with 50.0% of B. The concentration of B linearly increased to 100.0% in 20 min, continuing 100.0% of B to 30 min. Afterwards, the concentration of B was resumed to the original in order to prepare the column for the next analysis. The chromatograms were obtained at 254 nm and the peaks were compared to their EES and gut-A patterns, previously isolated at the laboratory.

2.4. Parasites

The *S. mansoni* LE strain used on the experiments of this study has been routinely maintained through serial passages in *Biomphalaria glabrata* models and *swiss* mice (Pellegrino et al., 1977). The procedures performed with animals were submitted to the Ethics Committee on Animal Use from Oswaldo Cruz Foundation (CEUA/FIOCRUZ).

2.5. Evaluation of the Activity of Gut-A, FHS, FAES and FAS Fractions and Ethanolic Extract of *Garcinia brasiliensis´*s Seed (EES) on *S. mansoni*

Mice infected with *S. mansoni* cercariae (LE strain) were sacrificed with 3.0% sodium pentobarbital, administered intraperitoneally (± 0.3 ml / mouse), and were perfused according to method of Smithers and Terry (1965).

The worms recovered were cultured in 6-well culture plates (4 couples per well) in RPMI-1640. The culture plates were supplemented with 5.0% fetal bovine serum (FBS) heat inactivated, and 1.0% penicillin (10,000 IU/ml) streptomycin / (10 mg/ml) (Sigma, USA).

The samples – *G. brasiliensis´*s ESS, its fractions obtained by partitioning (FHS, FAES and FAS) and the gut-A molecule isolated – solubilized in methanol at a concentration of 2.0 mg/ml, were added to the culture plates at different concentrations and maintained at 37°C and 5.0% CO_2. The cultures, already in contact with the substances, were analyzed in 2 and 24 hours.

After 24 hours, the wells were washed 5 times by removing the culture medium and extracts/compounds, followed by the addition of the same amount of sterile culture medium.

Praziquantel (PZQ) was used as a positive control, at a concentration of 2 μg/mL, and methanol was used as a negative control, at the same concentration (Oliveira et al., 2006). The worms in the control groups were kept under the same conditions, except for the presence of the compounds.

After 24 hours, the worms were washed with culture medium and maintained in the previous conditions, however without the addition of the extracts, for the remaining of the experiment. Inverted microscope observations were documented at 2 and 24 hours in contact with the extracts, and at 24 and 48 hours, when the worms were no longer in contact with the extracts. These observations were compared to the control groups, consisted of 3 wells, one with PZQ, other with supplemented culture medium, and the last with methanol, the solvent used in the dissolution of the extracts.

The worms were evaluated and compared with the controls, regarding the amount of coupled worms, movement, contraction/shortening, morphology, detachment of the tegument and oviposition. All tests were performed in triplicate, on three different occasions, and PZQ was used as the reference drug.

2.6. Excretory System Activity Evaluation of *S. mansoni* Exposed to Gut-A by Resorufin Labeling

Mice infected with *S. mansoni* cercariae (LE strain) were sacrificed according to the method of Smithers and Terry (1965). The worms were kept in 4 ml of RPMI-1640 supplemented culture medium.

Then, it was added into each well 10.0 µL of Resorufin (stock solution 10 mg/ml), and incubated at 37°C and 5.0% CO_2 for 30 min. After washing 5 times with 2 ml of RPMI to remove the excess of probe, were added to the wells 40.0 µL of methanol (control), 2.5 µL (2.0 µg/mL) of a PZQ stock solution at 8 mg/mL (control), and gut-A at concentrations of 16.0, 18.0 and 20.0 µg/mL, from a 2.0 mg/mL stock solution.

One of the wells was used as a control, remaining only with the RPMI-1640 supplemented medium. Subsequently, the wells were incubated at 37°C and 5.0 % CO_2, for 15 minutes. They were once again washed (5 times with culture medium) and transferred to glass slides delimited with vaseline, to prevent leakage, with a small quantity of culture medium supplemented with 1.0% of sodium pentobarbital at 3.0% (Hypnol – Fontoveter), to inhibit the parasites movements. These tests were performed in triplicate, on 3 different occasions.

Afterwards, the tests were analyzed in a fluorescence microscope (Nikon – Eclipse 80i) using Rhodamine filter for Resorufin (maximum excitation/emission of Resorufin at 571/585 nm).

2.7. Evaluation of Damage Caused to the Tegument of *S. mansoni* Exposed to Gut-A, by Hoechst 33258 Probe

The methods to detect the damage were described by Lima et al. (1994). In the wells were added 40.0 µL of methanol used as control, 2.0 µg/mL of a PZQ stock solution at 0.8 mg/mL (control), and gut-A at concentrations of 16.0, 18.0 and 20.0 µg/mL, from a 2.0 mg/ml stock solution. The culture plates were incubated for 24 hours at 37°C and 5.0% CO_2. After 24 hours, they were washed to remove the extracts. The worms were incubated for 15 min with 10.0 µL de Hoechst 33285 (stock solution at 10.0 mg/mL).

The damages were evaluated by fluorescence microscopy after washing the worms to remove the excess of probes, and transferred to glass slides delimited with vaseline, to prevent leakage. The worms were placed on the glass slides with a small quantity of culture medium supplemented with 1.0% of sodium pentobarbital at 3.0% (Hypnol – Fontoveter), to inhibit the parasites movements. Afterwards, the tests were analyzed with an optical fluorescence microscope (Nikon – Eclipse 80i) using DAP filter (maximum excitation/emission of Hoechst at 352/455 nm). These tests were performed in triplicate, on 3 different occasions.

2.8. Statistical Analyses

Statistical evaluation of the results was performed using the SISVAR 5.3 software, using analysis of variance (ANOVA) and applied the test of SNK to observe significant differences between mean values ($p < 0.05$).

3. Results

3.1. Chromatographic Analysis of the Extract, Fractions and Isolated Molecule

Chromatograms (Fig. 2) were obtained through analysis of *G. brasiliensis* EES, its fractions obtained by partition (FHS, FAES and FAS) and the isolated compound gut-A. Some compounds, flavonoids and others unidentified are being studied at the Laboratory of Phytochemistry and Medicinal Chemistry of UNIFAL-MG (LFQM).

EES chemical analysis identified gut-A as the major constituent at retention time of 25.425 (Fig. 2A). FHS, FAES and FAS chemical analysis identified gut-A with a percentage in area of 2.4980 (Fig. 2B), 54.0264 (Fig. 2C) and 11.0113 (Fig. 2D), respectively. In FAES analysis, gut-A molecule was found at a higher concentration, due its apolar characteristic. In FHS and FAS fractions, the gut-A concentration values found were not majorities.

3.2. *G. brasiliensis´*s EES and FHS, FAES and FAS Fractions Effect on *S. mansoni*

In vitro schistosomicidal effect of ESS and its fractions on the viability, amount of coupled worms, movement, contraction/shortening, morphology, tegument detachment and oviposition of the adult worms was analyzed regarding the concentration and incubation time (Table 1). All the adult worms were killed after 24 hours with 94.0, 97.0 and 89.0 µg/ml of EES, FHS and FAES, respectively. However, FAS was inactive at 200.0 µg/mL (highest dose tested). In contrast, the worms in the negative control group remained viable (RPMI-1640 supplemented medium and RPMI medium with methanol). In the positive control, PZQ at 2.0 µg/ml, death of all parasites occurred in 24 hours.

3.3. Gut-A Activity on *S. mansoni*

Activities in the EES compound of *G. brasiliensis* were observed, as well as in its fractions (FHS and FAES) obtained by partition, leading to the analysis of schistosomicidal activity of the major component, the gut-A.

The worms were exposed to different concentrations of gut-A, with wide variation in these concentrations, from 2.5 to 100.0 µg/m (Table 2). On the microscopic analyses, after contact with the isolated substance for 24 hours, the worms presented immobility, starting at a concentration of 18.0 µg/mL, and so remained throughout the experiment. In the analysis performed after 2 hours, contraction wasn´t noted, and this remained on further analyses. Blistering and tegument detachment were noted at the concentration of 18.0 µg/mL on readings performed 24 hours after exposure to gut-A. Higher concentrations were capable of causing death in 100.0% of parasites, with 24 hours of incubation, allowing to reach the ED_{50} at 21.8 µg/mL. There wasn´t oviposition of the females, even at the smallest concentrations tested. The worms on the control groups presented normal morphology and movements, and oviposition within the first 24 hours.

3.4. Excretory System Labelling with Resorufin Probe, After Exposure to Gut-A

It was evaluated the gut-A molecule activity on the excretory system of male and female *S. mansoni* adult worms. The worms' excretory system (main tubular and ramifications portions) presented fluorescence when labeled with Resorufin probe *in vitro*, without gut-A treatment, and the same occurred in the control (Fig. 3A and 3B). After exposure to 20.0 µg/mL gut-A, the excretory system showed diffusion of the Resorufin probe, and the visualization of the microtubules and peripheral ramifications wasn´t possible, equally seen in the PZQ treatment (Fig. 3C and 3D).

3.5. Tegument Damage Evaluation of *S. mansoni* Exposed to Gut-A, by Hoechst 33258 Probe

In the tests conducted with Hoechst 33258 probe, fluorescent areas are indicative of tegument injuries on the *S. mansoni* adult worms (Fig. 3). The parasites presented at Figures 3E and 3F were not exposed to gut-A in vitro, therefore didn´t present tegument damage. Figures 3G and 3H present male and female *S. mansoni* adult worms, labeled with Hoechst 33258 probe and exposed to 20.0 µg/mL of gut-A. The arrow shows fluorescence spots on the female tegument, indicative of injuries due the exposure.

4. Discussion

Schistosomiasis is a chronic disease caused by trematode worms of the genus *Schistosoma*, which represents a major public health issue, related to poverty in many tropical areas (Steinmann et al., 2006). The morbidity caused by schistosomiasis appears to be influenced by the nature of the induced immune response associated with the parasite, the illness effects on granuloma formation and the pathologies elicited by schistosomiasis in target organs (Cheever et al.,

2000). The WHO included schistosomiasis in its list of neglected diseases, one of the main causes of morbidity and mortality worldwide. Schistosomiasis incapacitates or kills thousands of people and represents a medical necessity that remains unmet (Chirac and Torreele, 2006)

In the absence of an effective vaccine against schistosomiasis, chemotherapy treatment is the most indicated. The drug of choice is Praziquantel (PZQ), but its use in large-scale control has accelerated the selection of resistant parasite strains (Bonesso-Sabadini and Dias, 2002; Katz et al., 1973; Tonuci et al., 2012)

The search for antiparasitic compounds from natural sources has increased over the last decade (Boissier et al., 2009; Botros et al., 2009; de Araújo et al., 2007; Magalhães et al., 2010, 2009; Mahran et al., 2007). Plants are an important source of biologically active compounds, which can provide structures for the development of new drugs (Tonuci et al., 2012). They have been used in all cultures as a source of natural medicine for health maintenance. Examples such as *Zingiber officinale*, *Nigella sativa* and *Asparagus officinalis*, *Chenopodium ambrosioides*, *Conyza dioscorides* and *Sesbania sesban*, *Allium cepa*, have been studied as natural products with schistosomicidal activity (Kamel et al., 2011; Mantawy et al., 2011)

In this context, the present study evaluated the schistosomicidal potential of the ethanolic extract of *G. brasiliensis´*s seed (EES) and its fractions – hexane (FHS), ethyl acetate (FAES) and aqueous (FAS) – obtained by partition, and the isolated molecule gut-A.

Experimental tests were performed *in vitro* in adult worms, males and females of *S. mansoni*, obtained by perfusion (Oliveira et al., 2006). The analyses were performed regarding the parasite´s motor activity, morphology and tegument alterations. All these effects occurred as dose-dependent. Analyses revealed morphological changes on the tegument that occurred after incubation of the *S. mansoni* adult worms at concentrations exceeding 50.0 µg/mL for extracts and fractions, 18.0 µg/mL for gut-A. Several parameters, such as changes on the integrity of the tegument, motility, interruption of oviposition and absence of mating, are frequently assessed as indicators of biological activity and toxicity in studies with species of *Schistosoma* (Boissier et al., 2009; Magalhães et al., 2010, 2009).

The results showed that the ethanolic extract of *G. brasiliensis´*s seed (EES) presented ED_{90} of 94.0 µg/mL. For FHS and FAES fractions, the ED_{90} was 97.0 e 89.0 µg/mL, respectively. The ED_{50} for EES was 64.0 µg/mL, and FAES and FSH fractions presented ED_{50} of 64.0 and 71.0 µg/mL, respectively. The aqueous fraction was inactive at 200.0 µg/mL, the highest dose tested.

Phyllanthus amarus extracts, popularly known as "quebra-pedra", commonly used to treat liver and urogenital illnesses, was used on *S. mansoni*, caused reduction up to 63.0% in the number of worms and ceased egg laying, indicating a potential schistosomicidal activity of the plant (Oliveira, 2008). Most biological activities of natural products in propolis extracts – antimicrobial, antitumor and trypanocidal activities – is mainly associated to their prenylated compounds, with the hypothesis that the same occurs with the benzophenones (Sasaki et al., 2012)

In the studies conducted with the isolated gut-A molecule, significant schistosomicidal activity was noted starting at a concentration of 18.0 µg/mL, in *in vitro* assays. Higher concentrations were capable of causing death in 100.0% of parasites, with 24 hours of incubation, allowing to reach the ED_{50} at 21.8 µg/mL. The ED_{50} is useful to establish and monitor drugs susceptibility and resistance profiles of the parasite strain (Cioli et al., 2004)

Microscopic analysis showed progressive damage to the surface, with blisters and detachment on the tegument. These alterations, such as the blister formation were observed by Aires et al., (2014). The tegument is extremely important to the success of the infection and survival in the host. This structure plays a fundamental part in protecting against the attack from the host´s immune system, in addition the capacity to absorb nutrients, molecules and synthesize some proteins (Lima et al., 1994).

Therefore, this structure has been an important target in developing schistosomicidal drugs, presenting a dose/dependent effect. The tegument damages reported were similar to those in studies with PZQ (William et al., 2001). They occur after incubations with doses less higher than lethal concentrations, suggesting that death of the worm is caused by different mechanisms.

Tegument damage may not always result in death (Shuhua et al., 2000) since severe injuries were reported in adult female worms of *S. mansoni*, when treated with artemether (Xiao et al., 2000, 1985). This action mechanism fundaments medications currently used against schistosomiasis like PZQ (Shuhua et al., 2000; William et al., 2001), and experimental drugs such as Mefloquine (Manneck et al., 2010) and Artemether (Xiao et al., 2000). In the present study, males treated with PZQ worms were more susceptible than females.

To verify the damages caused in the tegument of *S. mansoni* adult worms, it was used Hoechst 33258 probe, a specific marker for the cell's DNA, very sensitive in marking regions where there are injuries. Any type of tegumental lesion, however small, is accurately marked by this probe (Oliveira et al., 2006). The use of this fluorescent marker in the experiments performed demonstrated that concentrations of gut-A from 20.0 µg/mL, *in vitro*, were able to damage the parasite's tegument; in males the injuries were more evident than in females of *S. mansoni*.

The mechanisms by which the ethanolic extract of the seed, the fractions tested and the gut-A employ their effects in the tegument have not yet been elucidated. Sato et al., (2002) observed the modulating effect of some drugs, previously known to interact with transport proteins of animal cells, on the excretory system of the adult male worm of *S. mansoni*. For this purpose, they used a fluorescent probe named Resorufin that diffuses itself and is passively excreted through the intact tegument of the parasite, which in turn is a substrate for P-glycoprotein.

The parasites labelling with Resorufin probe showed that the concentration of 20.0 µg/mL of gut-A was capable to paralyze the excretory activity of *S. mansoni* adult worms. Such fact could be noted due the impediment of probe output (substrate of P-glycoprotein - PGP) to the external environment, caused by the presence of gut-A. Therefore, it is suspected that, somehow, gut-A acts on the excretory system, interfering with the activity of PGP. However, this mechanism has not been completely elucidated. The same result was noted by Sato et al., (2002) in the presence of amiloride, an inhibitor of the Na^+ pump and the Na^+/H^+ ATPase.

The gut-A is a promising natural agent that has been showing several biological activities. Furthermore, Pereira et al. (2010) evaluated the gut-A toxicity on macrophages, indicating that this compound has no toxic effects on mammalian cells. The same was demonstrated in cell viability assay by MTT (bromide 3-[4,5-dimethylthiazol-2-il]-2, 5-diphenyltetrazolium) reduction, where no cytotoxic effect was observed in human cells (Dias et al., 2012).

This study demonstrated that gut-A is an effective compound for use against *S. mansoni* adult worms, *in vitro*. With its extensive effects on the mortality rate, parasite tegument morphology and adult *S. mansoni w*orm excretory activity gut-A is a promising schistosomicidal composite. However, further studies are needed to elucidate the toxicity mechanism(s) and to evaluate the *in vivo* activity of this compound.

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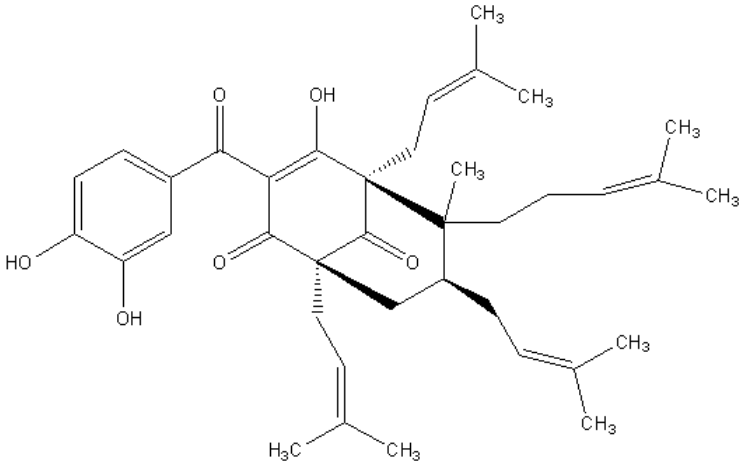

Fig.1. Chemical structure of Guttiferona-A.

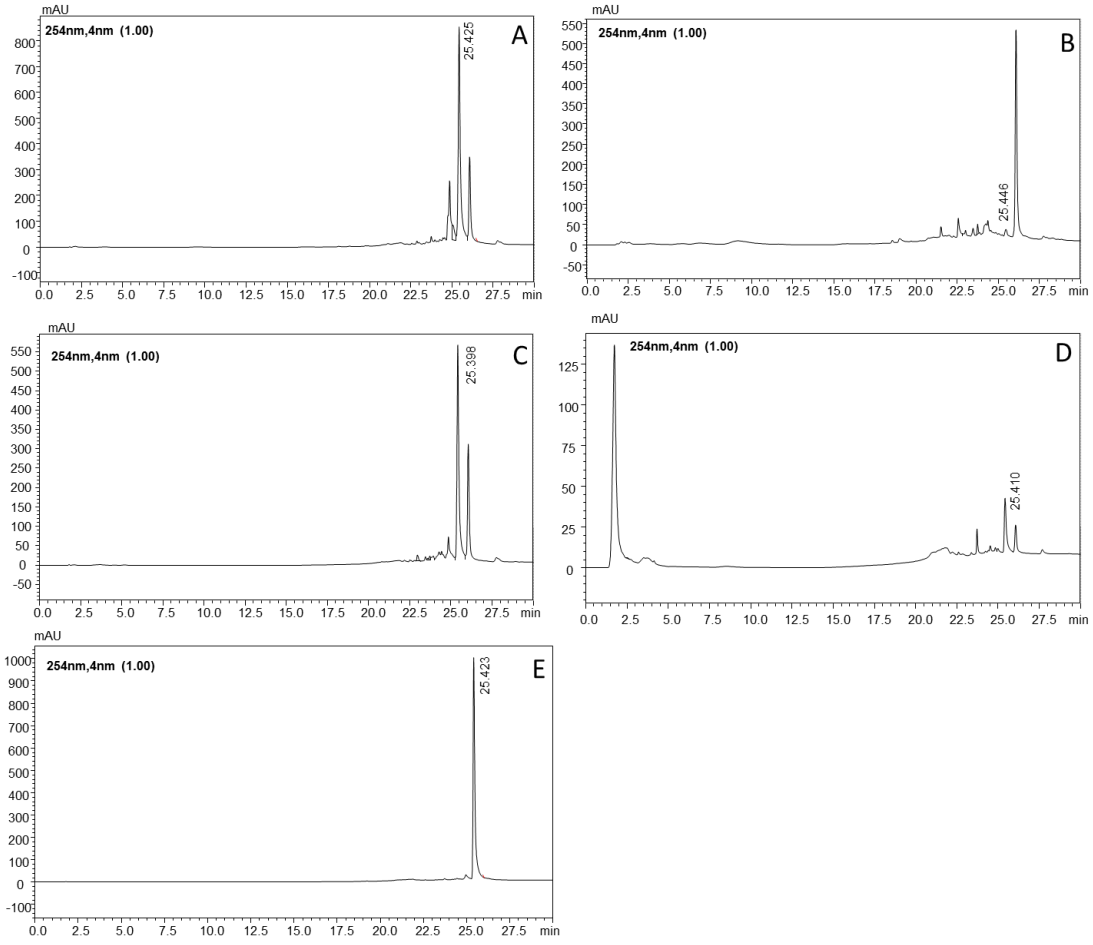

Fig. 2. Chromatograms obtained by high efficiency liquid chromatography: (A) EES, (B) FHS, (C) FAES, (D) FAS and (E) gut-A showing the separation of the gut-A with 25.425 min., 25.446 min., 25.398 min., 25.410 min. and 25.423 min, respectively.

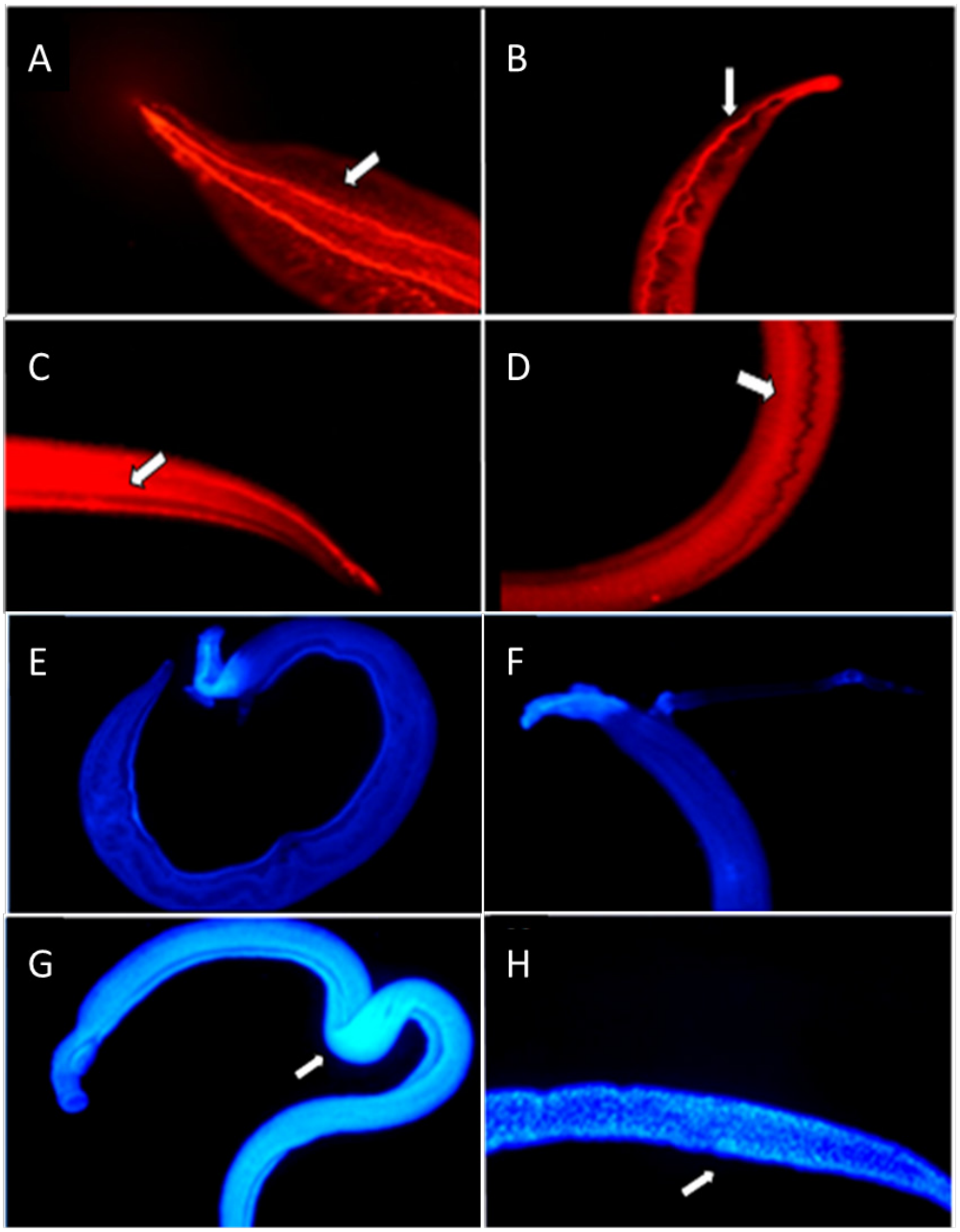

Fig.3. *In vitro* evaluation of the effect of the gut-A on the excretory system of adult worms of *S. mansoni:* Control (A and B) *S. mansoni* male and female labeled with Resorufin probe. (C and D) *S. mansoni* male and female labeled with Resorufin probe and exposed to 20.0 µg/mL of gut-A. Effect of the gut-A in the tegument of adult worms of *S. mansoni*: Control (E and F) Mated *S. mansoni* labeled with Hoechst 33258 probe. (G and H) *S. mansoni* male and female labeled with Hoechst 33258 probe and exposed to 20.0 µg/mL of gut-A.

Table 1. Analysis of the schistosomicidal parameters of EES and FHS, FAES and FAS fractions obtained from *Garcinia brasiliensis´s* seed.

Substance	ED_{90} (µg/mL)	Motility		Mating/ Morphology/ Eggs presence		Contraction and Shortening/ Tegument detachment	
		2h	≥ 24h	2h	≥ 24h	2h	≥ 24h
CONTROL	0	+++	+++	+/+/-	+/+/+	-/-	-/-
EES	94,0	+	-	-/-/-	-/-/-	+/-	+/+
FHS	97,0	+	-	-/-/-	-/-/-	+/-	+/+
FAES	89,0	+	-	-/-/-	-/-/-	+/-	+/-
FAS	200,0	+++	+++	+/+/-	+/+/-	-/-	-/-
PZQ	2,0	+	-	+/-/-	+/-/-	+/-	+/+

Table 2. *In vitro* effects of EES, FHS, FAES, FAS and gut-A against *Schistosoma mansoni*adult worm.

Group	Concentration (µg / mL)	% of dead worms
Control*		0 [d]
2% Methanol		0 [d]
PZQ	2	100,0[a]
EES	50	83,3[b]
	75	100,0 [a]
FHS	50	58,3[C]
	100	100,0 [a]
FAES	100	100,0 [a]
FAS	100	0 [d]
Gut-A	10	4,16[d]
	15	58,3[C]
	20	83,3[b]
	25	100,0[a]

Means followed by the same letter do not differ by SNK test, at 5% significance

*RPMI-1640 medium