



## In vitro evaluation of the schistosomicidal effect of the extracts, fractions and major 3-hydroxy-2,6-dialkyl-substituted piperidine alkaloids from the flowers of *Senna spectabilis* (Fabaceae)



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### ABSTRACT

In this work, we present the in vitro schistosomicidal activity evaluation of the most active dichloromethane fraction (FDM) (ED<sub>50</sub> = 83.5 µg/mL) and of a mixture of the major alkaloids ((-)-cassine/(-)-spectaline, C/E) (ED<sub>50</sub> = 37.4 µg/mL) from the flowers of *Senna spectabilis* against adult worms and cercariae. We also demonstrate other toxic effects including paralysis of the adult worms, inhibition of the secretory activity, tegument lesions and cercaricidal activity. In the association test of Praziquantel (PZQ)-C/E, we observed up to 80% mortality of *Schistosoma mansoni* in comparison to PZQ monotherapy. Due to the diversity of the toxic effects, the schistosomicidal activity of C/E is likely a result of a multitarget mechanism involving the tegument, secretory system and neuromotor action.

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Schistosomiasis is a chronic parasitic disease caused by parasites of the genus *Schistosoma*. It is included in the list of neglected diseases by the World Health Organization (WHO) and presents significant social and economic impacts.<sup>1–3</sup> It is estimated that more than 207 million people are infected, and approximately 250 thousand die each year worldwide by consequences of the infection of *Schistosoma*.<sup>1–4</sup>

Currently, chemotherapy is the first-line tool to minimize the prevalence and incidence of this disease worldwide,<sup>5</sup> but the only drug available and indicated by WHO for treatment is praziquantel (PZQ). However, the dependence on a single drug is worrying, especially in endemic areas where retreatment is constant and may favor the emergence of drug-resistant strains. Due to the warnings about drug resistance, possible infection recurrence, low efficacy in some cases and very restricted therapeutic alternatives, the search for new drug prototypes is a continuous challenge and must be encouraged in academia and the pharmaceutical industry.<sup>6,7</sup> In the past few years, the interest in natural products as a potential source for the treatment of parasitic diseases has

grown.<sup>8</sup> The objective of these studies is to propose alternative methods for the treatment of these diseases, especially for neglected ones, such as schistosomiasis,<sup>9</sup> since this has left researchers on alert regarding the development of resistance to the only drug used for its treatment. As part of the great effort in prospective studies on new drug candidates for all neglected diseases, several recent in vitro studies have been performed in the search for new schistosomicidal compounds from plants.<sup>9–12</sup>

Plants produce a great diversity of secondary metabolites with a very wide spectrum of biological activity, including cytotoxic, antiparasitic and antimicrobial properties. These compounds frequently interfere with central targets in the parasites, such as DNA (e.g., intercalation, alkylation activity), membrane integrity, microtubules and neuronal signal transduction.<sup>13</sup> The *Senna* genus comprises about 600 species distributed worldwide; some species have evoked interest due to the abundance of 2,6-dialkyl-3-*O*-substituted piperidine alkaloids in leaves, flowers and fruits. Biological studies with extracts from *Senna* species (specially *S. carnaval*, *S. excelsa* and *S. spectabilis*) have shown a number of relevant effects, such as antimicrobial, sedative, anticonvulsant, antioxidant, antinociceptive, anti-inflammatory and leishmanicidal effects.<sup>14–20</sup> In Brazil, *Senna spectabilis*, *S. carnaval* and

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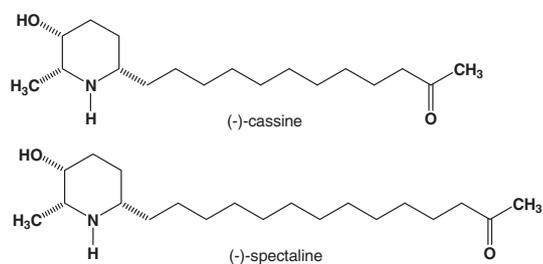
*S. excelsa* are endemic species of southeast states and have been identified as important sources of piperidine alkaloids, which have demonstrated sedative, anticonvulsant,<sup>14</sup> antimicrobial, antioxidant,<sup>21</sup> anti-inflammatory<sup>22</sup> and central nervous system effects,<sup>20,23</sup> including the inhibition of acetylcholinesterase and central analgesia. Recently, antiparasitic activity was also reported against *Leishmania major* promastigotes of the crude extract, fractions and a mixture of the two major alkaloids (–)-cassine/(–)-spectaline (C/E) from flowers of *S. spectabilis*.<sup>15</sup>

Due to our interest in the search for new bioactive compounds against schistosomiasis and other neglected parasitic diseases, and considering earlier reports about the promising antiparasitic activity of *S. spectabilis*, we decided to investigate the *in vitro* schistosomicidal activity of the crude extract, its fractions and a mixture of the two major alkaloidal constituents (Fig. 1).

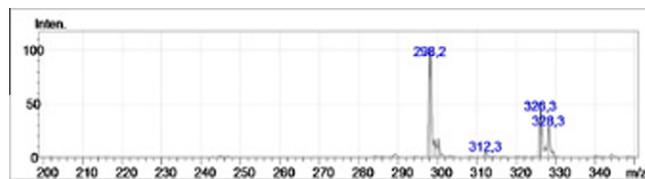
Flowers of *S. spectabilis* were collected in the region of Araraquara-SP (Brazil) during January 2013. A voucher specimen was deposited in the Herbarium of the Botanical Garden of São Paulo under registry number SP 370.917. Extraction procedures and the isolation of (–)-cassine and (–)-spectaline substances were performed as described by Albuquerque Melo and co-workers.<sup>15</sup> The alkaloidal portion was then purified by column chromatography (CC, neutral Al<sub>2</sub>O<sub>3</sub>) and eluted with EtOH/CHCl<sub>3</sub>/hexanes (1:7:2), providing a mixture of piperidine alkaloids (–)-cassine/(–)-spectaline (4.82 g) along with a complex mixture of other more polar alkaloidal metabolites. It is known that almost all alkaloids from *S. spectabilis* flowers occur in mixtures of two homologue-derivatives, differing only by two methylene units in the side chain.<sup>24</sup> Therefore, the mixtures of such homologues is very difficult to separate, and it was decided to evaluate the mixture of the most abundant alkaloids, (–)-cassine/(–)-spectaline. The C/E proportion in the mixture was determined by liquid chromatography coupled with mass spectrometry. The mass spectrum of the C/E mixture (Fig. 2) revealed that these two alkaloids are practically pure in the mixture, through identification of the respective molecular ions [M+H]<sup>+</sup> with 298.2 and 326.3 *m/z*, respectively, with (–)-cassine as the major constituent in the mixture at a proportion of 57% in relation to (–)-spectaline (Table 1).

The occurrence of mixtures of several homologous alkaloids, such as (–)-cassine and (–)-spectaline, with very similar chromatographic behavior in *S. spectabilis* had already been reported.<sup>24</sup> In this study, the authors have identified and characterized several of these metabolites, including their typical fragmentation data by mass spectrometry.<sup>20,24</sup> Therefore, this study was limited to a comparison of the chromatographic data obtained during the isolation and purification stage, using the mass spectrometry only for relative quantification among the most abundant alkaloids, (–)-cassine and (–)-spectaline.

To evaluate schistosomicidal activity, the parasites were recovered from experimental animals infected by the *S. mansoni* LE (Luiz Evangelista) strain. This strain has been routinely maintained by serial passages in *Biomphalaria glabrata* models and mice of Swiss



**Figure 1.** Chemical structures of (–)-cassine and (–)-spectaline, the two major alkaloids from flowers of *S. spectabilis*.



**Figure 2.** Mass spectrum of the mixture of (–)-cassine and (–)-spectaline isolated from *S. spectabilis* flowers.

**Table 1**

Relative abundance of (–)-cassine and (–)-spectaline in the evaluated mixture

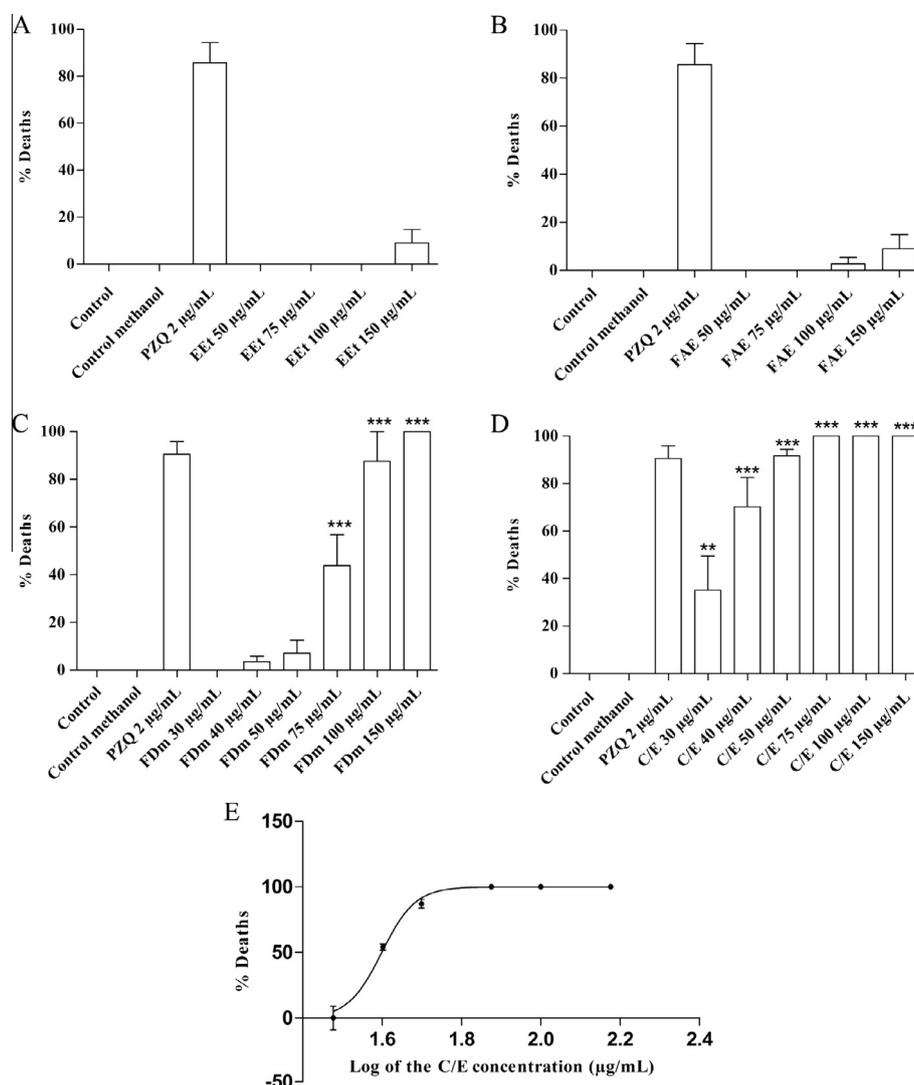
Substance	(–)-Cassine	(–)-Spectaline
<i>m/z</i>	298.20	326.25
Absolute intensity	15,206,026	6,596,588
Relative intensity	100.00	43.38
Main fragment	[M+H] <sup>+</sup>	[M+H] <sup>+</sup>
Fragmentation	279.6, 108.8	308.2, 141.0
Fragmentation pattern <sup>24</sup>	280 [M–H <sub>2</sub> O] <sup>+</sup> (relative intensity 100), 109 not assigned (relative intensity 10)	308 [M–H <sub>2</sub> O] <sup>+</sup> (relative intensity 10), 141 not assigned (relative intensity 8)

lineage for more than 30 years at the Research Center René Rachou/Oswaldo Cruz Foundation. The Ethics Committee on Animals at the Federal University of Alfenas (UNIFAL-MG) authorized all procedures, under registration number 544/2013 (on January 20th, 2014) in accordance with the ethical principles required for animal experimentation.

Mice infected with *S. mansoni* cercariae (LE strain) were sacrificed 45 days after infection by ‘overdose’ with 10.0% ketamine chloride (Ketamine Agener) and 2.0% xylazine hydrochloride (Rompun) dissolved in saline, which was administered intraperitoneally (±0.2 mL per animal). Subsequently, retrograde liver perfusion was performed according to the method of Smithers and Terry<sup>25</sup> to obtain the parasites. Subsequently, the *in vitro* activity of the ethanolic extract, its fractions, and the mixture of C/E against *S. mansoni* adult worms was evaluated using a methodology adapted from the literature.<sup>26</sup>

The recovered parasites were cultivated in six-well culture plates (four couples per well) in RPMI-1640 culture medium supplemented with heat-inactivated 5.0% fetal bovine serum with 1.0% penicillin (10,000 IU/mL) and streptomycin (10.0 mg/mL) (Sigma, USA). After thirty minutes of adaptation, the ethanolic extract (EEt), ethyl acetate fraction (FAE), dichloromethane fraction (FDm) and C/E samples were added to the cultures at different concentrations (EEt and FAE at 50.0; 75.0; 100.0; and 150.0 µg/mL; FDm and C/E at 30.0, 40.0, 50.0, 75.0, 100.0 and 150.0 µg/mL). The plates were incubated at 37 °C and 5.0% CO<sub>2</sub> and then analyzed within 2 and 24 h after contact with the substance samples. The test groups were compared with the controls of supplemented RPMI 1640 medium, methanol (used as solvent for the samples) and PZQ (2 µg/mL) under the same conditions described above.

The parasites in culture exposed to different samples were evaluated for effects on movement, mating, contraction, morphological tegument alterations and egg laying over the course of eight days. These parameters have also been used in previous studies as indicators of biological activity and toxicity studies with *Schistosoma* species.<sup>1,12,27–29</sup> After eight days of contact between the extracts/compounds and the parasites, the effective dose (ED<sub>50</sub>, ED<sub>90</sub> and ED<sub>100</sub>) were determined from sigmoidal regression curves. The ED<sub>50,90,100</sub> values were the concentration of C/E required bringing about 50.0, 90.0 and 100.0% death of adult worms of *S. mansoni*. These values were calculated from survival curves by sigmoidal



**Figure 3.** In vitro effects of the ethanol extract (A), ethyl acetate fraction (B), dichloromethane fraction (C) and (–)-cassine/(–)-spectaline (D) alkaloid mixture against adult *S. mansoni*  $p < 0.001$ . Sigmoidal curve obtained by plotting of the percentage of death of adult worms of *S. mansoni* versus the concentration (log) of compound C/E concerning the calculation of  $ED_{50,90,100}$  (E).

model. They took into account the percent of death of adult worms versus log C/E concentration, besides of the non-linear regression analyses by Graph Pad Prism 5.0 software (Graph Pad Software Inc., San Diego, CA, USA). The effective dose values were used to assess the potency of activity of compounds and to monitor the resistance and susceptibility profiles of the parasite strain in relation to the evaluated substance.<sup>8,28,30,31</sup>

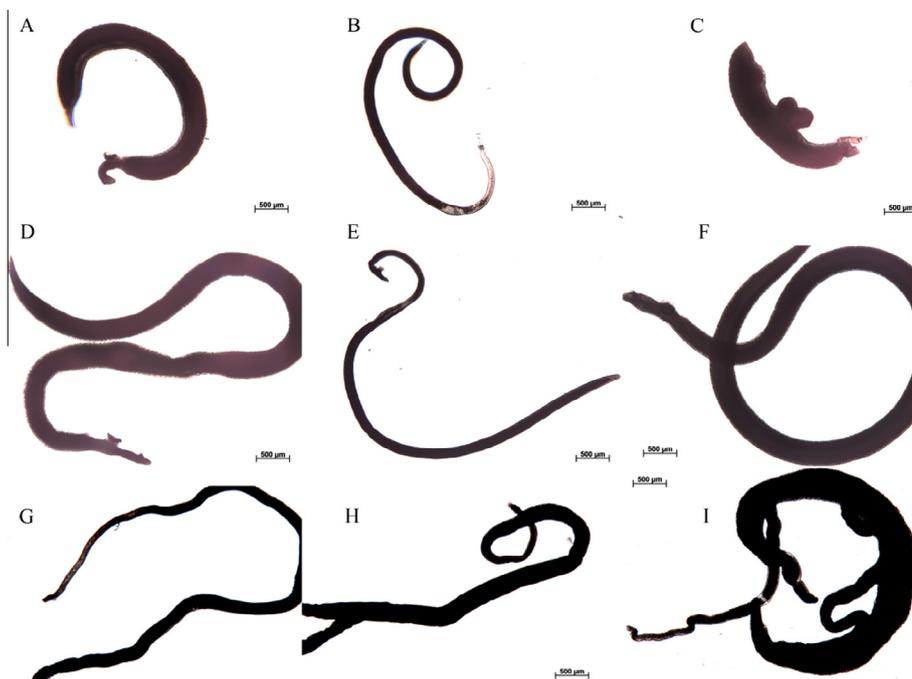
Adult worms exposed to concentrations of 50.0, 75.0 and 100.0 µg/mL of the EEt maintained normal movement and mating, with no evidence of tegument injury. In addition, egg laying was observed over eight days of experiment. However, the morphology of the parasites seemed to be altered, with elongated worms and some with stationary digestive systems, in addition to smaller quantities of egg laying compared to the control.

On the eighth day of the experiment, many eggs were unviable, and some parasites were immobile. The FAE fraction at the concentration of 50.0 µg/mL, after 24 h, did not alter parasite mating, although movement was decreased. At higher concentrations (100.0 and 150.0 µg/mL), there was tegument injury as well as paralysis of some parasites. The high inhibitory concentration for 50% ( $ED_{50}$ ) for EEt (495.4 µg/mL, Fig. 3A) and FAE (553.5 µg/mL, Fig. 3B) shows that the viability of the worms was not significantly different when compared to the controls RPMI 1640 medium and methanol.

In contrast, adult worms exposed to FDM for two hours, at all concentrations, showed flaccid paralysis. However, at lower concentrations (30.0; 40.0; and 50.0 µg/mL), after washing to remove the active compound, worms recovered even a small amount of egg laying. At concentrations of 75.0, 100.0 and 150.0 µg/mL, all parasites remained elongated until the eighth day and displayed tegument lesions. The  $ED_{50}$ ,  $ED_{90}$  and  $ED_{100}$  values for FDM were 83.5 µg/mL, 122.3 µg/mL and 132.0 µg/mL, respectively (Fig. 3C), which clearly show a promising anti-parasitic effect, with results statistically significant ( $p < 0.05$ ) compared to the control (methanol) at concentrations of 75.0, 100.0 and 150.0 µg/mL.

A comparison of the effects on parasites exposed to EEt, FAE and FDM clearly show that FDM caused greater damage to the worms than the other compounds tested. This effect could be explained by the results from a previous work,<sup>15</sup> in which the authors identified (–)-cassine and (–)-spectaline as the major constituents of the dichloromethane fraction of the crude ethanolic extract of flowers from *S. spectabilis*.

After two hours of administration of 150.0 µg/mL of C/E, parasites became stationary and elongated (Fig. 4). After 24 h, C/E was removed from the culture by washing and it was observed that the parasites exposed to lower concentrations (30.0 and 40.0 µg/mL) restored a little mobility. In contrast, those parasites exposed



**Figure 4.** Adult *S. mansoni* worms exposed to the C/E mixture with flaccid paralysis. (A and B) Adult worms, male and female, respectively. Control, RPMI. (C) Adult worms exposed to PZQ (2 µg/mL); (D) male worm exposed to 40 µg/mL of C/E; (E) female worm exposed to 40 µg/mL of C/E; (F) male worm exposed to 50 µg/mL of C/E; (G) female worm exposed to 75 µg/mL of C/E; (H) female worm exposed to 100 µg/mL of C/E; (I) mated worms exposed to 150 µg/mL of C/E. (Scale bar = 500 µm.)

to higher concentrations remained without movement, with ED<sub>50</sub>, ED<sub>90</sub> and ED<sub>100</sub> values of 34.7, 82.8 and 94.8 µg/mL, respectively (Fig. 3E). In all concentrations there was significant statistical difference ( $p < 0.05$ ) when compared with the control, as shown in the graph below (Fig. 3D). Additionally, it was observed that in all concentrations there were parasites with tegument lesions.

In fact, we observed a stronger effect on worm musculature for FDM and C/E than for EEt and FAE. Furthermore, flaccid paralysis as well as tegument damage was observed in parasites of both sexes, with a stronger effect on females. These effects occurred most strongly within 24 h of contact with the active substances, causing a flaccid paralysis identified by worm elongation (Fig. 4).

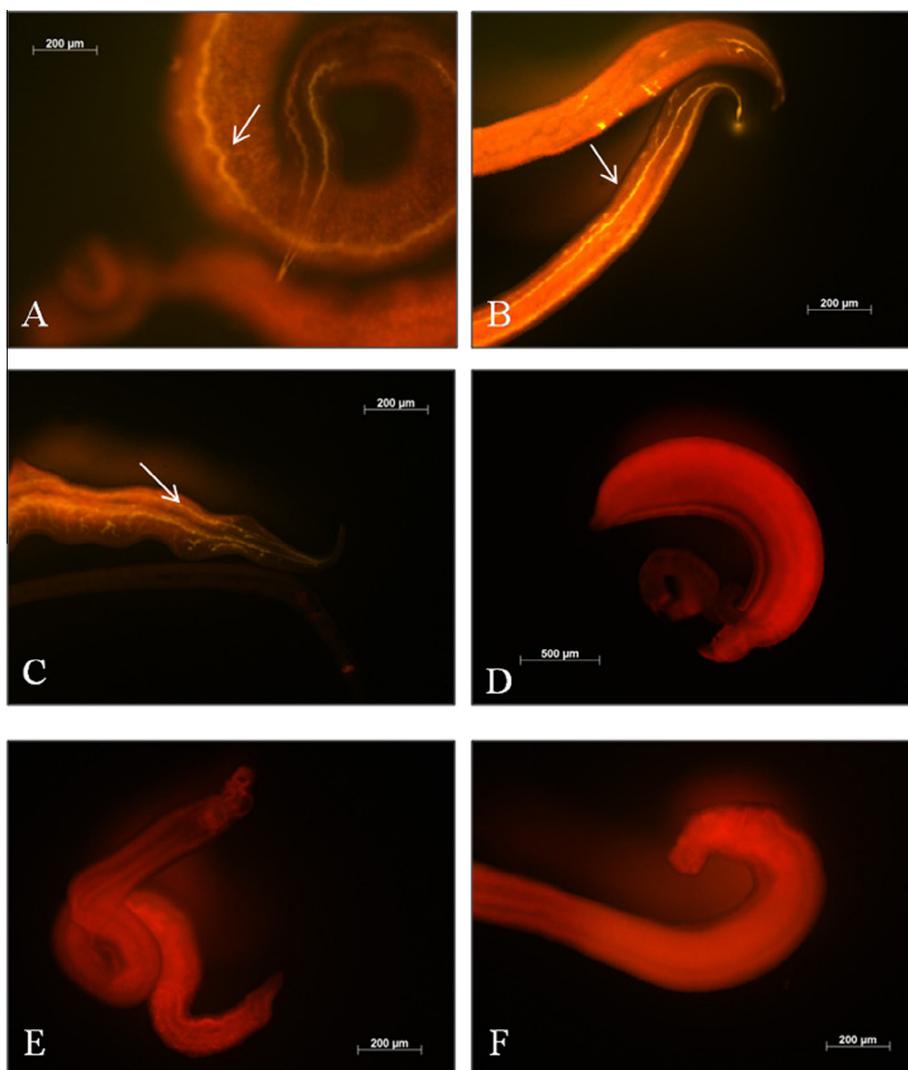
Our results were corroborated by previous studies that had reported the neurotoxic properties of these types of alkaloids, probably acting as agonists or antagonists of neuroreceptors or ion channels.<sup>13</sup> Multicellular parasites have a nervous system that utilizes neurotransmitters such as acetylcholine (ACh) and ACh receptors, which are responsible for controlling worm muscle activity.<sup>13</sup> Therefore, inhibition of the activity of these neuroreceptors may result in muscle paralysis, as was observed for PZQ, but involving a different mechanism. It is believed that PZQ blocks sodium and potassium channels responsible for neuronal signaling and also causes the direct paralysis and death of worms.<sup>32,33</sup>

It was reported that acetylcholinesterase enzyme (AChE) is found in cercariae and schistosomules<sup>34</sup> and is present in two forms in parasites. One is internally associated with muscle mass and has a functional role in the motor plates of parasites. The other form is external, associated with the tegument, and involved in signal transduction.<sup>35</sup> Therefore, the function of ACh as a neurotransmitter is reinforced by the fact that the worm possesses enzymes for its synthesis and degradation. Additional evidence for the physiological role of ACh in *S. mansoni* is that muscular activity of this worm is reduced by cholinomimetic agents such as carbachol, nicotine and AChE inhibitors that produce flaccid paralysis, which can be defined as a loss of motility accompanied by worm elongation due to relaxation of its longitudinal musculature.<sup>36,37</sup> In an in vivo study with a series of piperidine alkaloid

derivatives, it was found that these compounds could act as AChE inhibitors, with selectivity for cerebral AChE.<sup>38</sup> Thus, considering that 3-hydroxy-2,6-dialkyl-substituted piperidine derivatives could act as AChE inhibitors, with possible action on ACh receptors, our results may be explained by the action of C/E in direct muscle paralysis and parasite death.

In vitro evaluation of the excretory system activity in adult worms was also performed by using resorufin incubated with C/E, as described by Castro and co-workers.<sup>26</sup> Figure 5A–C shows normal functioning of the excretory system of adult *S. mansoni* worms from the RPMI and methanol control groups after labeling with the resorufin probe. In this case, fluorescence was clearly delimited along the parasite when expelling the probe from the organism. The parasites exposed to 2 µg/mL of PZQ (Fig. 5D) and 40 µg/mL of C/E (Fig. 5E and F) displayed excretory system damage, showing a pattern of differential fluorescence, which emissions scattered throughout the body. According to Sato et al.,<sup>39</sup> the resorufin probe is a salt of fluorescent nature besides a substrate P-glycoprotein (Pgp) modulator, a protein expressed in the excretory epithelium of *S. mansoni* adult worms. These proteins are associated with the absorption, elimination and distribution of many drugs.<sup>40</sup>

The in vitro assessment of tegument damage in adult worms exposed to a mixture of C/E was evaluated by the Hoechst 33258 probe, using a procedure adapted from Castro and co-workers.<sup>26</sup> The tegument of *S. mansoni* adult worms has been the subject of investigation as a promising target for schistosomicidal agents, since it is responsible for one of the parasite escape mechanisms from the immune system in the human body.<sup>41,42</sup> Hoechst 33258 is a hydrophilic probe that emits fluorescence when it binds to the cell DNA. So, in the presence of lesions, this probe diffuses itself, acting as an indicator of membrane integrity.<sup>43</sup> In Figure 6E and F, fluorescence is seen emitted from the parasite after exposure to C/E, which is compatible with observations of the worm under the action of PZQ (Fig. 6D). These effects were not observed in the RPMI medium and methanol group controls (6A, 6B and 6C). Once again, the tegument lesions were more significant in females,



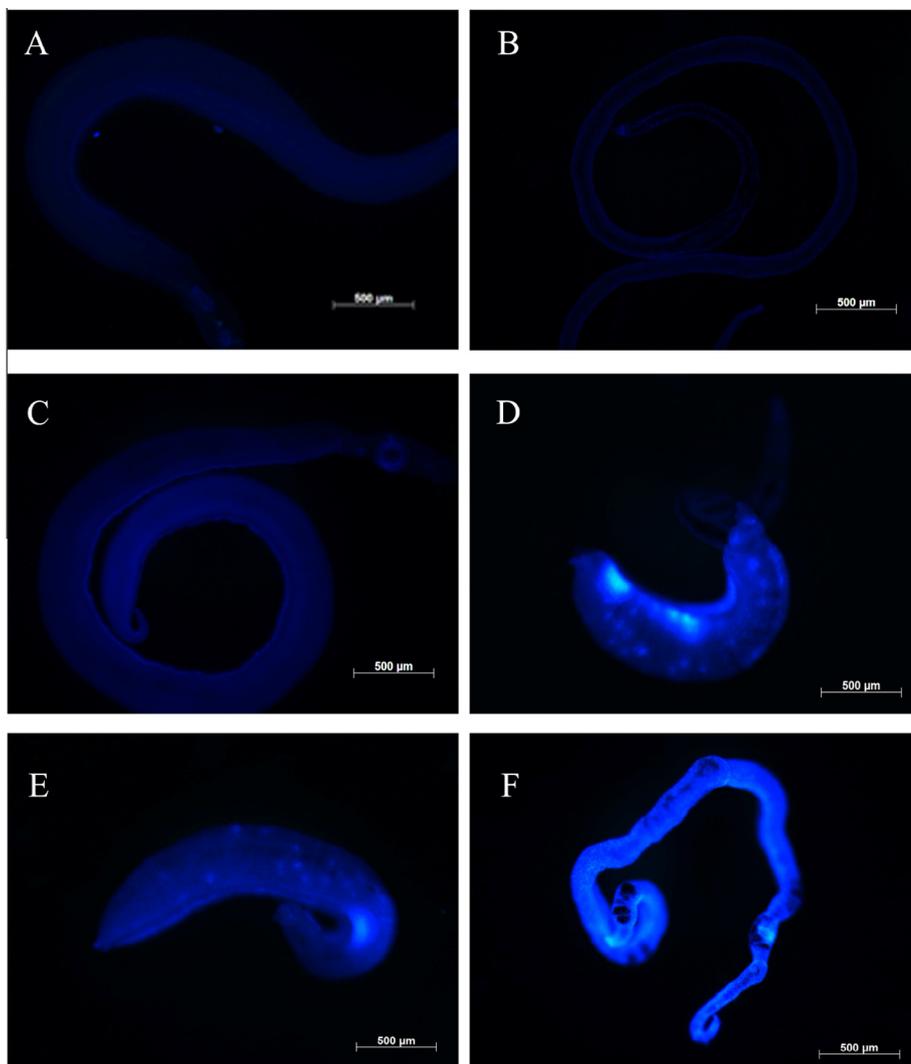
**Figure 5.** Marking of damage to the excretory system of adult worms of *S. mansoni* using a resorufin probe. (A and B) Males incubated with RPMI medium (negative control); (C) males exposed to methanol; (D) coupled worms exposed to 2.0 µg/mL PZQ (positive control); (E and F) males exposed to the C/E mixture at 40.0 µg/mL.

causing blistering in greater quantity and a greater proportion of the cutaneous surface to be stained by the probe (Fig. 6E and F). Therefore, these results make it clear that the C/E mixture is responsible for damage to the tegument of adult worms, because it is able to inflict damage on the *S. mansoni* excretory system.

We also evaluated the *in vitro* effect of an association of C/E and PZQ on *S. mansoni* adult worms, because chemotherapy with a combination of different drugs has been used for the treatment of infectious disease in order to delay or prevent an increase in drug resistance.<sup>43–45</sup> The methodology was modified from that proposed by Araujo and co-workers.<sup>44</sup> After thirty minutes of adaptation of the parasites, a solution of C/E in methanol (5.0 mg/mL) and PZQ solubilized in RPMI-1640 (0.8 mg/mL) was added to the cultures. For this purpose, we used associations of PZQ:C/E in the following proportions (µg/mL): 0.5:23.8, 0.5:11.9, 0.5:6.0; 0.25:23.8, 0.25:11.9, 0.25:6.0. Ten wells were used as controls, of which four contained PZQ (2.0, 0.5, 0.25 and 0.125 µg/mL), four contained C/E (47.5, 23.8, 11.9 and 6.0 µg/mL), one well contained only supplemented culture medium and another with methanol (1%) was used to solubilize the alkaloid mixture. The culture plates were maintained under the same conditions described previously, and the same procedure was performed to evaluate the interaction of these substances.

For monotherapy with PZQ at the concentration of 0.5 µg/mL, the percentage of dead adult worms was 33.0%. At the concentration of 0.25 µg/mL, there were no worms showing a total absence of movement. Upon exposure to C/E, the percentage of non-viability among parasites at the concentration of 23.8 µg/mL was 16%. There were viable parasites at the concentration of 11.9 µg/mL. In comparison to monotherapy, the association of PZQ:C/E exhibited a stronger effect ( $p < 0.05$ ) at the proportions of 0.5/23.8, 0.5/11.9 and 0.25/23.8 µg/mL, with a shutdown effect on 83%, 50% and 80% of worms, respectively (Fig. 7). As observed for PZQ, the C/E mixture also caused excretory system paralysis and damage to the parasite tegument. This result suggests a possible synergistic effect, increasing the action on these targets or acting concomitantly on different targets to enhance the effect.<sup>44,46–48</sup> Interestingly, it was observed that while PZQ caused an effect of muscle contraction, C/E induced relaxation in worms, but these effects were not observed for the PZQ:C/E association.

The drug association effect is also a very important strategy to minimize therapeutical doses of an active composition, aiming at the reduction of possible toxic effects of substances, which could be used in smaller doses than when administered as single compounds. Thus, the association of drugs with synergistic effect has been widely studied and can be a valuable way to improve the



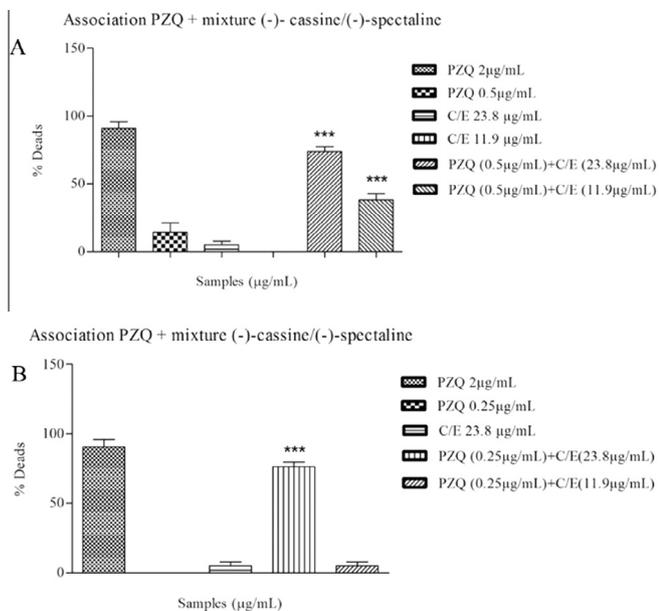
**Figure 6.** Injury by Hoechst 33258 probe marking the integument of adult worms of *S. mansoni*. (A and B) male and female, respectively, incubated with RPMI 1640 medium (negative control); (C) male incubated with methanol; (D) coupled worms exposed to 2.0 µg/mL PZQ (positive control); (E and F) male and female, respectively, exposed to the C/E mixture at 40 µg/mL.

treatment of a number of diseases such as malaria, Chagas disease, giardiasis and schistosomiasis.<sup>44,45,47,48</sup>

The cercaricidal activity of the C/E was also evaluated by investigating the loss rates of motility and tail, by an adapted methodology described earlier.<sup>26</sup> The loss of tail has been used as a measure of sensitivity to PZQ.<sup>49,50</sup> However, the mechanism that causes this effect remains unclear. It is known that the cercariae body narrows in a close folding connection with the tail, and it is believed that this bond can be easily broken.<sup>49</sup> Cercariae are the infective form of the parasite for the definitive host, and for the penetration through the skin to occur, they need the combined action of enzyme secretion and tail vibrating movements.<sup>51</sup> Consequently, the tail loss compromises the ability of cercaricidal infectivity. The tail loss induction by drug action is different from loss by physical stress, since the first occurs before the acetabular content is secreted, which was confirmed after exposure to PZQ. At concentrations of 12.5 and 25.0 µg/mL, C/E showed a weak effect. However, the effects were significantly higher from a concentration of 50.0 µg/mL ( $p < 0.05$ ), with 100% of loss in motility after 4 h and 75% of tail loss after the analysis time (Fig. 8). It might also be noted the formation of blisters and vacuolization in all concentrations. The effect of C/E in the three highest concentrations was

compatible or more efficient than the effect of PZQ. Thus, the action of the alkaloidal mixture C/E on larval stage is also proven, based on the same measure of sensitivity used for PZQ, as well as by other studies carried out with Hinokitiol, *Euphorbia conspicua* and *Garcinia braziliensis*.<sup>26,51,52</sup>

Parasites are eukaryotes as well as their hosts, and besides, they share most of the biochemical and molecular characteristics. This is an obstacle for researchers working in the field of medicinal chemistry to obtain new, effective and selective drug candidate prototypes. Attention should be paid to the limitation to find prototypes simultaneously effective against the parasite but not toxic to humans when in vitro tests are performed.<sup>13</sup> So, a cell viability assay was performed using the same methodology as described by Dias and co-workers.<sup>53</sup> Our results suggest that the natural C/E mixture could be a promising alternative against *S. mansoni*, since it has exhibited significant potency against adult worms ( $ED_{50} = 37.4$  µg/mL) and moderate cytotoxic potential against human monocytes with a  $CC_{50} = 132.1$  µg/mL and a safety index of  $SI = 3.5$ . This activity profile is significantly interesting, as the toxic concentration for human monocytes is 3.5 times higher than the effective dose to kill the parasites. In fact, many studies concerning the cytotoxicity of alkaloids of different types reveal that



**Figure 7.** Schistosomicidal activity of the association of PZQ:C/E in comparison to monotherapy with PZQ (0.5 µg/mL) (A) and PZQ (0.25 µg/mL) (B) and with C/E.

they do not show high safety indices. An example of this toxic profile can be seen in the work of Mengome and co-workers<sup>54</sup> in which monkey kidney cells were used for the cytotoxicity evaluation. In this same context, another recent study showed that 3-alkylpyridine marine alkaloid analogues showed significant toxicity against murine peritoneal macrophages.<sup>55</sup> Regarding to PZQ security index, it was conducted tests until the concentration of

500.0 µg/mL, but no toxicity was observed at murine macrophages (data not shown).

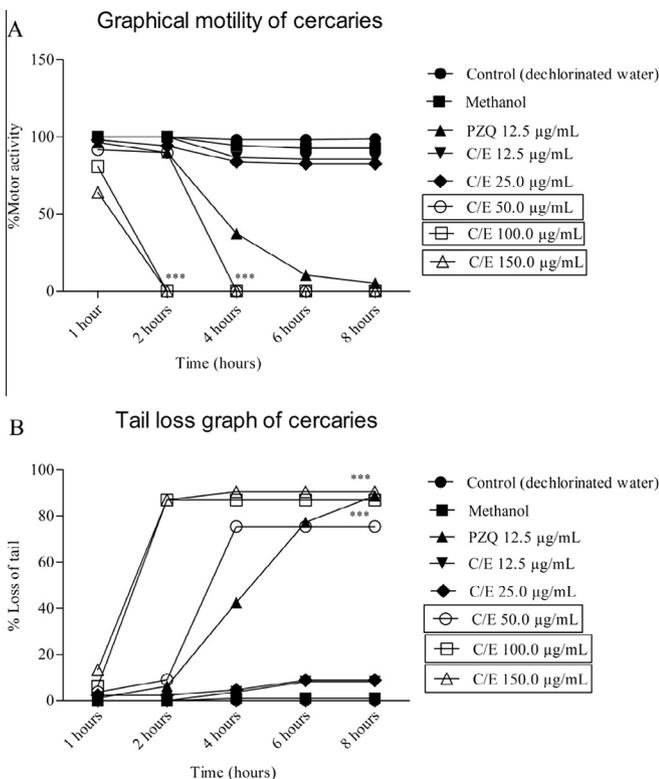
In conclusion, these data point to a promising schistosomicidal activity profile for this mixture of abundant natural alkaloids, (–)-cassine and (–)-spectaline. Moreover, C/E seems to act by a multi-target mechanism, which could be related to inhibitory effects on excretory activity, tegument lesions, and possible action on neuro-motor activity, as well as an effective toxic effect on the larval stage of cercariae. In addition, our results showed increased schistosomicidal activity when PZQ was used in combination with C/E. It suggests that C/E mixture or isolated (–)-cassine and (–)-spectaline compounds or other their semisynthetic derivatives could reinforce the therapeutic potency, specially against PZQ-resistant strains. Further studies of efficacy and toxicity are needed, as well as a deepened evaluation of the possible mechanisms involved. These findings could be of great value in the development of an innovative feature of these compounds for efficacy with a lower possibility of resistance or in the rational design of new drug candidates for schistosomiasis using (–)-cassine and (–)-spectaline as structural inspiration, considering that these two compounds are natural homologues with a great grade of structural similarity, differing only by two methylene groups on the lateral side chain. Finally, we have an ongoing project that aims to evaluate a series of C/E semi-synthetic derivatives for their schistosomicidal profile with higher potency and lower toxicity, which results will be published soon.

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**Figure 8.** Effect of different concentrations of the C/E mixture on the viability of cercariae, based on the rate (%) of movement (A) and loss of tail (B). Data are presented as the average of three experiments. \*\*\*  $p < 0.0001$ .

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