Anthelmintic, Antibacterial and Cytotoxicity Activity of Imidazole Alkaloids from *Pilocarpus microphyllus* Leaves

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Pilocarpus microphyllus **Stapf ex Wardlew (Rutaceae), popularly known as jaborandi, is a plant native to the northern and northeastern macroregions of Brazil. Several alkaloids from this species have been isolated. There are few reports of antibacterial and anthelmintic activities for these compounds. In this work, we report the antibacterial and anthelmintic activity of five alkaloids found in *P. microphyllus* leaves, namely, pilosine, epiisopilosine, isopilosine, epiisopiloturine and macaubine. Of these, only anthelmintic activity of one of the compounds has been previously reported. Nuclear magnetic resonance, HPLC and mass spectrometry were combined and used to identify and confirm the structure of the five compounds. As regards the anthelmintic activity, the alkaloids were studied using *in vitro* assays to evaluate survival time and damaged teguments for *Schistosoma mansoni* adult worms. We found epiisopilosine to have anthelmintic activity at very low concentrations (3.125 µg mL^{-1})**; at this concentration, it prevented mating, oviposition, reducing motor activity and altered the tegument of these worms. In contrast, none of the alkaloids showed antibacterial activity. Additionally, alkaloids displayed no cytotoxic effect on vero cells. The potent anthelmintic activity of epiisopilosine indicates the potential of this natural compound as an antiparasitic agent.

Keywords: pilocarpus microphyllus; imidazole alkaloids; epiisopilosine; *schistosoma mansoni*; anthelmintic activity.

Abbreviations: ^{13}C NMR, carbon 13 nuclear magnetic resonance; ^{1}H NMR, hydrogen 1 nuclear magnetic resonance; ATCC, American type culture collection; CID, collision induced dissociation gas; CLSI, Clinical Laboratory Standards Institute; DL, desolvation line; DMSO, dimethyl sulfoxide; EPI, epiisopiloturine; EPIIS, epiisopilosine; HPLC, high performance liquid chromatography; ISOP, isopilosine; LC50, letal concentrarion; MAC, macaubine; MIC, minimum inhibitory concentration; MRM, reaction monitoring mode; MS, mass spectrometry; PILO, pilosine; PZQ, prazinquantel; NMR, nuclear magnetic resonance; RT, retention time

INTRODUCTION

The Rutaceae family of flowering plants consists of around 161 genera and 2070 species and has a predominantly pantropical distribution (Stevens, 2016). *Pilocarpus microphyllus* Stapf ex Wardlew is a species native to Brazil and is found in the north (Pará State) and northeast (Maranhão and Piauí States) macroregions of Brazil. Also known as jaborandi, this shrub occurs in less dense forests, often on rocky outcrops and in habitats with high light intensity (Skorupa, 2000). Members of this family contain a wide variety of secondary metabolites such as alkaloids, coumarins, lignans and terpenoids (Guerreiro *et al.*, 2005). Fifteen alkaloids have so far been identified from various jaborandi species (*Pilocarpus* spp.) (Santos and Moreno, 2004). Pilocarpine from jaborandi leaves was the first alkaloid to be identified and is of great economic interest because it is a parasympathetic autonomic nervous system activator, commercially used for surgical eye procedures and

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treatment of glaucoma and xerostomia (Avancini *et al*., 2003). Pilocarpine is currently produced commercially by extraction from jaborandi leaves.

Besides pilocarpine, as many as 15 other alkaloids have been isolated from *P. microphyllus* including pilosine (Bento *et al*., 2010), isopilosine (Santos and Moreno, 2004), epiisopilosine (Bento *et al*., 2010 ; Lucio *et al*., 2000), epiisopiloturine (Veras *et al*., 2013; Voigtlander *et al*., 1978) and macaubine (Santos and Moreno, 2004).

Among these alkaloids, only pilocarpine and epiisopiloturine have so far been described as having biological activity. Epiisopiloturine has demonstrated effective activity against *Schistosoma mansoni* (Veras *et al*., 2013). Other studies reported that epiisopilosine is a peripheral parasympathetic nervous system stimulant (Lucio *et al*., 2000), and the physical properties of pilosine and epiisopilosine have been studied by Bento *et al*. (2010) using Raman spectroscopy. These alkaloids are structurally similar, and there are some studies demonstrating their biological activity (Santos and Moreno, 2004).

The antibacterial activity of many naturally occurring alkaloids has been reported in the literature (Navarro and Delgado, 1999 ; An *et al*., 2011), and some have been proposed for use as anthelmintic agents and to combat neglected diseases (Veras *et al*., 2013 ; Moraes, 2012; de Moraes, 2015). Table 1 summarizes previously known and now-reported data on the five alkaloids studied in this work.

Schistosomiasis, a disease brought about by blood flukes (genus *Schistosoma*) is common in low-income rural communities and has been relatively neglected by medical researchers. This disease, which occurs in more than 70 countries and affects more than 200 million people, is considered an extremely serious global public health problem (Steinmann *et al*., 2006). Praziquantel (PZQ), the only available drug for control of this disease, was developed in the 1970s and has been an essential factor in reducing mortality because of schistosomiasis. It is now known that these organisms have developed resistance to PZQ, resulting in the need for research into new drugs against schistosomiasis (Moraes, 2012; De Moraes, 2015).

Of the five alkaloids in Table 1, until now, only EPI has had the structure fully elucidated or had any biological activity demonstrated. In this case, EPI was shown to have promising activity against *S. mansoni* both *in vitro* and *in vivo*. The other alkaloids, three of which have structures extremely similar to that of EPI, have not been tested against any organisms. Therefore, in this work, we evaluated antibacterial and anthelmintic activities of pilosine (PILO), epiisopilosine (EPIIS), isopilosine (ISOP), epiisopiloturine (EPI) and macaubine (MAC) alkaloids from *P. microphyllus* leaves.

EXPERIMENTAL

Plant material

The alkaloids were obtained from *P. microphyllus* leaves, native to the municipality of Matias Olímpio, Piauí State, in 2011. Voucher specimens were identified and deposited in Graziela Barroso Herbarium (Teresina, Piauí, Brazil) under the code TEPB 27.152.

Isolation and characterization

The alkaloids were all obtained from a cultivated jaborandi acid solution as described in detail elsewhere (Veras *et al*., 2013). The samples were dissolved with methanol and vacuum filtrated through 0.20 μm nylon membrane filters (Supelco, Bellefonte, PA, USA) before injection into an HLPC system.

An HPLC system (Nexera Shimadzu, Tokyo, Japan) was used. The separation was carried out using a LiChrospher 60 RP C18 column (5 μm particle size, 250 × 4.6 mm i.d. manufactured by Merck, Darmstadt, Germany) maintained at 50°C. An injection volume of 5 μL was used. The mobile phase consisted of 0.1% acetic acid aqueous solution (solvent A) and acetonitrile (ACN) at 0.1% acetic acid (*v*/v) (solvent B) using gradient elution (5 min: 5% B; 6–25 min: 10% B; 30–35 min: 100% B; 40–45 min: 5% B) with 0.5 mL min^{-1} total flow. Chromatographic data were obtained and processed using Lab Solution LCMS workstation software.

The acquisition of mass spectral data was carried out using ESI inlet conditions as follows: nitrogen for nebulizing and drying, flow rate 2.5 and 12.0 L min^{-1}, respectively; interface voltage 4.5 kV; desolvation line temperature and heat block temperature was 230°C for both. The mass spectrometer was operated in multiple reaction monitoring mode (MRM) with argon as the collision induced dissociation gas (CID) at a pressure of 17 kPa; the detector voltage was set to 1.64 kV and ESI position at +1.2 mm. Each sample was prepared at 10 mg L^{-1} in methanol solution.

Other data from isolation and characterization of the alkaloids are described in the supplementary material.

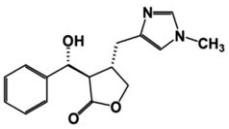
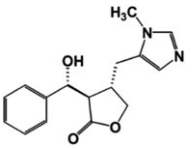
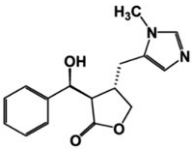
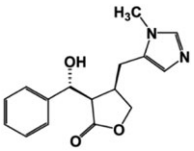
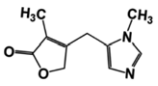
Antibacterial activity assay

To evaluate the antibacterial properties of the alkaloids, five bacterial strains were selected: *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228 and *Enterococcus faecalis* ATCC 29212 (Gram-positives); *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (Gram-negatives). The minimum inhibition concentration (MIC) was established following CLSI, (2016) with modifications, using 96-well microdilution plates where the strains were exposed to two-fold dilution series of the alkaloids with concentrations that varied from 50 to 400 μg mL^{-1}. Standard antibiotics, meropenen (Invitrogen, São Paulo, SP, Brazil) and oxacillin (Invitrogen, São Paulo, SP, Brazil), were also used as control. The MIC was defined as the lowest concentration of agent that inhibited growth to less than 0.05 at 600 nm (no growth visible).

Anthelmintic assay

The life cycle of *S. mansoni* (BH strain) was maintained in *Biomphalaria glabrata* snails and *Mesocricetus auratus* hamsters at the Adolf Lutz Institute (São Paulo, Brazil), according to standard procedures of our laboratory (Moraes, 2012; de Moraes *et al*., 2014). All animal

Table 1. Summary of available structural and biological information about the studied in this work

Name	Structure	Structural data	Biological activities
Episiopiloturine (EPI)		X-ray and NMR previously studied (Veras et al., 2013)	*In vitro* (Veras *et al.*, 2012) and *in vivo* (Guimaraes *et al.*, 2015) anti-*S. mansoni* previously studied. Antibacterial activity determined (this work).
Epiisopilosine (EPIIS)		NMR studies in this study to confirm the structure for further biological assays (this work).	*In vitro* anti-*S. mansoni* and antibacterial activity determined (this work).
Isopilosine (ISO)		NMR studies in this study to confirm the structure for further biological assays (this work).	*In vitro* anti-*S. mansoni* and antibacterial activity determined (this work).
Pilosine (PILO)		NMR studies in this study to confirm the structure for further biological assays (this work).	*In vitro* anti-*S. mansoni* and antibacterial activity determined (this work).
Macaubine (MAC)		NMR and X-ray studies to determine the structure (this work).	*In vitro* anti-*S. mansoni* and antibacterial activity determined (this work).

experiments were conducted in accordance with the Ethics Committee at the Instituto Adolfo Lutz, SP, Brazil (approval number 01/15). For schistosome preparation and culture, 49-day-old adult worms were selected and, after washing in RPMI 1640 medium, were kept at pH 7.5 with HEPES 20 mM, supplemented with 10% fetal bovine serum (GibcoBRL), 200 μg mL^{-1} streptomycin and 200 IU mL^{-1} penicillin (Invitrogen, São Paulo, SP, Brazil). After washing, one pair of adult worms was transferred to each well of a 24-well culture plate (TPP, St. Louis, MO, USA) in the same medium and maintained at 37°C in 5% CO_2.

The assays of *S. mansoni* performed *in vitro* were made as described previously (Veras *et al.*, 2013; de Moraes *et al.*, 2015). Solutions of the alkaloids were prepared in dimethyl sulfoxide (DMSO) or ethanol (0.5% final concentration) and used at concentrations varying from 1.5625 to 500 μg mL^{-1}. Each well held a final volume of 2 mL. The assay of the negative control group of worms was made in RPMI 1640 medium and RPMI 1640 with 0.5% DMSO or ethanol and that of the positive control group was made in 5 μg mL^{-1} praziquantel (Merck, São Paulo, Brazil). The parasites were kept for 120 h and monitored every 24 h. The drug's effect was observed under an inverted microscope (Nikon, Melville, NY, USA), observing motor activity changes in the worm, morphological/tegmental changes and mortality rate. Morphological tegument changes in adult parasites were observed after the *in vitro* assay, with a confocal laser scanning microscope, following described standard procedures (Moraes, 2012). After the drug treatment (120 h) or in case of death, parasites were fixed in FAA (formalin-acetic acid-alcohol solution) and examined with a confocal microscope (laser scanning microscope, LSM 510 META, Carl Zeiss, Standorf Göttingen, Vertrieb, Germany) (Moraes *et al.*, 2009). LC_{50} values were calculated by fitting the data to a sigmoidal dose–response curve using the GRAPHPAD PRISM (version 5.0) software (Graphpad, San Diego, CA, USA) (De Moraes *et al.*, 2015).

Cytotoxicity assay

The vero mammalian cells assay (African green monkey kidney fibroblast) used were from the American Type Culture Collection (ATCC CCL-81; Manassas VA); they were kindly provided by Ronaldo Z. Mendonça (Laboratory of Parasitology, Butantan Institute, São Paulo, Brazil). Cytotoxicity was measured using the crystal violet method as previously described (Silva *et al.*, 2015). Vero cells [kept in Dulbecco Minimum Essential Medium (Gibco BRL) with 10% heat-inactivated calf serum supplements] were seeded into 96-well culture microplates (Nalge Nunc International) at a density of 5×10^6 cells per milliliter. Control cells, and/or cells treated with different concentrations of EPIIS, were incubated at 37°C in 5% CO_2. After incubating for 2, 24, 48, 72 and 96 h, the supernatant was discarded, and the remaining living cells assessed by fixing and staining with crystal violet (0.2% in 20% methanol). Absorbance was measured by reading each well at 595 nm in a microplate reader (Quick Elisa, São José do Rio Preto, SP, Brazil).

RESULTS AND DISCUSSION

Isolation and characterization

Although four alkaloids have the same mass weight (286 Da), it was possible to combine HPLC, MS and nuclear magnetic resonance (NMR) techniques to identify each one. For MAC–[3-methyl-4-[(1-methyl-1H-

imidazol-5-yl)methyl]dihydrofuran-2(3H)-one (Fig. 1), the HPLC retention time (RT) was 9.680 min. The MRM analysis showed a pseudo molecular ion with *m/z* 192 Da $[M + H]^+$, and MS^2 transitions were *m/z* 96 and 83 Da. Pilocarpine had an RT of 14.393 min, its *m/z* ion was confirmed as 209 Da $[M + H]^+$ (data not shown), and in addition, its chemical structure was according as previously described (Van de Merbel *et al*., 1998). EPI–[(3S,4R)-3-[(S)-hydroxy(phenyl) methyl]-4-[(1-methylimidazol-4-yl)methyl]oxolan-2-one] had 15.623 min as RT, and the *m/z* was 287 Da $[M + H]^+$; these results are in accordance with the work of Veras *et al*. (2013) (data not shown). EPIIS–[3S-[3alpha(S*),4beta]-dihydro-3-(beta-hydroxy benzyl)-4-[(1-methyl-1H-imidazol-5 yl)methyl]furan-2(3H)-one, ISO–[3S-[3alpha(S*),4beta]-dihydro-3-(alpha-hydroxybenzyl)-4-[(1-methyl-1H-imidazol-5-yl) methyl]furan-2(3H)-one and PILO–[3R-[3alpha(R*), 4alpha]]-dihydro-3-(alpha-hydroxybenzyl)-4-[(1-methyl-1H-imidazol-5-yl)methyl]furan-2(3H)-one; for ISO and PILO, the identification was possible by HPLC through different RT, 17.816 and 19.245 min, respectively. For EPI and EPIIS, the identification was possible through their transitions obtained by MRM as 269 and 181 Da, respectively (Voigtlander *et al*., 1978).

The alkaloids EPI, ISO, PILO and MAC were characterized using NMR structural analysis, and the structure of MAC determined using X-ray diffraction. These data represent the first description of the structure of Macaubine, as well as the first NMR structural studies fully elucidating the structures of epiisopilosine, isopilosine and pilosine. The results are described thoroughly in the supplementary materials.

Antibacterial test

None of the alkaloids showed antibacterial activity at tested concentrations. Standard antibiotics meropenen and oxacilin showed MIC of <0.5. Some studies described antibacterial activity with values between 300 and 550 μg mL^{-1} using alkaloids extracted from *Holarrhena pubescens* (L.) Wall. (Apocynaceae) and *Bocconia arborea* Wat. (Papaveraceae) against Gram-negative strains such as *E. coli* and *P. aeruginosa*

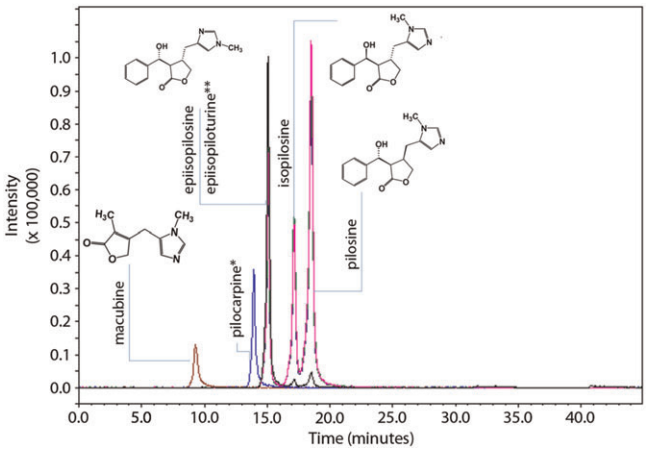


Figure 1. Chromatogram of alkaloids from jaborandi leaves with their chemical structures. Asterisk (*) indicates previously described compound, and chemical structure is not shown. [Colour figure can be viewed at wileyonlinelibrary.com]

(Navarro and Delgado, 1999). Other studies (An *et al*., 2011) found MICs values well below 100 μg mL^{-1} using alkaloids isolated from *Scutia buxifolia* Reiss. (Rhamnaceae) and *Hypericum japonicum* Thunb. ex Murray (Guttiferae), respectively, against various bacterial strains, such as *S. epidermidis*, *S. aureus* and *E. faecalis*. Despite the absence of antibacterial activity of the alkaloids tested, future assessments to evaluate their activity against other microorganisms such as fungi could be performed, because other work has shown that the presence of imidazole rings in isolated compounds can confer antifungal activity (Zhang *et al*., 2010).

Anthelmintic assays

The five tested alkaloids are structurally similar (Table 1), and all alkaloids were successfully isolated by HPLC with a purity above 98%. For EPI, it has previously been shown to possess activity against the flat worm *S. mansoni* (Veras *et al*., 2013). Despite displaying similar chemical structures, the alkaloids presented very different biological activity (Table 2).

The effect of the alkaloids on adult worms viability was studied *in vitro* by varying time of incubation and alkaloid concentration (Table 2). The effect of *in vitro* EPIIS treatment on *S. mansoni* showed 100% mortality of adult worms after 96 h incubation at a concentration of 3.125 μg mL^{-1} (Table 2). The worms remained viable in the negative control group (RPMI medium with 0.5% ethanol), while in the positive control group, with 5 μg mL^{-1} praziquantel, all parasites died within 24 h. No changes in motor activity or alterations to the tegument in the negative control group (0.5% ethanol) were observed, while in the positive praziquantel control, changes occurred in 100% of the individual worms.

When adult worms were incubated with doses of EPIIS above 1.5625 μg mL^{-1}, the male and female worms remained separate, the interruption of the mating process and oviposition was observed clearly, and there was a slightly decreased motor activity and changes to the tegument. The life cycle of *Schistosoma* is maintained, and the parasite is transmitted to other hosts as a direct consequence of the oviposition process. Moreover, the pathology effects of schistosomiasis are not the directly result of adult worms activity but are due to the large numbers of eggs that accumulate in the host tissues (De Moraes *et al*., 2013a). Thus, the results demonstrating interrupted oviposition would have a huge importance in this biological aspect of schistosomiasis. The obtained selectivity ratios concerning LC_{50} for EPIIS, in relation to anthelmintic assay in 24, 72 and 120 h are 9.38, 5.01 and 2.34 μM, respectively.

The anthelmintic effect of the alkaloid EPIIS was 100 times greater than that reported previously where the effect of the alkaloid EPI against *S. mansoni* was found to be 300 μg mL^{-1} (Veras *et al*., 2013). Antischistosomal activity has been described in the literature for various isolated molecules: the monoterpenes carvacryl acetate at ~30 μM (de Moraes *et al*., 2013a), 3,7-dimethyl-1-octanol at ~20 μM (Mafud *et al*., 2016) and (+)− limonene epoxide at ~165 μM (de Moraes *et al*., 2013b); the sesquiterpene nerolidol at ~30 μM (Silva *et al*., 2014); the diterpene alcohol phytol at ~170 μM (de Moraes *et al*., 2014); the alkaloids piperamide 1 at ~170 μM (Carrara *et al*., 2014) and piplartine at ~10 μM (Moraes

Table 2. ***In vitro*** **effects of epiisopilosine against** ***Schistosoma mansoni*** **adult worms**

Group	Period of incubation (h)	Dead worms (%)[a]	Motor activity reduction (%)[a]		Worms with tegumental alterations (%)[a]	
			Slight	Significant	Partial	Extensive
Control[b]	24	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
	96	0	0	0	0	0
	120	0	0	0	0	0
0.5% ethanol	24	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
	96	0	0	0	0	0
	120	0	0	0	0	0
Praziquantel 5 μg mL^{-1}	24	100	0	100	0	100
	48	100	0	100	0	100
	72	100	0	100	0	100
	96	100	0	100	0	100
	120	100	0	100	0	100
Epiisopilosine 1.5625 μg mL^{-1}	24	0	0	0	0	0
	48	0	0	0	0	0
	72	0	10	0	0	0
	96	0	40	0	40	0
	120	0	70	0	70	0
Epiisopilosine 3.125 μg mL^{-1}	24	0	50	0	0	0
	48	0	100	0	0	0
	72	30	0	100	70	30
	96	100	0	100	0	100
	120	100	0	100	0	100
Epiisopilosine 6.25 μg mL^{-1}	24	0	0	100	100	0
	48	40	0	100	60	40
	72	100	0	100	0	100
	96	100	0	100	0	100
	120	100	0	100	0	100
Epiisopilosine 12.5 μg mL^{-1}	24	100	0	100	0	100
	48	100	0	100	0	100
	72	100	0	100	0	100
	96	100	0	100	0	100
	120	100	0	100	0	100
Epiisopilosine 25 μg mL^{-1}	24	100	0	100	0	100
	48	100	0	100	0	100
	72	100	0	100	0	100
	96	100	0	100	0	100
	120	100	0	100	0	100

[a]Percentage relative to the total number of worms in each group (20).
[b]RPMI 1640.

et al., 2011; de Moraes *et al.*, 2012); the chalcone cardamonin at ~25 (de Castro *et al.*, 2015); and for the cationic antimicrobial peptide dermaseptin 01 at ~15 μM (de Moraes *et al.*, 2011). In comparison with these results, the alkaloid EPIIS at only 3.125 μg mL^{-1} (~10 μM) showed a strong effect against *S. mansoni*, and further investigation of this molecule as an anthelmintic agent is clearly desirable.

The assays for the alkaloid ISOP showed that there was an effect on the parasites at 500 μg mL^{-1} (data not show) with worm mortality, reduction of motor activity and changes in the tegument beginning after 96 h of incubation. The parasites remained attached, and no significant difference was observed in egg production, compared with the control group. The assay for the alkaloid MAC (data not shown) showed no significant effect against *S. mansoni* at the concentration tested. No significant difference was observed between the three tested alkaloids in mortality rate between male and female parasites.

Since EPIIS was the most promising alkaloid, a more detailed analysis on the effect of this compound on worm teguments was carried out using confocal microscopy. As shown in Fig. 2, compared with control worms, incubated only in culture medium, EPIIS caused morphological changes in the worms' tegument. Blebbing was visible on the helminth tegument, and this parameter is considered an indicator for stress, as described in previous studies (De Moraes *et al.*, 2013a). Furthermore, swelling was visible on the tegument of *Schistosoma*. The cause of such marked changes in the tegument of the worms is still unknown. It may be

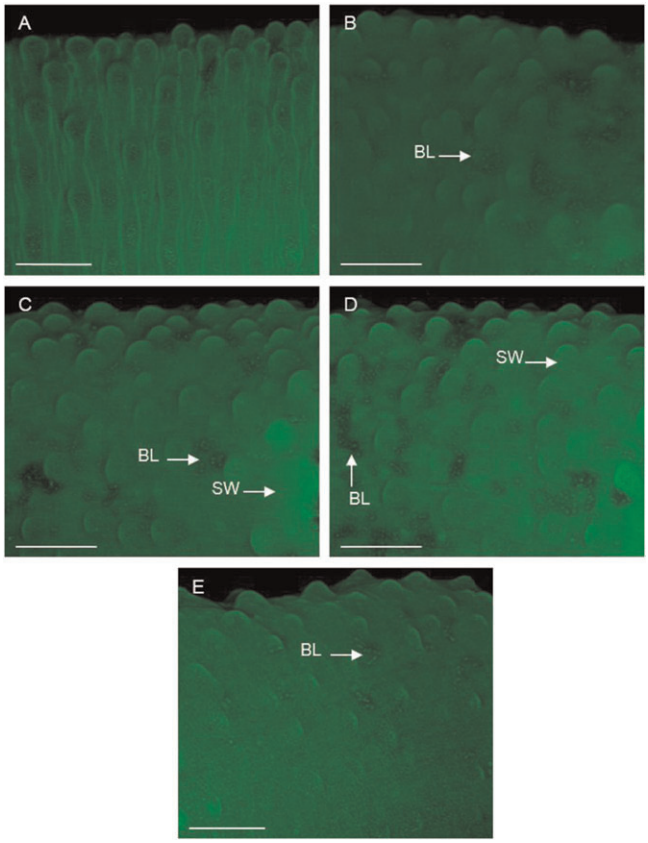


Figure 2. Confocal laser scanning microscopy investigations of the *in vitro* schistosomicidal effect of epiisopilosine. In these experiments, pairs of adult worms were incubated in culture plates and treated with different concentrations of epiisopilosine. After 120 h of incubation or in the case of death, adult male worms were fixed in FAA solution and the fluorescent images were obtained using confocal microscopy: (A) negative control worms; (B) worms treated with 3.125 μg mL^{-1} of epiisopilosine; (C) 6.25 μg mL^{-1} of epiisopilosine; (D) 12.5 μg mL^{-1} of epiisopilosine; (E) positive control worms (praziquantel 5 μg mL^{-1}) blebbing (BL) and swelling (SW) (arrows) on the dorsal mid-body tegument of a worm are visible. Scale bars, 50 μm. [Colour figure can be viewed at wileyonlinelibrary.com]

suggested that the mechanism of damage occurs from the inside-out in this study.

Although the antibacterial effect shown by these imidazole alkaloids was low, the alkaloid EPIIS was observed to have a strong anthelmintic activity. One reason that could explain this result might be the differences in composition and structure of the external surface of the organisms. The adult worms do not have a true epidermis, but are instead covered by a non-cellular cuticle produced by mesenchyme cells (Skelly and Alan, 2006). Bacteria, on the other hand, have a peptidoglycan cell wall and a lipoprotein plasma membrane (Bruner, 1993), which gives these organisms greater resistance to alkaloids despite being much smaller (0.2 to 5 μm), than the *S. mansoni* worms, in which adult females can attain up to 1.5 cm in length.

Cytotoxicity assay

Monkey kidney fibroblasts (vero cells) were incubated EPIIS at concentrations lethal to *S. mansoni* adult worms (1.5–12.5 μg mL^{-1}), in order to discover whether EPIIS is tolerated by mammalian cells.

After EPIIS treatment at the maximum dose, no cytotoxicity was detected in vero cells tested. Three independent experiments in triplicate were carried out, exposing cells to EPIIS at concentrations of 1.562, 3.125, 6.25 and 12.5 μg mL^{-1} for 2, 24 or 48 h. These treatments had no detectable effects on their viability, because a viability >95% has been observed. Similarly, PZQ treatment at the same concentrations failed to produce cytotoxicity (viability >95%). Schistosomicidal activity of EPIIS *in vitro* was not linked to cytotoxic effects in mammalian cells. No cytotoxic effect was observed for EPI (Veras *et al*., 2013), another alkaloid of *P. microphyllus* with a structure similar to EPIIS.

CONCLUSION

The structural characterization of the alkaloids MAC, PILO, ISOP and EPIIS isolated from *P. microphyllus* leaves was satisfactory; HPLC, MS and NMR techniques were combined to identify each one. The results showed that these molecules are not effective as antibacterial agents. On the other hand, the imidazole alkaloid EPIIS showed potent *in vitro* anthelmintic activity at low concentrations and may be considered a potential alternative for treating infections caused by *S. mansoni*. These promising anthelmintic results for EPIIS may open new avenues for research in this area, for example, *in vivo* assays could be performed, or testing against other Platyhelminth species.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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