the characteristic proton of –NH group of lactam, all the spectral data were similar to those of aristolactam BII described in the literature.[21,27,32] All the above information and data previously mentioned in the literature allowed us to identify the compound 9 as aristolactam BII acetylate (10-acetylamino-3, 4-dimethoxyphenanthrene-1-carboxylic acid lactam): a new derivative of aristolactam BII described for the first time.

The compound 9 together with compounds 4, 5, 6, 7, and 8 was evaluated for their antischistosomal (antiparasitic and/or enzymatic) and/or cytotoxic activities.

Antiparasitic assay *in vitro*

The extract and compounds 4, 5, 6, 8 and 9 were tested for their antischistosomal activity against *S. mansoni*. The stem bark extract showed interesting worm-killing capacity at concentration of 100 µg/mL with parasite rate survival of 81.8%. In addition, all compounds were less efficient than praziquantel (positive control) except gallic acid 5, which exhibited same activity as the positive control after 6 h at the concentration of 100 µM [Table 1]. To the best of our knowledge, this is the first time that gallic acid showed to be a potent antagonist against *S. mansoni*; on the contrary, piperolactam D 8 showed no activity at this concentration. On the other hand, the new compound aristolactam BII acetylate 9 displayed weak worm-killing capacity with a rate of 40% at the concentration of 100 µM [Figure 3]. Meanwhile, the study of structure–activity relationship of lactam compounds revealed that the most active was 6 at the concentration of 100 µM. This activity decreased when the hydroxyl group borne by carbon C-4 of 6 was replaced by a methoxyl (compound 4) and when carbon C-2 was substituted by a hydroxyl group in 4. However, an increase in activity was observed upon acetylation of the lactam nitrogen in 6 (compound 9). All compounds, for which an antiparasitic activity had been determined, were further subjected to enzymatic and cytotoxic assays.

Enzymatic assay *in vitro*

In 2010,[7] *Sm*NACE was used as a model in the context of screening for inhibitors, comprising thousands of molecules including natural products, using a high-throughput screening assay easily applicable in the laboratory. The high-throughput screening revealed a more or less marked inhibitory effect of natural products on *Sm*NACE (although cyanidin proved to be the best, but no specific). It was therefore interesting to test our extract and compounds on the activity of *Sm*NACE without presuming their action.

Results of enzymatic activity of some compounds are presented in Table 2. The stem bark extract was active on *Sm*NACE with an inhibition rate of 51.08% at 100 µg/mL. For isolated compounds, we noticed that only piperolactam D 8 presented a comparable activity to that of the reference *Sm*NACE inhibitors cyanidin and delphinidin, while the rest of evaluated molecules were shown to possess less IC_{50} values.

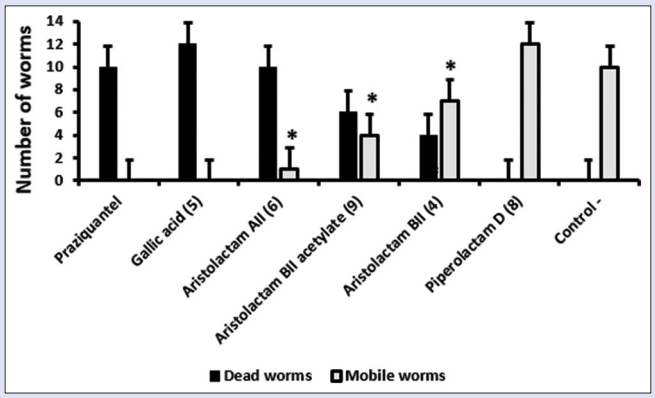

Figure 3: Survival of *Schistosoma mansoni* adult worms treated with the praziquantel (positive control). Worms were treated with compounds 4, 5, 6, 8, and 9 at 100 µM. Each test was performed in duplicate or triplicate. Parasites were subsequently observed for body contractility and movement each hour for 6 h. *$P < 0.05$

Cytotoxicity assay

The cytotoxicity assay was carried out with the same subset of extract and compounds (4, 5, 6 and 8) against Huh7 and A549 cells line. The human lung cancer (A549) and the human hepatocarcinoma (Huh7) cells line were chosen because lungs and liver are the focal points of pathogenic insult and subsequent pathological damage in schistosomiasis.[33] The results are consigned in Figure 4.

It showed that stem bark extract (AME) was not tested on Huh7 cells but showed toxicity on A549 cells line (with a percentage of cell viability of 37%). For isolated compounds, their percentage of cell viability on

Table 1: *In vitro* evaluation of the antischistosomal activity of compounds 1, 2, 3, 5, 8, and 10 at various concentrations; unless otherwise indicated, experiments were done in triplicates; for each experiment, 10-12 worms were used with equilibrated sex ratio

Extract/compound (number)	Concentration (µM)	Mobile worm after 6 h of incubation (%)
Stem bark of *Anonidium manniia*	100	81.8
Gallic acid (5)	100*	0
	50*	53.6
	10*	92
Aristolactam AII (6)	100	9.1
	50	66.7
	10	71
Aristolactam BII (4)	100	62.5
	50	77.3
	10	94.4
Aristolactam BII acetylate (9)	100	40
	50	95
	10	100
Piperolactam D (8)	100	100
	50	Not done
	10	Not done
Praziquantel	100	0
	50	7
	10	Not done
Control (RPMI)	-	100

*Experiments were performed in duplicates, [a]Concentration expressed in µg/mL. RPMI: Roswell Park Memorial Institute Medium

Table 2: IC_{50} values for the inhibition of *Schistosoma mansoni* nicotinamide adenine dinucleotide+ catabolizing enzyme by compounds

Compound (number)	IC_{50} (µM)
Aristolactam BII acetylate (9)	NT
Aristolactam AII (6)	>100
Aristolactam BII (4)	>100
Gallic acid (5)	>100
Piperolactam D (8)	10-20
Cyanidin*	2.3
Delphinidin*	6.0

*Cyanidin and Delphinidin are the natural products reference *Sm*NACE. NAD+: Nicotinamide adenine dinucleotide; *Sm*NACE: *Schistosoma mansoni* NAD+ catabolizing enzyme; NT: Not Tested

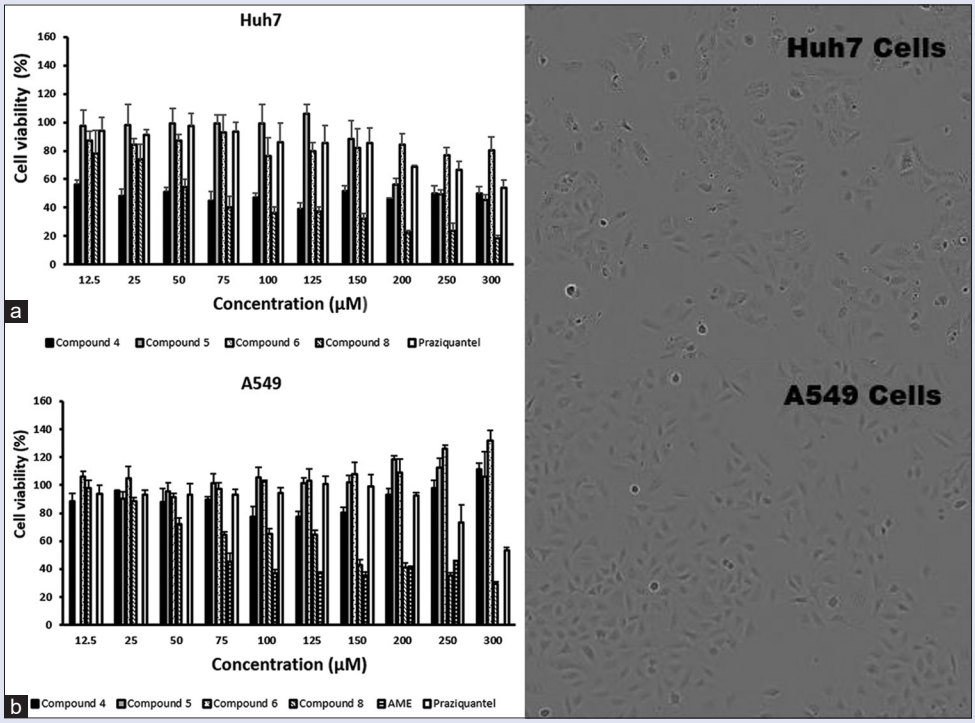

Figure 4: The cell viability rate of AME, compounds 4, 5, 6, 8 and praziquantel assayed by MTS. Notes: These two cell lines were continuously treated with different concentrations (12.5 μM, 25 μM, 50 μM, 75 μM, 100 μM, 125 μM, 150 μM, 200 μM, 250 μM, 300 μM) of AME, compounds 4, 5, 6, 8 and praziquantel for 48 h. (a) Huh7 cell line and (b) A549 cell line. Cell viability was then determined by MTS assay

Huh7 cells were 47% for 4, 99% for 5, 76% for 6, and 36% for 8 compared to reference drug (praziquantel, 86%). This revealed that gallic acid 5, which was a good antagonist against *S. mansoni*, did not exhibit toxicity on Huh7 cells line contrary to praziquantel (reference drug). Similarly, on A549 cells line, the percentage of cell viability of the tested compounds was 77% for 4, 100% for 5, 100% for 6, and 65% for 8 compared to drug (praziquantel, 94%). These results showed also that gallic acid 5 exhibited very good viability (100%) on A549 cell line.

CONCLUSION

The chemical investigation of stem bark of *A. mannii* led to the isolation and identification of eight compounds: β-sitosterol 1, stigmasterol 2, polycarpol 3, aristolactam BII 4, gallic acid 5, aristolactam AII 6, epicatechin 7, and piperolactam D 8. At the exception of compound 3, all these compounds were isolated and characterized from the genus *Anonidium* for the first time. Meanwhile, acetylation reactions were carried out on isolated compounds 4 and 6 to afford two semisynthetic derivatives: one known derivative aristolactam AII diacetylate 10 and one new aristolactam BII acetylate 9. Antischistosomal (antiparasitic and enzymatic) activity and cytotoxicity of *A. mannii* extract and some isolated compounds were also evaluated *in vitro* for the first time. As praziquantel (positive control), the only molecule used in Africa to cure schistosomiasis, gallic acid 5 was exhibited very promising worm killing capacity on *S. mansoni* after 6 h at the concentrations of 100 μM (all schistosomes were killed). Meanwhile, enzymatic activity testing on *Sm*NACE revealed that compound 8 showed a significant inhibition with IC_{50} around 10 μM compared to the cyanidin (IC_{50} = 2.3 μM), reference *Sm*NACE inhibitor. Moreover, the stem bark extract of *A. mannii* which was no tested on Huh7 cells showed less toxicity on A549 cells line all tested isolated compounds (4, 5, 6, and 8) showed little or no cytotoxicity activity on Huh7 cells and A549 cells line. Optimization of those two more bioactive compounds (5 and 8) could further improve their selectivity/effectiveness and so could be used as seed for the development of new remedies as well as the standardization of the stem bark extract of *A. mannii* which could be used to improved traditional medicine. In the future, we plan to biologically evaluate those extract and compounds on the two other pathogens species of *Schistosoma*: *S. haematobium* and *S. japonicum*.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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