

# Comparative Molluscicidal and Schistosomicidal Potentiality of Two *Solanum* Species and Its Isolated Glycoalkaloids

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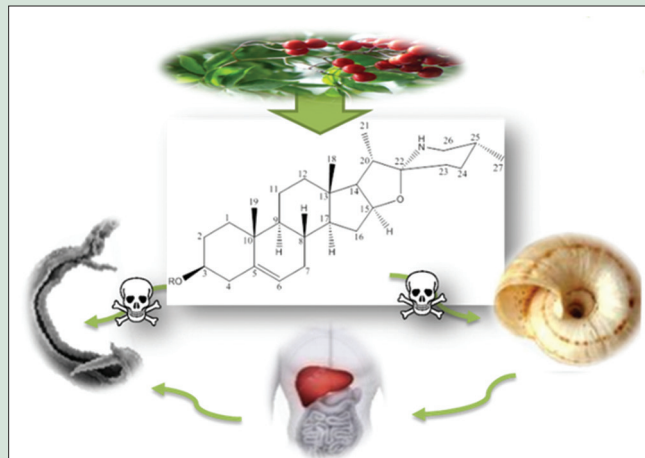
## ABSTRACT

Schistosomiasis is the most noteworthy parasitic disease after malaria. Furthermore, the significant activity of the genus *Solanum* against *Schistosoma* worms and its intermediate host snails reinforced the study of *Solanum seaforthianum* Andr. (SS) and *Solanum macrocarpon* L. (SM) for their molluscicidal and schistosomicidal potentiality. In this study, different extracts, fractions and isolated compounds of both *Solanum* species are evaluated for the molluscicidal and schistosomicidal potentialities. The niclosamide was used as positive molluscicide control against *Biomphalaria alexandrina* snails. Different extracts, fractions, or isolated compounds were used at a concentration of 100 µg/ml and dead snails were counted in each case. On the other hand, washed and sterilized *Schistosoma mansoni* adult worms were used in three replicates, and three worm pairs were placed in each well with 2 ml test solution of 100 µg/ml concentration. Positive (praziquantel [PZQ] 0.2 µg/ml) and negative controls were concurrently used and examined daily for 3 days for viability. The mortality rate was calculated and then both LC<sub>50</sub> and LC<sub>90</sub> were determined in triplicates. Highest potency was indicated to total glycoalkaloid (TGA) fraction of SM followed by TGA of SS. On the other hand, TGA fractions of both species showed higher potency than other extracts and isolated compounds. Meanwhile, solasodine-free aglycone showed declined activity compared to its glycosides. Promising molluscicidal and schistosomicidal activities were displayed which are attributed to the glycoalkaloid content. Therefore, this study can efficiently contribute toward validation of the traditional use of SS and SM in schistosomiasis control.

**Key words:** *Solanum seaforthianum*, macrocarpon, molluscicidal, schistosomicidal, glycoalkaloids, solamargine

## SUMMARY

- The current study evaluated the molluscicidal and schistosomicidal activities of different extracts and fractions of two *Solanum* species. The glycoalkaloids content depicted a promising activity against both the snails and the adult worms.



**Abbreviations Used:** PZQ; Praziquantel, SM; *Solanum macrocarpon*, SS; *Solanum seaforthianum*, TGA; total glycoalkaloid.

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## INTRODUCTION

Schistosomiasis is a parasitic infection caused by genus *Schistosoma* flatworms that affect 200 million people in diverse countries<sup>[1,2]</sup> while about 779 million people worldwide were at risk of infection.<sup>[3-5]</sup> It is claimed to be one of the most substantial mistreated diseases, with huge public health and economic consequences.<sup>[6]</sup> Among the infectious diseases of the tropical countries, schistosomiasis is well-thought-out as the second most significant parasitic disease after malaria.<sup>[7]</sup> Molluscicides use to exterminate the snail vector, which in turn disrupts the parasite life cycle, as a trial to spot the infection transmission, is the method of choice to eradicate schistosomiasis.<sup>[8]</sup> In poor countries, schistosomiasis is widely spread, so the snails control seemed practical and cost-effective procedure. On the other hand, synthetic molluscicides had been extensively used to control of vector snails effectively.<sup>[9,10]</sup> However, these molluscicides are considered harmful and nonspecific, especially to nontarget animals, and may have long-standing unfavorable effects on the aquatic environment.<sup>[11]</sup> That is why safer strategies are to be implemented to control snail populations.

PZQ is the drug of choice against all species of *Schistosoma*, with high efficacy and relative safety. However, it failed to prevent reinfection and is inactive against young schistosomes.<sup>[5]</sup> The developed schistosome-resistant strains reinforced the necessity for more effective, safe, biodegradable, and environment-friendly schistosomicidal drugs.<sup>[3,4]</sup>

Plants represent the oldest and most common medication form as a source of molluscicides and schistosomicidal agents, particularly when

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compared to the synthetic molluscicides in cost and safety.<sup>[8]</sup> Future tactics to control schistosomiasis involved the search for schistosomicidal and molluscicidal compounds from plants and other natural sources<sup>[5,12-18]</sup> which offer novel lead structures for efficient, less toxic, environment-friendly molluscicides, and schistosomicidal agents.

The *Solanum* species distributed all over the world, which are among the leading food plants of the human race with its remarkable biologically active glycoalkaloids content.<sup>[19,20]</sup> The most important of these are potato, eggplant, and tomato. Furthermore, it represented a potential source of molluscicidal and schistosomicidal agents. A significant literature review of genus *Solanum* activity against host snails and worms is summarized in Table 1. This study represents the evaluation of molluscicidal and schistosomicidal activity of *Solanum seaforthianum* Andr. (SS) and *Solanum macrocarpon* L. (SM) cultivated in Egypt. SS [Figure 1] is a flowering evergreen vine of the *Solanum* family native to tropical South America. SM [Figure 2] is a tropical perennial plant known as African eggplant or gboma. Macro- and micro-morphological studies, as well as DNA fingerprinting of both species under study, were also carried out.<sup>[29]</sup> Meanwhile, when reviewing the current literature, no data were found regarding the molluscicidal and schistosomicidal activity of SS and SM.

## MATERIAL AND METHODS

### Plant materials

SS and SM aerial parts used in this study were collected in the flowering stage from the Experimental Station for Aromatic, Medicinal and Toxic plants, Giza, Egypt. The plants were kindly authenticated by Prof. Dr. M. El-Gebaly, Botany Specialist, National Research Center (Dokki, Giza, Egypt). Voucher specimens (23082014 I and II, respectively) were kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

### Extracts and fractions preparation

Air-dried aerial parts powdered samples (1 kg each) of both species were soaked and homogenized in 70% ethanol until complete exhaustion was achieved. The extracts were evaporated to dryness under vacuum using Buchi Rotavapor R-210. Each ethanol extract was successively fractionated, using *n*-hexane, chloroform, ethyl acetate, and *n*-butanol saturated with water. On the other hand, other part of the air-dried

powdered samples (1 kg each) of both species is used to prepare the total glycoalkaloid fraction (TGA). The powder is soaked and homogenized with methanol. Subsequent filtration followed by the solvent elimination under vacuum takes place. The resulting dry extracts were dissolved in 1/2 L of 5% acetic acid thoroughly washed for several times with *n*-hexane. Then, it was extracted with CHCl<sub>3</sub>. Then, it was filtered and adjusted supernatant to 10.5–11.0 pH with NH<sub>4</sub>OH, kept in 70°C water bath for 10 min, and cooled and centrifuged. The residue is air-dried in a desiccator containing anhydrous calcium chloride. Then that, acid–base purification is repeated.<sup>[30]</sup>

Finally, the pure solasodine, solasonine, and solamargine were previously isolated from SS as shown in Figure 3.<sup>[31]</sup>

The different extracts and TGA fractions of both species with the isolated alkaloids were evaluated for molluscicidal and schistosomicidal potentiality.

### Evaluation of molluscicidal activity

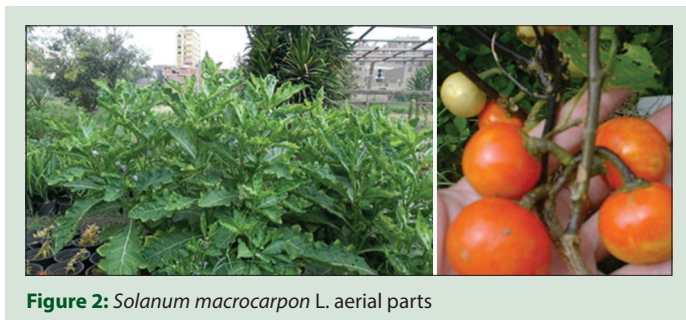
Adult *Biomphalaria alexandrina* (Ehrenberg) (*Planorbidae*) snails were obtained from the Schistosome Biological Supply Center at Theodor Bilharz Research Institute. It is the intermediate host of *Schistosoma mansoni* in Egypt. The potentiality of the plant extracts was mainly determined against the snails using the standard reported method,<sup>[10]</sup> whereas 1000 ml of the dechlorinated water (of 100 ppm concentration) of each compound was prepared followed by the addition of 10 snails. They were maintained in exposure period for 24 h at 25°C ± 1°C. The snails were subsequently washed carefully with dechlorinated water



**Figure 1:** *Solanum seaforthianum* Andr. aerial parts showing leaves, flowers, and fruits

**Table 1:** Molluscicidal and schistosomicidal activities of the genus *Solanum*

Activity	Plant names, parts and/or extracts	Notes	References
Molluscicidal	The methanolic extract of the fresh root bark and berries of <i>Solanum aculeastrum</i> Dun.	100% mortality at 20 ppm was indicated	[21]
	The root bark and berries of <i>Solanum aculeastrum</i> Dun.		
	<i>Solanum americanum</i> Miller	The screening for for Molluscicidal compounds led to isolation of solaculine A, solamargine and beta-solamarine	[22]
		The molluscicidal activity against intermediate host of <i>Schistosoma mansoni</i> was studied and 33% of the <i>Biomphalaria glabrata</i> snails were killed using 50 ppm extract	[23]
	<i>Solanum xanthocarpum</i> Schrad.	The extract had a significant effect on mature and young snails of the amphibious Asian freshwater and also on mature specimens of the snails	[24]
	The ethanol extract of <i>Solanum nigrum</i> L.	Molluscicidal activity seems to be directly proportional with the increase of temperature. Where sunlight, pH, and turbidity did not affect the activity of this extract	[25]
Schistosomicidal	The Glyco-alkaloid extracts from seeds and leaves of <i>Solanum sodomaicum</i> L. and berries of <i>Solanum elaeagnifolium</i> Cav.	The molluscicidal activity against <i>Bulinus truncates</i> was indicated	[26]
	The glyco-alkaloid mixture obtained from <i>Solanum mammosum</i> L. fruits	Revealed to be toxic to <i>Lymnaea cubensis</i> and <i>Biomphalaria glabratus</i> . The molluscicidal properties depend on the type of aglycones and on the glycoside bond	[27]
	The alkaloidal extract of <i>Solanum lycocarpum</i> fruits and its isolated steroidal alkaloids	Promising schistosomicidal activities against adult worms of <i>Schistosoma mansoni</i>	[28]



**Figure 2:** *Solanum macrocarpon* L. aerial parts

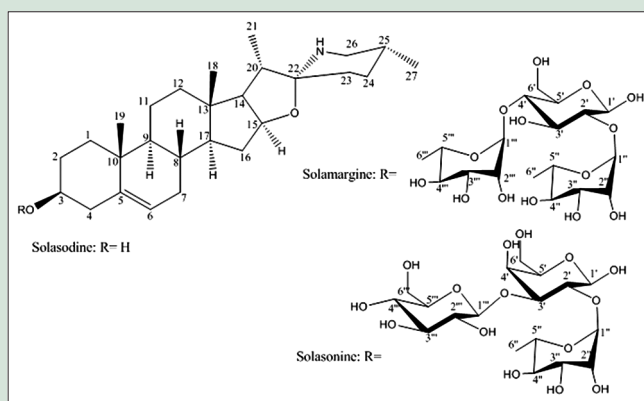
and maintained in freshwater for another 24 h for recovery. Three replicates were out and two groups of snails were used as negative control, whereas niclosamide (Sigma-Aldrich, USA) was used as positive control molluscicides. Dead snails were counted in each case. For LC determination of extract presented, a molluscicidal activity was retested by the same method using descending concentrations, and  $LC_{50}$  and  $LC_{90}$  were determined by IBM SPSS Statistics for Windows, Version 20 (Armonk, New York: IBM Corp.).

### Evaluation of schistosomicidal activity

The schistosomicidal effect of each plant was achieved in accordance with the reported method.<sup>[32]</sup> Thus, the fresh adult worms were obtained by perfusion from infected hamsters 7 weeks earlier. Worms were cleaned from blood in small sieves 20- $\mu$  mesh size using phosphate buffer. Then, they were quickly washed in the culture medium for more sterilization inside a sterilized laminar flow. A stock solution (500  $\mu$ g/ml) of each plant extract was prepared in dimethyl sulfoxide (DMSO) and then diluted with RPMI 1640 to produce 2 ml test solution of 100  $\mu$ g/ml final concentration. The culture medium used was PRMI 1640 containing 20% fetal calf serum, 300 mg streptomycin, 300 units penicillin, and 160  $\mu$ g gentamycin/100 ml medium. The worms were exposed to this concentration in sterilized tissue culture plates, 24 wells. Three replicates were used and three pairs of *Schistosoma* worms males and females equally represented were placed in each well using sterilized forceps. Positive and negative controls were concurrently used. The reference drug PZQ (Sigma-Aldrich, USA) 0.2  $\mu$ g/ml was used as the positive control. Tests and control wells were kept in an incubator at 37°C, examined daily for 3 days for worm viability using a stereomicroscope. Worms which did not show any sign of motility for 1 min were considered dead. The activity of the plant extract was measured by calculating the number of dead worms relative to the total number of worms and compared with the negative (DMSO) and positive (PZQ) controls. For determination of  $LC_{50}$  and  $LC_{90}$ , the same experiment was reported several times using several descending concentrations of the extract and the viability of worms was followed-up for 3 days. The worm mortality was recorded in each case, and the  $LC_{50}$  and  $LC_{90}$  were determined using IBM SPSS Statistics for Windows, Version 20 (Armonk, New York: IBM Corp.).

## RESULTS AND DISCUSSION

Percentage yield and organoleptic characters of the different extracts and fractions of the aerial parts of both *Solanum* species under study are listed in Table 2. The TGA percentage of 3.5 and 3.5 for SS and SM, respectively is indicated. Among different extracts and fractions, the highest molluscicidal potency is noticed for the TGA fraction of SM followed by the TGA fraction of SS ( $LC_{50}$  = 7.5 and 18.8 ppm, respectively) in comparison with niclosamide as positive control. On the other hand, the TGA fractions of both species show higher potency followed by *n*-butanol fractions, whereas the ethanol extracts show the lowest potency which is emphasizing the molluscicidal activity of the



**Figure 3:** Structures of the isolated solamargine, solasonine, and solasodine

glycoalkaloids which could be allocated in *n*-butanol fractions due to its polarity. The solamargine is the most potent isolated molluscicide followed by solasonine. The lowest potency is indicated for the free aglycone solasodine [Table 3 and Figure 4]. A result which is in agreement with the molluscicidal activity reported to solamargine isolated from *Solanum sisymbriifolium* against *Biomphalaria glabrata*.<sup>[33,28]</sup> Furthermore, A significant molluscicidal effect was indicated for various glycoalkaloids of *Solanum aculeastrum*<sup>[22]</sup> and *Solanum asperum*,<sup>[34]</sup> especially for the solamargine.

The highest schistosomicidal potency is noticed for the TGA fraction of SM followed by the TGA of SS ( $LC_{50}$  = 7.6 and 8.3 ppm, respectively) in comparison with PZQ as positive control. The inclined schistosomicidal activity of TGA fractions of both species augments the activity correlation to the total glycoalkaloid content. Moreover, the declined potency of solasodine aglycone versus the solamargine and solasonine glycosides [Table 4 and Figure 4] reinforces the importance of trisaccharide moiety as crucial part for the schistosomicidal activity. The Synergism between different types of glycoalkaloids of different *Solanum* species was observed for the cytotoxicity assay,<sup>[35]</sup> antifungal activity,<sup>[36]</sup> and schistosomicidal activity.<sup>[28]</sup> The declined schistosomicidal and molluscicidal activities of TGA fractions versus the individual glycoalkaloids which is contradictory with the concept of synergism may be attributed to the aglycone abundance and the hydrolysis of the glycosidic linkage of the glycoalkaloids. *Solanum* glycoalkaloids mechanism of action against schistosomes may be attributed basically to two features: its capability to bind the cell membrane components which in turn caused integrity and function disturbance of the cell membrane or by its inhibitory action to acetylcholinesterase enzyme.<sup>[37]</sup> The glycoalkaloids containing the chitotriose trisaccharide, as solamargine [Figure 3], are generally more active than alkaloids containing the solatriose trisaccharide, such as solasonine regarding the disruption of integrity and functionality of the cell membranes and acetylcholinesterase inhibition.<sup>[38]</sup>

Some of these aforementioned characteristics of the glycoalkaloids might subsidize the inhibition caused to adult worms of *S. mansoni*, on the other hand, it was concluded that the sugar moiety is essential for schistosomicidal activity as per solasodine did not kill the parasitic worms *in vitro* under these experimental conditions, which is in agreement with results gained formerly using *Solanum lycopersum*.<sup>[28]</sup>

## CONCLUSION

The data represented in this study showed that the TGA fraction of both SS and SM alongside with the isolated glycoalkaloids (solamargine and solasonine) displayed promising molluscicidal and schistosomicidal

**Table 2:** Percentage yield and organoleptic characters of the solvent extracts and fractions of the aerial parts of *Solanum seforthianum* Andr. and *Schistosoma mansoni* L.

Extractives	Percentage yield	Color	Taste	Odor
SS				
Ethanol (70%)	10.4	Dark green	NC	Faint
<i>n</i> -hexane	3.77	Dark green	Waxy	Faint
Chloroform	0.2	Dark green	NC	NC
Ethyl acetate	0.2	Brown	NC	NC
<i>n</i> -Butanol	3.58	Brown	NC	NC
TGA	3.4	Brown	NC	NC
SM				
Ethanol (70%)	12.35	Dark green	NC	Faint
<i>n</i> -hexane	3.5	Dark green	Waxy	Faint
Chloroform	0.3	Dark green	NC	NC
Ethyl acetate	0.4	Brown	NC	NC
<i>n</i> -Butanol	4.3	Brown	NC	NC
TGA	3.9	Brown	NC	NC

NC: Not characteristic; TGA: Total glycoalkaloid fraction; SM: *Solanum macrocarpon* L.; SS: *Solanum seforthianum* Andr.

**Table 3:** The molluscicidal effect of plant extracts on *Biomphalaria alexandrina* (mean±standard error, n=3)

Plant extract	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
SST	>100	-
SSB	30.4±1.3	46.8±1.9
SS TGA	18.8±0.9	33.5±1.2
SMT	>100	-
SMB	23.9±1.6	36.2±1.8
SM TGA	7.5±0.7	10.6±0.9
Solasodine	45.5±2.9	55.8±2.1
Solasonine	10.1±0.3	14.3±0.7
Solamargine	9.8±0.3	11.9±0.4
Niclosamide	0.2±0.1	0.6±0.2

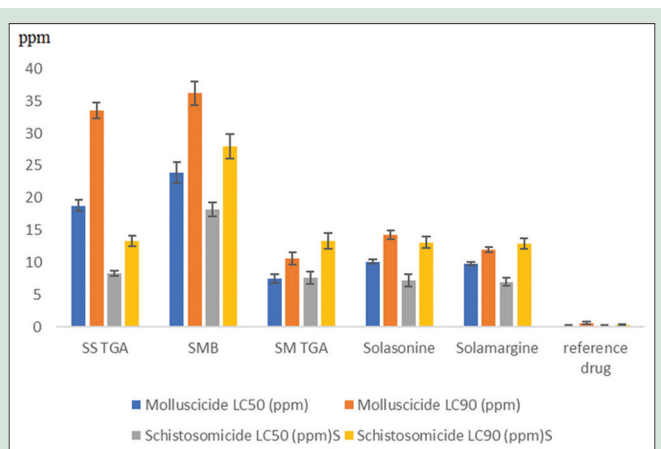
SMT: Total alcohol extract of SM; SMB: *n*-butanol fraction of SM; SM TGA: Total glycoalkaloid fraction of SM; SST: Total alcohol extract of SS; SSB: *n*-butanol fraction of SS; SS TGA: Total glycoalkaloid fraction of SS; SM: *Solanum macrocarpon* L.; SS: *Solanum seforthianum* Andr.

**Table 4:** *In vitro* schistosomicidal activity of plant extracts on *Schistosoma mansoni* (mean±standard error, n=3 after 3 days)

Plant extract	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
SST	>50	-
SSB	>50	-
SS TGA	8.3±0.4	13.3±0.8
SMT	>50	-
SMB	18.2±1.1	27.9±1.9
SM TGA	7.6±0.9	13.3±1.2
Solasodine	>50	-
Solasonine	7.2±0.9	13.1±0.9
Solamargine	7.0±0.6	12.9±0.8
PZQ	0.2±0.1	0.3±0.1

SMT: Total alcohol extract of SM; SMB: *n*-butanol fraction of SM; SM TGA: Total glycoalkaloid fraction of SM; SST: Total alcohol extract of SS; SSB: *n*-butanol fraction of SS; SS TGA: Total glycoalkaloid fraction of SS; PZQ: Praziquantel; SM: *Solanum macrocarpon* L.; SS: *Solanum seforthianum* Andr.

activity *in vitro* as shown in Figure 4 which is attributed to the glycoalkaloid content. The synergism of glycoalkaloids in TGA fractions and the sugar moiety effect are to be taken into consideration. However, additional studies, counting *in vivo* assays, are essential for the complete determination of the actual potentiality of these glycoalkaloids as a step to develop new therapeutics for schistosomiasis treatment.


**Figure 4:** The molluscicidal (using niclosamide as reference) and schistosomicidal (using praziquantel reference) potentiality of the isolated glycoalkaloids and total glycoalkaloid fraction of both species (SS: *Solanum seforthianum*; SM: *Solanum macrocarpon*)

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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