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 L50 and L90 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.

INTRODUCTION
Schistosomiasis is a parasitic infection caused by genus Schistosoma flatworms that affect 200 million people in diverse countries2,3 while about 779 million people worldwide were at risk of infection.4,5 It is claimed to be one of the most substantial mistreated diseases, with huge public health and economic consequences.6 Among the infectious diseases of the tropical countries, schistosomiasis is well-thought-out as the second most significant parasitic disease after malaria.7 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.8 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.9,10 However, these molluscicides are considered harmful and nonspecific, especially to nontarget animals, and may have long-standing unfavorable effects on the aquatic environment.11,12 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.13 The developed schistosome-resistant strains reinforced the necessity for more effective, safe, biodegradable, and environment-friendly schistosomicidal drugs.14,15 Plants represent the oldest and most common medication form as a source of molluscicides and schistosomicidal agents, particularly when

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

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compared to the synthetic molluscsicides in cost and safety.[36] Future tactics to control schistosomiasis involved the search for schistosomicidal and molluscicidal compounds from plants and other natural sources[3,12-18] 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.[19,20] 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 searforthianum 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.[29] 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 CHCl₃. Then, it was filtered and adjusted supernatant to 10.5–11.0 pH with NH₄OH, 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.[30]

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

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 ( Ehrenber) (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,[36] 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 searforthianum Andr. aerial parts showing leaves, flowers, and fruits](image)
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 restet 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.[25] Thus, the fresh adult worms were obtained by perfusion from infected hamsters 7 weeks earlier. Worms were cleaned from blood in small sieves 20-μm 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 μg/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 μg/ml final concentration. The culture medium used was PRMI 1640 containing 20% fetal calf serum, 300 μg streptomycin, 300 units penicillin, and 160 μg 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 μg/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} = 7.8 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 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 *Biophaliria glabrata].[33,28] Furthermore, A significant molluscicidal effect was indicated for various glycoalkaloids of *Solanum aculeastrum*[22] and *Solanum asperum*,[34] especially for the solasonine.

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,[35] antifungal activity[36] and schistosomicidal activity.[37] 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.[38] The glycoalkaloids containing the chitotriose trisaccharide, as solamargine [Figure 3], are generally more active than alkaldoids containing the solatriose trisaccharide, such as solasonine regarding the disruption of integrity and functionality of the cell membranes and acetylcholinesterase inhibition.[38]

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 lycopersicum*.[20]

**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
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 seaforthianum; SM: Solanum macrocarpon) 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.

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

<table>
<thead>
<tr>
<th>Extractives</th>
<th>Percentage yield</th>
<th>Color</th>
<th>Taste</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>10.4</td>
<td>Dark green</td>
<td>NC</td>
<td>Faint</td>
</tr>
<tr>
<td>n-hexane</td>
<td>3.77</td>
<td>Dark green</td>
<td>Waxy</td>
<td>Faint</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.2</td>
<td>Dark green</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.2</td>
<td>Brown</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>3.58</td>
<td>Brown</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>TGA</td>
<td>3.4</td>
<td>Brown</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>SM</td>
<td>12.35</td>
<td>Dark green</td>
<td>NC</td>
<td>Faint</td>
</tr>
<tr>
<td>n-hexane</td>
<td>3.5</td>
<td>Dark green</td>
<td>Waxy</td>
<td>Faint</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.3</td>
<td>Dark green</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.4</td>
<td>Brown</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>4.3</td>
<td>Brown</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>TGA</td>
<td>3.9</td>
<td>Brown</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>LC_{50} (ppm)</th>
<th>LC_{90} (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SST</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>SSB</td>
<td>30.4±1.3</td>
<td>46.8±1.9</td>
</tr>
<tr>
<td>SST TGA</td>
<td>18.8±0.9</td>
<td>33.5±1.2</td>
</tr>
<tr>
<td>SMT</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>SMB</td>
<td>23.9±1.6</td>
<td>36.2±1.8</td>
</tr>
<tr>
<td>SM TGA</td>
<td>7.5±0.7</td>
<td>10.6±0.9</td>
</tr>
<tr>
<td>Solasodine</td>
<td>45.5±2.9</td>
<td>55.8±2.1</td>
</tr>
<tr>
<td>Solasoline</td>
<td>10.1±0.3</td>
<td>14.3±0.7</td>
</tr>
<tr>
<td>Niclosamide</td>
<td>9.8±0.3</td>
<td>11.9±0.4</td>
</tr>
<tr>
<td>SMB n-butanol</td>
<td>0.2±0.1</td>
<td>0.6±0.2</td>
</tr>
</tbody>
</table>

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 seaforthianum Andr.

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

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>LC_{50} (ppm)</th>
<th>LC_{90} (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SST</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>SSB</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>SS TGA</td>
<td>8.3±0.4</td>
<td>13.3±0.8</td>
</tr>
<tr>
<td>SMT</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>SMB</td>
<td>18.2±1.1</td>
<td>27.9±1.9</td>
</tr>
<tr>
<td>SM TGA</td>
<td>7.6±0.9</td>
<td>13.3±1.2</td>
</tr>
<tr>
<td>Solasodine</td>
<td>&gt;50</td>
<td></td>
</tr>
<tr>
<td>Solasoline</td>
<td>7.2±0.9</td>
<td>13.1±0.9</td>
</tr>
<tr>
<td>Solamargine</td>
<td>7.0±0.6</td>
<td>12.9±0.8</td>
</tr>
<tr>
<td>PQZQ</td>
<td>0.2±0.1</td>
<td>0.3±0.1</td>
</tr>
</tbody>
</table>

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; PQZQ: Praziquantel; SM: Solanum macrocarpon L.; SS: Solanum seaforthianum Andr.

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 seaforthianum; SM: Solanum macrocarpon)

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Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES


