In Vitro Evaluation of Sida pilosa Retz (Malvaceae) Aqueous Extract and Derived Fractions on Schistosoma mansoni

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Abstract

Sida pilosa Retz. (Malvaceae) is a medicinal plant used in Africa for the treatment of dysmenorrhea, lower abdominal pains and intestinal helminthiasis. S. pilosa aqueous extract and derived fractions were investigated for their bioactivity against Schistosoma mansoni. The aqueous extract from S. pilosa aerial parts (1.25 - 40 mg/mL) and derived fractions (n-hexane, DCM, EtOAc and n-BuOH: 0.25 - 8 mg/mL) were tested on adult S. mansoni maintained in a GMEN culture medium. Praziquantel was used as the reference drug. After 24 h of incubation, worms were monitored for their viability and egg output. The antioxidant activity of S. pilosa was evaluated by the ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl free radicals. The chemical composition of the n-BuOH fraction was investigated by HPLC-MS analysis. S. pilosa aqueous extract and fractions significantly increased worm mortality in a concentration-dependent manner. The n-BuOH fraction was the most active with a LC50 of 1.25 mg/mL. Significant reduction of motor activity (25% to 100%) was

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recorded for surviving worms incubated in different concentrations of the extract and fractions. Incubation of *S. mansoni* in different concentrations of *S. pilosa* extract and fractions led to significant reduction of egg laying (52% to 100%). The aqueous extract and derived fractions exhibited antioxidant activity in a concentration-dependent manner. The highest antioxidant activity was found with the EtOAc fraction, followed by the DCM and *n*-BuOH fractions. HPLC-MS analysis of the *n*-butanol fraction revealed the presence of two indoloquinoline alkaloids. This study disclosed the schistosomicidal activity of the *n*-butanol fraction from *S. pilosa* aqueous extract. This activity is probably related to the indoloquinoline alkaloids identified in the fraction.

**Keywords**

*Sida pilosa*, Schistosomicidal Activity, Antioxidant Activity, Indoloquinoline Alkaloids, *Schistosoma mansoni*

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1. **Introduction**

Schistosomiasis is a chronic and debilitating disease affecting more than 200 million people in tropical and subtropical regions. A major problem in countries where schistosomiasis is endemic is the control of the disease. While preventive chemotherapy is the most important component in schistosomiasis control, other operational components, such as health education for behavioural change, provision of safe water and sanitation, environmental management and snail control, are necessary for a comprehensive control program. Although these actions were effective to decrease mortality and morbidity, schistosomiasis remains a public health concern in most endemic countries [1]. Chemotherapy with praziquantel, a low cost anthelmintic, is still the most effective treatment. Despite its benefits, the intensive use of praziquantel has resulted in reduced cure rates, treatment failure and development of resistant schistosomes strains [2] [3]. Therefore, there is an urgent demand for new effective schistosomicidal drugs. Schistosomiasis is responsible for oxidative damages in the vertebrate host through the release of reactive oxygen species (ROS) [4]. On the other hand, different antioxidant enzymes capable of degrading ROS produced by the host innate immune response have been identified in schistosomes [5]. Therefore, plant extracts or compounds with antioxidant properties in the vertebrate host or that induce oxidative stress on parasite can have antischistosomal activity for drug development.

Considered by the World Health Organization as a neglected tropical disease, little attention has been given to the research and development of new and effective antischistosomal drugs in the last decade [6]. Plants are regarded as a rich source of bioactive molecules, which have provided a number of useful clinical agents. Recently, several *in vitro* studies have been performed to search for new active compounds from medicinal plants against *S. mansoni* and promising results have been reported [7]-[10]. Within this context, we have been evaluating the role of plants as schistosomicidal agents. *Sida pilosa* Retz. (Malvaceae) is a creeping plant founded mainly on the outskirts of dwelling areas and on wastelands. It is empirically used for the treatment of intestinal helminthiasis and lower abdominal pains. To treat intestinal helminthiasis, it is recommended to squeeze the whole plant in water and to drink the macerate as often as possible until healed [11]. Our previous studies demonstrated the schistosomicidal activity of *Sida pilosa* aqueous extract using *in vivo* models. Moreover, phytochemical screening of *S. pilosa* aqueous extract revealed the presence of terpenoids, phenols, tannins and alkaloids [12]. This study was therefore designed to investigate the *in vitro* activity of *S. pilosa* aqueous extract and derived fractions towards *Schistosoma mansoni*.

2. **Materials and Methods**

2.1. **Plant Material**

The aerial parts of *S. pilosa* Retz. (Malvaceae) (Figure 1) were collected in March 2009 in the locality of Leboudi 2, near Yaoundé city in Cameroon. The authenticity of the material was confirmed against the specimen n° 86/399 (Lejoly) by Prof. Louis Zapfack, botanist at the University of Yaoundé I. A voucher specimen is conserved in the National Herbarium of Yaoundé, Cameroon, under the number 53202/HNC.
2.2. Extraction and Fractionation

Air dried and powdered aerial parts of *S. pilosa* were submitted to static maceration with water (100 g/L) for 24 hours, at room temperature. The solution was filtered, frozen and then lyophilized to give aqueous extract (AE), with a recovery rate of 13.6% w/w. The aqueous extract was fractionated by partition between immiscible solvents, as follows. Portions (5 g × 13) of the *S. pilosa* lyophilized extract were suspended in MeOH/water (1:11) and sequentially partitioned with equal volumes (4 × 50 mL) of n-hexane, DCM, EtOAc and n-BuOH. Solvents were removed in a rotatory evaporator, at maximum temperature of 50°C. The process allowed obtaining the n-hexane (93.30 mg), dichloromethane (345 mg), ethyl acetate (311 mg) and n-butanol (2760 mg) fractions.

2.3. HPLC-MS Analysis

Mass spectrometric analyses were performed at the Analytical Center from the “Universidade de São Paulo” (USP) using an Esquire 3000 plus quadrupole ion trap mass spectrometer (Bruker Daltonics, Billerica, USA). Nitrogen was used both as drying gas and as nebulizing gas (27 psi). The nebulizer temperature was set at 320°C and a potential of 4 KV was used on the capillary. Analyses were performed at room temperature on a Gemini ODS column (250 × 4.6 mm i.d., 5 μm; Phenomenex, Torrance, CA, USA), eluted with a gradient of water and ACN (0% to 20% ACN in 30 min, 20% to 40% ACN in 5 min and return to the initial condition in 5 min), at a flow rate of 0.5 mL/min and UV detection at 210 nm. Reagents used in these analyses were of HPLC grade. A flow rate of 90 μL/min was employed for the MS analyses and positive ion mass spectra were recorded in the range m/z 50 - 2000. Data analysis was carried out by the interpretation of spectral data and by comparison with literature records on the chemical composition of other *Sida* species [13]-[16].

2.4. Parasite Culture and Maintenance

SWISS mice obtained from the University animal facility (CEBIO, ICB-UFMG) were subcutaneously infected with 130 cercariae of the LE strain of *S. mansoni* released from experimentally infected *Biomphalaria glabrata* at the Laboratory of Schistosomiasis (ICB/UFMG). After 7 weeks of infection, adult worms were recovered under aseptic conditions by perfusion of the mesenteric veins and liver accordingly to the method described by Pellegrino and Siqueira [17]. The experimental procedures received prior approval from the local animal ethics committee and were in accordance with the ethical principles in animal research adopted by the Brazilian National Council on Animal Experimentation (CONCEA). Adult *S. mansoni* worms (male and female) recovered from infected animals were washed three times in a Glasgow Minimum Essential Medium (GMEM-Sigma, St Louis, USA) supplemented with an antibiotic-antimycotic solution (10,000 U/mL penicillin, 10,000 μg/mL streptomycin and 25 μg/mL amphotericin B (Atlanta Biologicals, Lawrenceville, USA) and gentamicine (40 μg/mL). To test the effect of *S. pilosa* extract and derived fractions on *S. mansoni* adult worms, the bioassay followed the standard operating procedures that recommended at least 5 females and 5 males per treatment [18]. In this bioassay, 10 male and 10 female adult worms were transferred to each well of a 24-well culture plate (NUNC) containing 1800 μL of complete GMEM culture medium (GMEM medium buffered to pH 7.5 con-
taining 20 mM of HEPES, 40 µg/mL gentamicine, 50 µg/mL penicillin, 50 µg/mL streptomycin, 100 µg/mL neomycin, 2 mM of L-glutamine and 5% heat-inactivated foetal bovine serum (GIBCO, Brazil)]. The plates were then incubated for 2 hours at 37°C in a humid atmosphere containing 5% CO₂ prior addition of products.

2.5. In Vitro Assays with Schistosoma mansoni

A wide range of concentrations (10 µg/mL to 50 mg/mL) is generally employed for in vitro screening of plants extracts or compounds for antischistosomal activity [7]-[10]. In this study, lyophilized crude extract of S. pilosa was initially dissolved in distilled water, filtered through a 0.2 µm sterile syringe filter and diluted in complete GMEM culture medium to a final concentration of 40, 20, 10, 5, 2.5 and 1.25 mg/mL. The n-hexane, DCM, EtOAc and n-BuOH fractions were dissolved in DMSO and diluted in the culture medium to final concentrations of 8, 4, 2, 1, 0.5 and 0.25 mg/mL. It is important to mention that the final volume was 2 mL/well and the maximum concentration of DMSO in each well was 0.5% v/v. Negative control for organic fractions was GMEN medium containing 0.5% of DMSO while GMEN medium was the negative control for the aqueous extract. Praziquantel was used as positive control at 100 µg/mL of final concentration. Quadruplicate measurements were carried out for each concentration and two independent experiments were performed for each sample. Culture plates were kept at 37°C for 24 h in a 5% CO₂ incubator; afterwards, worms were monitored to evaluate their viability and egg production by examination under an inverted microscope (Olympus CK × 41, Tokyo, Japan). Reduction of motor activity was defined as absence of worm motility apart from gut movements and occasional movement of head and tail of schistosome. Parasite death was defined as the absence of motor activity during 2 minutes. The median lethal concentration (LC₅₀) was calculated using the Trimmed Spearman-Karber (TSK) method [19], version 1.5 software downloaded from the US Environmental Protection Agency.

2.6. Radical Scavenging Activity—DPPH Assay

The antioxidant activity of S. pilosa aqueous extract and fractions was assessed using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay [20]. The crude extract was dissolved in distilled water and the fractions in methanol. Serial dilutions were carried out to give five concentrations in the range of 25 to 200 µg/mL for the crude extract and hexane fraction; and of 10 to 100 µg/mL for the other fractions. Each sample (2.5 mL) was mixed with 1 mL of a methanolic solution of 0.3 mMol DPPH. Rutin was employed as positive control (2.5, 10, 15, 20, and 25 µg/mL). The tests were carried out in quadruplicate. After 30 min of incubation at room temperature, in the dark, absorbance was recorded at 515 nm on a UV-VIS spectrophotometer (Hitachi U 2900, Tokyo, Japan). The antioxidant activity (AA%) was calculated according to the equation AA% = [(A_C – A_S)/A_C] × 100, where A_C is the absorbance of the control (DPPH solution) and A_S is the absorbance of the test sample. The antioxidant activity (AA%) was plotted against sample concentration, and a linear regression curve was established in order to calculate the effective concentration (EC₅₀) of the sample required to scavenge DPPH radical by 50% [20].

2.7. Statistical Analysis

Results were expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Newman-Keuls multiple comparison test, performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA). The level of significance was set at p < 0.05.

3. Results

3.1. Mortality of Schistosomes

The mortality rate of adult S. mansoni worms following in vitro exposure to different concentrations of S. pilosa aqueous extract and derived fractions is shown on Figure 2. There was a concentration-dependent increase in mortality of adult S. mansoni worms after incubation with the aqueous extract and fractions. After 24 h incubation of worms with either 20 or 40 mg/mL of the extract, 100% of the worms were dead. Moreover, we recorded mortality rates of 81%, 58%, 90%, and 100% for worms incubated respectively with 8 mg/mL of the n-hexane, DCM, EtOAc and n-BuOH fractions (p < 0.001). Incubation of the worms with the EtOAc (0.25 mg/mL), n-hexane and DCM fractions (0.25 to 2 mg/mL) did not promote any worm death. No death was observed in the
negative control groups (GMEN medium and 0.5% DMSO + GMEN medium). In the positive control group incubated with praziquantel (100 µg/mL), all parasites died within 24 h post-incubation. The evaluation of the median lethal concentration (LC₅₀) of S. pilosa aqueous extract and fractions, using the Trimmed Spearman-Karber method, disclosed the n-BuOH fraction as the most active one with LC₅₀ of 1.25 mg/mL (0.98 - 1.62 mg/mL) (Table 1).

3.2. Motor Activity

Absence of worm motility apart from gut movements and minimal motor activity marked by weak movement of the suckers and occasional sway of the body were recorded in surviving worms treated with S. pilosa aqueous extract and various fractions. The reduction of motor activity reached 100% for all the worms exposed to GMEM medium containing 10 mg/ml of S. pilosa aqueous extract or 8 mg/mL of n-hexane and EtOAc fractions and 4 mg/mL of the n-BuOH fraction. The addition of 8 mg/mL of the DCM fraction also resulted in reduction of worm motility by 80%. The reduction of motor activity varied from 27% to 40% with the crude extract (1.25 to 5 mg/mL) and from 25% to 79% with the n-BuOH fraction (0.25 to 2 mg/mL) (Figure 3). Adult S. mansoni worms belonging to the negative control groups (GMEN medium and 0.5% DMSO + GMEN medium) showed normal motor activity marked by undulatory movements of the body and peristaltic waves along the body.

3.3. Egg Output

Oviposition was followed and the number of eggs per female evaluated after 24 h of incubation with the extract

**Table 1.** Median lethal concentration (LC₅₀) values of Sida pilosa aqueous extract and various fractions.

<table>
<thead>
<tr>
<th>Sida pilosa</th>
<th>LC₅₀ (mg/mL)</th>
<th>95% low limit (mg/mL)</th>
<th>95% upper limit (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>8.57</td>
<td>6.88</td>
<td>10.74</td>
</tr>
<tr>
<td>n-Hexane fraction</td>
<td>5.70</td>
<td>5.10</td>
<td>6.38</td>
</tr>
<tr>
<td>DCM fraction</td>
<td>7.03</td>
<td>6.27</td>
<td>7.88</td>
</tr>
<tr>
<td>EtOAc fraction</td>
<td>4.54</td>
<td>3.78</td>
<td>5.45</td>
</tr>
<tr>
<td>n-BuOH fraction</td>
<td>1.25</td>
<td>0.98</td>
<td>1.62</td>
</tr>
</tbody>
</table>

DCM fraction: dichloromethane fraction; EtOAc fraction: ethyl acetate fraction; n-BuOH fraction: n-butanol fraction.

Figure 2. In vitro effect of Sida pilosa aqueous extract and various fractions on the mortality of Schistosoma mansoni after 24 h of incubation.

**Table 1.** Median lethal concentration (LC₅₀) values of Sida pilosa aqueous extract and various fractions.
or fractions (Figure 4). It generally appeared that *S. pilosa* aqueous extract and various fractions inhibited egg production. Egg laying was completely abolished by the aqueous extract at 40 mg/mL and concentrations from 20 to 2.5 mg/mL significantly reduced it by 99% to 66%. In comparison with the negative control group, treatment with the *n*-BuOH fraction from 8 to 0.25 mg/mL significantly decreased the number of eggs (97% to 85%) after 24 h of incubation (*p* < 0.001). Egg production was also reduced by the *n*-hexane, DCM and EtOAc fractions assayed at 8 mg/mL by 99%, 97% and 98% respectively. Praziquantel did not suppress the egg output, but reduced it by 98%. Reduction of oviposition was not only observed in wells containing dead worms, but also in wells where no mortality was recorded. For example, the *n*-hexane fraction (2 mg/mL) decreased egg production by 86%. The egg laying reduction also varied from 52% to 67% when worms were treated with DCM fraction (2 to 0.25 mg/mL). Significant negative correlations were established between mortality and egg output (*r* = −0.60, *r*² = 0.36, *p* < 0.001) and between decreased motor activity and egg output (*r* = −0.42, *r*² = 0.18, *p* < 0.05) for the

![Graphs showing motor activity and egg output](image)

***,**,**,* values are significantly different from controls (culture medium or culture medium + 0.5% DMSO) at *p* < 0.01 and *p* < 0.001 respectively.

**Figure 3. In vitro effect of *Sida pilosa* aqueous extract and various fractions on the motor activity of *Schistosoma mansoni* after 24 h of incubation.**

![Graphs showing motor activity and egg output](image)

* **,**,**,* values are significantly different from controls (culture medium or culture medium + 0.5% DMSO) at *p* < 0.05, *p* < 0.01 and *p* < 0.001 respectively.

**Figure 4. In vitro effect of *Sida pilosa* aqueous extract and various fractions on the egg output of *Schistosoma mansoni* after 24 h of incubation.**
aqueous extract. No significant correlation between decreased motor activity and egg output was established for worms incubated with the \( n \)-BuOH fraction, but a significant negative correlation between mortality and egg output (\( r = -0.45, r^2 = 0.20, p < 0.001 \)) was found.

3.4. DPPH Radical Scavenging Activity

The \textit{in vitro} antioxidant activity of \textit{S. pilosa} aqueous extract and fractions was evaluated by the capacity of samples to scavenge free radicals of DPPH. The aqueous extract and derived fractions exhibited antioxidant activity in a concentration-dependent manner. The EtOAc fraction exhibited the strongest antioxidant activity with an EC\(_{50}\) of 46.01 ± 0.63 µg/mL. The DCM and \( n \)-BuOH fractions also induced significant antioxidant response, with EC\(_{50}\) values of 60.85 ± 0.20 µg/mL and 81.14 ± 0.56 µg/mL, respectively. Although these fractions exhibited significant antioxidant activity, the EC\(_{50}\) value of the positive control rutin was 3 times lower than that of most active fraction (EtOAc fraction) (Table 2). The \( n \)-hexane fraction exhibited a poor radical scavenging activity (528.98 ± 3.91 µg/mL) and the aqueous extract was active at the highest concentration (150 µg/mL).

3.5. Mass Spectrometry Analysis of the \( n \)-Butanol Fraction from \textit{Sida pilosa} Aqueous Extract

As expected, the HPLC-MS chromatogram recorded for the \( n \)-butanol fraction was majorly composed by peaks of polar compounds, with retention times below 20 min (Figure 5(a)). The chemistry of \textit{S. pilosa} has never been investigated and TLC analyses of the \( n \)-butanol fraction revealed two major spots after spraying Dragendorff reagent (data not shown), suggesting the presence of alkaloids. Therefore, the MS data obtained for the constituents of the fraction were initially compared with literature records of alkaloids isolated from other \textit{Sida} species. None of the previously reported alkaloids was identified in the fraction, but it was possible to conjecture the chemical nature of two constituents. Hence, the minor peak eluted at 4.6 min (compound 1), corresponding to the molecular ion [M + H]\(^+\) detected at \( m/z \) 543 (Figure 5(b)), was credited to a diglycoside of an indolo[3,2-\( b \)]quinoline alkaloid, whose putative structure is presented in Figure 4(d). The proposed structure of compound 1 has a molecular mass of 542.19, compatible with the obtained HPLC-MS data. This hypothesis is based on the previous isolation of indoloquinoline alkaloids from \textit{Sida acuta} [13] [14] [16] and is also supported by the occurrence of some indolo[\( 3,2-b \)]quinoline alkaloid glycosides in other plant species [15].

A peak eluted at 9.3 min (compound 2) was also present in the HPLC-MS profile of the \( n \)-butanol fraction, which produced the molecular ion [M + H]\(^+\) detected at \( m/z \) 295 (Figure 5(c)). According to the obtained data, it was possible to infer the molecular mass of 294.14 for compound 2. Its putative structure (Figure 5(d)) was proposed by comparison with the chemical structure of compound 1 and by analysis of literature data reported for indoloquinoline alkaloids from \textit{S. acuta} [13] [14] [16]. The putative structure is supported by the fragment ion at \( m/z \) 276.9 ([M − 18]\(^+\)) resulting from the loss of H\(_2\)O, as well as by the parent ion at \( m/z \) 248.8 [M + H − 15 − 31]\(^+\), ascribed to the elimination of both a methyl and a methoxyl group (data not shown).

4. Discussion

Specie of the genus \textit{Sida} have been reported to have a wide range of biological activities that include analgesic, anti-inflammatory, antioxidant, antibacterial and anthelmintic activities [16] [21]-[23]. Jatsa \textit{et al.} [12] have previously shown the \textit{in vivo} schistosomicidal activity of \textit{S. pilosa} aqueous extract against \textit{S. mansoni}. With the perspective of searching new active compounds against \textit{Schistosoma} species, the activity of \textit{S. pilosa} aqueous

\begin{table}[h]
\centering
\caption{Antioxidant activity of fractions from \textit{Sida pilosa} aqueous extract on 2, 2-diphenyl-1-picrylhydrazyl (DPPH).}
\begin{tabular}{|l|c|}
\hline
\textit{Sida pilosa} & EC\(_{50}\) (µg/mL) \\
\hline
\textit{n}-Hexane fraction & 528.98 ± 3.91*** \\
Dichloromethane fraction & 60.85 ± 0.20*** \\
Ethyl acetate fraction & 46.01 ± 0.63*** \\
\textit{n}-Butanol fraction & 81.14 ± 0.56*** \\
Rutin & 14.81 ± 0.01 \\
\hline
\end{tabular}
\footnotesize{Results are mean ± SEM (n = 4). ***: values are significantly different from rutin (positive control) at p < 0.001.}
\end{table}
Figure 5. HPLC-MS chromatograms of the n-butanol fraction from *Sida pilosa* aqueous extract and the structure of indoloquinoline alkaloids identified in the fraction. (a) HPLC chromatogram of the n-butanol fraction from *Sida pilosa* aqueous extract; (b) Mass spectrum of compound 1 of the n-butanol fraction; (c) Mass spectrum of compound 2 of the n-butanol fraction; (d) Structure of the two indoloquinoline alkaloids (compound 1 and compound 2).
extract and fractions thereof were assayed in vitro at different concentrations.

The first parameter to consider was adult S. mansoni mortality. This bioassay clearly showed that the aqueous extract and various fractions were active against S. mansoni in a concentration-dependent manner. A similar concentration-dependent bioactivity has been reported for Sida acuta ethanol extract and Sida cordifolia aqueous and ethanol extracts against some helminths [22] [23]. Lethal concentrations of S. pilosa aqueous extract (20 mg/mL) and its n-butanol fraction (8 mg/mL) were in the range of lethal concentrations (0.6 to 25 mg/mL) of plant species popularly used against schistosomiasis in South Africa and Zimbabwe [7] [8]. In the present work, evaluation of the median lethal concentration (LC50) of the aqueous extract and derived fractions disclosed the n-butanol fraction as the most active, with a LC50 of 1.25 mg/mL. Many authors have reported that biological activities displayed by some species of Sida are generally attributed to alkaloids present in those plants [13] [16] [24] [25]. The chemical composition of the n-butanol fraction was therefore characterized by HPLC-MS analyses. None of the alkaloids previously isolated from other Sida species was identified in the fraction by comparison with their MS data. On the other hand, it was possible to propose the putative structures of two indoloquinoline alkaloids present in the fraction. These compounds will be isolated in the future for the unambiguous elucidation of their chemical structures by spectroscopic methods. Potential biological activities of alkaloids isolated from S. acuta have been described by Banzouzi et al. [14] which identified an indoloquinoline, the cryptolepine, as an active constituent associated with the antiplasmodial activity displayed by the extract of the aerial parts of S. acuta. Karou et al. [16] [26] also associated the antimalarial and antibacterial activities of the same specie to the presence of cryptolepine and quindoline. In view of these biological activities of alkaloids, it is possible that the schistosomicidal activity displayed by the n-butanol fraction from S. pilosa aqueous extract is related to the presence of indoloquinoline alkaloids in the fraction. This fraction could be considered as a promising source for schistosomicidal compounds.

Motor activity is often evaluated as indicator of biological activity of schistosome species. A concentration-dependent reduction of worm motor activity was observed, particularly with the n-BuOH fraction. Absence of motility apart from guts movement, feeble motor activity and reduction of peristaltic waves along schistosomes’ body after incubation in S. pilosa could be the consequence of the plant interference with the mechanism of contraction-relaxation of worm smooth muscles [27]. The reduction of worm motor activity was also reported after incubation of schistosomes with medicinal plants products [10] [28]-[31]. Results from this study showed that 18% of the variability of egg production was correlated to the reduction of motor activity after incubation of worms with the extract whereas worms’ mortality was correlated to 36% or 20% of the variability of egg laying after incubation of worms with the extract or the fraction. The reduction of egg output could be the consequence of cytotoxic damage or specific inhibition of worm reproductive processes by one or more compounds present in medicinal plants [9]. It has been demonstrated that the inhibition of larval migration, adult worm motility and egg excretion in helminths are associated with the consumption of tannins by their hosts [32]-[34]. It then appeared that tannins present in S. pilosa [12] could be involved in the reduction of worm motility and reproductive processes of schistosomes.

Reactive oxygen species contribute to a large variety of diseases, including schistosomiasis [4]. Extracts or compounds that induce oxidative stress on the parasite or which have antioxidant activity on the vertebrate host might represent a potential therapeutic approach for schistosomiasis. The DPPH assay was therefore used to evaluate the scavenging capacity of S. pilosa. The aqueous extract and derived fractions exhibited antioxidant activity in a concentration-dependent manner. The highest antioxidant activity was found with the EtOAc fraction, followed by the DCM and n-BuOH fractions. When Shah et al. [35] studied the DPPH radical scavenging activity of the methanolic extract of Sida cordifolia and S. pilosa fractions, the ethyl acetate fraction also show the best antioxidant activity. This antioxidant potential was correlate to the phenolic and flavonoid contents of the fraction. The presence of phenols in S. pilosa [8] could be responsible for its antioxidant potential.

5. Conclusion

Results from this study indicate that the n-butanol fraction from Sida pilosa aqueous extract possesses in vitro schistosomicidal activity against Schistosoma mansoni adult worms and antioxidant potential. This schistosomicidal activity is probably related to indoloquinolines alkaloids present in the fraction. Further studies are needed on the isolation and bioactivity evaluation of these compounds. Moreover, in vivo safety and efficacy of the n-butanol fraction and individual compounds on schistosomiasis mansoni also need to be investigated.
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