Arch Biol Sci. 2024;76(4):375-386 https://doi.org/10.2298/ABS240804027S

Biochemical and toxicological evaluation of *Solanum viarum* fruit extract on *Dugesia tigrina*

Felipe Schaly and Welligton Luciano Braguini*

Experimental Biochemistry and Toxicology Laboratory (BioTox). Midwestern Paraná State University, Guarapuava City, Paraná State, Brazil

***Corresponding author**: wbraguini@unicentro.br

Received: August 4, 2024; **Revised**: September 10, 2024; **Accepted**: September 12, 2024; **Published online**: September 20, 2024

Abstract: *Solanum viarum* has alkaloid-rich fruits. We evaluated the antioxidant and chelating activities of the aqueous extract fraction (AEF) from *S. viarum* fruits and its effects on the brown planarian *Dugesia tigrina*. The AEF demonstrated an iron-chelating effect comparable to ascorbic acid but lower than EDTA and a significant reducing power. The 96-h LC_{50} for planarians was 1.22 g/L. At a concentration of 0.50 g/L, the AEF reduced planarian locomotor velocity by 34.7%, while decreasing fecundity and fertility by 98.4% and 96.7%, respectively, leading to a significant decline in the number of cocoons and emerging planarians. Cocoon hatching time was increased at all AEF concentrations. Planarians exposed to 0.50 g/L AEF for 14 days showed a 62.3% reduction in glycogen content. After 14 days, catalase was inhibited at all concentrations, with 83.2% inhibition at 0.50 g/L. Superoxide dismutase (SOD) increased by 60.8% and 59.9% after 24 h and 14 days, respectively, at 0.50 g/L AEF. Catalase (CAT) was stimulated by 72.1% and 44% at 0.10 and 0.25 g/L AEF, respectively, but inhibited by 25.2% at 0.50 g/L after 24 h. The iron-chelating activity of the AEF reduces iron availability for catalase, an iron-dependent enzyme, leading to its inhibition, while also limiting Fenton and Haber-Weiss reactions, resulting in hydrogen peroxide accumulation and subsequent stimulation of SOD under prolonged exposure. Acetylcholinesterase (AChE) was stimulated by 40.6% and 44.8% after 24 h and 14 days, respectively, at 0.50 g/L AEF. These findings show that *S. viarum* is harmful to planarians and may pose environmental risks, highlighting the need for toxicity assessments in other species.

Keywords: *Solanum viarum*; environmental risk; reproductive toxicity; antioxidant enzymes; planarian

Abbreviations: AEF – aqueous extract fraction; AChE – acetylcholinesterase; ACh – acetylthiocholine; CAT – catalase; pLmV – planarian locomotor velocity; SOD – superoxide dismutase.

INTRODUCTION

Fruits from *Solanum* species are renowned for their diverse range of biological activities, including anthelmintic, wound healing, antipyretic, laxative, antiasthmatic, antibacterial, antioxidant, larvicidal, anti-inflammatory, antidiabetic, hepatoprotective, antiurolithiatic, antifertility, and aphrodisiac properties [1-3]. These fruits are rich in bioactive compounds, such as solasonine, solasodine, carpesterol, campesterol, daucosterol, caffeic acid, solamargine, lupeol, and diosgenin, which are recognized for their significant therapeutic potential [2,4].

Solanum viarum Dunal, commonly referred to as "juá" or "juá-bravo" in South America and tropical soda apple in the USA, is a member of the Solanaceae family known for its invasive behavior and significant ecological and agricultural impact [1-3]. Despite its toxicity to livestock, particularly cattle, *S. viarum* holds economic importance due to its production of solasodine, an alkaloid with applications in the synthesis of steroid hormones and various pharmacological derivatives [5,6]. This perennial plant thrives in warm climates, producing fruits rich in bioactive compounds, including solasodine and diosgenin. These compounds have been studied for their medicinal properties, including applications in cancer and arthritis treatments, as well as in the development of contraceptives (3,6-8). Despite its medicinal potential, the broader toxicological effects of *S. viarum*, particularly on non-mammalian species, remain underexplored.

In regions such as Brazil, Paraguay, and Argentina, *S. viarum* is prevalent in pastures, leading to frequent cases of cattle poisoning when the fruits are inadvertently consumed. The neurotoxicity of the fruits to ruminants has been documented, yet the broader toxicological effects, especially on non-mammalian species, remain underexplored. Solasodine, a steroidal alkaloid, is particularly noteworthy due to its role in the synthesis of steroid hormones and pharmacological derivatives [8-11]. Diosgenin, a sapogenin, is known for its anti-inflammatory and antioxidant properties, making it a key component in both medicinal and toxicological contexts [11-12]. Other significant compounds include lupeol, which exhibits anti-inflammatory and hepatoprotective activities, and solamargine, a glycoalkaloid with demonstrated anticancer potential [13]. These compounds are believed to contribute substantially to different biological effects, including reproductive toxicity [8-12]. However, the toxicological profile of *S. viarum* fruit extracts, particularly in invertebrate models, has been inadequately characterized.

To address this gap, our study investigates the biochemical and toxicological effects of the aqueous extract fraction (AEF) of *S. viarum* fruits on the invertebrate model *Dugesia tigrina*, a planarian species known for its regenerative capabilities. *D. tigrina*, a freshwater platyhelminth, is widely recognized in toxicological research due to its sensitivity to environmental toxins and its capacity to reflect broader ecological impacts [14-16]. While previous research has focused on the toxicity of *S. viarum* to livestock, the effects on smaller organisms such as planarians have not been thoroughly studied.

This study addresses the knowledge gap by conducting a comprehensive phytochemical analysis of the AEF, with a particular focus on its iron ion chelation ability and reducing power. It also includes an assessment of the extract toxicity through bioassays, measuring endpoints such as median lethal concentration, locomotor activity, reproductive outcomes, glycogen content, and the activities of key enzymes like superoxide dismutase, catalase, and acetylcholinesterase. By employing sublethal concentrations, the research seeks to elucidate the broader biochemical and physiological effects of *S. viarum* on *D. tigrina*, focusing on the antioxidant, neurological, and reproductive parameters. This investigation advances our understanding of the toxicological risks of invasive species like *S. viarum*. It also demonstrates the usefulness of *D. tigrina* as a model organism for environmental toxicity studies. The work sheds new light on the potential ecological consequences of the spread of *S. viarum*, emphasizing the importance of evaluating its impact across various levels of biological organization.

MATERIALS AND METHODS

Chemicals

Acetylthiocholine (ACh), ascorbic acid, tannic acid, ascorbic acid, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), bovine serum albumin, nitroblue tetrazolium chloride (NBT), EDTA, ferrozine [3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine], $K_4[Fe(CN)_6]$, quercetin, Folin-Ciocalteu reagent, and Triton X-100 were from Sigma Chemical, St. Louis, MO, USA and from Merck, Darmstadt, West Germany. All other reagents used were of analytical grade.

Collection of *S. viarum* fruits and preparation of the AEF

Green fruits of *Solanum viarum* were collected in November 2023 on a farm in the Pinhão District Municipality, Paraná, Brazil (latitude 25°42'06.7" and longitude 51°38'20.6"). The plant was identified, and a voucher specimen (HUCO-7408) was prepared and deposited in the Herbarium of the Midwestern Paraná State University (Unicentro), HUCO Herbarium, Campus of Irati, Paraná, Brazil. After collection, the green fruits were rinsed with deionized water, gently dried with paper towels, cut into smaller pieces, and then dried in an oven at 40°C for 72 h until reaching a constant weight. After drying, they were ground in a GM 200 knife mill (Retsch®) and passed through a 2.0-mm sieve. The AEF from the fruits was prepared from 5 g of plant material and 250 mL of distilled water as a solvent (20 g/L). Extraction was performed in a Soxhlet apparatus for 5 h. The extracts were stored at 4-8°C and used within 3 days.

ADLabMET 3.0 analysis

The molecular formulas in SMILES format correspond to the molecules present in the *S. viarum* extract that

were analyzed using the ADMETlab 3.0 software, which can be accessed at https://admetlab3.scbdd.com/server/evaluation (Supplementary Table S1). The data for each molecule is available for anyone interested in conducting their analysis.

Assessment of the chelating capacity of the AEF from *S. viarum* fruit

The chelating capacity of Fe^{2+} ions was evaluated according to the methodology described by Tang et al., with modifications [17]. An aliquot of up to 1 mL of the samples was transferred to 25-mL amber test tubes, resulting in final concentrations of 0.40 and 0.60 g/L of the extract. The chelating effect of EDTA and ascorbic acid at concentrations of 0.40 and 0.60 g/L was also compared. To each aliquot, 3.7 mL of deionized water, 0.1 mL of 2 mM $FeSO_4$, and 0.2 mL of 5 mM ferrozine were added. One milliliter of distilled water was used as a control instead of *S. viarum* extract. The mixture was shaken, and after 20 min, the absorbance was read at 562 nm, with the instrument adjusted using a blank (sample replaced with EDTA). The reduction in absorbance indicates the metal chelating effect, determined by the following equation:

$$CC(\%) = 100 - [(\frac{Ap - At}{Ap}) \times 100]$$

where CC(%) is the metal chelating effect, Ap is the absorbance of the standard, and At is the absorbance of the test samples.

Determination of the reducing power of the AEF from *S. viarum* fruit

The reducing power was determined using the method described by Oyaizu [18]. A volume of 125 µL of the extract solutions, at concentrations of 0.005 to 0.050 g/L of AEF, along with 500 µL of 0.2 M sodium phosphate buffer (pH 6.0), and 500 µL of 1% (w/v) aqueous solution of potassium ferrocyanide were pipetted into test tubes, which were then placed in a water bath at 50°C for 20 min. Subsequently, 500 µL of 10% (w/v) aqueous solution of trichloroacetic acid, 1500 µL of distilled water, and 300 µL of ferric chloride solution were added to the mixture. A volume of 1000 µL was transferred to the cuvette, and the intensity of the blue-green color was measured at 700 nm. Ascorbic acid was used as the standard, and five assays were performed in triplicate.

Animals

Dugesia tigrina planarians have been maintained and propagated in the Experimental Biochemistry and Toxicology Laboratory (BioTox) since 2017. The planarians are fed once a week with bovine liver in planarian culture water (PCW) consisting of distilled water and 6 mM NaCl, 0.13 mM $CaCl_2$, and 0.12 mM $NaHCO_3$ [19].

Determination of the median lethal concentration of the AEF from *S. viarum* fruit

The AEF was dissolved in PCW at concentrations of 1.0 to 1.5 g/L. In 150×15 mm Petri dishes, 50 mL of PCW with the AEF concentrations and 6 planarians per dish were added. The planarians were kept in the dark for 96 h without food. Mortality was checked at 24, 48, 72, and 96 h. The LC_{50} was determined at 96 h using the Probit method (Supplementary Fig. S1).

Assessment of AEF impact on planarian locomotor velocity

The planarian locomotor velocity (pLmV) was evaluated according to Raffa and Valdez with modifications [20]. Each planarian was placed in a Petri dish containing PCW, with a paper divided into 0.5 cm^2 quadrants placed under the dish. Mobility was expressed as the number of lines crossed and re-crossed by the planarian during 5 min of filming with a high-speed NEOCoolcam-Webcam® camera with a varifocal zoom lens. Planarians were pre-incubated for 15 min in the *S. viarum* AEF at concentrations of 0.10, 0.25, and 0.50 g/L. Planarians were used for each extract concentration, and each planarian was used only once in the experiment.

Assessment of AEF impact on planarian reproduction

Reproductive performance was evaluated using randomly selected planarians with an average size of 1.2 ± 0.2 cm in length in containers containing 1000 mL of planarian water. Planarians were treated with three

concentrations of *S. viarum* AEF (0.10, 0.25, and 0.50 g/L) for 4 weeks. They were evaluated for the number of cocoons per planarian per week. Fertility was measured as the number of viable cocoons per week per planarian (viable cocoons that generated viable individuals after hatching). Reproductive indices were calculated as described by Knakievicz et al. [16] with modifications.

Assessment of AEF impact on planarian glycogen levels

The total glycogen determination was performed according to the methodology of Kemp et al. [21]. Planarians exposed to 0.10, 0.25, and 0.50 g/L of the *S. viarum* AEF were incubated for 14 days and assessed for glycogen concentration. A deproteinizing solution was prepared by dissolving 5 g of trichloroacetic acid and 0.1 g of $AgSO_4$ in water to a final volume of 100 mL. Control and AEF-treated planarians were weighed and placed in a Potter-Elvehjem tissue grinder with 5 mL of deproteinizing solution. After maceration, the mixture was transferred to a glass centrifuge tube, sealed, and the volume was marked. The tube was placed in a water bath at 100°C for 15 min, then cooled in an ice bath and topped off with a deproteinizing solution to compensate for any evaporation. The sample was centrifuged at 1025×*g* for 5 min. One milliliter of the supernatant was transferred to a new tube to which 3 mL of H_2SO_4 was added and mixed vigorously. The mixture was then heated in a water bath at 100°C for 5-6 min and cooled under running water. The pink color intensity was measured spectrophotometrically at 520 nm, with glycogen concentration determined from a glucose calibration curve (0.025, 0.050, 0.100, 0.150, and 0.200 g/L). The pink color intensity is proportional to glucose concentration, up to 0.2 g of glucose/L.

Evaluation of AEF effects on antioxidant enzymes and acetylcholinesterase

Planarians exposed to the AEF for 24 h and 14 days were used for enzyme tests. A cellular extract was obtained from 8 planarians subjected to the treatments. After pre-incubation in the AEF, the planarians were mechanically homogenized on ice using a mortar in cold potassium phosphate buffer (50 mM, pH 7.0, with 0.5 mM EDTA and 10 µM Triton X-100) for superoxide dismutase (SOD) and catalase (CAT) assays, and in Tris-HCl buffer (20 mM, pH 8.0, with 1 mM EDTA and 10 µM Triton X100) for the acetylcholinesterase (AChE) assay. The suspension was vigorously mixed in a test tube to a final volume of 3 mL. The solution was then centrifuged at 12000×g for 20 min at 4°C in a refrigerated Loccus® benchtop centrifuge, model L3024R, and the supernatant was used to determine SOD, CAT, and AChE enzymes.

Determination of SOD (E.C. 1.15.1.1)

SOD was determined according to the method of Kono (1978). The reaction medium consisted of sodium bicarbonate buffer (100 mM pH 10.2), 17.5 µM EDTA, and 1 mM nitroblue tetrazolium chloride (NBT)). The reaction was initiated by the addition of 1 mM hydroxylamine. The increase in absorbance was measured at 560 nm using a spectrophotometer. One unit of SOD was defined as the amount of enzyme that inhibits 50% of NBT reduction, and the results were expressed in $U \times min^{-1} \times mg^{-1}$ protein [22].

Determination of CAT (E.C. 1.11.1.6)

CAT from *D. tigrina* was determined according to the method described by Aebi [23]. The assay principle was based on measuring the decomposition rate of 5 mM H_2O_2 at 240 nm in potassium phosphate buffer (50 mM pH 7.0). An equivalent amount of CAT obtained from the planarian extract was added to achieve a final buffer volume of 1.5 mL. The results were expressed as µmol H_2O_2 consumed$\times min^{-1} \times mg^{-1}$ protein.

Determination of AChE (E.C. 3.1.1.7.)

AChE was assayed using the method described by Ellman et al. [24]. The method is based on the hydrolysis reaction between AChE present in the sample and the substrate acetylthiocholine. The byproduct thiocholine interacts with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), allowing the detection of its products 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate at 412 nm [24]. In a cuvette, 1.5 mM ACh and 50 µM DTNB were added, and the reaction was initiated by the addition of 1 mg/mL of enzyme obtained from the planarian in Tris-HCl buffer (20 mM pH 7.0), 1 mM EDTA and 10 µM Triton x100, to a final

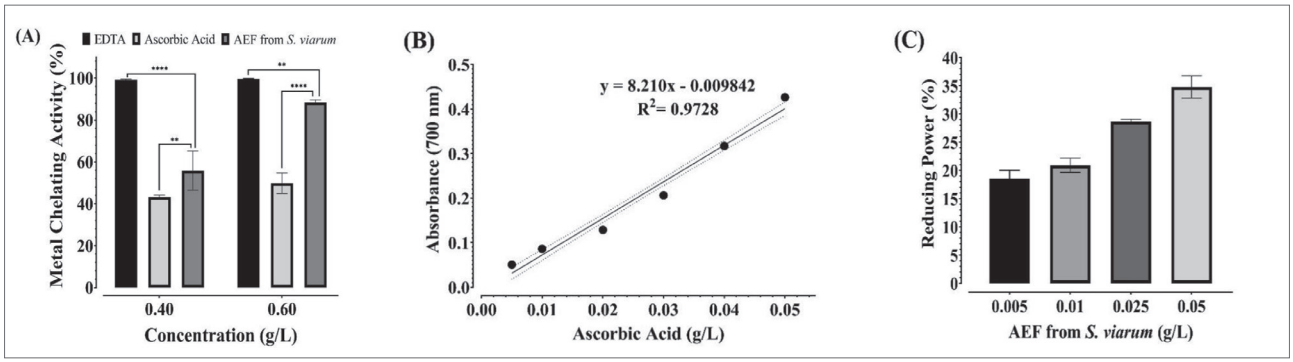


Fig. 1. A – Metal chelating effect of AEF; **B** – Reducing power of ascorbic acid; **C** – reducing power of *S. viarum* AEF. Data are the mean±standard deviation of 5 experiments performed in triplicate. **A:** **** P<0.0001; ** P=0.0029.

volume of 1.5 mL. The enzyme effects were expressed in μmoles of ACh hydrolyzed×min^{-1}×mg^{-1} protein.

Protein quantification

Protein quantification for the enzymatic assays was determined using the Bradford method [25] with a calibration curve ranging from 50 to 1000 μg/mL.

Statistical analysis

The reduction power and chelating effect of the AEF were determined from the best-fit line obtained by regression analysis. The median lethal concentration (LC_{50}) and 95% confidence intervals of the test samples were calculated using the probity analysis method described by Finney [26] as the measure of the toxicity of the plant extract. For statistical significance, the study was carried out in 5 experiments in triplicate. The differences between the groups were determined by analysis of variance (ANOVA) followed, when detected, by Dunnett's test. The analysis was performed using GraphPad Prism® ver. 8.01 for Windows®, Graph Pad Software®, San Diego, California, USA. The results were considered statistically significant when P<0.05.

RESULTS

Phytochemical evaluation

Fig. 1A demonstrates that the AEF from *S. viarum* exhibited a higher iron chelating effect compared to ascorbic acid but was less effective than EDTA (~100%), a well-known iron chelator. Specifically, the chelating effect of AEF was 60.1±9% and 88.4±1.2% at concentrations of 0.40 and 0.60 g/L of AEF, respectively, while ascorbic acid showed effects of 47.9±4.7% and 45.6±5%, respectively. Chelating compounds often possess antioxidant properties because of their ability to bind metal ions involved in reactive oxygen species (ROS) formation, thus reducing cellular oxidative stress [27]. Such chelating effects highlight the potential of plant extracts in pharmacological research for drug discovery and development [33]. The results indicate that the *S. viarum* AEF has significant chelating effects at concentrations of 0.40 and 0.60 g/L (P=0.0029 and P<0.0001, respectively), with EDTA showing the highest efficacy, followed by the *S. viarum* AEF and ascorbic acid.

The *S. viarum* AEF demonstrated a significant ability to reduce ferric ions (Fe^{3+}) and donate electrons to neutralize free radicals, resulting in the formation of stable products. However, its reducing power was lower than the standard antioxidant ascorbic acid. Fig. 1B presents the curve for ascorbic acid, and Fig. 1C shows the reducing power of the *S. viarum* AEF. The results show that the AEF demonstrated reducing power of 18.5±1.9, 21±1.7, 28.7±0.2, and 34.9±2.5% at concentrations of 0.005, 0.01, 0.025, and 0.05 g/L, respectively.

Evaluation of AEF toxicity

The 96-hour LC_{50} of AEF for planarians was 1.22 g/L (Table 1), determined by Probit analysis from five experiments conducted in triplicate. According to Braguini et al., AEF is moderately toxic to *Artemia salina* [1]. Given that *A. salina* is much smaller and inhabits seawater

Table 1. LC_{50} of the AEF from *S. viarum* in *D. tigrina*.

	Equation	LC_{50} (g/L)	95% Confidence Intervals	
24 h	y=25.992x+1.075	1.42	1.35	1.49
48 h	y=26.672x+1.6158	1.34	1.27	1.41
72 h	y=25.523x+2.2884	1.28	1.22	1.34
96 h	y=31.759x+2.318	1.22	1.16	1.28

Data represents the best fit of five experiments conducted in triplicate.

environments unlike the freshwater *D. tigrina*, this comparison highlights species-specific differences in sensitivity to the extract. The LC_{50} values for planarians and *A. salina* demonstrate significant variability in their responses to the extract. Assessing the LC_{50} at various time intervals (24 h, 48 h, 72 h, and 96 h) allows for an evaluation of toxicity over time and reveals potential cumulative effects of the extract. This temporal variation in LC_{50} values emphasizes the need to consider time-dependent dynamics in toxicity assessments as the effects may differ at various exposure durations.

Effects of *S. viarum* AEF on planarian locomotor velocity

Fig. 2 shows the mean±standard deviation of pLmV after 15 min exposure to ***S. viarum* AEF**. The 0.10 g/L concentration of AEF did not significantly impact planarian locomotion. However, there was a significant difference (P<0.0001) between the control and the 0.25 and 0.50 g/L concentrations of AEF [$F_{(2.207, 15.45)}$=22.87]. The results indicated a significant interaction between pLmV time and AEF concentration (P<0.0001) on planarian locomotion [$F_{(2.350, 16.45)}$=15.51]. At the 5-min mark, pLmV inhibition was 21.5±3.5% at a concentration of 0.25 g/L and 34.7±2.0% at 0.50 g/L of AEF.

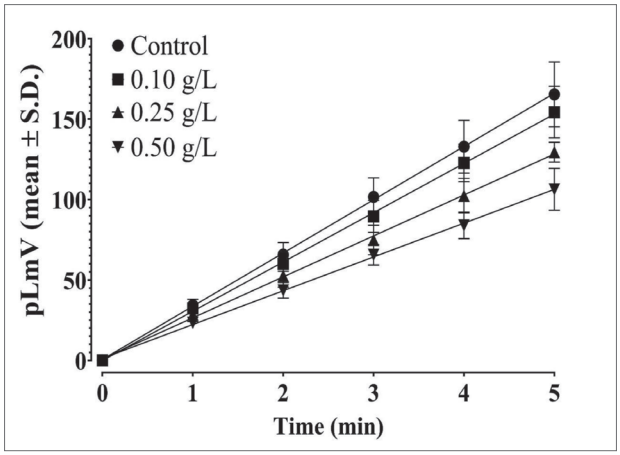


Fig. 2. Effect of a 15-min exposure to *S. viarum* AEF on pLmV. The data are the mean±standard deviation of 6 independent experiments performed in triplicate.

Effects of *S. viarum* AEF on reproduction

Planarian reproduction can be either sexual or asexual [29]. This study aimed to assess sexual reproduction by measuring fecundity (the number of cocoons per week per planarian) and fertility (the number of cocoons hatching viable individuals per week per planarian) when exposed to the AEF at concentrations of 0.10, 0.25, and 0.50 g/L. Controls were performed using only PCW, as described in the materials and methods section. Fig. 3A shows that the control group had a fecundity of 0.903±0.21 cocoons per week per planarian. In contrast, the groups treated with 0.10, 0.25, and 0.50 g/L AEF produced 0.472±0.17, 0.264±0.08, and 0.014±0.02 cocoons per week per planarian, respectively. This resulted in reductions in fecundity of 47.7, 70.8, and 98.4% at the respective AEF concentrations (P<0.05).

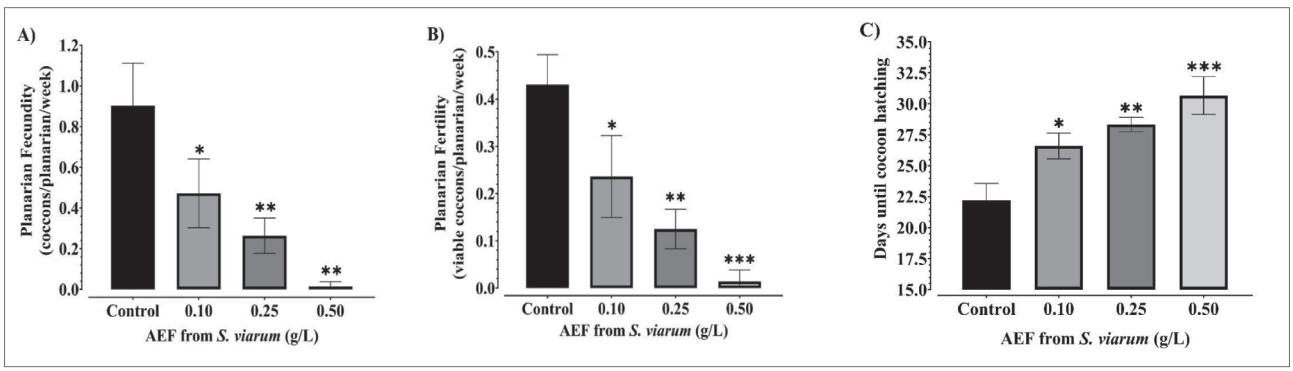


Fig. 3. A – Planarian fecundity; **B** – Planarian fertility; **C** – Days until cocoons hatch from planarian exposure to *S. viarum* AEF for 30 days. Data are the mean±standard deviation of 3 experiments. * P≤0.0342; ** P≤0.0057; *** P≤0.0003.

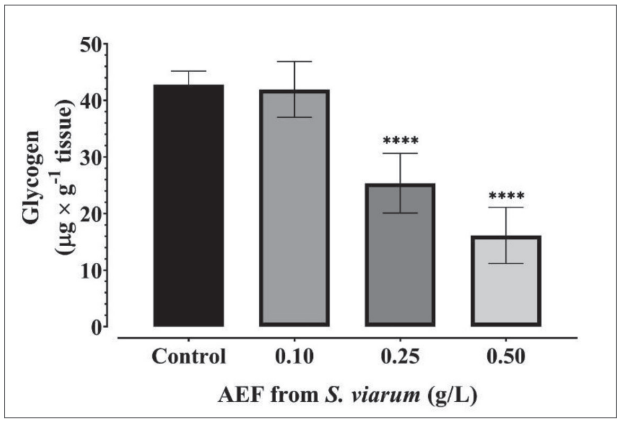


Fig. 4. Glycogen concentration in planarians exposed to *S. viarum* AEF after 14 days. Data are the mean±standard deviation of 5 experiments performed in triplicate. **** P<0.0001.

Fig. 3B revealed a significant reduction in planarian fertility, as indicated by the number of viable planarians emerging from cocoons. The control group averaged 0.4307±0.06 viable cocoons per week per planarian. In comparison, the treated groups showed reductions to 0.236±0.08, 0.125±0.04, and 0.014±0.03 viable cocoons per week per planarian at concentrations of 0.10, 0.25, and 0.50 g/L of AEF, respectively (P<0.05). The reduction in viable planarians emerging from cocoons was 45.2, 71.0, and 96.7% for the 0.10, 0.25, and 0.50 g/L of AEF treatments, respectively (P<0.05).

The hatching time of cocoons from the control group was 22.2±1.4 days (Fig. 3C). For the treated groups, hatching times increased to 26.6±1.0 (+19.8%), 28.3±0.6 (+27.5%), and 30.7±1.5 days (+38.3%) at concentrations of 0.10, 0.25, and 0.50 g/L of AEF, respectively, compared to the control (P<0.05).

Effects of *S. viarum* AEF on the glycogen content

Glycogen is a critical energy reserve in planarians, enabling them to maintain essential functions during periods of food scarcity or environmental stress [30-32]. It plays a crucial role in helping planarians adapt to such changes and maintain metabolic homeostasis [32]. In this study, the glycogen concentration of planarians was measured after 14 days of exposure to 0.10, 0.25, and 0.50 g/L of *S. viarum* AEF. The data provided in Fig. 4 show that while the glycogen content remained unchanged at 0.10 g/L, it was significantly reduced by 40.8% and 62.3% at concentrations of 0.25 and 0.50 g/L compared to the control, respectively (P<0.0001).

Effects of *S. viarum* AEF on antioxidant enzymes and AChE

SOD is crucial for regulating superoxide radicals and preventing oxidative damage. Fig. 5A shows that SOD was stimulated by the AEF at both 24 h and 14 days. Specifically, SOD increased by 25.6%, 18.5%, and 60.8% (P≤0.0018) after 24 h of exposure to 0.10, 0.25, and 0.50 g/L of AEF, respectively. After 14 days, SOD increased by 78.0%, 79.2%, and 59.9% at the same concentrations (P<0.0001). Statistical analysis indicated significant effects of AEF concentration [$F_{(3, 43)}$=117.0] and incubation time [$F_{(1, 39)}$=355.3] on SOD, highlighting the combined impact of concentration and time [$F_{(3, 39)}$=52.64].

Exposure to lower concentrations of the *S. viarum* AEF at 0.10 and 0.25 g/L significantly stimulated CAT enzyme by 72.1% and 44%, respectively. However, the highest concentration of 0.50 g/L inhibited CAT by 25.2% (Fig. 5B). Prolonged exposure (14 days) resulted

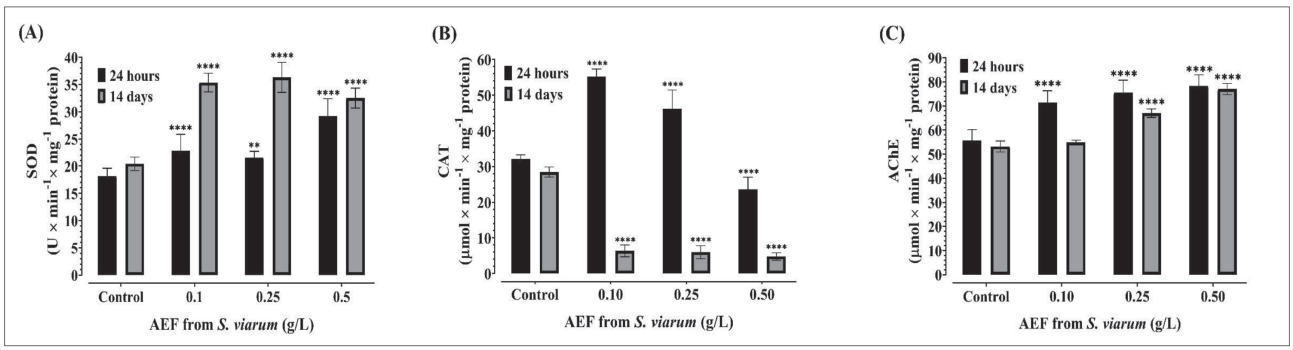


Fig. 5. Effects of *S. viarum* AEF on SOD **(A)**, CAT **(B),** and AChE activities **(C)** from *D. tigrina* after 24 h, and 14 days of incubation. Data are the mean±standard deviation of 5 experiments performed in triplicate. Significance levels: ** P≤0.0028; **** P<0.0001.

in a substantial decrease in CAT by 77.7%, 78.9%, and 83.2% at 0.10, 0.25, and 0.50 g/L of AEF, respectively. Statistical analysis confirmed significant effects of both AEF concentration [$F_{(3, 44)}$=60.56] and incubation time [$F_{(1, 11)}$ = 997.8] on CAT, with both factors significantly influencing the enzyme [$F_{(3, 11)}$ = 120.8].

Fig. 5C demonstrates the effects of the AEF on AChE. After 24 h of exposure, AChE increased by 28.2%, 35.5%, and 40.6% at 0.10, 0.25, and 0.50 g/L of AEF, respectively. After 14 days, AChE increased by 26.1% and 44.8% at 0.25 and 0.50 g/L. Significant differences were observed across AEF concentrations [$F_{(3, 87)}$=179.7] and incubation time [$F_{(1, 87)}$=91.10], with a combined effect of concentration and time on AChE [$F_{(3, 87)}$=21.72]. These results highlight the significant impact of AEF concentration and incubation duration on AChE.

DISCUSSION

In the present study, we explored the effects of the aqueous extract of *S. viar*um on the physiological and biochemical parameters of *D. tigrina* planarians, aiming to deepen our understanding of extract toxicity in aquatic organisms. Our results indicate that even at sublethal concentrations significantly below those causing toxicity in livestock, the extract induced notable alterations in planarian locomotion, reproduction, and enzyme activities without resulting in mortality. This underscores the heightened sensitivity of planarians to the extract and suggests that even minimal quantities could have significant toxic effects, potentially impacting other organisms and ecosystems.

Building on this, our study specifically assessed the impact of aqueous extracts from *S. viarum* fruits on *D. tigrina* planarians. As an invasive species, *S. viarum* poses a significant risk to livestock due to its known toxicity. The concentrations used in this study (0.10-0.50 g/L or 0.01-0.05%) are considerably lower than those observed to cause toxicity in cattle [34]. In planarians, the LC_{50} was determined to be 1.22 g/L (0.122%), whereas in cattle, concentrations of 4.9 g/kg (0.49%) cause toxicity without mortality, and the LC_{50} ranges from 7.5-10 g/kg (0.75 to 1%). Therefore, the lethal concentration in planarians is approximately four times lower than the concentration that causes toxicity symptoms in cattle; the lethal concentration in cattle is 6.2 to 8.2 times higher than that for planarians. By employing lower concentrations in our experiments, we observed different effects, including reduced locomotion, decreased reproduction, and enzymatic changes, without inducing mortality.

Phytochemical analysis revealed that AEF has remarkable antioxidant and metal-chelating properties even at low concentrations. The presence of polyphenols and flavonoids points to potential applications in various fields, including industrial, medicinal, and environmental. These properties could help reduce oxidative stress, prevent metal-catalyzed oxidation, and treat heavy metal poisoning. However, high antioxidant levels might have pro-oxidant effects, necessitating sustainable management of *S. viarum* extraction to prevent ecological impacts. Comprehensive investigations would evaluate the stability, efficacy, and economic feasibility of these compounds.

Our study included a long-term assessment of planarian reproduction over 30 days, exposing them to different concentrations of the aqueous extract. This period allowed us to collect and analyze cocoons, observing the number of cocoons formed per week per planarian and the number of individuals generated weekly per cocoon, thereby capturing the complete reproductive cycle. Since the hatching period for *D. tigrina* cocoons is approximately 22 days at 20°C [16], short-term studies cannot adequately capture reproductive trends and potential sublethal effects of the extract. Extending the study period provides detailed data on planarian reproductive patterns and the ecological effects of the extract. However, as the findings are based solely on planarian reproduction, further research on other organisms and different ecological contexts is needed to understand the potential environmental impact.

Biochemical analyses revealed that the extract affects glycogen metabolism in *D. tigrina*. A significant decrease in glycogen concentration over 14 days suggests increased glycogen catabolism and decreased glycogen synthesis. This metabolic stress might be due to the increased energy demands of survival in a toxic environment. The similarity of planarian metabolism to vertebrates implies that these effects could be relevant across different species. However, focusing solely on glycogen levels may not capture the full spectrum of metabolic changes induced by the extract.

The reproductive toxicity observed in planarians, including reduced cocoon numbers (fecundity), decreased hatchling rates (fertility), and delayed cocoon hatching times in the presence of the AEF, suggests a disruption of key physiological processes essential for reproduction. Although the exact mechanisms remain unclear, it is plausible that exposure to the extract may interfere with the energy metabolism required for gametogenesis and embryonic development. Since reproduction is highly energy-dependent, any disruption in energy supply, whether through direct effects on ATP synthesis or oxidative stress, could account for the patterns observed. Studies on AEF effects on specific aspects of energy metabolism, such as mitochondrial function, will be crucial in elucidating the mechanisms underlying these reproductive alterations.

The iron-chelating activity of the *S. viarum* AEF may exacerbate oxidative stress by reducing the availability of iron for metal-dependent reactions, such as the Fenton and Haber-Weiss reactions [33,34]. When the extract chelates iron, it decreases hydroxyl radical formation due to reduced iron availability. This can result in an accumulation of H_2O_2 [35]. An increase in H_2O_2 may indicate elevated SOD activity in response to superoxide accumulation, as SOD converts superoxide radicals into H_2O_2. This explains the observed stimulation of SOD activity.

Lower iron availability due to the AEF chelating activity directly affects CAT, which requires iron in its active site to decompose H_2O_2. Without iron, CAT activity is compromised, particularly at higher extract concentrations and with prolonged exposure. This explains the inhibition of catalase at higher concentrations after 24 h and after 14 days of exposure. The oxidative stress resulting from H_2O_2 accumulation, combined with the inability of CAT to function effectively without iron, likely leads to its progressive inactivation, a phenomenon often associated with sustained oxidative stress [36]. The disruption of iron-dependent reactions, such as those described by the Fenton and Haber-Weiss mechanisms [34], further exacerbates the oxidative damage.

The iron-chelating activity of the *S. viarum* AEF contributes to the observed effects on antioxidant enzymes. The continued stimulation of SOD is likely a response to the accumulation of superoxide due to increased hydrogen peroxide, which cannot be efficiently converted into hydroxyl radicals due to iron chelation. Conversely, the inhibition of CAT under higher extract concentrations and prolonged exposure may be attributed to iron deprivation necessary for its function, leading to impaired hydrogen peroxide degradation and subsequent enzyme inactivation due to oxidative stress. In the absence of iron CAT is less effective in decomposing H_2O_2, leading to oxidative stress and progressive inactivation [34,37-39].

Acetylcholinesterase (AChE) is essential for maintaining brain function in planarians. AChE degrades acetylcholine at synaptic connections, thereby controlling nerve transmission. Increased AChE can help restore normal nerve function and mitigate the negative effects of excess acetylcholine in vertebrates, such as muscle fatigue and weakness. Planarians exposed to AEF have demonstrated increased AChE, which reduces acetylcholine levels and has short-term physiological consequences. However, persistent or prolonged exposure may result in sustained neurotransmitter breakdown, potentially affecting organismal health and long-term brain signaling. This extension provides more background information on the role of AChE in planarians, the effects of elevated AChE, and the implications of AEF exposure on neurotransmitter dynamics and organismal health.

This study on the effects of the AEF from *Solanum viarum* fruits on *Dugesia tigrina* revealed significant antioxidant and metal-chelating properties at low concentrations. Acute exposure to the extract led to notable reductions in planarian mobility, while long-term exposure resulted in impaired reproduction, evidenced by delayed cocoon hatching, reduced fecundity, and decreased fertility. Biochemical analyses further revealed a significant decline in glycogen levels, suggesting heightened catabolism and metabolic stress triggered by the extract. The impact on antioxidant enzymes was also concentration-dependent: lower concentrations stimulated CAT, while higher concentrations and prolonged exposure led to its inhibition. Meanwhile, SOD increased in response to oxidative stress, likely as an adaptive mechanism against glycoalkaloid-induced damage. In addition, the extract stimulated AChE, potentially disrupting normal neural function by decreasing ACh levels.

These findings illustrate the complex, concentration-dependent effects of the AEF from *S. viarum* fruits on planarian physiology, emphasizing the need for further research to clarify the underlying mechanisms. This work highlights the ecological and toxicological significance of *S. viarum*, with potential implications for both environmental and biomedical applications. It also highlights the importance of sustainable management to minimize ecological disruptions caused by this invasive species.

CONCLUSIONS

This study offers a detailed assessment of the biochemical and physiological effects of the aqueous extract fraction (AEF) from *Solanum viarum* fruits on *Dugesia tigrina*, demonstrating their increased sensitivity to even low concentrations of the extract. The AEF significantly impacted the locomotion, reproduction, and the enzymatic activities of SOD and CAT, without causing mortality. The findings reveal the powerful antioxidant and iron-chelating properties of the extract. They also highlight the potential for pro-oxidant effects due to iron depletion and hydrogen peroxide accumulation. These results underscore the ecological risks of extensive *S. viarum* fruit use, necessitating careful management of its applications. Future research should explore the specific molecular mechanisms of these effects and evaluate their environmental implications. This study contributes to a better understanding of the effects of natural compounds on aquatic organisms and the environment. It offers valuable information for potential biomedical applications of *S. viarum*.

Funding: The authors received no specific funding for this work.

Author contributions: All listed authors have contributed sufficiently to the work be included as co-authors. Conceptualization: FS and WLB; investigation: FS and WLB; data processing: FS and WLB; writing of the original draft: FS and WLB; revision and editing: FS and WLB.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: Data underlying the reported findings have been provided as a raw dataset available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Schaly%20and%20Braguini%20Raw%20Dataset.docx

REFERENCES

1. Nithya M, Ragavendran C, Natarajan D. Antibacterial and free radical scavenging activity of a medicinal plant Solanum xanthocarpum. Int J Food Prop. 2018;21(1):313-27. https://doi.org/10.1080/10942912.2017.1409236
2. Tekuri SK, Pasupuleti SK, Konidala KK, Amuru SR, Bassaiahgari P, Pabbaraju N. Phytochemical and pharmacological activities of Solanum surattense Burm. f - A review. J Appl Pharm Sci. 2019;9(3):126-36. https://doi.org/10.7324/JAPS.2019.90318
3. Pereira APA, Lauretti LBC, Alvarenga VO, Paulino BN, Angolini CFF, Neri-Numa IA, Orlando EA, Pallone JAL, Sant'Ana AS, Pastore GM. Evaluation of fruta-do-lobo (Solanum lycocarpum St. Hill) starch on the growth of probiotic strains. Food Res Int. 2020;133. https://doi.org/10.1016/j.foodres.2020.109187
4. Jayakumar K, Murugan K. Solanum alkaloids and their pharmaceutical roles: a review. J Anal Pharm Res. 2016;3(6):397-408. https://doi.org/10.15406/japlr.2016.03.00075
5. Moyo SM, Serem JC, Bester MJ, Mavumengwana V, Kayitesi E. The impact of boiling and in vitro human digestion of Solanum nigrum complex (Black nightshade) on phenolic compounds bioactivity and bioaccessibility. Food Res Int. 2020;137:109720. https://doi.org/10.1016/j.foodres.2020.109720
6. Akanda RU, Mullahey JJ, Shilling DG. Environmental factors affecting germination of tropical soda apple (Solanum viarum). Weed Sci. 1996;44(3):570-4. https://doi.org/10.1017/S0043174500094352
7. Mullahey JJ, Shilling DG, Mislevy P, Akanda R. Invasion of tropical soda apple (Solanum viarum) into the US: lessons learned. Weed Technol. 1998;12(4):733-6 https://doi.org/10.1017/S0890037X00044638
8. Al Sinani SSS, Eltayeb EA. The steroidal glycoalkaloids solamargine and solasonine in Solanum plants. South African J Bot. 2017;112:253-69. https://doi.org/10.1016/j.sajb.2017.06.002
9. Mullahey J. Tropical soda apple (Solanum viarum Dunal), a biological pollutant threatening Florida. Castanea. 1996;61:255-60.
10. Saha M, Datta BK. Reproductive biology of Solanum viarum Dunal (Solanaceae) in Northeast India. Pleione. 2014;8(2):258-66.
11. Manrique-Moreno M, Londoño-Londoño J, Jemioła-Rzemińska M, Strzałka K, Villena F, Avello M, Suwalsky M. Structural effects of the Solanum steroids solasodine, diosgenin and solanine on human erythrocytes and molecular models of eukaryotic membranes. Biochim Biophys Acta - Biomembr. 2014;1838(1 Pt B):266-77. https://doi.org/10.1016/j.bbamem.2013.08.003
12. Javed M, Saleem A, Akhtar MF. Diosgenin, a steroidal sapogenin, arrests arthritis through modulation of inflammatory cytokines and oxidative stress biomarkers in Wistar rats. Inflammopharmacology. 2023;31(4):1951-66. https://doi.org/10.1007/s10787-023-01244-7
13. Winkiel MJ, Chowański S, Słocińska M. Anticancer activity of glycoalkaloids from Solanum plants: A review. Front Pharmacol. 2022;13:979451. https://doi.org/10.3389/fphar.2022.979451

14. Torres-Da Matta J, Kanaan S, Nery da Mata A. Changes in glycolytic metabolism during the regeneration of planarians Dugesia tigrina (Girard). Comp Biochem Physiol Part B Comp Biochem. 1989;93(2):391-6. https://doi.org/10.1016/0305-0491(89)90097-7
15. Nery da Matta A, Giovanni De Simone S, Kanaan S, Torres-da Matta J. Aminotransferase activity during the regeneration of planarians Dugesia tigrina (Girard). Comp Biochem Physiol Part B Comp Biochem. 1992;101(3):323-6. https://doi.org/10.1016/0305-0491(92)90007-E
16. Knakievicz T, Vieira SM, Erdtmann B, Ferreira HB. Reproductive modes and life cycles of freshwater planarians (Platyhelminthes, Tricladida, Paludicula) from southern Brazil. Invertebr Biol. 2006;125:212-21. https://doi.org/10.1111/j.1744-7410.2006.00054.x
17. Tang SZ, Kerry JP, Sheehan D, Buckley DJ. Antioxidative mechanisms of tea catechins in chicken meat systems. Food Chem. 2002;76(1):45-51. https://doi.org/10.1016/S0308-8146(01)00248-5
18. Oyaizu M. Studies on Products of Browning Reaction Antioxidative Activities of Products of Browning Reaction Prepared from Glucosamine. Japanese J Nutr Diet. 1986;44(6):307-15. https://doi.org/10.5264/eiyogakuzashi.44.307
19. Ted E, McConnell J. A Manual of Psychological Experimentation on Planarians. 1st ed. McConnel J, editor. Ann Arbor, Michigan; 1965. 118 p.
20. Raffa RB, Valdez JM. Cocaine withdrawal in Planaria. Eur J Pharmacol. 2001;430(1):143-5. https://doi.org/10.1016/S0014-2999(01)01358-9
21. Kemp A, Van Heijningen AJ. A colorimetric micro-method for the determination of glycogen in tissues. Biochem J. 1954;56(4):646-8. https://doi.org/10.1042/bj0560646
22. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 1978;186(1):189-95. https://doi.org/10.1016/0003-9861(78)90479-4
23. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6. https://doi.org/10.1016/S0076-6879(84)05016-3
24. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88-95. https://doi.org/10.1016/0006-2952(61)90145-9
25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54. https://doi.org/10.1016/0003-2697(76)90527-3
26. Finney DJ. Probit Analysis. J Pharm Sci. 1971;60(9):1432. https://doi.org/10.1002/jps.2600600940
27. Rodríguez-Arce E, Saldías M. Antioxidant properties of flavonoid metal complexes and their potential inclusion in the development of novel strategies for the treatment against neurodegenerative diseases. Biomed Pharmacother. 2021;143:112236. https://doi.org/10.1016/j.biopha.2021.112236
28. Braguini WL, Pires NV, Alves BB. Phytochemical analysis, antioxidant properties and Brine Shrimp lethality of unripe fruits of Solanum viarum. J Young Pharm. 2018;10(2):159-63. https://doi.org/10.5530/jyp.2018.10.36
29. Sarnat HB. Book Review: The First Brain: The Neuroscience of Planarians. J Child Neurol. 2015;30(9):1230-1. https://doi.org/10.1177/0883073814542952
30. da Matta AN, Kanaan S, Silva CB, Santos DO, Côrte-Real S, Simone G De, Torres-da-Matta J. Changes in energetic substrates during the regeneration of planarians Dugesia tigrina (Girard). Comp Biochem Physiol Part A Physiol. 1993;105(2):341-5. https://doi.org/10.1016/0300-9629(93)90218-S
31. Adeva-Andany MM, González-Lucán M, Donapetry-García C, Fernández-Fernández C, Ameneiros-Rodríguez E. Glycogen metabolism in humans. BBA Clin. 2016;5:85-100. https://doi.org/10.1016/j.bbacli.2016.02.001
32. Tokarnia C, Canella C, Döbereiner J. Experimental poisoning by the fruits of Solanum aculeatissimum in cattle. Pesq Agropec Bras. 1973;8:35-9.
33. Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. Nat Chem Biol. 2008;4(5):278-86. https://doi.org/10.1038/nchembio.85
34. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 5th ed. Oxfor: Oxford Academic Press; 2015. https://doi.org/10.1093/acprof:oso/9780198717478.001.0001
35. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev. 1979;59(3):527-605. https://doi.org/10.1152/physrev.1979.59.3.527
36. Kehrer JP. The Haber-Weiss reaction and mechanisms of toxicity. Toxicology. 2000;149(1):43-50. https://doi.org/10.1016/S0300-483X(00)00231-6
37. Valko M, Morris H, Cronin M. Metals, toxicity and oxidative stress. Curr Med Chem. 2005;12(10):1161-208. https://doi.org/10.2174/0929867053764635
38. Wang J, Zhang H, Zhang T, Zhang R, Liu R, Chen Y. Molecular mechanism on cadmium-induced activity changes of catalase and superoxide dismutase. Int J Biol Macromol. 2015;77:59-67. https://doi.org/10.1016/j.ijbiomac.2015.02.037
39. Wang Y, Branicky R, Noë A, Hekimi S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol. 2018;217(6):1915. https://doi.org/10.1083/jcb.201708007

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Molecular formulas for ADMETlab 3.0 analysis.
The molecular formulas in SMILES format correspond to the molecules contained in the *S. viarum* extract analyzed using the ADMETlab 3.0 software. The software can be accessed at https://admetlab3.scbdd.com/server/evaluation. The data for each molecule is available for anyone interested in conducting their analysis.

Caffeic Acid
SMILES 1: O=C(O)C=Cc1ccc(O)c(O)c1

Campesterol
SMILES 2: CC(C)C(C)CCC(C)C1CCC2C3CC=C4CC(O)CCC4(C)C3CCC12C

Carpesterol
SMILES 3: CCC(CC(O)C(C)C1CCC2C3=CC(=O)C4C(C)C(OC(=O)c5ccccc5)CCC4(C)C3CCC21C)C(C)C

Daucosterol
SMILES 4: CCC(CCC(C)C1CCC2C3CC=C4CC(OC5OC(CO)C(O)C(O)C5O)CCC4(C)C3CCC12C)C(C)C

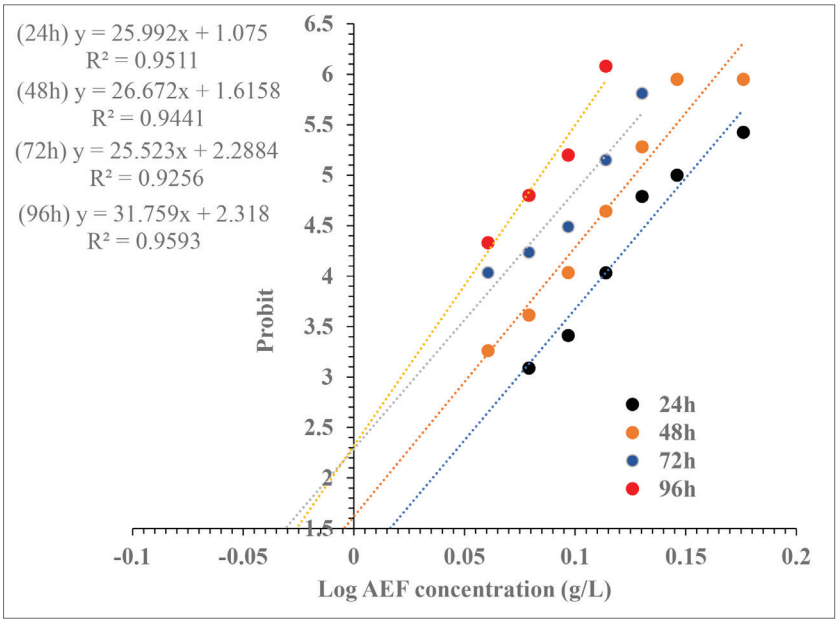
Diosgenin
SMILES 5: CC1CCC2(OC1)OC1CC3C4CC=C5CC(O)CCC5(C)C4CCC3(C)C1C2C

Lupeol
SMILES 6: C=C(C)C1CCC2(C)CCC3(C)C(CCC4C5(C)CCC(O)C(C)(C)C5CCC43C)C12

Solomargine
SMILES 7: (OC6OC(CO)C(OC7OC(C)C(O)C(O)C7O)C(O)C6OC6OC(C)C(O)C(O)C6O)CCC5(C)C4CCC3(C)C1C2C

Solanosine
SMILES 8: OC6OC(CO)C(O)C(OC7OC(CO)C(O)C(O)C7O)C6OC6OC(C)C(O)C(O)C6O)CCC5(C)C4CCC3(C)C1C2C

Solasodine
SMILES 9: CC1CCC2(NC1)OC1CC3C4CC=C5CC(O)CCC5(C)C4CCC3(C)C1C2C



Supplementary Fig. S1. Graph obtained from the LC_{50} data of AEF from *S. viarum* in planarians.