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Leaf and fruit extracts of *Solanum betaceum* Cav.: Antioxidant potential and embryotoxicity using a zebrafish model

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ABSTRACT

This study aimed to evaluate the antioxidant potential and toxicological effects of *Solanum betaceum* leaf and fruit extracts on zebrafish embryos. Leaf and fruit pulp extracts were prepared by turbo extraction using distilled water or methanol as solvent. The levels of total phenols and flavonoids were quantified. The antioxidant potential was assessed by the total antioxidant capacity test and two free radical scavenging tests. The toxic effects of *S. betaceum* species were assessed using an acute toxicity test on zebrafish embryos exposed to *S. betaceum* extracts. The leaves of *S. betaceum* were found to have high total phenolic and flavonoid content, high total antioxidant capacity and high toxicity to developing zebrafish embryos, with a teratogenic index (TI) equal to 17.65. The leaves are used topically in folk medicine, which may allow for safer utilization of phenolics compared to oral ingestion. The fruits had higher free radical scavenging activity and lower toxicity for the embryos than the leaves, with the TI around 2.2. Water was a more suitable solvent than methanol for obtaining safer bioactive compounds from *S. betaceum* leaf and fruit. This work provides an initial basis for pharmacological studies of *S. betaceum* species in animal models.

1. Introduction

Solanum betaceum Cav. (syn. *Cyphomandra betacea* Sendt.) is a species native to the Andes that is known as the tree tomato or 'tamarillo'. It has traditionally been cultivated to produce fruit for fresh consumption in some South American countries, such as Peru, Bolivia, Colombia, Ecuador, and Venezuela. Bittersweet fruits are used in a variety of culinary recipes. Additionally, the juice of the ripe fruit is used in Ecuadorian folk medicine to treat tonsillitis, high cholesterol, and stomach pain [1]. In Colombia, after the fruit is cooked over hot coals, the fruit pulp is used as a poultice for inflamed tonsils. In Costa Rica, fresh fruit is eaten on an empty stomach in cases of flu and is also used as a remedy for liver problems. *S. betaceum* leaves are also heated for topical use on the neck, around the throat to treat inflamed tonsils, and on the chest for angina pain [2,3]. In Brazil, *S. betaceum* is considered exotic and is typically grown in backyards for non-commercial purposes, classifying it as an unconventional vegetable. Its medicinal, nutritional, and technological properties are still largely unknown in the country [4,5].

Biological activities, such as antioxidant [6], anti-inflammatory, antidiabetic [7], antinociceptive [8], improve cognitive performance and prevent cognitive deterioration raised by several neurotoxins [9,10], and antiobesity effects, have been demonstrated *in vivo* and *in vitro* in tree tomato fruit [11]. However, the chemical composition of tree tomato fruits varies depending on factors such as fruit color, maturity stage, fruit parts (pulp, mucilage, seeds), genetics, and environmental conditions [12].

Studies reporting the biological activities of tree tomato leaves are scarce. Chemical analysis of the aqueous extract of the leaves showed the presence of hydroxycinnamic phenolic acids, antioxidant properties, and inhibitory action of aldose reductase [13]. Leaves from species of the genus *Solanum* are widely used in folk medicine and have numerous biological properties. The biological activities have been attributed to some steroidal saponins, steroidal alkaloids, and phenols [14,15].

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In light of the traditional use of the tree tomato as a medicinal plant, it is important to assess the toxicity of this fruit to assess its safety for human health; notably, with the use of herbal medicines in general, there have been cases of adverse drug interactions and mutagenic, carcinogenic, and teratogenic effects reported in the literature [16].

However, it is known that *Solanum* species can present toxic alkaloids, mainly in their leaves. Human toxicological studies have shown that solamargine and solasonine used at certain levels cause such toxic effects as cell-membrane disruption, acetylcholinesterase inhibition, liver damage, heart damage, teratogenicity, and embryotoxicity [17]. In tomatoes, enzymatic degradation of glycoalkaloids occurs when tomatoes ripen [18]. Further studies should focus on analyzing the alkaloid composition and content of *S. betaceum* fruit and leaves better to understand its potential biological effects and toxicological implications.

However, studies on the toxicity and safety of tree tomatoes have been rare. Thus far, tomato tree fruit extracts have been found to be largely noncytotoxic to normal cells, with an $IC_{50} > 200.00$ μg mL^{-1} [19]. In an animal model, daily treatment of male *Sprague—Dawley* rats with 300 mg kg^{-1} fruit extract for 7 weeks did not cause toxicity [20].

The use of animal models in toxicology tests has a substantial advantage over *in vitro* tests since the animal model is more closely correlated with toxicity in humans and more reliably incorporates pharmacokinetics, absorption, distribution, and metabolism. Among animal models, the zebrafish embryo toxicity model, due to its high genetic similarity to humans (87 % similarity), has been widely used in screening the toxic effects of bioactive compounds and, more recently, in toxicological evaluations of herbal medicines (Jayasinghe and Jayawardena, 2019). The fish embryonic toxicity (FET) test [21], carried out with what is popularly known as zebrafish (*Danio rerio*), a species of tropical freshwater fish native to Asia, has several advantages for studies on the pharmacological effects of plants: small size, short life cycle, rapid development, fecundity, and transparency of eggs and embryos that allows the internal organs to be observed. Moreover, these animals are easy to maintain, and their behavior is readily observable [22].

Thus, this study aimed to evaluate the antioxidant potential of fruit pulp and leaf extracts from *S. betaceum* plants, as well as their toxicological effects on zebrafish embryos. This article is part of the doctoral thesis of Karina Schulz Borges, defended at Federal University of Lavras in 2021 [23].

2. Materials and methods

2.1. Plant material

2.1.1. Sample collection

Samples of fresh leaves and ripe fruit were collected from *S. betaceum* plants (with red-fleshed fruits) in the Germplasm Collection of Unconventional Vegetables of the Agriculture Department of the Federal University of Lavras (UFLA) (latitude 21°13'21" S, longitude 44°58'12" O, 922 m altitude), located in Lavras, MG, Brazil. According to Sá Júnior et al. [24] and the Köppen classification, the region has a Cwa climate, characterized by a humid temperate conditions with hot summers and dry winters.

2.1.2. Extraction

Extracts were prepared using two parts of the plant — leaves or fruits — and two different solvents — distilled water or pure methanol (99.8 % purity) — resulting in a total of four extracts. The fresh leaves were washed in running water and chopped. The fruit was washed, peeled with a knife, and cut lengthwise, and the seeds were removed manually to obtain the pulp (mesocarp).

The extracts were prepared by turbo extraction using 10 g of plant material (leaves or fruit pulp) and 100 mL of solvent (distilled water or pure methanol). Each plant sample was placed in a plastic beaker in an ice bath and subjected to turbo extraction for three cycles of 10 min each, with a one-minute interval between cycles. After these procedures, the extracts were vacuum filtered and stored at −20°C.

2.2. Antioxidant tests

2.2.1. Preparation of extracts

An aliquot of each *Solanum betaceum* extract was centrifuged, and from the supernatant, serial dilutions were prepared for each test.

2.2.2. Determination of total phenols

Total phenolic compounds were determined by the colorimetric method using the Folin-Ciocalteu reagent described by Slinkard and Singleton [25]. The reactions were carried out in microtiter plates using 20 μL of the extracts, 125 μL of 7 % sodium carbonate (Na_2CO_3), and 100 μL of 10 % ethanolic Folin-Ciocalteu solution. The plate was stored for 2 h in the dark at room temperature, after which the absorbance was measured on a spectrophotometer (TECAN Infinity microplate reader® M200 PRO) at 760 nm. Total phenolic contents were determined using a calibration curve, which was generated with gallic acid at eight concentration points (1, 0.5, 0.25, 0.125, 0.062, 0.0312, 0.0156, and 0.0078 μg mL^{-1}). The results were calculated according to the straight-line equation (y = 3.887x - 0.1545, where y is the absorbance and x is the concentration; $R^2 = 0.9943$). The tests were carried out in triplicate, and the results are expressed as milligram equivalents of gallic acid per g of fresh sample material (mg EAG g^{-1}).

2.2.3. Quantification of flavonoids

The quantification of flavonoids in the extracts was determined using the method of Ahn et al. [26]. One hundred microliters of 10 % aluminum chloride solution ($AlCl_3$) (prepared with 70 % ethanol) was mixed with 100 μL of each extract. After 60 min in the dark at room temperature, the absorbances of the samples were read in a spectrophotometer at 420 nm against a blank of 100 μL of solvent + 100 μL of $AlCl_3$. Flavonoids contents were determined using a calibration curve, which was generated with quercetin at eight concentrations (1, 0.5, 0.25, 0.125, 0.062, 0.0312, 0.0156, and 0.0078 μg mL^{-1}). The results were calculated according to the equation of the line y = 12x + 0.0648, where y is the absorbance and x is the concentration ($R^2 = 0.999$). The tests were carried out in triplicate, and the total flavonoid content was expressed as milligram equivalents of quercetin per gram of fresh sample weight (mg EQ g^{-1}).

2.2.4. Total antioxidant capacity

The total antioxidant capacity (TAC) was established according to the ammonium molybdate reduction method described by Prieto et al. [27]. For the TAC test, 20 μL of the extracts mixed in 1.5 mL of reagent solution (28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulfuric acid) was used. The solutions were allowed to react for 90 min at 90ºC, and then, after allowing them to cool to room temperature, 100 μL of each solution was applied to microtiter plates, and the absorbance was measured at 695 nm in a spectrophotometer. TAC was determined using a calibration curve, which was generated with ascorbic acid at seven concentrations (0.65, 0.32, 0.16, 0.08, 0.04, 0.02, and 0.010 mg mL^{-1}). The results were calculated according to the equation of the line y = 3.4675x + 0.1722, where y is the absorbance and x is the concentration ($R^2 = 0.9951$). The analysis was carried out in triplicate, with results expressed as milligram equivalents of ascorbic acid per g of fresh weight of the sample (mg EAC g^{-1}).

2.2.5. DPPH free radical scavenging activity

The DPPH free radical scavenging activity was determined according to Brand-Williams et al. [28]. Fifty microliters of the samples at different concentrations (100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 mg mL^{-1}) were added to the microtiter plates with 250 μL of 0.2 mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The solutions were then placed in the dark at room temperature for 60 min, after which

spectrophotometric readings were taken at 517 nm, with 3,5-di-tert-4--butylhydroxytoluene (BHT) used as a positive control and methanol used as a negative control. The free radical scavenging activity was expressed as the IC_{50} (mg mL^{-1}), equivalent to the concentration of the extract capable of inhibiting 50 % of DPPH radicals, which was calculated using the formula (%) = (A0 - A1)/A0 * 100, where A0 is the absorbance of the negative control, and A1 is the absorbance of the samples.

2.2.6. ABTS free radical scavenging activity

The elimination of free radicals by the ABTS radical cationic decolorization test was carried out using the method described by Ling et al. [29] and Re et al. [30], with a few modifications. The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS (ABTS-+) solution, produced by adding 0.0768 g of ABTS salt and 0.0132 g of potassium persulfate to 20 mL of distilled water, was left to stand at room temperature in the dark for 16 h before use. The ABTS-+ solution was diluted in ethanol until it reached an absorbance of 0.7 ± 0.02 at 734 nm. Ethanol was used as a negative control, while Trolox was used as a positive control. Thirty microliters of sample extract at different concentrations (100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 mg mL^{-1}) was added to 270 µL of diluted ABTS solution in microtiter plates. The solution was allowed to react for 6 min at room temperature, after which the absorbance was measured at 734 nm. The free radical scavenging activity was expressed as the IC_{50} (mg mL^{-1}), equivalent to the concentration of the extract capable of inhibiting 50 % of the ABTS radicals, which was calculated using the formula (%) = (A0 - A1)/A0 * 100, where A0 is the absorbance of the negative control, and A1 is the absorbance of the samples.

2.3. Embryotoxicity tests

2.3.1. Handling zebrafish matrices and facilities

All the experimental procedures in this study were carried out in strict accordance with the institutional rules of the Ethics Committee on Animal Experimentation of the Federal University of Lavras (UFLA), Lavras, MG, Brazil, under No. 014/19 and in compliance with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) for the maintenance and use of laboratory animals and the OECD guidelines [21].

The experiment was carried out in the Fish Ward of the Central Bioterium of the Department of Veterinary Medicine at UFLA.

The zebrafish (*Danio rerio*), approximately five months old, were kept in aquariums (Hydrus-Alesco) designed specially for the species in a recirculation system with sixty aquariums of 3 L each, with ten animals per aquarium. All the animals were kept under a 14:10 photoperiod (light:dark) [31] and a temperature of 28°C, and were fed with Nutrifish-Floculada twice a day. The aquariums were cleaned using an automated raking system.

Water quality parameters such as temperature, dissolved oxygen content, ammonia concentration, and pH were measured daily.

2.3.2. Embryo obtention

The adult zebrafish were separated according to sex (80 males and 80 females) the day before each round of breeding in small brooders. The brooders were kept in 40 L aquariums with a constant oxygenation system and a controlled temperature of 28ºC, with a photoperiod of 14 h light/10 h dark. The following morning, the males were placed in a female brooder (2 males/1 female) one or two hours before the end of the dark period. These changes stimulated mating behavior and the release of gametes in the next light cycle. Brooders with a grid bottom were used so that the eggs could pass through the bottom.

The embryos were collected using a siphon and were kept in E3 medium, which allowed for the standardization of zebrafish embryonic development (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, 0.33 mM MgSO4, and 0.1 % methylene blue) [32].

2.3.3. Acute embryo toxicity test (FET)

The extract of each *S. betaceum* sample was rotavaporated to remove the solvent, and the dry extract was weighed on a precision balance. Then, each extract was diluted in E3 medium, to ensure standardized preparation for all samples. Serial dilutions were prepared in a falcon tube using a sonicator. The diluted sample was then centrifuged, and the supernatant was used.

Zebrafish embryos and larvae were exposed to the extract in 96-well plates according to the method described by the OECD [21] (test no. 236). Forty fertilized eggs were selected for each treatment, placed in Petri dishes, and treated with 15 mL of extract at different dilutions. The control group was exposed to E3 medium. The embryos were kept in Petri dishes in an oven at 28°C for 24 h. After this period, 20 healthy embryos (n = 20) were selected from each treatment and placed in 96-well plates, with one embryo per well and 200 µL of diluted extract or E3 medium.

The embryos were analyzed one by one every 24 h until 120 h after fertilization (HAF). The developmental parameters evaluated in embryos and larvae during the 120-hour exposure were analyzed, as shown in Table 1. The embryos and larvae were subsequently examined using an inverted microscope (Olympus CX22LED) and a magnifying glass (Motic BA310) to assess somatic development at each extract concentration over a 120-h exposure period. Two tests were carried out in which *S. betaceum* extracts were tested for their embryotoxic and teratogenic effects on the development of zebrafish embryos and larvae at the following concentrations: 10, 5, 2.5, 1.25, and 0.625 mg mL^{-1} and 2, 1, 0.5, 0.25, 0.125, 0.062, and 0.031 mg mL^{-1}.

2.3.4. Teratogenic index (TI) evaluations

The method described by Selderslaghs et al. [33] was used to evaluate the data. At 24, 48, 72, 96, and 120 HAF, embryo mortality/embryotoxicity and morphological changes were assessed using an inverted microscope (Olympus CX22LED). Scores were assigned for each characteristic in a binominal manner ('1' was assigned for abnormal, and '0' was assigned for normal characteristics). On the basis of the score assigned to certain morphological characteristics, an overall score for the percentage effect was created for each treatment in the experiment. In addition, the effects were evaluated as a function of time.

Each individual in the experiment was assigned a score every 24 h for malformation (morphological deformity) and mortality, and the effective percentage of each concentration at each time point was determined. The extract toxicity percentage was determined as the ratio of dead embryos and/or larvae to the initial total number of embryos (20 fertilized eggs). The malformation percentage at 24, 48, 72, 96, and 120 HAF was determined as the proportion of malformed embryos/larvae (with edema, lordosis, or failure to hatch at 120 HAF) to the total number of initial embryos (n = 20).

The TI values were calculated as the ratio of the LC_{50}/EC_{50} for each treatment at 120 HAF. This index is used to classify the teratogenic effects of any toxic compound; in other words, the greater the TI value is,

Table 1
Morphological characteristics evaluated as measures of the teratogenic potential of *S. betaceum over* 120 h.

			HAF				
Stage of life	Embryotoxicity	Features	24	48	72	96	120
Egg	Egg coagulation		V	V	V	V	V
		Somites	V	V	V	V	V
		Tail detachment	V	V	V	V	V
		Eyes	V	V	V	V	V
		Pigmentation	X	V	V	V	V
Hatching (Larva)	Live larva	Lordosis	X	X	V	V	V
		Edema	X	V	V	V	V

HAF: Hours after fertilization. V: characteristic that was evaluated at a given time. X: characteristic that was not evaluated at a given time.

the greater the teratogenic potential of the substance [33].

2.3.5. Evaluation of the dose-response curve

The dose-response analysis was performed according to the procedures described by Alafiatayo et al. [34]. The *Graph Pad Prisma* program, version 8.0, was used to construct concentration-response curves for malformation and mortality data for each treatment, each with 20 replicates per concentration at 120 HAF. The data are presented in the form of sigmoidal curves. The lower and upper curves were set at 0 and 100, respectively, with the requirement that the percentages close to 0 and 100 for effects fall within the concentration range. This concentration-response curve was used to determine the concentration of *S. betaceum* extracts required to cause mortality in 50 % of zebrafish embryos/larvae (LC_{50}) and to induce malformations in 50 % of zebrafish embryos/larvae (EC_{50}) at 120 HAF.

Using the calculated LC_{50} and EC_{50} values, a teratogenic index (TI) was calculated as the ratio of LC_{50}/EC_{50} for the 120 HAF time point. The greater the TI was, the greater the teratogenic effect of the tested extract, in contrast to general embryotoxicity, as measured by organism mortality.

2.4. Statistical analysis

For the antioxidant tests, the experimental design used was entirely randomized, in a 2 × 2 factorial arrangement, with two parts of the plant (leaf and fruit) and two types of solvent (water and methanol). All tests were carried out in triplicate, and the R program was used for the statistical analysis of the data. The means between the treatments were subjected to analysis of variance using the F test and grouped using the Scott–Knott test at 5 % probability. For the embryotoxicity test, dose-response curves were generated using *GraphPad Prism* version 8. The concentrations were transformed into the logarithm of the concentrations to analyze embryotoxicity.

3. Results

3.1. Phenolic compounds and antioxidant activity of S. betaceum extracts

The results revealed the presence of phenols and flavonoids and antioxidant activity in the *S. betaceum* leaf and fruit pulp extracts (Table 2). The leaf showed higher levels of total phenols and flavonoids than the fruit. The methanol-extracted leaves had the highest total phenolic content, and the water-extracted leaves had the highest flavonoid content. The difference in polarity between the solvents—water is more polar than methanol—may imply different extraction powers for the components in the sample.

Concerning the antioxidant activities, the methanolic extract showed greater free radical inhibition activities in both the leaves and the fruit pulp. For the TAC test, the methanolic leaf extract showed the best activity (2.04 mg EAC g^{-1}). In the ABTS and DPPH free radical scavenging tests, the aqueous fruit pulp extracts reduced these radicals by less than 50 %, making it impossible to calculate the IC_{50}. However, the methanolic leaf extract captured 50 % of the DPPH and ABTS free radicals present in the sample at significantly lower concentrations than the methanolic extract of the fruit pulp. Regardless of the plant organ (leaf or fruit pulp), the methanolic extract exhibited higher antioxidant activity and was more effective in extracting total phenols and flavonoids.

3.2. Acute toxicity test of zebrafish embryos exposed to S. betaceum extracts

For the initial test (*S. betaceum* leaf and fruit extracts at concentrations between 0.625 and 10 mg mL^{-1}), there was 100 % mortality at 24 h after fertilization (HAF) for the concentrations between 2.5 and 10 mg mL^{-1} for the four extracts tested. A second test was therefore carried out at lower concentrations (0.031–2 mg mL^{-1}) to better observe the development of the embryos and calculate the TI. At a concentration of 2 mg mL^{-1}, there was still 100 % mortality for the leaf extracts at 24 HAF and 100 % mortality for the fruit extracts up to 72 HAF.

Embryos incubated with *S. betaceum* extracts at concentrations less than 1 mg mL^{-1} were observed at 24 HAF (Fig. 1). There was no hatching at this point, and it was possible to observe changes in the development of the embryos. At a concentration of 1 mg mL^{-1}, for the four extracts tested, there was a delay in the development of the embryos, most of which did not show tail detachment or the development of somites and eyes (characteristics of the embryos in the control group between 11 and 14 HAF).

At 48 HAF, there was no hatching. The embryos incubated with the highest concentration of the extracts (1 mg mL^{-1}) still showed delayed development, independent of the solvent or part of the plant. At concentrations less than 0.5 mg mL^{-1}, the embryos were identical to those in the control group (E3 medium), with somite formation, tail detachment, formation of eyes, and presence of pigmentation.

At 72 and 96 HAF, most of the larvae incubated at a concentration of 1 mg mL^{-1} had not yet hatched and had no or little pigmentation. The eyes and tail had developed, except for the methanolic extract of the fruit, and only the larvae from the aqueous extract of the fruit presented development similar to the control group. At a concentration of 0.5 mg mL^{-1}, the larvae hatched with the expected pigmentation and development, but some showed edema in the yolk sac or pericardium. At values less than 0.5 mg mL^{-1}, the embryos/larvae were identical to those in the control group, with no morphological changes.

At 120 HAF, at the highest concentration (1 mg mL^{-1}), the larvae from the methanolic extracts of the leaf and fruit pulp had not hatched, and those from the fruit did not show any pigmentation. Additionally, at a concentration of 1 mg mL^{-1}, the larvae hatched and showed edema in the yolk sac or pericardium for both aqueous extracts. At values less than 0.5 mg mL^{-1}, the larvae showed the expected development and no alterations at 120 HAF, identical to the control group.

The toxicity effect on each individual was concentration-dependent (dose-dependent) for all tested extract concentrations. Concentration-response curves were produced for 120 HAF using the percentage of embryos affected (lethality or malformation for the observed characteristics) for each concentration (Figs. 2 and 3). The data for the LC_{50} (concentration of *S. betaceum* extracts required to cause mortality in 50 % of zebrafish embryos/larvae at 120 HAF) and EC_{50} (concentration

Table 2
Phenolic constituents and antioxidant activity of *S. betaceum* leaf and fruit pulp extracts.

	Total phenols (mg EAG g^{-1})		Flavonoids (mg EQ g^{-1})		TAC (mg EAC g^{-1})		ABTS (IC_{50} = mg mL^{-1})		DPPH (IC_{50} = mg mL^{-1})	
	Water	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water	Methanol
Leaf	2.64 Ab	3.03 Aa	1.20 Aa	0.73 Ab	1.67Ab	2.04 Aa	0.64 a	0.22 Bb	3.65 a	0.74 Bb
Fruit	0.89 Bb	1.80 Ba	0.03 Ba	0.02 Ba	1.33 Bb	1.57 Ba	*NA*	5.49 A	*NA*	4.88 A

Averages followed by the same uppercase letter in the column and lowercase letter in the row were grouped according to the Scott–Knott test ($p < 0.05$). Total phenols: expressed in mg of gallic acid equivalent per 1 g of fresh matter. Flavonoids: expressed in mg of quercetin equivalent per 1 g of fresh matter. TAC: total antioxidant capacity in mg of ascorbic acid equivalent per 1 g of fresh matter. ABTS: The ABTS free radical scavenging capacity is expressed as the IC_{50}. DPPH: DPPH free radical scavenging capacity expressed as the IC_{50}. *NA*: not available.

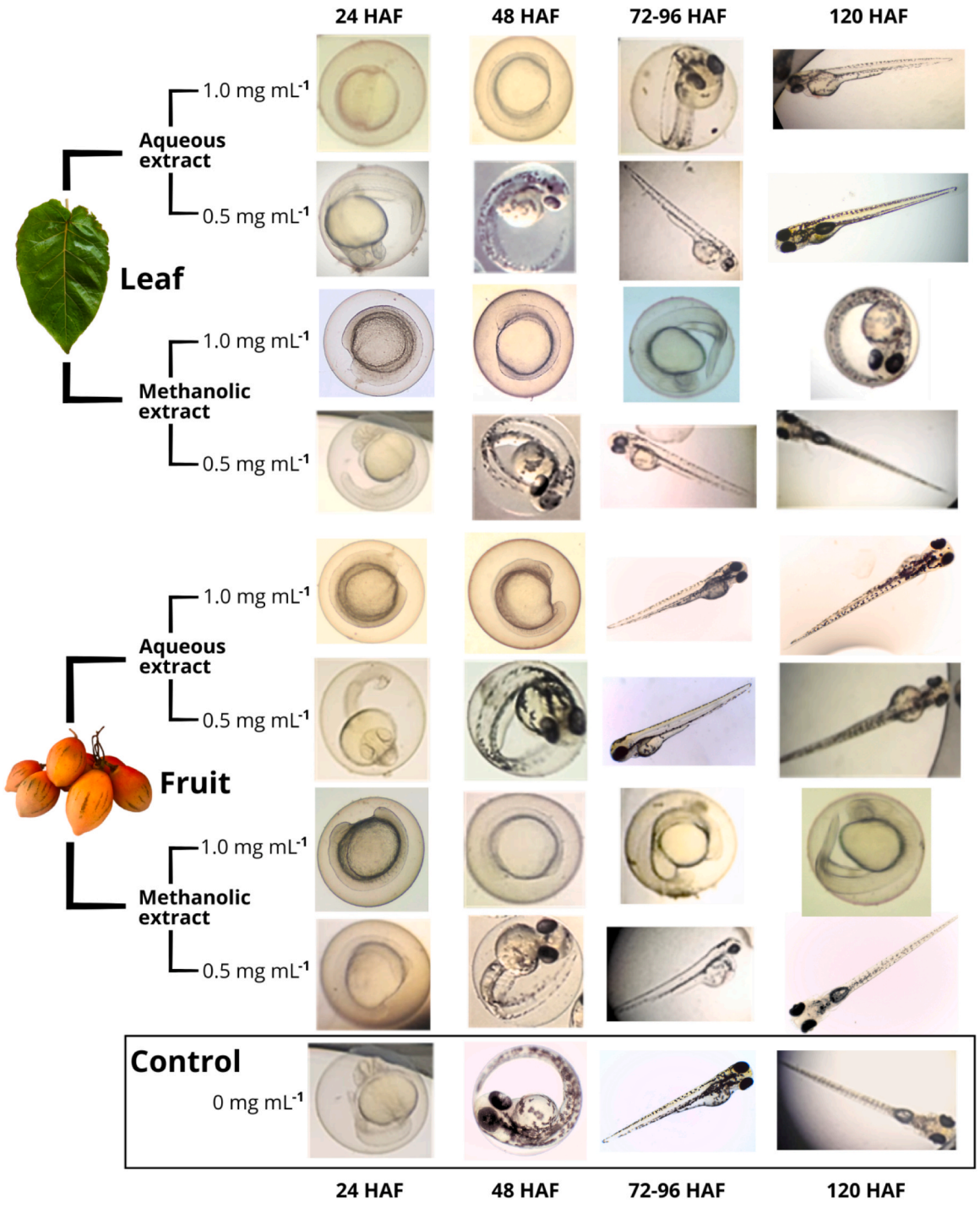

Fig. 1. Morphological characteristics of zebrafish larvae exposed to the four different *S. betaceum* extracts from 24 to 120 h after fertilization (HAF) is shown. At a concentration of 1 mg mL^{-1}, there was no disruption of the chorion for the methanol extracts of the leaf and fruit until 120 HAF. At concentrations less than 0.5 mg mL^{-1}, the larvae looked similar to the control at 120 HAF.

of *S. betaceum* extracts required to induce malformations in 50 % of zebrafish embryos/larvae at 120 HAF) were obtained from the concentration-response curves at 120 HAF (Table 3). However, it was not possible to calculate the LC_{50} for the aqueous leaf extract at the concentrations evaluated (up to 1 mg mL^{-1}). The teratogenic index (TI) was calculated using the LC_{50} and EC_{50} values obtained at 120 HAF (Table 3).

At 120 HAF, larvaes exposed to all concentrations higher than 1 mg mL^{-1} had 100 % mortality. At concentrations lower than 1 mg mL^{-1}, however, there was low or absence of lethality. However, it was possible to observe that the malformation percentage gradually increased as the dose of the extract increased.

The concentrations of *S. betaceum* extracts required to cause the death of 50 % of zebrafish embryos/larvae (LC_{50}) ranged from 1.088 to 1.425 mg mL^{-1} (Table 3). The methanolic extract of the fruit had the lowest concentration and the highest lethality at 120 HAF, with an LC_{50} equal to 1.088 mg mL^{-1}. The aqueous extract of the fruit was the least toxic (LC_{50} equal to 1.425 mg mL^{-1}).

The malformation effects (EC_{50}) at 120 HAF were detected in extracts with concentrations equal to or less than 1 mg mL^{-1} since the

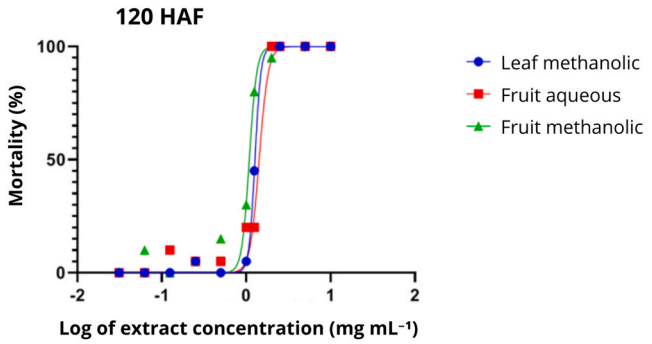

Fig. 2. Concentration-response curve for mortality of zebrafish larvae exposed to different concentrations of *S. betaceum* extracts (0.031, 0.062, 0.125, 0.25, 0.5, 1, 2, 2.5, 5, and 10 mg mL^{-1}) 120 h after fertilization. It was not possible to calculate the LC_{50} for the aqueous leaf extract at the concentrations evaluated.

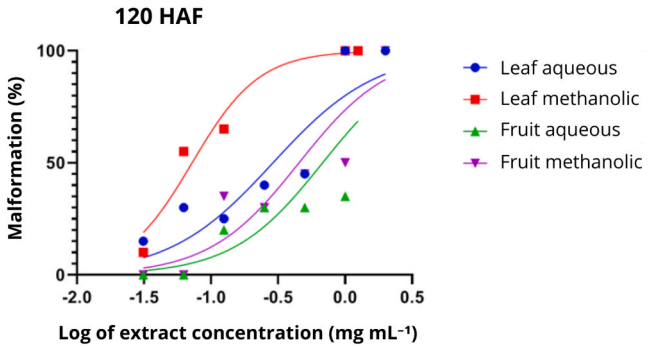

Fig. 3. Concentration-response curve for malformation in zebrafish larvae exposed to *S. betaceum* extracts at different concentrations (0.031, 0.062, 0.125, 0.25, 0.5, and 1 mg mL^{-1}) 120 h after fertilization.

lethality was 100 % for concentrations above this value (Fig. 1). The EC_{50} values ranged from 0.072 to 0.68 mg mL^{-1}. The lowest concentration capable of causing morphological changes in 50 % of zebrafish embryos/larva was found for the methanolic leaf extract (EC_{50} equal to 0.072 mg mL^{-1}).

The fruit extracts showed the lowest teratogenic index (TI), while the leaf extract had a sevenfold higher TI.

The aqueous fruit extract had the lowest EC_{50} and the lowest LC_{50}.

4. Discussion

Tree tomato is an underutilized species despite its richness in bioactive compounds associated with several health applications, including antioxidant, antiproliferative, antinociceptive, anti-inflammatory, antidiabetic, anti-allergenic, and antiobesity properties, and it may aid in the treatment of cognitive impairment associated with neurodegenerative diseases [7,9,35]. The fruits are consumed fresh and are rich in polyphenols and flavonoids, exhibiting antioxidant properties as demonstrated in this study and corroborated by other authors [11, 35].

The variety of phytochemicals in *S. betaceum* fruit is responsible for its numerous pharmacological and nutritional properties, as well as its pronounced antioxidant activity [11,36]. Do Nascimento et al. [8] extracted a polysaccharide from fruit pulp (arabinogalactan) that showed an antinociceptive effect. To date, chlorogenic acid has been found to be the main phenolic compound in fruit (peel and pulp) regardless of the type of cultivar and tissue analyzed [35,37]. Tree tomato fruit has a great capacity for retaining pigments due to the presence of hydrocolloids such as anthocyanins and carotenoids [35], as well as being an excellent food source of soluble fiber and starch [38]. Moreover, *S. betaceum* peels can be used as a natural food coloring to reduce waste and encourage sustainable production [39].

Numerous studies report the functional properties of the fruits; however, research on the leaves remains limited. In this study, high levels of phenols, flavonoids, and antioxidant activity were detected in *S. betaceum* leaves, significantly exceeding those found in the fruit pulp. Similar results were found by Gobikanila and Jeyaramraja [7] when evaluating phenols and flavonoids content and antioxidant activity in leaves and fruits of *S. betaceum* red-fruit and yellow-fruit cultivars. The presence of flavonoids, catechins, phenolic acids, as well as organic acids, reducing sugars, proteins, amino acids, and tannins in hydroalcoholic extracts of *S. betaceum* leaves has also been reported [13,40]. A study using extracts from the fruit peel, pulp, and seeds reported low toxicity in experiments with *Artemia salina* larvae, demonstrating the absence of a genotoxic effect [41], as found in this study with zebrafish embryos.

The leaves are used topically by populations in some Andean countries to treat tonsil inflammation and angina pain [2,3]. The phenolic compounds in the leaves, recognized for their antioxidant and anti-inflammatory properties, may contribute to these traditional therapeutic effects. Chemical analysis of the aqueous leaf extract demonstrated inhibitory action on aldose reductase, suggesting potential use in treating microvascular complications, such as neuropathy and nephropathy [13]. However, the leaf extract exhibited a teratogenic index (TI) up to seven times higher than that of the fruit pulp extract, resulting in a significantly higher percentage of malformations in developing zebrafish embryos. Toxic and teratogenic activities against the zebrafish embryo model were also reported by Sabarinath et al. [42] for *Solanum xanthocorpum*. The leaves of *Solanum nigrum* are also toxic, mostly because of the glycoalkaloid solanine, and despite that toxicity, *S. nigrum* is utilized to make various external therapeutic products [43,44]. In this context, the leaf of *S. betaceum* may be toxic to humans, and it is recommended not to ingest it. In folk medicine, the leaves are used topically probably because of their toxicity, so the anti-inflammatory therapeutic effects of phenolic compounds can be applied more safely than when

Table 3
Toxicity and teratogenicity parameters (LC_{50}, EC_{50}, and TI) of *Solanum betaceum* extracts in zebrafish embryos/larvae at 120 h after fertilization.

	LC_{50}[a] (mg mL^{-1})			EC_{50}[b] (mg mL^{-1})			TI[c] (LC_{50}/EC_{50})
Treatment	Mean	LCL[d]	UCL[e]	Mean	LCL	UCL	Mean
Leaf aqueous	*NA*[f]	*NA*	*NA*	0.290	0.114	0.630	*NA*
Leaf methanolic	1.271	1.254	1.294	0.072	0.043	0.135	17.653
Fruit aqueous	1.425	1.310	1.580	0.680	0.251	8.110	2.096
Fruit methanolic	1.088	1.027	1.151	0.453	0.209	0.884	2.402

[a] LC50: concentration of *S. betaceum* extracts required to cause mortality in 50 % of zebrafish embryos/larvae at 120 HAF;
[b] EC_{50}: concentration of *S. betaceum* extracts required to induce malformations in 50 % of zebrafish embryos/larvae at 120 HAF;
[c] TI: teratogenic index (LC_{50}/EC_{50});
[d] LCL: lower confidence limits;
[e] UCL: upper confidence limits;
[f] *NA*: not available.

ingesting them. This finding underscores the importance of carefully regulating the doses of remedies used in health treatments, independently of whether they are natural or synthetic.

The *Solanum* genus is rich in bioactive compounds in the form of steroids and alkaloids, which can have both therapeutic applications and toxic effects, most often dose-dependent [15]. The potato *S. tuberosum* contains glycoalkaloids that can cause vomiting, diarrhea, and abdominal pain. Other species of the Solanaceae family have alkaloids that act on the central nervous system and can lead to various side effects and even lethal poisoning depending on the dosage [45]. Friedman et al. [46], evaluating the relative embryotoxicity of structurally different compounds from *Solanum* species, detected toxic and teratogenic effects for some compounds, as well as dose-dependent effects for others in the frog embryo teratogenesis assay using Xenopus (FETAX).

The aqueous extract of *S. betaceum* leaf and fruit was less toxic than the methanolic extract, suggesting that water is a more suitable solvent than methanol for obtaining safer bioactive compounds from *S. betaceum* leaf and fruit. Previous studies have indicated that methanol at a concentration of up to 1 % is not toxic to zebrafish larvae, and since methanol is present in rotavaporated extracts at a concentration of less than 1 %, the malformation effects are due to the phytocompounds present in the leaf [47].

No studies have investigated the presence of alkaloids in *S. betaceum*, and the identification of these compounds could be important for determining the toxicological potential of this species. However, further research is needed to assess its safety for topical use. Evaluating the toxicity of medicinal plants during embryonic development is important once products derived from these plants, which have reported pharmacological effects, may gain popularity in the market despite a lack of information regarding their toxicological profiles and health effects [34].

5. Conclusion

The *S. betaceum* leaf extract showed greater total antioxidant capacity than the fruit extract, while the fruit extract showed greater free radical scavenging activity and lower toxicity in developing zebrafish embryos than the leaf extract. Zebrafish model toxicity was concentration-dependent (dose-dependent) for *S. betaceum* leaf and fruit extracts. Water was a more suitable solvent than methanol for obtaining safer bioactive compounds from *S. betaceum* leaf and fruit. This work provides an initial basis for pharmacological studies on *S. betaceum* in animal models, making it possible to plan future research on the therapeutic effect of the *S. betaceum* leaves and fruit.

CRediT authorship contribution statement

Aazza Smail: Supervision, Methodology. **Cavalcanti Vytória Piscitelli:** Writing – review & editing, Visualization. **Virote Bárbara do Carmo Rodrigues:** Methodology. **Borges Karina Schulz:** Writing – original draft, Methodology, Investigation, Formal analysis. **Resende Luciane Vilela:** Writing – review & editing, Resources, Project administration. **Murgas Luis David Solis:** Resources, Conceptualization. **Bertolucci Suzan Kelly Vilela:** Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data will be made available on request.

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