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Phytochemicals and Bioactivities of *Tradescantia zebrina* Bosse: A Southern Mexican Species with Medicinal Properties

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Abstract Research was conducted on the biological potential and phenolic composition of *Tradescantia zebrina* Bosse. Through aqueous and hydroalcoholic extracts of leaves of the species, the total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) were determined; the antioxidant activity by DPPH•, ABTS•+ and FRAP methods, as well as the biological activities, antibacterial by agar diffusion and minimum inhibitory concentration (MIC), and anti-inflammatory under the 12-O-tetradecanoylphorbol-13-acetate (TPA) method in Institute of Cancer Research (ICR) mice. The hydroalcoholic extract presented the highest TPC (73.07 ± 0.78 mg EAG/g), TFC (24.34 ± 0.68 mg EC/g) and TTC (26.96 ± 0.18 mg EAG/g), and a relevant antioxidant activity with the FRAP assay (353.92 ± 3.20 mM ET/g). In addition, this extract was the only one that presented an inhibition zone of 8.5 ± 1.4 and 8 ± 0.0 mm, against *Bacillus cereus* and *Staphylococcus aureus*, respectively. As well as a higher percentage of inhibition of inflammation (47.99%), an approximate value to the reference drug used. The evidence indicates that this plant possessing pharmaceutical potential that can be further explored as a natural source of new drugs.

Keywords: *Tradescantia zebrina, antioxidant, antibacterial, anti-inflammatory, extracts*

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1. Introduction

Herbal medicine and medicinal plants are playing an integral role in the modern healthcare system. Their acceptance and utilization are increasing every day because of their better compatibility, less side effect and economical health management [1]. Based on the World Health Organization (WHO), herbs or herbal products are used by many populations for basic health care needs due to the side effects of modern drugs, failure of modern therapies against chronic diseases and microbial resistance [2]. Traditionally, our ancestors have provided us with medicinal knowledge, which includes herbs, herbal materials (plant parts), preparations, and processed and finished herbal products [1,3,4]. Therefore, there is a great need to discover bioactive compounds that can be used for a variety of medicinal purposes, such as antibacterial, antioxidant and anti-inflammatory treatments [5,6].

Several studies have proven the biological potential of many medicinal plants, highlighting the need to analyze their active components [7,8]. Different investigations have reported that their bioactive potential is attributed to the presence of phenols, flavonoids, lignins, terpenoids, carotenoids, among others [9,10,11]. However, phenolic compounds are probably the most explored natural compounds due to their potential health benefits, as demonstrated in several studies [12]. Phenolic compounds are associated with antioxidant, anti-inflammatory, anticancer, antiallergic, antihypertensive, cardioprotective, and antimicrobial activities [13,14,15,16,17,18].

These bioactive compounds thus represent an alternative to develop new antimicrobial agents that are

effective against microorganisms and less harmful to the host, due to the negative effects of synthetic drugs and the constant development of bacterial resistance [19]. Furthermore, as natural antioxidants they play an important role in protecting the cell against reactive oxygen species and free radicals [20]. Since, excess free radicals in cells cause oxidative stress, leading to premature aging, development of diabetes, as well as cardiovascular, neurological diseases and cancer [21,22].

The species *Tradescantia zebrina* Bosse is a very familiar and commonly cultivated plant in tropical and temperate regions. It is native to Mexico and Central America but is also widely escaped and naturalized in many tropical and subtropical areas in both hemispheres [23]. It is characterized by green leaves on top with broad, whitish longitudinal stripes, purple or purple undersides with stripes, and purple flowers [24]. It is a medicinal plant whose use is diverse, according to the different traditional systems of medicine. In the south of Mexico, it is mainly consumed as fresh water, lemon and honey or sugar are added, it can be prepared by cooking its leaves or by maceration, and the people who consume it attribute medicinal properties to it, especially for treating kidney diseases [25]. In eastern Cuba, *T. zebrina* extract is used as a treatment for conjunctivitis [26]. In Jamaica, it is used for the treatment of tuberculosis, arterial hypertension, and cough. In addition, the leaves are applied for the management of swelling and hemorrhoids [27]. In Guyana, the leaves are used as a tea for blood cleansing and treatment of influenza [28]. It is even reported to be used as a poultice, to treat burns and combat skin irritation [29].

On the other hand, scientific reports on the plant have demonstrated significant pharmacological activities. The antioxidant activity of *T. zebrina* has been analyzed, showing good potential as a free radical eliminator [30]. Moreover, there are reports of methanolic extracts exhibited antibacterial activity against Gram-positive and Gram-negative bacteria [31]. In addition, it exhibited a high total content of phenols, flavonoids, and tannins. There are even reports about the treatment of bone fractures, as well as to reduce inflammations caused by blows [32,33].

However, reports that evaluate aqueous and hydroalcoholic extracts of leaves of this plant are still scarce. Therefore, the objective of this work was that, through aqueous and hydroalcoholic extracts of *T. zebrina*, phenols, flavonoids and tannins, as well as antioxidant activity were quantitatively determined by DPPH•, ABTS•+ and FRAP assays. In addition, an evaluation of the biological properties of the species was also carried out, through antimicrobial and anti-inflammatory activities.

2. Materials and Methods

2.1. Chemical Reagents

Absolute ethyl alcohol, deionized water and double distilled water were acquired from Wöhler® (Mex., Mexico); absolute methanol and Folin-Ciocalteu reagent were obtained from Hycel® (Mex., Mexico); sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), hydrochloric acid (HCl) and sodium acetate were obtained from J. T. Baker® (Mex., Mexico); aluminum chloride ($AlCl_3$) and acetic acid were purchased from Reasol® (Mex., Mexico); Gallic acid, catechin, polyvinylpolypyrrolidone (PVPP), 2,2-Diphenyl-1-picrylhydrazyl (DPPH•), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+), trolox, potassium persulfate, 2,4,6-tripyridyl-S-triazine (TPTZ), Tween 80, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich®, iron chloride ($FeCl_3$) was acquired from Meyer® (Mex., Mexico) and brain-heart infusion broth was obtained from BD Dixon™ (Mex., Mexico).

2.2. Bacterial Pathogens

The bacteria used were *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6639 and *Staphylococcus aureus* ATCC 25923, donated by the Department of Food and Biotechnology, Faculty of Chemistry, National Autonomous University of Mexico.

2.3. Plant Sample

T. zebrina Bosse plants were collected in November 2019 at the farmers' market "Jesús Taracena Martínez" in Villahermosa, Tabasco, Mexico (17°59'50 "N 92°54'50 "W). The leaves were washed and dehydrated in a room equipped with an Environmental Dehumidifier (LG® LHD45EL) at room temperature for 72 h. The leaves were then shredded using a dehumidifier (LG® LHD45EL). They were then crushed using an electric grinder (Krups® F2034251) and sieved through a 60-mesh sieve. The powder obtained was deposited in coated jars and protected from light and humidity until the extraction process.

2.4. Extracts Preparation

The preparation of the extracts was carried out by the following method. 9 g of dry powder was placed in 300 mL of each extraction solvent: deionized water (aqueous extract) and ethanol: deionized water (hydroalcoholic extract, 70:30 v/v). The aqueous extract was prepared in a water bath with periodic shaking, for 10 min at 90°C. It was filtered twice: using Whatman No. 1 filter paper, then using Millipore® equipment with a nitrocellulose membrane with a pore diameter of 0.45 µm. It was lyophilized and stored until use. The hydroalcoholic extract was obtained by macerating it in an orbital shaker (AOSHENG® OS-200-100) at 150 rpm for 48 h. It was filtered following the procedure described above. Using a rotary evaporator at 50 °C (BUCHI Rotavapor™ R-210) the ethanol was removed, then in a vacuum oven at 55 °C (Felisa® FE-295VD) the remaining water was removed. The dried extract was ground and stored at 4°C in coated vials, protecting them from light and humidity until use.

2.5. Phenolic Compound Determination

2.5.1. Determination of Total Phenolic Content (TPC)

The spectrophotometric method described by [34] with modifications was used. 0.125 mL of extract was mixed

with 0.625 mL of Folin-Ciocalteu reagent diluted with deionized water (1:10) and 0.5 mL of 7.5% Na_2CO_3 solution. After 45 min of incubation, absorbance was measured at 760 nm with a spectrophotometer (RAYLEIGH® UV-1800), at room temperature and protected from light. The TPC was calculated with a calibration curve using gallic acid as standard and the results were reported as mg gallic acid equivalents per gram of dry extract (mg GAE/g dry extract). Measurements were performed in triplicate.

2.5.2. Determination of Total Flavonoid Content (TFC)

It was determined using the method of [35], based on the formation of a flavonoid-aluminum complex. An aliquot of 0.5 mL of the extract solution was mixed with 2 mL of distilled water and 0.15 mL of a $NaNO_2$ (5%) solution, stirred and allowed to stand for 6 min. Then, 0.15 mL of $AlCl_3$ (10%) was added, again stirred, and allowed to stand for a further 6 min. Finally, 2 mL of NaOH (4%) was added to the mixture and it was completed with distilled water to obtain a final volume of 5 mL. The absorbance of the reaction was measured at 510 nm. A catechin calibration curve was used to determine the flavonoid content, which was expressed in mg catechin equivalents per g of dry extract (mg CE/g dry extract).

2.5.3. Determination of Total Tannin Content (TTC)

The total tannin content was determined based on the method described by [36] with modifications. It is obtained from the difference between TPC and non-tannin phenols by precipitating tannin phenols with polyvinylpolypyrrolidone (PVPP). TPC and non-tannin phenols were obtained by the Folin-Ciocalteu method described above. To the previously diluted sample (0.2 mL) 100 mg of PVPP and 1 mL of distilled water were added, vortexed and incubated at 4°C for 15 min. Again, vortexed and centrifuged (3000 rpm, 4°C, 10 min). The supernatant, consisting of simple phenolics other than tannins, was collected and the phenolic content was determined. Total tannins were expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g dry extract).

2.6. Determination of Antioxidant Activities

2.6.1. Free Radical Scavenging Activity DPPH•

The antioxidant activity of the extracts was examined by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity following the method proposed by [37] with modifications. 2 mL of DPPH solution (125 µM in 100 mL of 80% methanol) was added to 200 µL of the extract and allowed to react in the dark for 60 min at room temperature. The absorbance of the solution was measured at 520 nm.

IC_{50} values (concentration of extract required to inhibit 50% radical, µg/mL) were calculated by linear regression analysis. A calibration curve with trolox as reference standard was used. Percent radical uptake was calculated using the formula: % inhibition = (Abs control - Abs sample) / (Abs control) × 100. Where, Abs control is the absorbance of the reagent mixture without adding sample or standard; and Abs sample is the absorbance of the mixture added with sample or standard reagent.

2.6.2. Free Radical Scavenging Activity of ABTS•+

Free radical scavenging activity was determined following the ABTS•+ [2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization assay of [38] with modifications. The ABTS-+ radical solution was prepared with 2.45 mM potassium persulfate and 7 mM ABTS•+ (1: 1) and allowed to react for 12 - 16 hours at room temperature protected from light. The absorbance of the ABTS•+ solution was adjusted with methanol to obtain an absorbance of 0.800 ± 0.05 at 734 nm. The radical scavenging activity was determined by mixing 10 µL of the extract with 990 µL of the adjusted solution and allowing to react for 90 min. The mixed absorbance was read at 734 nm. The results were reported as IC_{50} (µg/mL), which was calculated following the same procedure as in the DPPH• assay.

2.6.3. Reduced Ferric Ion Antioxidant Strength (FRAP) Assay

This method was used as an indicator of antioxidant activity based on the reducing power of a sample from [39] with modifications. FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in the ratio of 10:1:1 at room temperature. Next, 1200 µL of fresh FRAP reagent was mixed with 40 µL of extract and 120 µL of double distilled water. The absorbance of the mixture was measured at 593 nm after 30 min of reaction. A standard curve was prepared using Trolox as a reference standard, and the results were expressed as mM Trolox equivalent per g dry extract (mM ET/g dry extract). All solutions used in the assay were prepared on the same day of analysis.

2.7. Determination of Antibacterial Activity

2.7.1. Agar Diffusion Technique

Antibacterial activity was evaluated by the technique by [40] of disk diffusion against *S. typhimurium*, *E. coli*, *B. cereus*, *B. subtilis* and *S. aureus*. The microorganisms were activated in brain heart infusion broth (BHI) from aliquots in glycerol stored at -20 °C. Petri dishes (0.1 mL at a concentration of 10^8 CFU/mL) were inoculated [41]. Four 6 mm diameter Whatman no. 1 paper disks were placed on the inoculated plates. Two discs were impregnated with 5 and 10 mg of extract, one disc with 20 µg of antibiotic (amikacin) as a positive control [41], and the last one with the corresponding solvent of each extract as a negative control. After standing at 4 °C for 30 min, they were incubated at 37 °C for 24 h. The zone of inhibition was measured in mm. All tests were performed in duplicate.

2.7.2. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the microplate dilution technique. For this purpose, the bacterial strains (*S. typhimurium*, *E. coli*, *B. cereus*, *B.*

subtilis and *S. aureus*) were activated in Mueller Hinton (MH) broth. Sterile 96-well microplates of 500 µL capacity were used. Each extract was applied at a serial dilution rate (1:1) of 240 to 1.875 mg/mL. The aqueous extract was diluted with sterile double-distilled water. The 70% ethanolic extract was diluted with sterile Tween 80 (10%) solution. To each well was added 100 µL of MH broth inoculated with the respective bacterial strain and 100 µL of diluted extract or control (positive or negative). Amikacin was used as a positive control [42] at a dilution rate of 1.5 to 0.0117 mg/mL. The negative control was sterile double distilled water with Tween 80 (10%). Each microplate was shaken for 5 min at 150 rpm on a vortex shaker (IKATM) and incubated for 24 h at 37°C.

To evaluate bacterial growth, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazole) (MTT) bromine solution was prepared by dissolving 25 mg in 5 ml of PBS solution. As an indicator of viability, which changed the color of the solution from yellow to violet after 1 h [41], by adding 10 µL to each well of the plate. The concentration of the well that did not change color was considered the MIC. Finally, 10 µL were taken from the well with MIC, inoculated into a Petri dish containing MH agar and incubated for 24 h. The results were reported as bacteriostatic activity if there was bacterial growth; if there was no bacterial presence they were reported as bactericidal activity [43].

2.8. Determination of Anti-inflammatory Activity

2.8.1. Evaluation of anti-inflammatory Activity in Mice with TPA

It was performed according to the method described by [44], with modifications. ICR (Institute of Cancer Research) mice of both sexes, weighing between 29 and 30 g, were used. Experiments were performed in strict accordance with the official requirements of the Mexican Standard for the Care of Experimental Animals (NOM-062-ZOO-1999), as well as the international ethical guidelines for the care and use of experimental animals. Mice were maintained at a temperature of 24 ± 3 °C, 70 ± 5% humidity with 12 h light/dark cycle and food/water ad libitum. The control group received acetone as vehicle and dexamethasone was used as an anti-inflammatory positive control.

Inflammation in mice was induced using 12-O-tetradecanoylphorbol-13-acetate (TPA), according to the method described by [45]. Mice (seven individuals) were grouped and TPA (2.5 µg) dissolved in acetone (20 mL) was topically applied to the inner and outer surface of the right ear to cause edema. Doses of 1.6 mg/ear of each treatment were applied to each individual's ear. 1 mg/ear of the reference anti-inflammatory drug was administered. All treatments were dissolved in acetone and applied topically to both ears after TPA administration. Four hours after administration of the inflammatory agent, the animals were sacrificed by cervical dislocation. Circular sections of 6 mm diameter were taken from the treated (t) and untreated (nt) ears and immediately weighed to determine the extent of inflammation. The percentage inhibition was obtained by the following expression: inhibition (%) = [Δw control– Δw treatment] x 100, where $\Delta w = wt – wnt$ is the weight of the treated ear section; *wnt* is the weight of the untreated ear section.

2.9. Statistical Analysis

Analysis were carried out using a completely randomized experimental design. Each test was performed in triplicate, and the results were expressed as mean ± standard deviation. Statistical variations were determined using a one-way analysis of variance (ANOVA) and means were compared using Tukey ($p < 0.05$) multiple range test. Pearson's coefficients ($p < 0.05$) were calculated between the antioxidant activity values and the total phenolic content values of the extracts. Statistical analysis was performed using the Statgraphics CENTURION XVI software.

3. Results and Discussion

3.1. Extraction Yield

Extraction is the first step in the analysis of medicinal plants to obtain the desired chemical constituents from plant materials. The extraction efficiency will depend on the solvent with varying polarity, pH, temperature, extraction time, and sample composition [46,47]. Table 1 shows the yield percentages of *T. zebrina* extracts, where the aqueous extract obtained 31.10% yield, while the hydroalcoholic extract at 70% presented 20.15%. According to the statistical analysis, there are significant differences. The values exceed those reported by [48] in a methanolic extract of the same species, indicating a 6.18% extraction yield. This shows that the extraction yield depends on the polarity of the solvent [41], likewise, the yields are variable and depend on their composition, place of development, part of the plant under study (root, leaf, stem, fruit), as well as the type and conditions of extraction [49].

Table 1. Extraction yield of *T. zebrina* leaves extracts

Extract	Extraction yield (%)
Aqueous	31.10 ± 3.23^{b}
Hydroalcoholic	20.15 ± 2.49^{a}

Values are means ± standard deviations of triplicates (n = 3). Means in the same column followed by a lowercase letter in different superscript are significantly different ($p < 0.05$).

3.2. Phenolic Compounds

3.2.1. Total Phenolic Content (TPC)

As shown in Table 2, the TPC (expressed in mg gallic acid equivalents per gram of dry extract), was higher for the hydroalcoholic extract compared to the aqueous extract, with values of 73.07 ± 0.78 and 41.67 ± 0.12 mg EAG/ g dry extract, respectively. Statistical analysis showed significant differences ($p < 0.05$) between both extracts. Moreover, the values obtained agree with previous research, indicating the presence of phenolic compounds in extracts of *T. zebrina* leaves. Cheah *et al.* [48] reported 33.5 mg GAE/g in methanolic extracts. Sanchez-Chino *et al.* [25] obtained 146.35 and 88.38 mg

EAG/g of sample in 80% aqueous and ethanolic extracts, respectively. Olivo-Vidal *et al.* [50] indicated a concentration of 70.2 and 47.2 mg EAG/g in 80% ethanolic and aqueous extracts, respectively. While Silva *et al.* [51] reported a concentration of 67.68 ± 0.12 mg EAG/g in methanolic extracts. According to aforementioned, the highest concentration tends to occur in ethanolic extracts, this may be due to the possible formation of complexes of some phenolic compounds, which can be higher in molecular weight than phenols present in aqueous extracts [46]. It has also been reported that the duration of extraction plays an important role, because after 48 h all the cell walls are destroyed, so that all the plant material is present in very small particles [52]. In addition, phenolic compounds have a wide spectrum of biological effects, being related to antioxidant, antimicrobial, anti-inflammatory, and antihypertensive activities, among others. Phenols can be classified according to different systems, but they all have an aromatic ring with at least one hydroxyl substituent. The number and position of hydroxyl groups in the chemical structure determine the potential of phenolic compounds as antioxidant molecules [53].

Table 2. Total Phenolic Content, Total Flavonoid Content and Total Tannin Content of *T. zebrina* leaves extracts

Extract	TPC (mg GAE/g dry extract)	TFC (mg CE/g dry extract)	TTC (mg GAE/g dry extract)
Aqueous	41.67 ± 0.12a	13.12 ± 0.06a	14.04 ± 0.07a
Hydroalcoholic	73.07 ± 0.78b	24.34 ± 0.68b	26.96 ± 0.18b

GAE: gallic acid equivalent; CE: catechin equivalent. Values are means ± standard deviations of triplicates (n = 3). Means in the same column followed by a lowercase letter in different superscript are significantly different (p <0.05).

3.2.2. Total Flavonoid Content (TFC)

Table 2 shows the TFC (expressed in mg catechin equivalents per gram of dry extract). The hydroalcoholic extract obtained the highest concentration before the aqueous extract (24.34 ± 0.68 and 13.12 ± 0.06 mg EC/G dry extract, respectively). The analysis showed significant differences (p < 0.05) between both extracts. Previous research has verified the presence of flavonoids in *T. zebrina* species in different extracts. Authors such as [48], reported 9.4 ± 1.06 mg EC/ g of extract in methanolic extracts. While [31] also obtained outstanding results about the quantification of flavonoids, however, the units in which they reported them are different from those used in this study, so a comparison is not possible. Olivo-Vidal *et al.* [50] have reported the presence of flavonoids in 80% ethanolic and aqueous extracts, with concentrations of 16.8 and 21.1 mg EC/g, respectively. However, genetic diversity and biological, environmental, and seasonal variations can significantly affect the flavonoid content of plants [54]. These compounds constitute the main group of phenolic compounds [53] and possess a wide spectrum of biological activities including antioxidant and free radical scavenging properties [55], as they could form complexes with metal ions and act as antioxidants and bind to proteins such as structural proteins and enzymes [56]. This antioxidant activity depends on the presence, number, and position of hydroxyl groups in the chemical structure of these compounds [57].

3.2.3. Total Tannin Content (TTC)

As well as flavonoids and total phenols, TTC was higher in the hydroalcoholic extract, with a concentration of 14.04 ± 0.07 mg EAG/g dry extract, while the aqueous extract presented 26.96 ± 0.18 mg EAG/g dry extract (Table 2). Statistical analysis showed significant differences (p < 0.05) between the two extracts. Few investigations have carried out the quantification of tannins in extracts of the genus *Tradescantia*. For example, [31] obtained a significant value (57.6 mg EAT/100 g in terms of fresh weight) but reported in units different from those presented in this study. On the other hand, authors such as [32] and [58] have identified tannins in species of the same genus, although qualitatively together with other phytochemical compounds. The importance of these compounds lies in the fact that they possess antimicrobial activity, through the inactivation of adhesins, cell envelope, enzymes, and different transport proteins [59]. Until this moment, these results are considered the first ones obtained in aqueous and hydroalcoholic extracts of the species *T. zebrina* indicating a new line of research for a better extraction of these compounds, and thus develop novel pharmaceuticals.

3.3. Antioxidant Activities

3.3.1. Free Radical Scavenging Activity of DPPH•

The DPPH• radical scavenging activity is shown in Table 3. The hydroalcoholic extract (IC_{50} 831.09 ± 71.61 µg/mL) showed more effective radical scavenging activity compared to in the aqueous extract (IC_{50} 1146.90 ± 92.53 µg/mL). The results show significant differences between the antioxidant activity of both extracts (p < 0.05). The IC_{50} indicates half of the maximum inhibitory concentration [60] of a compound is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant needed to decrease the concentration of the radical by 50%, which is obtained by interpolation of a linear regression to decrease the concentration of the radical by 50%, which is obtained by interpolation of a linear regression analysis [61]. That is, a lower IC_{50} indicates a higher antioxidant activity of a compound.

Table 3. Antioxidant activity by DPPH•, ABTS•+ and FRAP assays of *T. zebrina* leaves extracts

Extract	DPPH• (µg/mL, IC50)	ABTS•+ (µg/mL, IC50)	FRAP (mM TE/g dry extract)
Aqueous	1146.90 ± 92.53b	1060.32 ± 171.58a	318.97 ± 0.37a
Hydroalcoholic	831.09 ± 71.61a	1033.67 ± 149.91a	353.92 ± 3.20b

Values are means ± standard deviations of triplicates (n = 3). Means in the same column followed by a lowercase letter in different superscript are significantly different (p <0.05).

Research by [31], [48] and [51] proved the antioxidant activity of methanolic extracts of *T. zebrina* leaves, demonstrating the ability of the species to inhibit DPPH• radicals. However, these results have been reported in percentages and not in IC_{50} µg/mL units. It is important to recognize that comparison of antioxidant values between different authors is not straightforward, given the wide variety of antioxidant assays available, the potential

incompatibility between the same plant species grown at different conditions, and minor modifications to the assays that however, made them less comparable [62]. Furthermore, the difference in efficacy in capturing the DPPH• radical between extracts is due to the existence of phenolic and polyphenolic compounds with different polarities and chemical characteristics, depending on the characteristics of the solvent used [63].

3.3.2. Free Radical Scavenging Activity of ABTS •+

Table 3 shows the results of the ABTS•+ assay. Both the hydroalcoholic and aqueous extracts obtained similar concentrations, with an IC_{50} of 1033.7 ± 149.91 and 1060.32 ± 171.58 µg/mL, respectively. Furthermore, they did not present significant differences ($p > 0.05$). Highlighting that the results obtained may be very high values due to the extraction method used, as it is carried out by conventional methods, which implies some inconveniences before the non-conventional methods used in other investigations.

3.3.3. Ferric reducing antioxidant potential (FRAP)

The FRAP assay results (Table 3) show significant differences between the antioxidant activity of the extracts ($p < 0.05$). The hydroalcoholic extract showed a higher antioxidant capacity (353.92 ± 3.20 mM TE/ g dry extract) followed by the aqueous extract (318.97 ± 0.37 mM TE/ g dry extract). Generally, reducing properties are associated with compounds contained in a sample capable of donating hydrogen atoms to break free radical chains [55]. According to an investigation by [31], methanolic extracts of *T. zebrina* achieved the highest ferric reducing power against the antioxidant capacity of five species of the Commelinaceae family. Therefore, our results indicate that aqueous and hydroalcoholic extracts of *T. zebrina* possess the ferric ion reducing capacity, so these results could indicate a new line of research.

3.4. Correlation between Antioxidant Activity and Total Phenolic Content

Table 4. Correlation between antioxidant activity and total phenolic content of *T. zebrina* leaves

Assay (expressed in units)	Equation	Pearson´s coefficient (*r*)	Significance
DPPH•	$y = 1570.7 - 10.14x$	-0.927051	$p < 0.05$ ($p = 0.0078$)
ABTS•+	$y = 1087.8 - 0.7121x$	-0.084652	$p > 0.05$ ($p = 0.8733$)
FRAP	$y = 272.53 + 1.114x$	0.995578	$p < 0.01$ ($p = 0.00001$)

Many investigations have correlated the activity of phenolic compounds with the antioxidant properties of plants [64]. In this work, Pearson's correlation coefficient was significant between TPC and antioxidant activity assays (Table 4). However, the coefficients were low between TPC and ABTS assays ($r = -0.0844$), and high for DPPH and FRAP assays ($r = -0.9270$ and $r = 0.9955$).

Negative values of the correlation coefficient indicate that the lower the IC_{50} values, the higher the antioxidant activity [41]. Moreover, the antioxidant activity of the extracts does not depend only on the vegetal material, but also on the interaction of the phenolic compounds present in the sample, as well as on their structure and concentration [65,66]. Thus, it is proved that the species has low antioxidant activity, having very high IC_{50} values.

3.5. Antibacterial Activity

3.5.1. Agar Diffusion Technique and Minimum Inhibitory Concentration (MIC)

The antibacterial activity of the aqueous and hydroalcoholic extracts of *T. zebrina* was evaluated by the disc diffusion method (DD) and the minimum inhibitory concentration (MIC) versus Gram-positive (*B. cereus*, *S. aureus* and *B. subtilis*) and Gram-negative (*S. typhimurium* and *E. coli*) bacteria. However, only the hydroalcoholic extracts achieved a zone of inhibition against *B. cereus* and *S. aureus* bacteria, with 8.5 ± 1.4 and 8 ± 0.0 mm in diameter, respectively (Table 5). However, the bacteria did not show susceptibility to the extracts by the MIC method. These results agree with those reported by [67], who analyzed the antibacterial capacity of ethanolic extracts against several Gram-positive and Gram-negative bacteria, obtaining only the inhibition of *S. aureus* with a halo of 14.2 mm in diameter. Thus, a null susceptibility of Gram-negative bacteria can be attributed to the higher natural permeability of the bacterial cell wall [68]. In addition, the results obtained may be due to seasonal interference, seeding process and sample collection [51]. It should be mentioned that the plant was obtained from a local market, so the time and storage conditions of the collected specimens until pretreatment are unknown.

Table 5. Antibacterial activity of extracts of *T. zebrina* leaves

Microorganisms	Aqueous		Hydroalcoholic	
B. cereus	–	–	8.5 ± 1.4	–
S. aureus	–	–	8 ± 0.0	–
B. subtilis	–	–	–	–
S. typhimurium	–	–	–	–
E. coli	–	–	–	–

DD: disc diffusion, zone of inhibition expressed in mm; MIC: minimum inhibitory concentration, expressed in mg/mL. Values are means ± standard deviations of triplicates (n=3).

3.6. Anti-inflammatory Activity

The results indicate that the species has anti-inflammatory activity. The application of the aqueous and hydroalcoholic extracts of *T. zebrina* was performed at a dose of 1.6 mg/ear, achieving 47.99 % and 42.16 % inhibition of edema, respectively. These values are approximately the percentage obtained by the reference agent dexamethasone (Table 6). According to the statistical analyses, there are no significant differences ($p > 0.05$). It is important to emphasize that these results are the first performed in the species with the TPA model. The TPA model of ear inflammation is useful for screening potential topical anti-inflammatory compounds or botanical extracts that act at a variety of levels [69]. Because it promotes mast cell infiltration with the release of mediators that increase vascular permeability and

promote neutrophil influx [70]. Although the anti-inflammatory potential of *T. zebrina* has been previously analyzed, [25] evaluated ethanolic and aqueous extracts of *T. zebrina*, through erythrocyte membrane stability and protection from thermal denaturation of albumin, obtaining positive and significant values, thus proving the anti-inflammatory activity that this species possesses. Baghalpour *et al.* [71], prepared ethyl acetate, n-hexane and chloroform fractions from an ethanolic extract to evaluate the anti-inflammatory potential by formalin and carrageenan tests, indicating positive results to this test. Likewise, it has been proved that other species of the Commelinaceae family have this potential. Ethanolic extracts at 80% of *T. fluminensis* have been evaluated by [72] through the egg albumin-induced edema model, demonstrating a dose-dependent inhibition of inflammation and proving its anti-inflammatory capacity.

Our results indicate that *T. zebrina* possesses anti-inflammatory activity. According to [72] the extracts could have elicited the anti-inflammatory activity by inhibiting the release or action of serotonin or histamine, because these are inflammatory mediators that are released in response to the inflammatory inducer. Also, the anti-inflammatory effects can be attributed to phytochemicals present in the leaves of the plant, previous studies have shown that, compounds such as tannins and flavonoids, possess anti-inflammatory activities [73,74,75]. But for a greater understanding of the mechanisms by which the bioactive components of *T. zebrina* act, further studies are suggested.

Table 6. Anti-inflammatory activity of crude extracts of *T. zebrina* leaves by TPA-induced edema in ICR mice

Treatment	Dose (mg/ear)	Edema (mg)	Edema inhibition (%)
TPA	2.5 (µg)	10.81 ± 0.87	–
Dexametasona	1	4.16 ± 1.60[a]	54.51
Hydroalcoholic	1.6	5.62 ± 0.86[a]	47.99
Aqueous	1.6	6.25 ± 0.89[a]	42.16

Values are means ± standard deviations (n = 5). TPA (12-O-tetradecanoylphorbol-13-acetate): negative control. Dexamethasone: positive control.

4. Conclusions

The present investigation revealed that the aqueous and hydroalcoholic extracts of *T. zebrina* Bosse leaves possess phytochemicals such as phenols, flavonoids, and tannins with important biological and pharmacological activities, although the hydroalcoholic extracts had the highest values. Although, in this case, the extracts showed antibacterial activity against two of the five strains used, it is proved that the species can inhibit bacteria such as *B. cereus* and *S. aureus*. However, relevant results were obtained against the anti-inflammatory activity with the TPA method of ear inflammation, with values close to the reference drug used. Therefore, in-depth research is required for the extraction of bioactive compounds that represent a development of new drugs to treat inflammation.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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