Scientific research article / http://dx.doi.org/10.15446/rcciquifa.v51n3.101403

Determination of phenolic bioactive compounds and evaluation of the antioxidant and hemolytic activities in the methanolic extracts of *Tradescantia zebrina*

Vagner Cardoso da Silva[1], Bárbara Elizabeth Alves de Magalhães[2], Tatiana Barbosa dos Santos Magalhães[1], Elisalva Teixeira Guimarães[1], Alessandra da Silva Guedes[1], Milleno Dantas Mota[1], Walter Nei Lopes dos Santos[2,3], Bruno Antonio Veloso Cerqueira[1], Aníbal de Freitas Santos Júnior[1]*

[1] Department of Life Sciences (DCV), State University of Bahia (UNEB), Campus I, 2555 Silveira Martins Street, Cabula, 41150-000, Salvador, Bahia, Brazil. Tel: +5571 31175313.

*Corresponding author: afjunior@uneb.br

[2] Institute of Chemistry, Federal University of Bahia (UFBA), 147 Barão de Jeremoabo Street, Ondina, 40170-115, Salvador - Bahia, Brazil.

[3] Department of Exact and Earth Sciences (DCET), State University of Bahia (UNEB), Campus I, 2555 Silveira Martins Street, Cabula, 41150-000, Salvador, Bahia, Brazil.

Received: March 8, 2022
Corrected: April 22, 2022
Accepted: April 28, 2022

Summary

Introduction: *Tradescantia zebrina* is a species used mainly in South America, with few reports about its chemical composition and bioactivity. **Aim:** To determine phenolic bioactive compounds, for the first time in the literature, and the antioxidant and hemolytic activities of extracts (dried and *in natura*) of this species. **Methods:** The extracts were obtained by maceration in methanol and the total phenolic and flavonoid contents were expressed in mg equivalent of gallic acid (EAG)/g and quercetin (EQ) of extract, respectively. The antioxidant potential was evaluated by DPPH• and ABTS•+ radical capture methods. The identification and quantification of phenolic bioactive compounds was performed by high performance liquid chromatography coupled to a diode detector (HPLC-DAD) and, the hemolytic activity assay was performed by diffusion in blood agar, using the disk antiobiogram technique. **Results**: The total phenolic and flavonoid contents in the extracts (*in natura* and dried) were 67.68 and 233.94 mg EAG/g and 29.70 and 10.99 mg EQ/g of concentrated extract, respectively. Phenolic acids (caffeic,

ellagic, ferulic, ρ-coumaric and protocatechuic), flavonoids (chrysin and rutin) and a phenolic aldehyde (vanillin) were determined by high performance liquid chromatography-diodearray detector (HPLC-DAD). The antioxidant activities were confirmed by inhibition of DPPH$^{\bullet}$ (44.08%) and ABTS$^{\bullet+}$ (46.99%) radicals for *in natura* samples, respectively. **Conclusion:** The tested methanolic extracts of *Tradescantia zebrina* did not present toxicities when tested for hemolytic activity after 72 hours. Therefore, *Tradescantia zebrina* is a potential source of bioactive substances.

Keywords: Tradescantia zebrina, antioxidants, phenolic compounds, HPLC-DAD, biological activity.

Resumen

Determinación de compuestos bioactivos fenólicos y evaluación de las actividades antioxidante y hemolítica en los extractos metanólicos de *Tradescantia zebrina*

Introducción: *Tradescantia zebrina* es una especie utilizada principalmente en Sudamérica, hay pocos reportes sobre su composición química y bioactividad. **Objetivo:** determinar compuestos bioactivos fenólicos, por primera vez en la literatura, y las actividades antioxidante y hemolítica de extractos (secos e *in natura*) de esta especie. **Métodos:** los extractos se obtuvieron por maceración en metanol y los contenidos de fenoles y flavonoides totales se expresaron en mg equivalentes de ácido gálico (EAG)/g y quercetina (EQ) de extracto, respectivamente. El potencial antioxidante fue evaluado por los métodos de captura de radicales DPPH$^{\bullet}$ y ABTS$^{\bullet+}$. La identificación y cuantificación de compuestos bioactivos fenólicos se realizó por cromatografía líquida de alta resolución acoplada a detector de diodo (HPLC-DAD) y el ensayo de actividad hemolítica se realizó por difusión en agar sangre, utilizando la técnica de antiobiograma de disco. **Resultados:** los contenidos de fenoles y flavonoides totales en los extractos (*in natura* y secos) fueron 67,68 y 233,94 mg EAG/g y 29,70 y 10,99 mg EQ/g de extracto concentrado, respectivamente. Los ácidos fenólicos (cafeico, elágico, ferúlico, ρ-cumárico y protocatequiico), los flavonoides (crisina y rutina) y un aldehído fenólico (vainillina) se determinaron mediante cromatografía líquida de alta resolución-detector de diodos (HPLC-DAD). Las actividades antioxidantes

fueron confirmadas por la inhibición de los radicales DPPH$^{\bullet}$ (44,08%) y ABTS$^{\bullet+}$ (46,99%) para muestras *in natura*, respectivamente. **Conclusión:** los extractos metanólicos de *Tradescantia zebrina* probados no presentaron toxicidades cuando se probaron para la actividad hemolítica después de 72 horas. Por lo tanto, *Tradescantia zebrina* es una fuente potencial de sustancias bioactivas.

Palabras clave: Tradescantia zebrina, antioxidantes, compuestos fenólicos, HPLC-DAD, actividad biológica.

Resumo

Determinação de compostos fenólicos bioativos e avaliação das atividades antioxidante e hemolítica em extratos metanólicos de *Tradescantia zebrina*

Introdução: *Tradescantia zebrina* é uma espécie utilizada principalmente na América do Sul, há poucos relatos sobre sua composição química e bioatividade. **Objetivo:** determinar compostos bioativos fenólicos, pela primeira vez na literatura, e as atividades antioxidante e hemolítica de extratos (secos e *in natura*) desta espécie. **Métodos:** os extratos foram obtidos por maceração em methanol e os teores de compostos fenólicos e flavonóides totais foram expressos em mg equivalente de ácido gálico (EAG)/g e quercetina (EQ) de extrato, respectivamente. O potencial antioxidante foi avaliado pelos métodos de captura do radical DPPH$^{\bullet}$ e ABTS$^{\bullet+}$. A identificação e quantificação de compostos bioativos fenólicos foi realizada por cromatografia líquida de alta eficiência acoplada a detector de diodo, na região do ultravioleta e o ensaio de atividade hemolítica foi realizado por difusão em ágar sangue, pela técnica de antiobiograma em discos. **Resultados:** os teores de fenóis totais e flavonoides nos extratos (*in natura* e seco) foram 67,68 e 233,94 mg EAG/g e 29,70 e 10,99 mg EQ/g de extrato concentrado, respectivamente. Ácidos fenólicos (caféico, elágico, ferúlico, ρ-cumárico e protocatecúico), flavonoides (crisina e rutina) e um aldeído fenólico (vanilina) foram determinados por cromatografia líquida de alta eficiência-detector de diodo (CLAE-DAD). As atividades antioxidantes foram

confirmadas pela inibição dos radicais DPPH$^{\bullet}$ (44,08%) e ABTS$^{\bullet+}$ (46,99%) para amostras *in natura*, respectivamente. **Conclusão:** os extratos metanólicos de *Tradescantia zebrina* testados não apresentaram toxicidade quando testados para atividade hemolítica após 72 horas. Portanto, *Tradescantia zebrina* é uma fonte potencial de substâncias bioativas.

Palavras-chave: Tradescantia zebrina, antioxidantes, compostos fenólicos, HPLC-DAD, atividade biológica.

Introduction

The use of medicinal plants for the prevention, treatment, and cure of various diseases is as old and universal as the history of humanity. *Tradescantia* is a genus of herbaceous plants found mainly in South America, being abundant and easily accessible in Brazil, more specifically in the state of Bahia. Reports of the bioactivity of the species *Tradescantia zebrina*, popularly known as "egenda", "trapoeraba", "viuvinha", "lambari" or sea wave, are distant from each other, as there is not enough data in the scientific literature regarding its chemical composition, biological activities, effectiveness, and therapeutic safety. The plant is traditionally used in Mexico as a drink capable of treating various diseases, including cancer [1, 2].

Phenolic compounds are secondary metabolites of plants and are subclassified into flavonoids (polyphenols: flavones, flavanones, flavanols, isoflavones, anthocyanins, etc.) and non-flavonoids (phenolic acids, tannins, coumarins and lignans) [3]. Currently, phenolic compounds have been the substances of greatest interest to science, due to their beneficial characteristics for health, such as antioxidant, antimicrobial and antineoplastic activities [4-8].

Few studies have evaluated total phenolic and flavonoid levels in *Tradescantia zebrina* [1, 9-11]. To guarantee the phytochemical profile of a species or its extracts, a chromatographic *fingerprint* is essential to ensure the quality and reproducibility of the chemical composition, concentration of chemical substances, and, subsequently, therapeutic efficacy. Therefore, high-performance liquid chromatography (HPLC) is an alternative analytical tool for the separation, identification, quantification, and quality control of medicinal plants [12-16].

Although very important, the use of medicinal plants can present risks, since many plants can have a variable chemical composition and relative toxicity [17]. The possibility of

hemolysis is one of the most relevant problems in the use of plants since the saponins or alkaloids present can cause risks to consumption. The in vitro hemolysis test has been used frequently in studies that evaluate the toxicity of medicinal plants [18, 19].

This study aims to expand the knowledge about the species *Tradescantia zebrina*, contributing to the chemical and biological prospection of substances with antioxidant and non-hemolytic potential. For the first time, bioactive phenolic compounds were identified and quantified in methanolic extracts from samples (dried and *in natura*) in this species, promoting greater pharmacological interest and its traditional use

Materials and methods

Plant material

Tradescantia zebrina specimens were collected in Aporá/Bahia/Brazil within the same terrain (11.6605° S, 38.0803° W) at autumn (May 2021), in the full moon. The dried samples (whole plant) were collected, cleaned of dirt and earth remains, and dried in the shade until they became friable. Then, the material was ground in a knife mill (OMDR100, Oster®, São Paulo, Brazil) with a power of 150 W. The process was carried out in 3 cycles of comminution to powder and then they were stored until the extracts were prepared. The *in natura* plant was collected, cleaned, scraped, and weighed for the preparation of extracts.

The botanical identification of the species was carried out by comparison with the characterization described in taxonomic reviews and scientific botanical literature (botanical atlases and photos of the species) and, from traditional native knowledge. A branch of the species was used to identify the macromorphological aspects and preparation of the exsiccate, observing division, leaf blade shape, blade base, type of enervation, presence of petiole, sheath, and stipule, and also the morphological characteristics of the flowers.

Extraction of samples

The extracts obtained from dried samples were performed by maceration in methanol and a re-maceration with solvent exchange in 24 hours and, the extract from fresh samples (*in natura*) of *Tradescantia zebrina*, was obtained by dynamic maceration in methanol, using a blender for 4 hours. The crude extracts were concentrated in a rotary evaporator (Quimis®, São Paulo, Brazil) under vacuum (40 mbar) at a temperature below 35 °C. After complete evaporation, the adhered material was reconstituted with approximately 3.0 mL of methanol to wash the collection flask, until complete drying. The concentrated extracts (dried and *in natura*) were placed in clean and identified

eppendorfs and stored in the freezer until the standardized extracts were prepared at a concentration of 1 mg·mL^{-1}. The unconcentrated extract was standardized at a concentration of 75 000 mg·L^{-1}, being used in a maximum of 3 days. Extracts were filtered and stored at 4 °C when not in use. All analyses were conducted in triplicates.

Determination of total phenolic and flavonoids contents

To quantify the total phenolic compounds, the methodology proposed by Bonoli *et al.* (2004) and the contents of total phenolic compounds were expressed in mg gallic acid equivalent (GAE)/g of extract. All analyses were conducted in triplicates [20].

The determination of total flavonoid levels was performed in triplicate, according to the method described by Woisky & Salatino in 1998 and the total flavonoid levels were expressed in mg quercetin equivalent (EQ)/g of extract the [21].

In vitro assays for evaluating the antioxidant activity

DPPH radical scavenging activity: It was performed according to Brand-Williams *et al.* (1995) with some modifications [22].

ABTS radical scavenging activity: It was performed according to Nenadis *et al.* (2004) with some modifications [23].

Chromatographic analysis

To extract the phenolic compounds, about 10 mg of the crude extract of *Tradescantia zebrina* was solubilized in 1 mL of methanol (HPLC grade). Analyzes were performed in HPLC with a Prominence-i LC-2030C 3D Plus liquid chromatography system (Shimadzu®, Kyoto, Japan), equipped with a diode array detector (DAD) (model SPD-20A), high-pressure quaternary (model LC-20AD), heating oven (model CTO-20A), automatic sampler (model SIL-20A) and communication module (CBM-40Life), all treatment and acquisition of samples were controlled by the LCsolutions software (Shimadzu®, Kyoto, Japan).

Chromatographic separation was performed using a Lichrospher® C18 column (5 μm, 4.0 × 250 mm) from Sigma-Aldrich® (USA), at 40 °C and flow rate of 1.0 mL·min^{-1} with analytical solvents for binary elution mixture consisting of (A) ultrapure water acidified with 1% glacial acetic acid (v·v^{-1}) and (B) HPLC grade methanol according to gradient program: 0-10 min, 100% A; 10-20 min, 30% A and 70% B; 20-21, 25% A and 75% B; 21-25, 100% A. Prior to injection (20 μL) into the HPLC-DAD system, extract solutions were filtered through a PTFE syringe filter (0.45 μm) [12, 24].

A sample of each extract (dried and *in natura*) was fortified with a 0.005 $mg{\cdot}mL^{-1}$ solution containing a mix of 17 phenolic standards for identification and quantification from its spectrum (chromatogram). Identification and qualitative analyzes were performed by comparison with standard spectra at each retention time (Sagdic *et al.*, 2011). The standards of phenolic compounds that were tested show absorbances at the following wavelengths: Caffeic acid (330 nm), Chlorogenic acid (330 nm), Ellagic acid (260 nm), Ferulic acid (330 nm), Gallic acid (272 nm) , ρ-coumaric acid (310 nm), Protocatechuic acid (260 nm), Sinapic acid (330 nm), Syringic acid (272 nm), Trans-cinnamic acid (280 nm), Catechin (280 nm), Chrysin (272 nm)), Kaempferol (360 nm), Naringenin (280 nm), Quercetin (360 nm), Rutin (360 nm) and Vanillin (280 nm). The identified analytes were quantified using external calibration curves (0.1 - 2.5 $mg{\cdot}L^{-1}$) and the results were expressed in $mg{\cdot}g^{-1}$ of extract.

The proposed method was validated according to Brazil in 2017 and the main analytical parameters normally found for method validation are: linearity, precision, limits of detection (LOD) and of quantification (LOQ) [25]. Linearity was evaluated according to the coefficient of determination (R^2) of the analytical curves. Precision was demonstrated by the dispersion of the results, calculating the relative standard deviation (RPR) of the measurements. The limits of detection (LOD) and quantification (LOQ) were calculated based on the parameters of the analytical curves, relating the standard deviation of the intersection (s) and the slope of the curve (S).

Hemolytic activity test

The hemolytic activity assay was performed according to CLSI in 2015 with modifications, by diffusion in blood agar using the disc antibiogram technique [26]. Filter paper discs were impregnated with samples (1 $mg{\cdot}mL^{-1}$ of dried extract) of *Tradescantia zebrina*. After impregnation, the discs were distributed on the plates and incubated at 35 °C for 24, 48 and 72 hours. As a positive control, a solution of the detergent Triton X-100 1 % ($v{\cdot}v^{-1}$) was used. Methanol (0.1%) and saline solution (20%) were used. After incubation, the plates were inspected for the presence of halos of hemolysis between 24 and 72 hours.

Statistical analysis

Data are given as mean ± SD of three measurements for each experiment, using GraphPad InStat (Graph software Inc., V 3.05, Ralph Stahlman, Purdue University, Lafayette, IN). IC_{50} were calculated by nonlinear regression analysis with Graph Pad Prism version 5 for Windows (Graph Pad Inc., USA).

Results and discussion

Macroscopic analysis, total phenolic and flavonoids contents, and antioxidant activities

The macroscopic characteristics of the specimens analyzed were in agreement with the family Comelinaceae [27]. The analysis of the architecture of the leaves showed a parallel pattern, presence of hermaphroditic inflorescences and protection by bracts recorded for the species. It was possible to identify a stoloniferous stem with creeping growth, with the presence of nodes from which adventitious roots emerged and the formation of new branches.

Tradescantia zebrina is a species that offers easy adaptation in any space, in addition to developing throughout the year, both outdoors and in greenhouses [28]. The environmental influences that most interfere with its growth and development are photoperiod, radiation and temperature [29]. Therefore, the place and time of planting are essential to obtain a high yield of the species and substances of secondary metabolism. Phenolic acids and flavonoids have several functions for plant growth and metabolism (defense against insects, fungi, viruses and bacteria and protection against ultraviolet radiation). In addition, these compounds show antioxidant, antimicrobial, anti-inflammatory and antineoplastic activities, as well as in the prevention of neurodegenerative diseases [30-34].

In the literature, some studies have indicated the presence of phenolic compounds in *Tradescantia zebrina*, as well as its antioxidant potential [1, 2, 35]. Table 1 presents the comparative average values (found in this study and in the scientific literature) for phenolic compounds and total flavonoids and, also for the antioxidant activity of extracts of *Tradescantia zebrina*.

The results indicated variations in the contents of phenolic compounds and total flavonoids, in the dried and *in natura* extracts of the *Tradescantia zebrina* specimens studied when compared with a few data from the literature. A reduction in the concentration of phenolics and total flavonoids was observed in the dried sample, probably due to the drying process of the plant, associated with the process of rotaevaporation of the crude extract. The drying temperature can decrease the polyphenol content of plant extracts, as well as their antioxidant activity [37]. The results indicated concentrations of total phenolic compounds and flavonoids higher than those found by Cheah *et al.* in 2017 for dried methanolic extracts of *Tradescantia zebrina*, in Malaysia [11].

Table 1. Comparative mean values (found in this study and in the scientific literature) for total phenolics and flavonoids and, antioxidant activity for extracts of *Tradescantia zebrina*.

Studies/country	Total Phenolics (mg EAG/g) (sample type)	Total flavonoids
Present study/Bahia, Brazil	67.68 ± 0.12* (dried) 233.94 ± 0.71* (*in natura*)	10.99 ± 0.08* (dried) 29.70 ± 0.04* mg EQ/g (*in natura*)
Cheah et al. in 2017 [11]/ Kuala Lumpur, Malaysia	33.50 ± 2.58* (dried)	9.40 ± 1.06* mg ECAT/g (dried)
Olivo-Vidal et al. in 2020 [10]/Tabasco, Mexico	70.20 ± 1.20** (*in natura*)	16.80 ± 0.50 mg ECAT/g** (*in natura*)
Baghalpour et al. in 2021 [9]/ Alborz, Iran	0.08 ± 0.14**	-
To in 2020 [36]/Kuala Lumpur, Malaysia	694.86 ± 54.46 (stem)** (*in natura*) 510.70 ± 31.45 (leaf)** (*in natura*)	496.22 ± 16.56 mg ECAT/g (stem)** (*in natura*) 410.27 ± 8.34 mg ECAT/g (leaf)** (*in natura*)
Tan et al. in 2014 [1]/Kuala Lumpur, Malaysia	6.21 ± 0.40* (*in natura*)	17.10 ± 2.80 mg ER/100 g (*in natura*)
Studies/country	Antioxidant activity (%) (DPPH)	Antioxidant activity (%) (ABTS)
Present study/Bahia, Brazil	44.08 (ExC: 45.00 mg mL^{-1})*	46.99 (ExC: 0.80 mg mL^{-1})*
Cheah et al. in 2017 [11]/ Kuala Lumpur, Malaysia	18.10 (ExC: 0.10 mg mL^{-1})*	-
Olivo-Vidal et al. in 2020 [10]/Tabasco, Mexico	84.00 (ExC: 100 mg mL^{-1})**	16.00 (ExC: 100 mg mL^{-1})**
To in 2020 [36]/Kuala Lumpur, Malaysia	26.19 (stem) (ExC: not informed)** 18,78 (leaf) (ExC: not informed)**	-

EAG: gallic acid equivalents; ECAT: catechin equivalents; EQ: quercetin equivalents; ER: rutin equivalents;*: methanolic extract; **: ethanol extract; ExC: extract concentration; -: not available.

The results obtained for the *in natura* samples were also superior to those found by Tan *et al.* in 2014 in methanolic extracts of this species, in Malaysia [1] and, by Olivo-Vidal *et al.* in 2020 [10] and Baghalpour *et al.* in 2021 [9], who determined phenolic compounds in ethanolic extracts of this species in Mexico and Iran, respectively. However, were inferior (about 10 times) to those found by To (2020), in ethanol extracts (stem and leaf) of *Tradescantia zebrina*, in Malaysia [36].

Thus, secondary metabolites represent a chemical correlation between the species and the environment where they are planted. Environmental stimuli can alter the biosynthesis of different compounds, such as seasonality, collection location, the incidence of solar radiation, rainfall, plant interactions with microorganisms, insects and/or other plants, circadian cycle, nutrition, time and time of collection and, sample preparation method [38, 39].

The DPPH$^{\bullet}$ radical was inhibited (44.08%) in 45 g of plant (in natura) extracted in 1 liter of methanol. To prepare the drink "Agua de Matalí", popular among Mexicans according to the cultural tradition typical of Tabasco, about 100-150 g of plant is used infused in 1 liter of boiling water (± 1 liter) and, after cooling, it is added with lemon juice and honey, serving ice cold or hot [40]. Thus, the consumption of soft drinks, in this proportion, promotes the intake of antioxidant compounds in the diet.

Olivo-Vidal *et al.* (2020) [10] compared the contents of phenolic compounds and total flavonoids, as well as the determination of antioxidant activity by the methods of DPPH, ABTS and by the antioxidant power capable of reducing Iron III (FRAP) with extracts (dynamic maceration in 80% ethanol; aqueous maceration at 22 °C; and aqueous infusion, in the proportion of 1 g of leaf for 10mL of solvent) prepared with fresh leaves of *Tradescantia zebrina*. The authors indicated that the aqueous extract, obtained by maceration, showed a greater ability to capture the radical ABTS$^{\bullet+}$ and also a greater dosage of total flavonoids (27.8 mg ECAT/g). However, the total phenolic compounds showed lower concentrations (51 mg EAG/g) when compared to the ethanolic extract (70.2 mg EAG/g); and this, in turn, had lower flavonoid levels (16.8 mg ECAT/g) and higher DPPH sequestration activity (84%). The extract obtained by infusion showed better results by the FRAP method, being equivalent to the extract obtained by aqueous maceration, in total phenolic contents.

The results of the present study, when compared to those of Cheah *et al.* (2017) [11], in Malaysia, showed higher concentrations of phenolic compounds and total flavonoids for the methanolic extract of *Tradescantia zebrina*. Also, were comparable with those of Olivo-Vidal *et al.* in 2020, in Mexico [10]. This may be due to seasonal interference, the planting process, and the collection and preparation of samples, as the authors

reported that only the leaves were used to obtain the extracts. Also, it is noteworthy that the Mexican plant was acquired at a local fair and the conditions of time and storage of the specimens after collection until analysis were not informed.

The phytochemical assessment investigated by Tan *et al.* (2014) [1] highlighted the presence of phenolic compounds and the antioxidant activity of the *Tradescantia zebrina* species when compared to others of the Tradescantia family, *Tradescantia spathacea* var. variegata, *Tradescantia spathacea* "Hawaiian Dwarf" and *Tradescantia pallida*. Among these species, *Tradescantia zebrina* showed the highest antioxidant activity (about nine times greater) than the other species studied. This observation was also confirmed by Cheah *et al.* (2017) [11] and Baghalpour *et al.* (2021) [9].

Chromatographic analysis

To validate the proposed method, a solution containing 17 phenolic compounds (0.005 mg·mL^{-1}) was prepared and, from the chromatograms, the analytical parameters were obtained (Table 2). Then, the method was applied to analyze the methanolic extracts of the samples (dried and *in natura*) of *Tradescantia zebrina*.

Table 2. Analytical validation parameters for the proposed method by HPLC-DAD.

Analyte	Regression equation	R^2	LOD (mg·L^{-1})	LOQ (mg·L^{-1})
Caffeic acid	y = 149675x – 15785	0.9999	0.04	0.13
Chlorogenic acid	y = 65056x + 5481.7	0.9973	0.14	0.41
Ellagic acid	y = 180471x – 6206.8	0.9992	0.13	0.39
Ferulic acid	y = 106019x – 10833	0.9998	0.06	0.17
Gallic acid	y = 69058x – 987.92	0,9988	0.16	0.48
ρ-Coumaric acid	y = 173234x – 16450	1	0.03	0.09
Protocatechuic acid	y = 132697x – 8605	0.9997	0.08	0.24
Sinapic acid	y = 107178x – 10354	0.9998	0.06	0.18
Syringic acid	y = 73260x – 8065.3	0.9999	0.04	0.11
trans-Cinnamic acid	y = 156741x – 16963	1	0.03	0.08
Catechin	y = 11344x + 3841.1	0.9979	0.21	0.63
Chrysine	y = 23736x – 10191	0.9903	0.44	1.34
Kaempferol	y = 23417x – 4108.7	0.9925	0.40	1.20
Naringenin	y = 63685x – 6429.1	0.9995	0.10	0.29

(Continued)

Analyte	Regression equation	R^2	LOD ($mg \cdot L^{-1}$)	LOQ ($mg \cdot L^{-1}$)
Quercetin	y = 45981x – 10078	0.9985	0.18	0.54
Rutin	y = 31435x – 3903	1	0.01	0.04
Vanillin	y = 92846x – 620.8	1	0.02	0.07

The identification of phenolic compounds by HPLC-DAD was based on the comparison between the peaks obtained from the respective analytical standards and the extracts, regarding retention times and UV spectra. To confirm the presence of phenolic compounds, the samples (dried and *in natura*) were fortified with the standard mixing solution (5.00 $\mu g \cdot mL^{-1}$) and the chromatograms obtained were compared (Fig. 1).

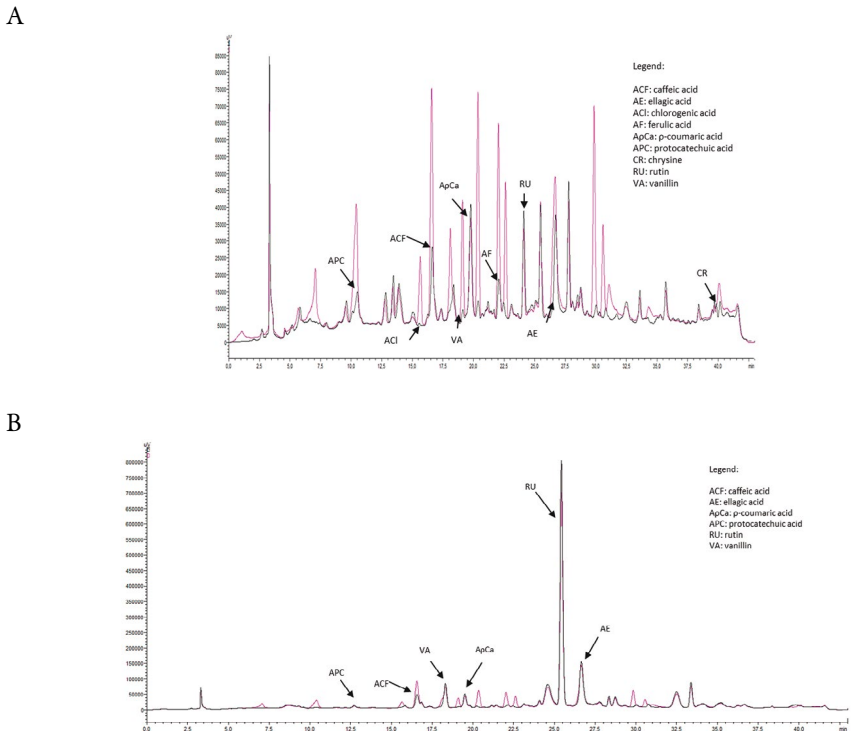

Figure 1. Chromatograms obtained from pure methanolic extract (black line) and enriched with analytical standards (5 $\mu g \cdot mL^{-1}$) of phenolic compounds (pink line) from samples of *Tradescantia zebrina* (A – dried and B – *in natura*) by HPLC-DAD.

It was possible to identify nine phenolic compounds for the dried extract: six phenolic acids (caffeic, ellagic, chlorogenic, ferulic, ρ-coumaric and protocatechuic), two flavonoids (chrysin and rutin) and one a phenolic aldehyde (vanillin) and, of these, eight analytes were quantified (Table 3). Ellagic acid, caffeic acid, and rutin showed higher concentrations. Chlorogenic acid, identified in the extract from its analytical standard, presented amounts below the detection limit (LOD). For extracts *in natura* a change in the chromatogram profile was observed (Fig. 1B). It was possible to identify six phenolic compounds for the dried extract: four phenolic acids (caffeic, ellagic, ρ-coumaric and protocatechuic), one flavonoid (rutin) and one phenolic aldehyde (vanillin) and, of these, five analytes were quantified (Table 4).

Table 3. Phenolic compounds quantified from the dried methanolic extract of *Tradescantia zebrina* by HPLC-DAD.

Analyte	Wavelength	Retention time	Integrated area	Conc. ($mg \cdot L^{-1}$)	Conc. ($\mu g \cdot g^{-1}$)
Caffeic acid	330	16.636	257002	1.82	182.25
Chlorogenic acid	330	15.566	5636	nd	nd
Ellagic acid	260	26.717	521488	2.92	292.40
Ferulic acid	330	22.136	134203	1.37	136.80
ρ-Coumaric acid	310	20.394	95896	0.65	64.85
Protocatechuic acid	260	10.512	103298	0.84	84.33
Chrysine	272	40.168	17318	1.16	115.90
Rutin	360	25.495	107323	3.54	353.83
Vanillin	280	19.139	38708	0.42	42.36

nd: not determined; Conc: concentration.

A high intensity peak was obtained, with a larger integrated area, for the flavonoid rutin, with a concentration much higher than the sample enriched with the analytical standard. After calculating the purity of this substance (from the integrated area of the peak), a value of 70% was obtained. Therefore, it was decided not to predict the actual content of this flavonoid, since other compounds (present in *in natura* sample, not contained in the standard solution used in this study) may be eluting together with rutin, by absorbing radiation at 360 nm.

Table 4. Phenolic compounds quantified from the *in natura* methanolic extract of *Tradescantia zebrina* by HPLC-DAD.

Analyte	Wavelength	Retention time	Integrated area	Conc. ($mg\ L^{-1}$)	Conc. ($\mu g\ g^{-1}$)
Caffeic acid	330	16.580	427860	2.96	296.41
Ellagic acid	260	26.649	2253336	12.52	1,252.03
ρ-Coumaric acid	310	20.232	87347	0.60	59.92
Protocatechuic acid	260	10.433	38817	0.36	35.74
Rutin	360	25.434	2534792	nd	nd
Vanillin	280	19.147	15829	0.18	17.72

nd: not determined; Conc: concentration.

The nature and amount of chemical compounds identified in plants can vary according to several factors [41]. No data were found in the scientific literature on the identification and quantification of phenolic compounds in *Tradescantia zebrina* by HPLC-DAD. Therefore, this study fills a gap because, for the first time, it demonstrated the relevance of this species to science, confirming the chemical, biological, and therapeutic potential of *Tradescantia zebrina*.

Hemolytic activity test

In tests of biological and, especially, toxicological activities of plant extracts, there is a need to evaluate hemolytic activity. With the rupture of red blood cells, the iron released from the heme group is harmful and can cause damage to essential organs, such as the liver and kidneys, resulting in hemoglobinemia [42, 43]. When this value exceeds 100 mg%, hemoglobin begins to be filtered by the kidneys and, when excessively high (above 3000 mg%), it can promote kidney damage with hemoglobinuria [19, 44].

From the results obtained in the hemolytic activity test through the diffusion of the extract in blood agar, no sample showed formation of a hemolytic halo after 72 hours (Fig. 2). This confirms no toxicity to the erythrocyte membrane, from the methanolic extracts of samples (dried and *in natura*) of the species. Only, there was the formation of a halo of 2 cm of hemolysis with the use of the positive control, a solution of the detergent Triton X-100 1 % ($v{\cdot}v^{-1}$). Therefore, it is suggested that, preliminarily, *Tradescantia zebrina* does not indicate toxicological risks for use in living organisms, despite the saponins present in the plant.

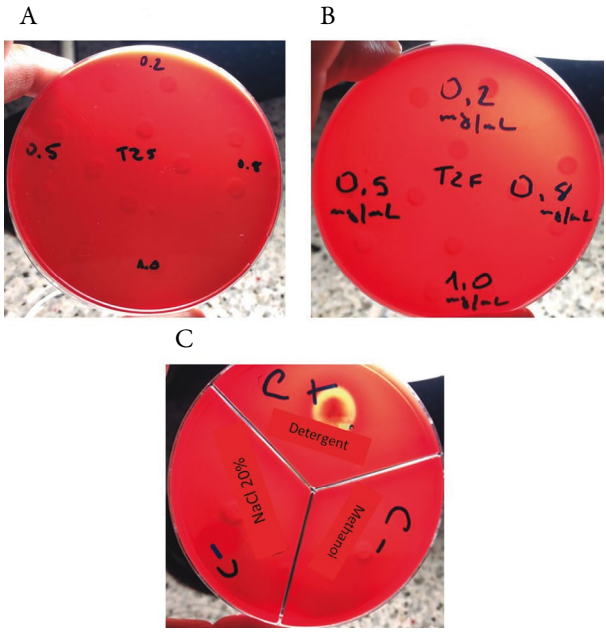

Figure 2. Evaluation of the presence of hemolysis halos, in plates, with controls (positive and negative), after 72 hours in methanolic extracts of *Tradescantia zebrina*. A: dried extract; B: *in natura* extract and C: negative and positive controls.

Conclusion

The methanolic extracts of samples (dried and *in natura*) of *Tradescantia zebrina* showed considerable levels of phenolic compounds and antioxidant activities. From a preliminary fingerprint obtained by HPLC-DAD, the presence of phenolic compounds in this species was confirmed, for the first time described in the literature. It was possible to identify eight phenolic compounds in the samples, being five phenolic acids (caffeic, ellagic, ferulic, ρ-coumaric and protocatechuic), two flavonoids (chrysin and rutin) and one a phenolic aldehyde (vanillin). The proposed and validated chromatographic method was linear, with good precision and accuracy and presented low detection and quantification limits, confirming its sensitivity and applicability. The absence of toxicity observed in the hemolytic activity assays suggests the performance of additional studies to determine the therapeutic properties of this species.

Therefore, from the observation of the botanical and morphological aspects, from the chemical and biological prospection, it is possible to conclude that *Tradescantia zebrina*

is a species of interest, as a potential source of bioactive substances. Gaps are still present in this knowledge and will serve as a starting point for the continuity of these studies.

Disclosure statement

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are grateful for State University of Bahia (UNEB), Coordination for the Improvement of Higher Education Personnel-Brazil (CAPES), Brazilian National Council for Scientific and Technological Development (CNPq) and Research Group: "Biopharmaceutics and Drugs".

References

1. J.B.L. Tan, W.J. Yap, S.W. Tan, Y.Y. Lim, S. M. Lee, Antioxidant content, antioxidant activity, and antibacterial activity of five plants from the Commelinaceae family, *Antioxidants*, **3**, 758-769 (2014).

2. A.J. Alonso-Castro, M.L. Villarreal, L.A. Salazar-Olivo, M. Gomez-Sanchez, F. Dominguez, A. Garcia-Carranca, Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies, *J Ethnopharmacol.*, **133**, 945-972 (2011).

3. O.R. Alara, N.H. Abdurahman, C.I Ukaegbu, Extraction of phenolic compounds: a review, *Curr. Res. Food Sci*., **4**, 200-214 (2021).

4. S.V.S. da Silva, O.M. Barboza, J.T. Souza, É. N. Soares, C.C. dos Santos, L.V. Pacheco, I.P. Santos, T.B.S. Magalhães, M.B.P. Soares, E.T. Guimarães, C. S. Meira, S.L. Costa, V.D.A. da Silva, L.L.B. de Santana, A. de Freitas-Santos Júnior, Structural design, synthesis and antioxidant, antileishmania, anti-inflammatory and anticancer activities of a novel quercetin acetylated derivative, *Molecules*, **26**, 6923 (2021).

5. D. Stagos, Antioxidant activity of polyphenolic plant extracts, *Antioxidants (Basel)*, 9, 19 (2020).

6. C. Manach, A. Mazur, A. Scalbert, Polyphenols and prevention of cardiovascular diseases, *Curr. Opin. Lipidol.*, **16**, 77-84 (2005).

7. E. Niki, Assessment of antioxidant capacity *in vitro* and *in vivo*, *Free Radic. Biol. Med.*, **49**, 503-515 (2010).

8. A.R. Tapas, D.M. Sakarkar, R.B. Kakde, Flavonoids as nutraceuticals: a review, *Trop. J. Pharm. Res.*, **7**, 1089-1099 (2008).

9. N. Baghalpour, S.A. Ayatollahi, N. Naderi, T. Hosseinabadi, Y. Taheri, J. Mahroo-Bakhtiyari, Z.K. Shinwari, A.T. Khalil, J. Sharifi-Rad, Antinociceptive and anti-inflammatory studies on *Tradescantia zebrina*, *Pak. J. Bot.*, **53**, 357-365 (2021).

10. Z. Olivo-Vidal, J. Ruiz-Ruiz, V.S. Mayday, C. Irecta-Nájera, D.L.H. Ochoa, X.M. Sánchez-Chino, Pharmacological potential of *Tradescantia zebrina* leaf extracts, *J. Biomed. Eng. Res.*, **4**, 31-37 (2020).

11. S.Y. Cheah, C.Y. Magdalene, C.Z. Eldwin-Lim, M.H. Wong, S. Amir, S. Daniel, Y.C. Liow, S. Ketnipha, K. Karenjit, G.A. Gabriel, J.H. Chin, *In-vitro* antioxidant and acetylcholinesterase inhibitory activities of *Tradescantia zebrina*, *Res. J. Pharm. Biol. Chem. Sci.*, **8**, 82-87 (2017).

12. H.F.S. Moura, F.S. Dias, L.B. Souza e Souza, B.E.A. Magalhães, C.A. Tannus, W.C. Carvalho, G.C. Brandão, W.N.L. dos Santos, M.G.A. Korn, D.C.M.B. Santos, M.V. Lopes, D.A. Santana, A.F. Santos Júnior, Evaluation of multielement/proximate composition and bioactive phenolics contents of unconventional edible plants from Brazil using multivariate analysis techniques, *Food Chem.*, **363**, 129995 (2021).

13. H. Nybom, K. Weising, B. Rotter, DNA fingerprinting in botany: past, present, future, *Investig. Genet.*, **5**, 1 (2014).

14. R.R. Sá, R.A. Matos, V.C. Silva, J.C. Caldas, M.C.S. Sauthier, W.N.L. dos Santos, H.I.F. Magalhães, A.F. Santos Júnior, Determination of bioactive phenolics in herbal medicines containing *Cynara scolymus*, *Maytenus ilicifolia* Mart ex Reiss and *Ptychopetalum uncinatum* by HPLC-DAD, *Microchem. J.*, **135**, 10-15 (2017).

15. R.R. Sá, J.C. Caldas, D.A. Santana, M.V. Lopes, W.N.L. dos Santos, M.G.A. Korn, A.F. Santos Júnior, Multielementar/centesimal composition and determination of bioactive phenolics in dried fruits and capsules containing Goji berries (*Lycium barbarum* L.), *Food Chem.*, **273**, 15-23 (2019).

16. L. Yun, S. Yao, Y. Chang-Liang, G. De-An, Quality assessment of herbal medicines based on chemical fingerprints combined with chemometrics approach: a review, *J. Pharm. Biomed. Anal.*, **185**, 113215 (2020).

17. J. Felix-Silva, I.M. Tomaz, M.G. Silva, K.S.C.R. Santos, A.A. Silva-Júnior, M.C.R.D. Carvalho, L.A.L. Soares, M.F. Fernandes-Pedrosa, Botanical and chemical identification of plant species of popular use in Rio Grande do Norte, Brazil, *Rev. Bras. Plantas Med.*, **14**, 548-555 (2012).

18. C.S. Paula, V.C.D. Canteli, M.C.S. Verdam, M. Kalegari, R. Campos, B.C.K. Hirota, O.G.M. Miguel, M.D. Miguel, Antioxidant activity and preliminary toxicity of the extracts and fractions obtained from the leaves and stem bark of *Dasyphyllum tomentosum* (Spreng.) Cabrera, *Rev. Bras. Plantas Med.*, **16**, 189-195 (2014).

19. M. Kalegari, M.D. Miguel, J.D.F.G. Dias, A.L.L. Lordello, C.P.D. Lima, C.M.S. Miyazaki, S.M. Zanin, M.C. Verdam, O.G. Miguel, Phytochemical constituents and preliminary toxicity evaluation of leaves from *Rourea induta* Planch. (Connaraceae), *Braz. J. Pharm. Sci.*, **47**, 635-642 (2011).

20. M. Bonoli, V. Verardo, E. Marconi, M.F. Caboni, Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds, *J. Agric. Food Chem.*, **52**, 5195-5200 (2004).

21. R.G. Woisky, A. Salatino, Analysis of propolis: some parameters and procedures for chemical quality control, *J. Apic. Res.*, **37**, 99-105 (1998).

22. W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, *LWT - Food Sci. Technol.*, **28**, 25-30 (1995).

23. N. Nenadis, L.F. Wang, M. Tsimidou, H.Y. Zhang, Estimation of scavenging activity of phenolic compounds using the ABTS(*+) assay. *J. Agric. Food Chem.*, **52**, 4669-4674 (2004).

24. B.E.A. Magalhães, D.A. Santana, I.M.J. Silva, L.A.C. Minho, M.A. Gomes, J.R.G.S. Almeida, W.N.L. dos Santos, Determination of phenolic composition of oilseed whole flours by HPLC-DAD with evaluation using chemometric analyses, *Microchem. J.*, **155**, 104683 (2020).

25. Brazilian Health Surveillance Agency (ANVISA), Resolution of the Collegiate Board of Directors - RDC, No. 166 of July 24, 2017, Providing for the validation of analytical methods and other provisions, Ministry of Health of Brazil, URL: https://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/19194581/do1-2017-07-25-resolucao-rdc-n-166-de-24-de-julho-de-2017-19194412, acessado em janeiro de 2022.

26. Clinical and Laboratory Standards Institute (CLSI), Performance standards for antimicrobial disk susceptibility tests; approved standard—eighth edition, NCCLS document M2-A8 (2015), URL: https://clsi.org/media/1631/m02a12_sample.pdf, accessed em janeiro de 2022.

27. P.M. Elbl, G.F. Melo-de-Pinna, N.L. Menezes, Morphology and anatomy of leaf miners in two species of Commelinaceae (*Commelina diffusa* Burm. f. and *Floscopa glabrata* (Kunth) Hassk), *Acta Bot. Bras.*, **24**, 283-328 (2010).

28. H.A. Carvalho, Use of *Tradescantia* to monitor the clastogenic effects of ionizing radiation, *Rev. Radiol. Bras.*, **38**, 459-462 (2005).

29. J.A. Marchese, G.M. Figueira, The use of pre and post-harvest technologies and good agricultural practices in the production of medicinal and aromatic plants, *Rev. Bras. Plantas Med.*, **7**, 86-96 (2005).

30. E. Ahmed, M. Arshad, M.Z. Khan, M.S. Amjad, H.M. Sadaf, I. Riaz, S. Sabir, N. Ahmad, Sabaoon, Secondary metabolites and their multidimensional prospective in plant life, *J. Pharmacogn. Phytochem.*, **6**, 205-214 (2017).

31. D. Esquenazi, M.D. Wigg, M.M. Miranda, H.M. Rodrigues, J.B. Tostes, S. Rozental, A.J. Da Silva, C.S. Alviano, Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract, *Res. Microbiol.*, **153**, 647-652 (2002).

32. R. Gutam, S.M. Jachak, Recent developments in anti-inflammatory natural products, *Med. Res. Rev.*, **29**, 767-820 (2009).

33. M. Negri, T.P. Salci, C.S. Shinobu-Mesquita, I.R.G. Capoci, T.I.E. Svidzinski, E.S. Kioshima, Early state research on antifungal natural products, *Molecules*, **19**, 2925-2956 (2014).

34. O. Sagdic, I. Ozturk, G. Ozkan, H. Yetim, L. Ekici, M.T. Yilmaz, RP-HPLC–DAD analysis of phenolic compounds in pomace extracts from five grape cultivars: Evaluation of their antioxidant, antiradical and antifungal activities in orange and apple juices, *Food Chem.*, **126**, 1749-1758 (2011).

35. C.S.M. Alaba, C.L. Chichioco-Hernandez, 15-Lipoxygenase inhibition of *Commelina benghalensis*, *Tradescantia fluminensis*, *Tradescantia zebrina*, *Asian Pac. J. Trop. Biomed.*, **4**, 184-188 (2014).

36. S.K. To, Stability of plant pigments in *Tradescantia zebrina* and *Ixora coccinea* Linn as potential food colorant with antioxidant activity (2020), URL: https://eprints.tarc.edu.my/14408/#:~:text=For%20Tradescantia%20Zebrina%20showed%20the,0.91%20%25)%20at%20pH%207.&text=Tradescantia%20Zebrina%20and%20Ixora%20Coccinea%20Linn%20extracts%20have%20lower%20viability,used%20as%20a%20food%20colorant, acessado em janeiro de 2022.

37. T. Katsube, Y. Tsurunaga, M. Sugiyama, T. Furuno, Y. Yamasaki, Effect of air-drying temperature on antioxidant capacity and stability of polyphenolic compounds in mulberry (*Morus alba* L.) leaves, *Food Chem.*, **113**, 964-969 (2009).

38. M. Gomes, B. Magalhães, W. Santos, J.R. Almeida, Influence of seasonality on phytochemical composition, phenolic content and antioxidant activity of *Neoglaziovia variegata* (Bromeliaceae), *Biointerface Res. Appl. Chem.*, **12**, 2889-2904 (2021).

39. S. Kabtni, D. Sdouga, I. Bettaib Rebey, M. Save, N. Trifi-Farah, M.L. Fauconnier, S. Marghali, Influence of climate variation on phenolic composition and antioxidant capacity of *Medicago minima* populations, *Sci. Rep.*, **10**, 8293 (2020).

40. M.A. Guadarrama-Olivera, De los "Matalis", "señoritas embarcadas" y otras Commelinas en Tabasco, *Kulxulkab,* **17**, 79-86 (2010).

41. L. Gobbo-Neto, N.P. Lopes, Medicinal plants: factors of influence on the content of secondary metabolites, *Quim. Nova*, **30**, 374-381 (2007).

42. T. Afsar, S. Razak, M.R. Khan, S. Mawash, A. Almajwal, M. Shabir, I.U. Haq, Evaluation of antioxidant, anti-hemolytic and anticancer activity of various solvent extracts of *Acacia hydaspica* R. Parker aerial parts, *BMC Complement. Altern. Med.*, **16**, 258 (2016).

43. T.D. Coates, Physiology and pathophysiology of iron in hemoglobin-associated diseases, *Free Radic. Biol. Med.*, **72**, 23-40 (2014).

44. E.B. Carvalho, E.L. Borges, L.M.B. Carlos, M.A.M. Silva, S.M.M. Magalhães, F.V.B.A.F. Gomes, M.J.C. Carvalho, A.T.S. Quixadá, Effect of the infusion pumps of solutions on the degree of hemolysis, *Rev. Bras. Hematol. Hemoter.*, **29**, 149-152 (2007).

How to cite this article

V.C. Silva, B.E.A. Magalhães, T.B.S. Magalhães, E.T. Guimarães, A.S. Guedes, M.D. Mota, W.N.L. dos Santos, B.A.V. Cerqueira, A. de Freitas Santos Júnior, Determination of phenolic bioactive compounds and evaluation of the antioxidant and hemolytic activities in the methanolic extracts of *Tradescantia zebrina*, *Rev. Colomb. Cienc. Quím. Farm*., **51**(3), 1341-1361 (2022). http://dx.doi.org/10.15446/rcciquifa.v51n3.101403