Tradescantia spathacea extract as biostimulating in growth and biological activities of *Lactobacillus acidophilus* and *Lacticaseibacillus rhamnosus*

Extracto de *Tradescantia spathacea* como bioestimulante en el crecimiento y actividades biológicas de *Lactobacillus acidophilus* y *Lacticaseibacillus rhamnosus*

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Scientific article
Received: January 16, 2025
Accepted: April 04, 2025

ABSTRACT. Medicinal plants are an essential source of bioactive compounds with health benefits; however, their impact on probiotic properties remains largely unknown. The aim of this study was to evaluate the biostimulating effect of aqueous extracts of *Tradescantia spathacea* (EAT) on the development and probiotic properties of *Lacticaseibacillus rhamnosus* HN001 and *Lactobacillus acidophilus* La-14. For this purpose, the total phenol content (TPC), total flavonoid content (TFC), and antioxidant activity of EAT were evaluated by spectrophotometric methods (DPPH and ABTS). Subsequently, the biostimulating effect on the growth (BG), antimicrobial activity (AA), cholesterol consumption (CC), and biofilm formation (BFF) of *Lacti. rhamnosus* and *Lacto. acidophilus* were evaluated. The EAT presented a TPC of 68.98 ± 5.01 mg GAE g^{-1} dry extract and TFC of 4.54 ± 0.62 mg CE g^{-1} dry extract and antioxidant activity with IC_{50} values of 778.81 ± 60.80 and 4068.16 ± 206.8 μg mL^{-1}, for DPPH and ABTS respectively. The correlation between CFT and antioxidant activity was low (r = -0.7948 and -0.7208; for DPPH and ABTS, respectively). In the BG study, the data showed that EAT stimulated the growth of *Lacti. rhamnosus* and *Lacto. acidophilus* and that AA was higher in biostimulated probiotic bacteria. However, CC and FBP were also stimulated by the symbiotic interaction between probiotics and EAT. The aqueous extract of *T. spathacea* had a biostimulating effect on the probiotic properties of *Lacti. rhamnosus* HN001 and *Lacto. acidophilus* La-14.
Keywords: Biostimulation, phenols, medicinal plant, probiotic.

RESUMEN. Las plantas medicinales son fuente importante de compuestos bioactivos con beneficios a la salud; sin embargo, poco se conoce sobre su impacto sobre las propiedades probióticas. El objetivo del presente trabajo fue evaluar el efecto bioestimulante de los extractos acuosos de *Tradescantia spathacea* (EAT) sobre el desarrollo y las propiedades probióticas de *Lacticaseibacillus rhamnosus* HN001 y *Lactobacillus acidophilus* La-14. Para ello se evaluó el contenido de fenoles totales (CFT), flavonoides totales (TF) y la actividad antioxidante mediante métodos espectrofotométricos (DPPH y ABTS) del EAT. Posteriormente, se evaluó el efecto bioestimulante en el crecimiento (BC), la actividad antimicrobiana (AA), el consumo de colesterol (CC) y la formación de biopelículas (FBP) de *Lacti. rhamnosus* y *Lacto. acidophilus*. El EAT presentó un CFT de 68.98 ± 5.01 mg GAE g^{-1} dry extract y TF de 4.54 ± 0.62 mg CE g^{-1} dry extract y actividad antioxidante con valores de IC_{50} de 778.81 ±60.80 y 4068.16 ± 206.8 μg mL^{-1}, para DPPH y ABTS respectivamente. La correlación entre CFT y la actividad antioxidante fue baja (r = -0.7948 y -0.7208; para DPPH y ABTS, respectivamente). En el estudio del CB los datos muestran que el EAT estimula el crecimiento de *Lacti. rhamnosus* y *Lacto. acidophilus* y que la AA es mayor en bacterias probióticas bioestimuladas. Por otro lado, el CC y FBP también se ven estimulados por el efecto simbiótico entre los probióticos y el EAT. El extracto acuoso de *T. spathacea* tienen un efecto bioestimulante sobre las propiedades probióticas evaluada en *Lacti. rhamnosus* HN001 y *Lacto. acidophilus* La-14.
Palabras clave: Bioestimulación, fenoles, planta medicinal, probiótico.

Como citar: Cruz-Maldonado J, Velázquez-Martínez JR, Pozo-Santiago CO, Guzmán-Ceferino J, Ochoa-Flores AA, Hernández-Gallegos MA (2025) *Tradescantia spathacea* extract as biostimulating in growth and biological activities of *Lactobacillus acidophilus* and *Lacticaseibacillus rhamnosus*. Ecosistemas y Recursos Agropecuarios 12(1): e4452. DOI: 10.19136/era.a12n1.4452.

INTRODUCTION

The human microbiota is an ecosystem comprised of trillions of microorganisms, including bacteria, viruses, fungi, and archaea (Nazzaro *et al*. 2020). Most of them are resident principals of the gastrointestinal tract (Thursby and Juge 2017). However, some nutrients in the diet have been associated with an imbalance in gut microbiota compositions, i.e., dysbiosis (Milutinović *et al*. 2021, Rosales-Bravo *et al*. 2021); it is also related to some disorders, such as obesity, diabetes, and metabolic disorders (Federico *et al.* 2017). Positive and negative interactions have been described between the metabolites found in different foods and the intestinal microbiota. These metabolites modulate the microbiota through their interactions, which can confer health benefits or endanger human health (Milutinović e*t al*. 2021, Tannock 2021, Steinert *et al*. 2025). Phenolic compounds are secondary metabolites of plants, vegetables, fruit, herbs, and spices whose chemical structures contain one or more aromatic rings with one or more hydroxyl groups (Catalkaya *et al*. 2020). Phenols are known for their health-promoting properties, such as antioxidants, antimicrobial, cytotoxic, hepatoprotective, antiviral, anti-inflammatory, and antiapoptotic activities. In addition, they prevent chronic diseases, such as diabetes, obesity, neurodegenerative diseases, and cardiovascular diseases (López-Escamilla *et al*. 2024). However, when consumed, their activity is mediated by the intestinal microbiota, as the small intestine reabsorbs a part of the phenols, and the remaining compounds reach the large intestine and interact with the intestinal microbiota to be absorbed (Ozdal *et al.* 2016). Many studies have shown that some polyphenols stimulate the growth of beneficial bacteria, mainly of the genre *Bifidobacterium* and *Lactobacillus*, in addition to improving probiotic activity (Cheng *et al.* 2023, Makarewicz *et al*. 2021). Medicinal plants are a source of bioactive compounds, such as terpenes, phenolic compounds, glycosides, and alkaloids, related to bioactive properties (López-Escamilla *et al*. 2024). *Tradescantia spathacea*, commonly known as "purple maguey", belonging to the Commelinaceae family, is a wild native plant from southern México that can be found in the states of Chiapas, Tabasco, and Yucatan Peninsula and is recognized for its healing activities, such as antioxidant, anticancer, immunomodulatory, antimicrobial, and antiviral activities (Castillo *et al.* 2024). In this study, the antioxidant activity and the phenolic and flavonoid contents of the aqueous extract of *T. spathacea* were first identified. The biostimulating effects of this extract on the growth, biofilm formation, and biological activities, such as antimicrobial activity and cholesterol consumption, of the probiotic bacteria *Lactobacillus acidophilus* La-14 and *Lacticaseibacillus rhamnosus* HN001 were also evaluated.

MATERIALS AND METHODS

Biological Material

Tradescantia spathacea leaves were collected from Parrilla, Centro, Tabasco, México (17° 54' 28.2" N 92° 54' 57.8" W) in April 2023. The leaves were washed with distilled water and dehydrated at room temperature using a dehumidifier. The dried leaves were ground with a blender (Oster ®, México), and the powder was sieved (No. 60 mesh) and stored in amber bottles at 20 °C until use. *Lacticaseibacillus rhamnosus* HN001 was obtained from the collection of the Laboratorio de Biotecnología of the Escuela Nacional of Ciencias Biológicas at the Instituto Politécnico Nacional in

México, and *Lactobacillus acidophilus* La-14 was purchased from Nature's Bounty (The Bountiful Company, US). The pathogenic strains used were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6639), and *Salmonella typhimurium* (ATCC 14028), donated by the Departamento de alimentos y Biotecnología, of the Falcultad de Química, Universidad Nacional Autónoma de México.

Preparation of aqueous plant extracts

Extraction was performed with deionized water and 2% of the leaf powder, which were macerated at room temperature for 48 h. The macerate was filtered with Whatman No. 1 filter paper and subsequently microfiltered with a nitrocellulose membrane (Millipore®, Darmstadt, Germany) with a 0.45 µm pore diameter. The filtrate was freeze-dried (Freeze Dryer, FreeZone 4.5, LABCONCO, USA) and stored at 4 °C in amber flasks until use (Sánchez-Zarate *et al*. 2020). For use of the extract in subsequent assays, it was dissolved in water, and for use in culture media, it was sterilized by microfiltration (Millipore®, membrane, 0.45 µm pore diameter).

Growth conditions of the strains

The probiotic strains were preserved in Man, Rogosa, and Sharpe broth (MRS, Bioxon®, México), and the pathogenic strains were stored in brain heart infusion broth (BHI, Bioxon®, México) with 50% glycerol in both cases at −20 °C. For the studies, *Lacti*. *rhamnosus* HN001 and *Lacto*. *acidophilus* La-14 were grown in MRS-Cys broth (MRS enriched with 0.5 g L-cysteine L^{-1}) at 37 °C for 12–48 h, and counts were performed on MRS agar plates. The pathogenic strains were grown in BHI broth or agar at 37 °C under aerobic conditions for 24 h.

Phytochemicals content

Phytochemicals (phenols and flavonoids) were determined by spectrophotometric methods using a spectrophotometer (UV-1800, Rayleig®, China).

The total phenol content (TPC) was determined using the Folin–Ciocalteu reagent using the method proposed by Sánchez-Zarate *et al*. (2020). Briefly, 125 µL of extract (adjusted concentration), 625 µL of Folin–Ciocalteu diluted in deionized water (1:10), and 500 µL of 7.5% Na_2CO_3 were mixed. The mixture was incubated in the dark for 45 min. Absorbance was measured at 760 nm, and the results were expressed as mg of gallic acid equivalents per g of dry extract (mg GAE g^{-1} dry extract) using a gallic acid curve.

The total flavonoid content (TFC) was determined by the aluminum chloride ($AlCl_3$) method developed by (Zhishen *et al*. 1999). Briefly, 200 µL of the extract, 1.2 mL of distilled water, and 150 µL of $NaNO_2$ (5%) were mixed. The mixture was allowed to react for 6 min. Subsequently, 150 µL of $AlCl_3$ (10%) was added to the mixture and left to stand for 6 min, and 800 µL of NaOH (10%) was added. The absorbance was measured at 510 nm. A catechin curve was used, and the results were expressed in mg of catechin equivalents per g of dry extract (mg CE g^{-1} dry extract).

Antioxidant activity

The antioxidant activity was evaluated by determining the DPPH• and ABTS•+ free radical scavenging capacity of the extract following the methods described by Sánchez-Zarate *et al*. (2020).

For the DPPH assay, 2 mL of DPPH solution (125 µM in 80% methanol, v/v) was added to 200 µL of the extract and allowed to react at room temperature in the dark for 60 min. The absorbance of the solution was measured at 520 nm.

The ABTS•+ assay was performed by preparing a solution with 2.45 mM potassium persulfate and 7 mM ABTS•+ (1:1) and allowing it to react at room temperature and away from light for 12–16 h. 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 it to react for 6 min. The absorbance was read at 734 nm.

The results in both methods were expressed as the IC_{50} (extract concentration required to inhibit 50% of free radicals, µg mL^{-1}) and were calculated using linear regression analysis. The percentage of free radical scavenging was calculated using the following formula: % inhibition = ((Control Abs − Sample Abs) / (Control Abs)) × 100, where Control Abs is the absorbance of the reagent mixture without adding sample or standard and Sample Abs is the mixture absorbance added with sample or standard reagent.

Effect of extracts on the growth of probiotic strains

The biostimulating effects of the aqueous extract of *T. spathacea* on the growth of *Lacto. acidophilus* La-14 and *Lacti. rhamnosus* HN001 was evaluated using the methodology of (Lu *et al.* 2017), with some modifications. MRS-cys broth was prepared with the addition of *T. spathacea* extract, so that the final concentration in the broth was 2 mg mL^{-1}. The extract was added after the medium was sterilized. At the same time, MRS-cys broth without extract was prepared as a control. Both media were inoculated with 1% standardized probiotic strain inoculum (10^8 CFU mL^{-1}) and then incubated at 37 °C under aerobic conditions. The samples were taken at 12 and 24 h, and growth was determined by plate counting in triplicate on MRS-cys agar after incubation under the same conditions.

Antibacterial activity

The antibacterial activity of the probiotic strains was evaluated according to the plate diffusion method described by (Jorgensen and Turnidge 2015), with modifications. Unsolidified BHI agar (40 °C) was inoculated with 1% of the inoculum of the pathogenic strains (*E. coli*, *St. aureus*, *B. subtilis*, and *Sa. typhimurium*) previously standardized (10^8 CFU mL^{-1}). The inoculum was dispersed in BHI agar by shaking, and the agar was poured onto plates. The inoculated plates were prepared by depositing 10 µL of the probiotic bacteria culture previously grown (12 h, 10^8 CFU mL^{-1}) in MRS-cys broth with (as mentioned in the previous section) and without extract in triplicate. On 6-mm filter paper disks, 50 µg of amikacin was used as a positive control, and 10 µL of sterile distilled water was used as a negative control. The plates were incubated at 37 °C under aerobic conditions, and the inhibition zones were measured after 14 h using a vernier caliper and recorded in mm.

Cholesterol reduction assay

Cholesterol reduction was assessed using MRS-cys broth with cholesterol (MRS-cys-Ch) at a 180 mg/dL concentration. Cholesterol was homogenized (Homogenizer, POLYTRON®, Swiss) in

propanol (20% of the final volume of the medium). MRS-cys-Ch broth was prepared in duplicate with and without the extract (80% of the volume of the medium) at an extract concentration of 2 mg mL^{-1}. Cholesterol homogenized in propanol was added to the medium after sterilization. MRS-cys-Ch broth with and without extract was inoculated with 1% of the previously standardized inoculum of probiotic bacteria strains (10^8 CFU mL^{-1}). The inoculated media were incubated at 37 °C under aerobic conditions, and samples were collected at 0, 3, 6, 12, and 24 h in duplicate. Samples were centrifuged at 6 000×g (15 min at 4 °C). The cholesterol content was determined using an *in vitro* liquid cholesterol determination kit (Pointe Scientific Inc.). The plate count of the samples was determined, and the growth (CFU mL^{-1}) and cholesterol consumption (mg dL^{-1}) kinetics were constructed (Kumar *et al*. 2013).

Biofilm formation assay

The capacity to form a biofilm was determined using a previously described method (Gómez *et al*. 2016, Sanchez *et al*. 2013), with some adjustments. The culture medium used was MRS-cys with plant extract (2 mg mL^{-1}) and without plant extract. In a 96-well microplate, 200 µL of medium and 10 µL of previously standardized inoculum (10^8 CFU mL^{-1}) were added to each well. The microplate with the cultures was incubated at 37 °C under aerobic conditions for 14 h. The absorbance was then measured at 620 nm in a microplate reader (Thermo Fisher Scientific, MultiSkan GO, Finland). The plate was washed with sterile saline solution (0.9%) to remove non-adhered bacteria. The plate was then dried with air flow for 30 min, and 200 µL of 0.3% crystal violet was added to the wells for 1 min. The wells were washed with distilled water, dried for 1 h, and then filled with 200 µL of 96% ethyl alcohol, and the absorbance was read at 540 nm. The biofilm forming capacity (BFC) was calculated using the following equation: BFC = (AB-CW) / G. AB is the absorbance at 540 nm of the solution containing the bacteria that remained adhered to the microplate, CW is the absorbance at 540 nm of the control medium (MRS without bacteria), and G is the absorbance at 620 nm of the medium with bacterial growth.

Statistical analysis

Descriptive statistics were used to report on the phytochemical content and antioxidant activity in the plant. A completely randomized experimental design was used for the rest of the experiments. Each test was performed in triplicate, and the results were expressed as the mean ± standard deviation. A one-way analysis of variance (ANOVA) was performed, and the means were compared using the Tukey test ($p < 0.05$). Pearson's correlation coefficients ($p < 0.05$) were determined between the antioxidant activity values and the total phenolic content of the extracts.

RESULTS

Phytochemical content and antioxidant activity of *T. spathacea* aqueous extract

The phytochemical composition and antioxidant activity of the *Tradescantia spathacea* aqueous extract are presented in Table 1. The TPC present in the extract was significant, and the TFC was lower since they comprise a portion of the TPC. However, the *T. spathacea* extract showed antioxidant activity with a better capacity to capture the DPPH radical than the ABTS+ radical by

presenting a lower IC_{50} value for DPPH than for ABTS. The IC_{50} was determined as the concentration of extract necessary to capture 50% of the free radicals present in the solution.

Table 1. Phytochemical content and antioxidant activity of *Tradescantia spathacea* aqueous extract.

Phytochemical content		Antioxidant activity	
Phenols (mg GAE g^{-1} dry extract)	Flavonoids (mg CE g^{-1} dry extract)	DPPH (μg mL^{-1}, IC_{50})	ABTS (μg mL^{-1}, IC_{50})
68.98 ± 5.01	4.54 ± 0.62	778.81 ±60.80	4068.16 ± 206.8

GAE: gallic acid equivalent; CE: catechin equivalent. The values shown are means ± standard deviations of triplicates (n = 3).

The *T. spathacea* aqueous extract presented a low correlation between the TPC and the antioxidant activity determined by the DPPH and ABTS radical scavenging methods, with Pearson coefficients (r) of 0.7208 and 0.7948, respectively (Table 2). This indicates that the antioxidant activity presented by the extract did not depend entirely on the TPC, suggesting that other metabolites were involved in this activity.

Table 2. Correlation between antioxidant activity and the total phenolic content in *Tradescantia spathacea* aqueous extract.

Assay	Equation	Pearson´s coefficient (r)	Significance
DPPH• (IC_{50})	y = -240.949−0.226631x	−0.7948	p > 0.1 (p = 0.1081)
ABTS •+ (IC_{50})	y = 300.532−0.060163x	−0.7208	p > 0.1 (p = 0.1695)

Biostimulation of the growth of *Lacto. acidophilus* La-14 and *Lacti. rhamnosus* HN001 with *T. spathacea* aqueous extract

Figure 1 shows the growth of the strains *Lacti. rhamnosus* HN001 and *Lacto. acidophilus* La-14 at 12 and 24 h with and without the *T. spathacea* aqueous extract. The extract biostimulated the growth of *Lacti. rhamnosus* HN001 (Figure 1a) and *Lacto. acidophilus* La-14 (Figure 1b) with statistically significant differences ($p < 0.05$) at both 12 and 24 h.

Biostimulation effects of *T. spathacea* aqueous extract on the antimicrobial activity of *Lacto. acidophilus* La-14 and *Lacti. rhamnosus* HN001

Table 3 shows the antimicrobial activity of *Lacto. acidophilus* La-14 and *Lacti. rhamnosus* HN001 with and without *T. spathacea* extract. *Tradescantia spathacea* extract significantly improved ($p < 0.05$) the antimicrobial activity of *Lacto. acidophilus* against all strains of pathogenic bacteria evaluated. However, biostimulation significantly improved ($p < 0.05$) the antimicrobial activity of *Lacti. rhamnosus* HN001 against *E. coli* and *St. aureus*. The results for *Sa. typhimurium* and *B. subtilis* were similar with and without extract. Therefore, the *T. spathacea* aqueous extract had a biostimulating effect on the antimicrobial activity of *Lacto. acidophilus* La-14 and *Lacti. rhamnosus* HN001.

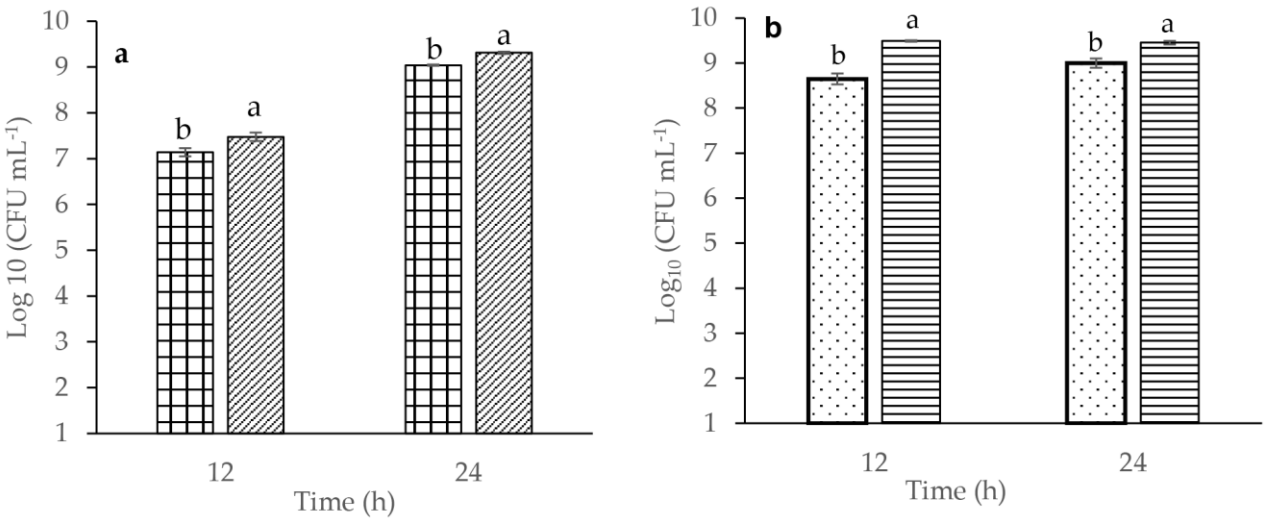

Figure 1. Biostimulating effect of *Tradescantia spathacea* aqueous extract on *Lacticaseibacillus rhamnosus* HN001 and *Lactobacillus acidophilus* La-14 growth. Figure 1a. ▦ indicates *Lacti. rhamnosus* growth without the extract, ▩ indicates *Lacti. rhamnosus* growth with the extract. Figure 1b. ⁙ indicates *Lacto. acidophilus* growth without the extract, ▤ indicates *Lacto. acidophilus* growth with the extract. Different letters between growth values at 12 and 48 h indicate significant differences between media with or without extract. Significant differences between the biostimulation of probiotic bacteria by *T. spathacea* aqueous extract were found with a $p < 0.05$ according to Tukey's HSD test.

Table 3. Antimicrobial activity of *Lactobacillus acidophilus* La-14 and *Lacticaseibacillus rhamnosus* HN001 on pathogenic bacteria with and without *Tradescantia spathacea* extract.

Probiotic strain	**Pathogenic bacteria (Diameters of inhibition halos, in mm)**			
	E. coli	***Sa. typhimurium***	***St. aureus***	***B. subtilis***
Lacto. acidophilus	8.73 ± 0.40[a]	11.3 ± 0.178[a]	9.3 ± 0.19[a]	20.28 ± 0.85[a]
Lacto. acidophilus + extract	11.92 ± 0.28[b]	12.24 ± 0.67[b]	10.53 ± 0.34[b]	22.69 ± 1.25[b]
Lacti. rhamnosus	13.64 ± 0.62[a]	12.97 ± 0.57[a]	20.07 ± 0.94 [a]	24.08 ± 2.16[a]
Lacti. rhamnosus + extract	15.82 ± 0.75[b]	13.74 ± 0.42[a]	24.31 ± 0.07 [b]	24.19 ± 1.69[a]

Data represents the diameters of inhibition halos (mm) ± standard deviation. Different superscript letters within a column indicate statistically significant differences ($p < 0.05$) between the diameters of the inhibition zones caused by the probiotic strains (*Lacto. acidophilus* and *Lacti. rhamnosus*) with and without extract against each strain of pathogenic bacteria tested (*Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Bacillus subtilis*).

Cholesterol-lowering capacity of *Lacti. rhamnosus* HN001 and *Lacto. acidophilus* La-14 stimulated with *T. spathacea* aqueous extract

Figure 2 shows the growth and cholesterol consumption of *Lacti. rhamnosus* HN001 (Figure 2a) and *Lacto. acidophilus* La-14 (Figure 2b) with and without *T. spathacea* aqueous extract. Both strains are capable of decreasing cholesterol levels. However, *Lacti. rhamnosus* HN001 and *Lacto. acidophilus* La-14 with the extract increased cholesterol consumption ($p < 0.05$).

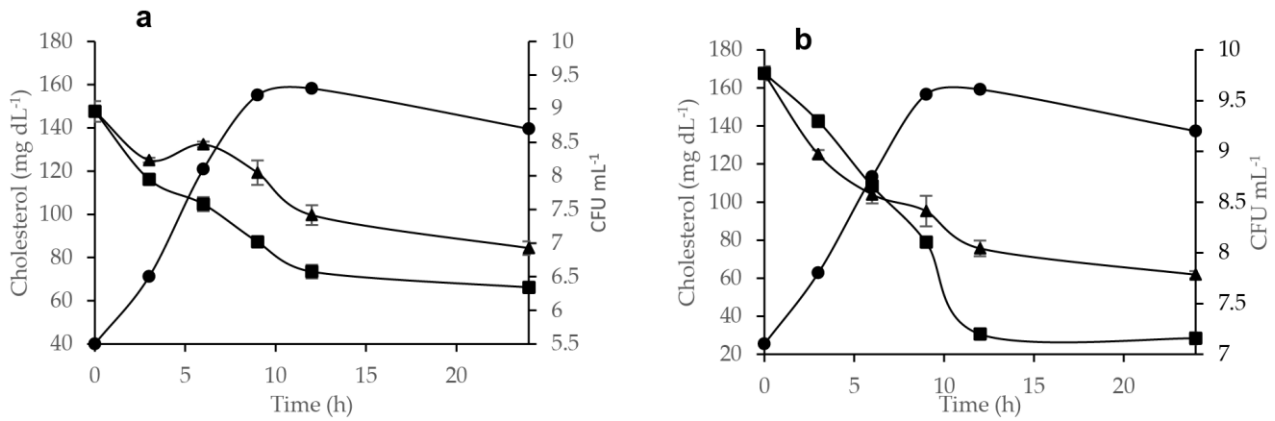

Figure 2. Biostimulation effects of *Tradescantia spathacea* aqueous extract on *Lactobacillus acidophilus* La-14 and *Lacticaseibacillus rhamnosus* HN001 in cholesterol consumption. Figure 2a. *Lacto. acidophilus* La-14 cholesterol consumption (▲), *Lacto. acidophilus* La-14 cholesterol consumption biostimulated with *T. spathacea* (■), and cinetic growth of probiotic bacteria (●). Figure 2b. *Lacti. rhamnosus* HN001 cholesterol consumption (▲), *Lacti. rhamnosus* HN001 cholesterol consumption biostimulated with *T. spathacea* (■), and cinetic growth of probiotic bacteria (●).

Biostimulation of biofilm formation of probiotic bacteria by *T. spathacea* aqueous extract

Lacticaseibacillus rhamnosus HN001 and *Lacto. acidophilus* La-14 had the capacity for biofilm formation (Table 4). However, the *T. spathacea* aqueous extract offered significant biostimulation ($p < 0.05$) for biofilm production in *Lacti. rhamnosus*.

Table 4. Biofilm formation of *Lactobacillus acidophilus* La-14 and *Lacticaseibacillus rhamnosus* HN001 with and without *Tradescantia spathacea* extract.

Probiotic bacteria	Biofilm formation without extract (DO_{520nm})	Biofilm formation with extract (DO_{520nm})
Lacti. rhamnosus	0.061 ± 0.009^{b}	0.68 ± 0.097^{a}
Lacto. acidophilus	0.031 ± 0.007^{a}	0.024 ± 0.014^{a}

Values with different superscript letters indicate significant differences ($p < 0.05$) between biofilm formation by the bacteria with and without extract.

DISCUSSION

The TPC and TFC of medicinal plants is related to antioxidant activity and biological activities, such as anti-inflammatory, vasodilatory, analgesic, anticancer, antioxidant, and antimicrobial activities (Catalkaya *et al*. 2020). Some studies support these properties, both *in vitro* and *in vivo* (Amarowicz and Pegg 2019). In this sense, the TPC and TFC in *T. spathacea* could be related to antioxidant activity and other biological activities. Lopes *et al.* (2024) reported a TPC in *T. spathacea* lower values. However, the differences can be attributed to the climatic conditions of the collection sites, soil conditions, and growth (Bozhüyük *et al.* 2022, Ramos-Arcos *et al.* 2023). Some phenolic compounds are recognized for their antioxidant activity; thus, their importance lies in the elimination of reactive oxygen species (ROS) and free radicals, thus preventing oxidative stress in cells (Vázquez-Ovando *et al*. 2022). A similar study reported IC_{50} values for DDPH of 1146.90 ±

92.53 µg mL^{-1} in *Tradescantia zebrina*, demonstrating that antioxidant activity is a characteristic shared by plants of the same family (Ramos-Arcos *et al*. 2023). The values obtained in the present study showed that the *T. spathacea* aqueous extract had antioxidant activity, which may be differ from that reported for other plants in the family due to the type of extract and origin of the plant. However, the correlation between the TPC and the antioxidant activity of *T. spathacea* obtained was relatively low; this may be because the antioxidant activity does not depend only on the presence of phenolic compounds since there may be other non-phenolic metabolites (Zejli *et al*. 2024). As mentioned previously, phenolic compounds from medicinal plants are associated with health benefits. Additionally, the redefinition of prebiotics now includes phenolic compounds, as they can resist host digestion, can be fermented by probiotic bacteria, and exert a stimulating effect on them (Plamada and Vodnar 2022). Some studies point to phenols as stimulants of probiotic bacteria growth, as well as the inhibitory effect of pathogens (Duda-Chodak *et al.* 2015). However, several authors have reported that this stimulation is selective due to studies that demonstrate its effects on the growth, proliferation, and survival of *Lactobacillus* and *Bifidobacterium* (Hervert-Hernández and Goñi 2011, Milutinović *et al*. 2021). Similarly, Alberto *et al.* (2001) reported that the phenolic compounds catechin and gallic acid stimulated the growth of *Lactobacillus hilgardii*. However, Tian *et al.* (2023) evaluated the stimulation of persimmon leaf extract on the growth of *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae* and reported better stimulation on the growth of *Saccharomyces cerevisiae*. Haș *et al.* (2023) verified the stimulating effect of the methanolic extract of *Sambucus nigra* L. on the growth of *Lactiplantibacillus plantarum*, *Lactobacillus casei*, *Lacti. rhamnosus*, *Lactocacillus fermentum*, and *Saccharomyces bulardii*. Similar results were observed in the present study, where *T. spathacea* had a stimulating effect on the growth of *Lacti. rhamnosus* HN001 and *Lacto. acidophilus* La-14. The effects of phenolic compounds on growth have been attributed to the ability of some probiotic bacteria to metabolize them; thus, their health benefits are linked to the modulation of intestinal microbiota (Catalkaya *et al*. 2020, Dueñas *et al.* 2015). The modulation of the intestinal microbiota by phenols, in addition to promoting the growth of probiotic bacteria, also affects the inhibition of pathogenic bacteria (Catalkaya *et al.* 2020). Some reports have confirmed this effect. For example, (Liang *et al*. 2021), who demonstrated that aqueous extracts of *Taraxacum officinale* and *Astragalus membranaceus* stimulate the antimicrobial activity of *Lacto. acidophilus* and *B. subtilis* against *E. coli*. However, Banerjee and Dhar (2019) mentioned that the antimicrobial activity of plant extracts and probiotic bacteria against Gram-positive and/or Gram-negative pathogenic bacteria could depend on the synergy that exists between these two factors. Yang *et al.* (2018) demonstrated that black tea fermented by *Lacto. acidophilus* had better antimicrobial activity on *E. coli* due to the endogenous oxidative stress caused by biotransformed phenolic compounds. Therefore, some authors attribute this stimulation to the ability of some probiotic bacteria to biotransformed phenolic compounds from complex to simple, which could represent a higher level of absorption, bioactivity, and bioavailability (Sharma *et al*. 2022). In the present study, *T. spathacea* showed positive stimulation both in the growth of probiotic bacteria and in the inhibition of pathogens so that phenolic compounds could be related to beneficial effects in protecting the intestinal barrier. The symbiotic effect between probiotics and prebiotics on the lipid profile, such as total cholesterol, low-density lipoprotein (LDL), high-density lipoproteins (HDL), and triglycerides, has been widely studied, and some *in vitro* studies have demonstrated the reducing effects of some probiotic and/or prebiotic strains (Ooi and Liong 2010). In a study conducted by

Kumar *et al.* (2013) in murine animals with hypercholesterolemia, a drink based on *Lacti*. *rhamnosus* and aloe vera extract had a positive effect on serum cholesterol levels, which was attributed to synergism between the probiotic bacteria and the plant. The *T*. *spathacea* extract exerted a biostimulating effect on cholesterol consumption in *Lacti*. *rhamnosus* HN001 and *Lacto*. *acidophilus* La-14, which could be considered an effect of symbiosis and the phenolic compounds of the extract as prebiotics. The formation of biofilms in probiotic bacteria provides several benefits, including enhanced cell adhesion capacity, increased antibiotic resistance, and promoted colonization and permanence in the colon; however, biofilm development in probiotic bacteria depends on several factors, such as the bacterial strain and environmental conditions, such as pH, nutrient concentration, and temperature (Salas-Jara *et al*. 2016). Additionally, some authors have reported that phenolic compounds affect biofilm formation, mainly described in pathogenic bacteria, due to the obstruction of a bacterial quorum (Plamada and Vodnar 2022). The data obtained in the present study indicate (HDL) that the *T*. *spathacea* aqueous extract stimulates biofilm production by *Lacti*. *rhamnosus* HN001 and *Lacto*. *acidophilus* La-14, which could guarantee bacterial establishment in the colon and the health benefits of the consumer. Recently, several authors have carried out in-depth reviews of the mechanisms that trigger the biostimulation of phenolic compounds in probiotic bacteria; however, there is a limitation in the studies thus far for understanding the biostimulation of phenolic compounds in probiotic bacteria (Ozdal *et al.* 2016, Nazzaro *et al.* 2020, Plamada and Vodnar 2022, Sharma *et al*. 2022).

CONCLUSION

The *T*. *spathacea* aqueous extract presents antioxidant compounds that have a biostimulating effect on the growth, antimicrobial activity, cholesterol consumption, and biofilm formation of *Lacti*. *rhamnosus* HN001 and *Lacto*. *acidophilus* La-14. These findings suggest that medicinal plant extracts can improve the biological activities of probiotics. However, it is advisable to continue with studies on the biostimulation of biological activities, both *in vitro* and *in vivo*, in the bacteria studied.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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