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SONICATION-EXTRACTED POLYPHENOLS FROM TOMATILLO CALYX WASTE: A NOVEL APPROACH TO ENHANCING ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES FOR FOOD PRESERVATION

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ABSTRACT

The increasing demand for natural food preservatives is driven by growing consumer concerns regarding health and environmental sustainability, posing a significant challenge in food preservation technology. Although synthetic additives have long been used to extend the shelf life of foods, the health risks they pose have led to a search for safer alternatives. This study explores the potential of tomatillo calyx extracts (*Physalis ixocarpa* Brot ex. Hornem.) as natural preservatives, addressing the knowledge gap regarding their efficacy and safety. Sonication was employed as a novel extraction method to enhance polyphenol yield and improve bioactivity of the extracts. Antioxidant activity was evaluated using in vitro assays ($ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP). Antimicrobial efficacy was assessed against pathogenic and food-spoiling microorganisms. In addition, toxicity was evaluated using the *Artemia salina* nauplii assay. The results demonstrated that sonication significantly increased the extraction of phenolic compounds, leading to robust antioxidant activity. The extracts effectively inhibited *S. aureus* and *A. niger*, showing potential as antimicrobial agents, although no activity was observed against *E. coli* and *C. albicans*. The brine shrimp assay revealed median lethal doses below 100 µg/mL, indicating potential bioactivity. Hence, tomatillo calyx extracts exhibit promising antioxidant and antimicrobial properties, suggesting their utility in food preservation. These findings contribute to the valorization of agricultural waste and propose a sustainable alternative to synthetic preservatives. Future research should focus on optimizing extraction methods, expanding the analysis of the antimicrobial spectrum, and evaluating the extracts in food models.

Keywords: Husk tomato, *Physalis ixocarpa*, Calyx, Antioxidant activity, Antimicrobial activity, Agro-industrial waste, Polyphenols

INTRODUCTION

Food loss and waste represent critical challenges in the quest for global sustainability and food security. The Food and Agriculture Organization (FAO) reports that approximately one-third of all food produced worldwide, equating to 1.3 billion tons, is wasted annually, resulting in staggering economic losses of around 750 billion dollars **(Bhatia *et al.*, 2024)**. Microbial contamination is one of the main causes of food spoilage and is mainly due to bacteria, yeasts, and molds. These microorganisms thrive in food environments, breaking down organic compounds and resulting in unpleasant flavors, odors, sometimes toxic byproducts and foodborne outbreaks **(Mafe *et al.*, 2024)**. Microorganisms represent one of the main factors affecting food safety due to outbreaks associated with the consumption of contaminated food and spoilage occurring during production, storage and distribution. Their presence not only compromises quality, availability, and safety, but also poses a significant risk to public health. It is estimated that the consumption of contaminated food causes approximately 600 million cases of illness and 420,000 deaths worldwide each year **(Chen *et al.*, 2025)**, being primarily responsible: *Salmonella* spp., *Campylobacter* spp., *Escherichia coli, Bacillus cereus, Listeria monocytogenes* y *Staphylococcus aureus* **(Gutiérrez del Río *et al.*, 2018)**.

In response to these situation, traditional methods of prolonging the shelf life of food products have relied heavily on chemical preservatives such as benzoates, nitrites, nitrates, and synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and TBHQ (*tert* –butylhydroquinone). These compounds are effective in inhibiting microbial growth and slowing lipid oxidation, thereby extending the shelf life of food **(Thbayh and Fiser, 2022)**. However, there is growing concern about the health implications of these additives, with reports linking their excessive consumption to allergies, dermatitis, asthma, cancer, and liver damage **(Karwowska and Kononiuk, 2020; Wang *et al.*, 2021)**. This has led to increased consumer demand for safer and healthier food products derived from natural sources, promoting the use of plant extracts, essential oils, and bioactive phytochemicals as alternatives to synthetic preservatives **(Gutiérrez del Río *et al.*, 2018; Bukvicki *et al.*, 2023)**. Recent research has focused on organic compounds that exhibit antimicrobial and antioxidant properties, even at low concentrations, without altering the sensory qualities of food products **(Zhang *et al.*, 2023; Upadhyay *et al.*, 2024)**. For this reason, studies evaluating the properties of extracts obtained from agricultural by-products or waste products are becoming increasingly common, whether it be bitter orange peel (*Citrus aurantium*) **(García-Juárez *et al.*, 2024)**, tomato plant residues (*Lycopersicon esculentum*) **(Silva-Beltrán *et al.*, 2015)**, husk of pecan nut (*Carya illinoinensis*) **(Flores-Estrada *et al.*, 2019)**, and aguaymanto or capulí seeds (*Physalis pubescens* L.) **(Zimmer *et al.*, 2021)**, with the aim of developing plant-based food preservatives and adding value to agricultural waste due its antimicrobial capacity.

In this regard, one of the agricultural byproducts that has been studied for its potential bioactivity is the calyx of the tomatillo or husk tomato (*Physalis ixocarpa* Brot. ex Hornem.). It is an annual plant native to Mexico and Guatemala, member of the *Solanaceae* family and is commercially important due to its fruit, which is protected by a calyx during growth (González and Guerrero, 2021). Once the fruit is harvested, calyx is considered agro-industrial waste which represents approximately 4% of fruit´s total weight. Previous studies have reported bioactivity in extracts obtained from tomatillo calyx. For instance, some authors demonstrated the antimicrobial activity of tomatillo calyx extracts against various microorganisms **(Khan *et al.*, 2016)**. Additionally, the antioxidant and hypoglycemic potential of these extracts were evaluated, identifying significant concentrations of phenolic acids and flavonoids and reporting health benefits such as the inhibition of lipid oxidation in human plasma **(Guerrero-Romero *et al.*, 2021)**.

Consequently, the aim of this study was to evaluate the in vitro antioxidant potential, toxicity (using brine shrimp), and antimicrobial activity against selected foodborne and spoilage microorganisms in extracts obtained from the tomatillo

calyx using different extraction techniques. Additionality, this study aimed to valorize the agricultural byproduct such as the tomatillo husk or calyx, contributing to the circular economy model; and to investigated its bioactive properties in the order to assess its potential as a natural food preservative and a substitute for chemical preservatives to mitigate the effects caused by microorganisms on the food industry and consumers, based on the ability of the phenolic compounds present to inhibit microbial growth and slow oxidative spoilage.

MATERIAL AND METHODS

Chemicals

Ethanol was procured from MEYER (Mexico), while acetic acid was obtained from Fermont (Mexico). $AlCl_3$ and Na_2CO_3 were supplied by CTR SCIENTIFIC (Mexico). Additional reagents included Potassium persulfate ≥ 99% purity, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) ≥ 98% purity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) ≥ 90% purity, 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) ≥ 97% purity, Folin-Ciocalteu reagent (2N), and quercetin ≥ 98% purity were all acquired from Sigma-Aldrich (USA), with gallic acid (97.5 - 102.5%) from Sigma-Aldrich (China). The culture media Agar Mueller Hinton and Potato Dextrose Agar were purchased form BD Bioxon (Mexico), while Czapek Dox Agar Sigma-Aldrich (Switzerland). *Artemia salina* eggs were purchased commercially.

Plant Material

The calyxes of the husk tomato (*P. ixocarpa* Brot ex. Hornem.) were collected from Altamirano de Marcos farm, located on the west coast of Hermosillo, Sonora, Mexico (Latitude N: 29°11'02.226" and Longitude W: -111°57'47.898", 320 m above sea level) in December 2023, during the fall-winter harvest. The selection criteria for the plant material included uniformity in size and maturity to ensure consistency across samples. The calyxes were thoroughly washed with distilled water to remove any surface contaminants, dried at 45 °C for 24 h in a convection oven (VWR Scientific Products Model 1320), and subsequently milled using a 2 mm sieve (Thomas-Wiley Model 4, USA) to achieve a consistent particle size for extraction.

Preparation of extracts

The extraction process was designed to maximize the yield of bioactive compounds from the plant material. Two 17 g samples of the sieved plant material were mixed with 500 mL of ethanol and 5% acetic acid (95:5 v/v). The extraction was performed using maceration and ultrasonication techniques, with specific modifications to optimize efficiency. Ultrasound-assisted extraction was carried out for 10 minutes at 550 W and 20 kHz (**Robles-Apodaca *et al.*, 2024**), using a Branson SFX 550 Digital Sonicator (Mexico). Maceration was carried out for 72 h on a constant agitation plate, at 28 °C and in total darkness **(Silva-Beltrán *et al.*, 2015)**. After extraction, both solutions were vacuum filtered with Whatman No. 1 paper to remove plant material, and the collected solvent was evaporated using a rotary evaporator at 40 °C and 200 rpm (RE100-S, Science Med, Finland). Finally, the macerated extract (ME) and sonicated extract (SE) were resuspended in ethanol and stored at -20 °C for preservation until further evaluation.

Total phenolic content

The total phenolic content was quantified in microplate using the Folin-Ciocalteu method **(Magalhães *et al.*, 2010)**, with slight modifications. An aliquot of 10 µL of each extract was mixed with 25 µL of 1N Folin-Ciocalteu reagent. After 5 min, 25 µL of Na_2CO_3 (20% w/v) and 140 µL of distilled water were added, to achieve a final volume of 200 µL per well. Then, the mixture was incubated for 30 min at room temperature and darkness for development of a blue color. Absorbance was measured at 765 nm using a spectrophotometer (Thermo Fisher Scientific Inc. Multiskan GO, NY, USA) Ethanol was used as the negative control. The phenolic content was calculated using a gallic acid standard curve (0.02 - 0.8 mg/mL) and expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (GAE /g). All analyzes were conducted in triplicate.

Total flavonoid determination

For flavonoid determination, in a 96-well microtitter plate, 75 µL of each extract was mixed with 75 µL of $NaNO_2$ (6 g/L). After 5 min at room temperature, 75 µL of $AlCl_3$ (22 g/L) was added, followed by incubation for 6 min. Subsequently, 75 $AlCl_3$ µL de NaOH (0.8 M) was added, and the reaction was left in dark for 10 min. The absorbance was measured at 510 nm using a spectrophotometer. Flavonoid content was calculated using a quercetin standard curve (0.01 - 0.2 mg/mL) and expressed as milligrams of quercetin equivalent (QE) per gram of extract (QE/g). Each measurement was performed in triplicate to ensure accuracy and reproducibility **(Magalhães *et al.*, 2012)**.

Antioxidant activity assessment

ABTS Free radical scavenging activity

The ABTS assay was performed according to following method **(González-Vega *et al.*, 2021)**. $ABTS^{\bullet+}$ radical cations were generated mixed 5 mL of 7.04 mM ABTS solution with 88 µL of a 0.139 mM sodium persulfate solution ($K_2S_2O_8$) and left to stand for 16 h at room temperature in dark. The $ABTS^{\bullet+}$ solution was diluted with ethanol, and their absorbance was adjusted to 0.700 ± 0.05 at 734 nm. Subsequently, 270 µL of free radical were combined with 20 µL of each extract and incubated for 30 min. The absorbance was read in spectrophotometer (Thermo Fisher Scientific Inc. Multiskan GO, NY, USA) at 734 nm. The control sample was prepared with 20 µL of ethanol and mixed 270 µL of $ABTS^{\bullet+}$ solution. The antioxidant activity against $ABTS^{\bullet+}$ radical was measured using a Trolox curve standard and expressed as Trolox equivalents (TE) per gram of extract (mmol TE/g). Analyses were carried out in triplicate.

DPPH Free radical scavenging activity

A stock solution of 2.2-Diphenyl-l-picrylhydrazyl ($DPPH^{\bullet}$) was prepared (0.025 mg/mL) using ethanol as solvent, and its absorbance was adjusted at 0.700 ± 0.05 at 515 nm. An aliquot of 200 µL of $DPPH^{\bullet}$ radical and 20 µL of each extract were mixed in microplate and incubate at room temperature for 30 min. The absorbance of samples was read (Thermo Fisher Scientific Inc. Multiskan GO, NY, USA) at 515 nm. For the control, 200 µL of $DPPH^{\bullet}$ solution and 20 µL ethanol were added. The antioxidant activity against the $DPPH^{\bullet}$ radical was determined using a Trolox standard curve and expressed as Trolox equivalents per gram of extract (mmol TE/g) **(Robles-Apodaca *et al.*, 2024)**. Analyses were carried out in triplicate.

FRAP Assay

Initially, FRAP stock solution was prepared from sodium buffer acetate (300 mM, pH: 3,6), ($FeCl_3$) (20 mM) and 2,4,6-tripyridyl-s-triazine (TPTZ) (10 mM) in hydrochloric acid solution (40 mM); in a relation 10:1:1 (Buffer: $FeCl_3$: TPTZ). For samples analysis, a volume of 20 µL of each extract was placed in 280 µL of FRAP stock solution and incubated in microplate for 30 min at room temperature. For the control, 280 µL of FRAP stock solution and 20 µL ethanol were added. Absorbance was measured using a spectrophotometer (638 nm) and a Trolox standard curve was made. The results were expressed as Trolox equivalent per gram of extract (mmol TE/g) **(García-Juárez *et al.*, 2024)**. Measuring was carried out in triplicate.

Antimicrobial activity testing

The antimicrobial activity of the extracts was individually evaluated by disc diffusion and radial growth assay against two bacterial strain and two fungi. The strains of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Aspergillus niger* (NRRL3) and *Candida albicans* (ATCC 14053) were provided by the Microbiology and Mycotoxins Laboratory of the Department of Research and Postgraduate Studies in Food of the University of Sonora and the Biomedical Engineering Laboratory of the Sonora State University. These microbial strains were selected with the aim of achieving representativeness of the four most common microbial groups (Gram (+) and Gram (-) bacteria, filamentous fungi, and yeast). In addition, the bacteria evaluated are associated with outbreaks caused by the consumption of contaminated food. *A. niger* is known as a mycotoxigenic fungus that frequently appears in stored cereals. Instead, *C. albicans* is a drug-resistant yeast that has been associated with contamination in the dairy industry due to cases of mastitis and has also been found in raw chicken and lamb meat.

Inoculum preparation

The bacterial strains were grown on Muller Hinton agar and incubated for 24 h at 37 ± 2 °C to obtain young colonies in optimal metabolic condition. Working inoculum solutions were prepared in tryptone soy broth (TSB) with a turbidity equivalent to 0.5 McFarland (1×10^6) colony-forming units (CFU/mL) **(Zimmer *et al.*, 2021)**. The yeast strains got reactivated in potato dextrose broth (PDB) and incubated for 48 h at 37 ± 2 °C. After the incubation period, a yeast suspension was prepared using a Neubauer chamber at a concentration of 1×10^6 yeast cells/mL **(Hernández-Abril *et al.*, 2024)**.

Disk diffusion assay

The disk diffusion assay was employed for the determination of antimicrobial activity of the extracts against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *C. albicans* (ATCC 14053) **(Wangi *et al.*, 2024)**. The inoculum solutions were placed and spread with a sterile cotton swab on a Petri dish (90 mm) containing 20 mL of Muller Hinton agar for bacterial strains and Potato Dextrose Agar for yeast strain. Sterile disks (6 mm diameter) made with filter paper were impregnated with 10 µL of each extract (36.5, 17.8, 8.9 and 4.45 mg/mL). For negative control was used 10 µL of ethanol (50% v/v), while the positive control was Amoxicillin (50

mg/mL) for bacterial strain and Fluconazole (25 µg/mL) for yeast strain. Each extract was dissolved in ethanol (50% v/v) for the measures. Each test was performed in triplicate. All of plates were incubated at 37 ± 2 °C for 24 h. Then, the inhibition zones were measured with a Vernier calibrator. The analyses were carried out in triplicate.

Fungistatic activity of extracts on *Aspergillus niger*

Growth conditions

The strain of *A. niger* (NRRL 3) was activated on Potato Dextrose Agar and incubated for 5 days at 30 ± 2 °C. Spores were collected with Tween 80 sterile solution of (0.1% v/v) and a magnetic stir bar for 5 min. The spore solution concentration was determined using a Neubauer chamber and adjusted at concentration of 1×10^5 spores/mL **(Martínez-Camacho *et al.* 2010)**. The antifungals tests with *A. niger* were carried out using Czapek Agar medium.

Radial growth

Petri dishes (55 mm) containing Czapek Agar medium supplemented with 500 µL of each extract (17.8 and 4.45 mg/mL) were inoculated with 1×10^5 spores/mL of *A. niger* at a central puncture point. Additionally, 500 µL of ethanol (50% v/v) was used as negative control, and 500 µL of distilled water as growth control. All plates were incubated at 30 ± 2 °C. Colony diameter was measured every 12 h with a Vernier calibrator and the growing was compared with the control until the fungus control reached the edge of the plate **(Cota-Arriola *et al.*, 2013)**. The determinations were made in triplicate.

Spore germination

Petri dishes containing 20 mL of Czapek liquid medium were inoculated with 300 µL of 1 x 10^6 spores/mL of *A. niger* solution and incubated at 30 ± 2 °C. Moreover, the liquid medium was mixed with 500 µL of distilled water as growing control, ethanol (50% v/v) as negative control and each extract at two concentrations (35.6 and 17.8 mg/mL). The number of germinated spores was counted each 5 h using a microscope (10× objective) (Olympus CX31, Japan). A total of 200 spores were evaluated to ensure statistical representativeness. Each germination experiment was made in duplicated. Spore germination (%) was determined with the equation **(Cota-Arriola *et al.*, 2011)**:

$$\text{Spore germination (\%)} = \left(\frac{S_i}{S_c}\right) \times 100$$

where S_1 represent number of germinated spores in the extract treated samples and S_c the number of spores counted.

Morphometric analysis of hyphae and spores

The diameter of fungi spores and hyphal length were measured each 5 h during 20 h trough image analysis using Infinity Software V6.5.4 (2016, Lumenera Corporation, Canada), using the 40× objective of an optical microscope (Labomed Lx 400, USA), coupled with Infinity camera (2008 Media Cybernetics Inc, USA) **(Martínez-Camacho *et al.* 2010)**. Morphometric analysis was performed by taking 30 independent measurements (n = 30) for each parameter per treatment.

Acute toxicity in *Artemia salina* nauplius

The acute toxicity of extracts was assessed utilizing *Artemia salina* nauplius via the brine shrimp lethality assay **(Rivas-Gastélum *et al.*, 2025)**, with some modifications. Brine shrimp eggs were incubated in sterile sea water at room temperature, controlled illumination, agitation and aeration until the hatching (24 - 36 h). The test was reproduced three-fold using sterile tubes with 5 mL of sea water with 10 nauplii incubated for 24 h and 5 concentrations (500, 100, 50, 25 and 10 µg/mL) of each extract. Ethanol (50% v/v) was used as a negative control; it was the solvent used to dissolve the extracts. In addition, growth control containing only seawater was used. Afterward, the surviving was counted with a stereoscope (Motic SMZ-171, China). Percentage mortality was determined and median lethal doses (LD_{50}) of each extract were deduced with Probit Analysis Regression (IBM SPSS Statistics 22, USA) at 95% confidence intervals.

Experimental Design and Statistical Analysis

A completely randomized experimental design with a three-factor layout was used. The factors and their respective levels varied depending on the assay: extract concentration (2 to 5 levels, depending on the assay), incubation time (4 to 9 levels), and extraction method (2 levels: maceration and sonication). The data were subjected to one-way analysis of variance (ANOVA), and statistical analysis was performed using InfoStat software (version 2020, Argentina). Mean comparisons were carried out using Tukey's multiple comparison test at 95% confidence level to determine significant differences among means. All data are presented as mean ± standard deviation (SD).

RESULTS

Phenolic composition and antioxidant activity of extracts

Table 1 shows the total phenolic compounds and flavonoids contents of tomatillo calyx extracts obtained by maceration and sonication methods. As for the metabolite content, both analyses were significantly higher ($p < 0.05$) in sonicated extract (SE) in comparison with macerated extract (ME). Table 1 also shows the in vitro antioxidant activity of the extracts. For each of these assays, sonicated extract was significantly ($p < 0.05$) higher ($ABTS^{\bullet+}$: 86.64 ± 2.19, $DPPH^{\bullet}$: 49.90 ± 1.08, FRAP: 116.40 ± 2.63) compared to the extract obtained by the maceration technique ($ABTS^{\bullet+}$: 51.71 ± 2.10, $DPPH^{\bullet}$: 29.60 ± 1.45, FRAP: 61.85 ± 1.64). These results are based on the notable differences between the two extraction methods, demonstrating that sonication is a more efficient method, allowing higher yields in terms of extraction of bioactive metabolites of vegetable origin.

Table 1 Total phenols and flavonoids content of *P. ixocarpa* Brot ex. Hornem. calyx extracts obtained by different methods and in vitro antioxidant activity.

Method of obtaining	Total phenols (mg GAE/g of extract)	Total flavonoids (mg EQ/g of extract)	$DPPH^{\bullet}$ (mmol ET/g of extract)	$ABTS^{\bullet+}$ (mmol ET/g of extract)	FRAP (mmol ET/g of extract)
Maceration	23.37 ± 0.74^{a}	0.91 ± 0.27^{a}	51.71 ± 2.10^{a}	29.60 ± 1.45^{a}	61.85 ± 1.64^{a}
Sonication	48.21 ± 1.64^{b}	6.63 ± 0.34^{b}	86.64 ± 2.19^{b}	49.90 ± 1.08^{b}	116.40 ± 2.63^{b}

Values represent the mean of three replicates ± standard deviation. Means with a common letter are not significantly different ($p > 0.05$) in the same column.

Antimicrobial activity

Disk diffusion assay

Figure 1 shows the results of the evaluation of the antimicrobial activity of the macerated and sonicated extracts of tomatillo calyx by the disk diffusion method against *S. aureus, E. coli* and *C. albicans*. The results obtained indicate that the macerated and sonicated extracts were able to inhibit the growth of the Gram (+) strain *S. aureus*, no significant differences ($p < 0.05$) were observed between similar concentrations of both extracts, the inhibition halos obtained were: Macerated extract: M_1 (36.8 ± 3.3^{a} mm), M_2 (35.4 ± 2.8^{a} mm), M_3 (33.0 ± 2.7^{ab} mm) and, M_4 (23.3 ± 2.8^{c} mm) and Sonicated extract: S_1 (33.5 ± 2.6^{ab} mm), S_2 (36.0 ± 2.3^{a} mm), S_3 (30.3 ± 2.7^{b} mm) and, S_4 (22.8 ± 1.9^{c} mm). In the case of the Gram (-) bacteria *E. coli* and the yeast *C. albicans*, no inhibition halos were observed in the Petri dishes, which means that none of the extracts were able to inhibit the growth of these microorganisms.

Normalization of phenolic content (mg GAE/mL of extract) at the different concentrations used:

$$N_{phenols} = C_{extract} \times \frac{TPC}{1000}$$

Sonicated extract: S1 (35.6 mg/mL): 1.72; S2 (17.8 mg/mL): 0.86; S3 (8.9 mg/mL): 0.43; S4 (4.45 mg/mL): 0.21

Macerated extract: M1 (35.6 mg/mL): 0.83; M2 (17.8 mg/mL): 0.42; M3: (8.9 mg/mL): 0.21; M4 (4.45 mg/mL): 0.10

Radial growth

The radial growth of *A. niger* were 39.5 ± 0.87 and 44 ± 1.00 mm in mediums combined with the macerated and sonicated extracts respectively, at a concentration of 17.8 mg/mL at the conclusion of the kinetics at 108 hours (Figure 2.a). At this concentration, the effect caused by the macerated extract was significantly greater ($p < 0.05$) than that caused by the sonicated extract. The colonies that grew in the presence of the extracts showed variations in the morphology of the aerial mycelium compared to the controls, atypical cream-

colored colonies were observed with a higher compact and concentrated mycelium in the center of the plate.

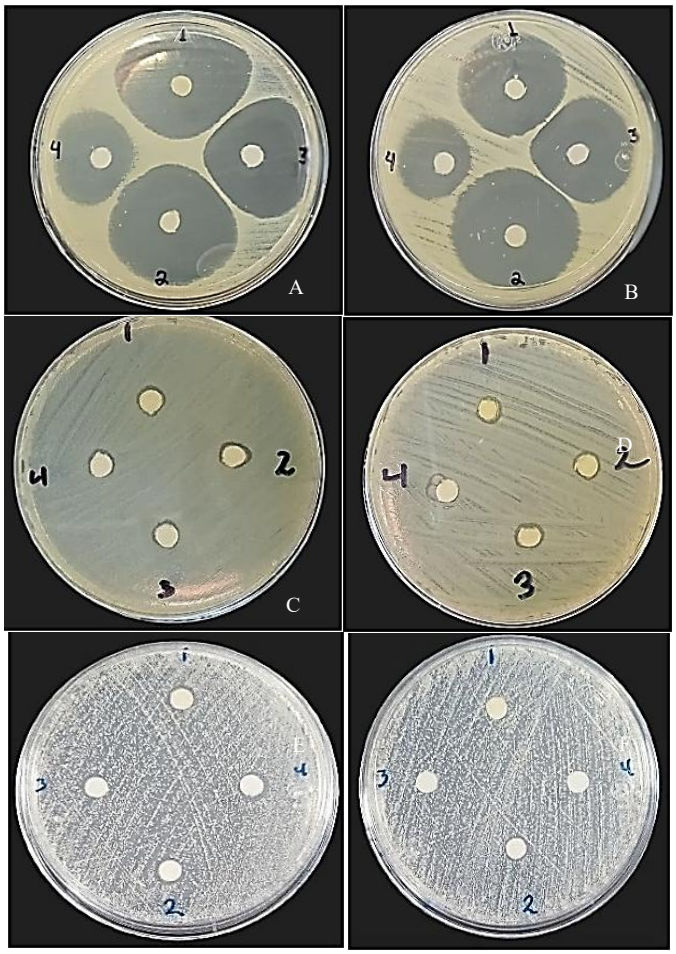

Figure 1 Disc diffusion assay against *S. aureus* (A: macerated) (B: sonicated), *E. coli* (C: macerated) (D: sonicated) and *C. albicans* (E: macerated) (F: sonicated) after 24 h of incubation. Four concentrations were evaluated: 1 (35.6 mg/mL), 2 (17.8 mg/mL), 3 (8.9 mg/mL) and 4 (4.45 mg/mL).

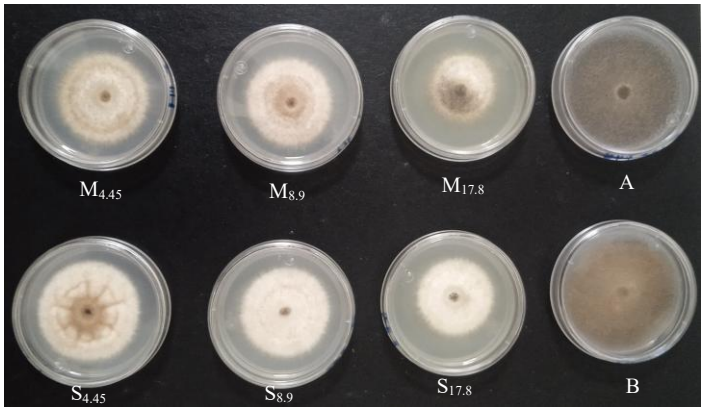

Figure 2.a Radial growth assay of *A. niger* in Petri dishes with Czapek agar. Control (A) and solvent (B), macerated extracts at different concentrations $M_{17.8}$ (17.8 mg/mL), $M_{8.9}$ (8.9 mg/mL), and $M_{4.45}$ (4.45 mg/mL) and sonicated extracts at different concentrations $S_{17.8}$ (17.8 mg/mL), $S_{8.9}$ (8.9 mg/mL) and $S_{4.45}$ (4.45 mg/mL) are shown at 108 h.

Figure 2.b shows that on Czapek agar combined with the highest concentration fractions (17.8 mg/mL) of each of the extracts, there was no macroscopic growth of *A. niger* until 48 h after inoculation, delaying growth by 12 hours compared to the rest of the treatments and controls. There was no total inhibition of the growth of *A. niger*, which assures the inexistence of a fungicidal effect. However, a significant decrease ($p < 0.05$) was observed between the diameters of the control colonies and those treated with the extracts during the entire growth kinetics, which demonstrates the fungistatic effect of the extracts.

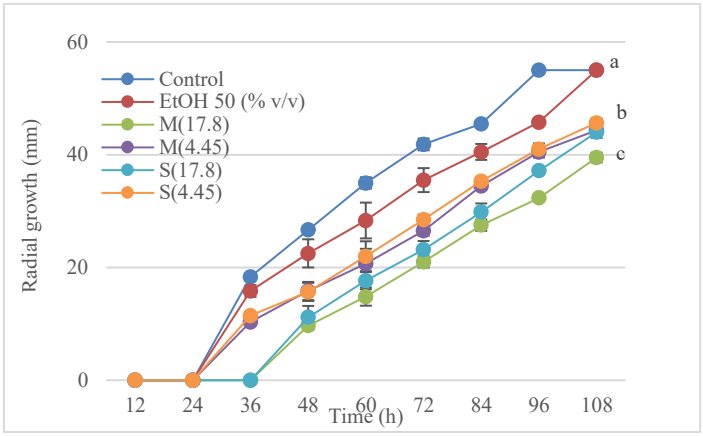

Figure 2.b Radial growth kinetics of *Aspergillus niger* colonies in Czapek medium mixed with tomatillo peel extracts at different concentrations. Macerated extract: M (17.8 mg/mL); M (4.45 mg/mL) and for the sonicated extract: S (17.8 mg/mL); S (4.45 mg/mL).

Spore germination

Figure 3 shows the percentage of spore germination (%) of *A. niger* in Czapek liquid medium, there were no significant differences ($p > 0.05$) between the germination percentages of the control plates and the solvent control during all the germination kinetics. After 10 hours, germination began to be observed in the spores exposed to the extracts, a significant difference ($p < 0.05$) was observed between the percentage of spore germination (%) corresponding to the plates containing solvents and those containing the extracts, however, no statistical differences were observed between treatments. At 15 hours there were significant ($p < 0.05$) differences between all treatments and controls, but it was M (35.6 mg/mL) that most effectively inhibited the germination process (6.25 ± 1.06), even compared to its sonicated (S) counterpart (18.25 ± 1.77). At 20 hours, germination values increased considerably, all treatments and controls showed rates higher than 85% germination, with the except for treatment M (35.6 mg/mL) which showed 78.75 ± 1.77% spore germination. This was the only treatment statistically different from the controls ($p < 0.05$), reducing the germination efficiency of *A. niger* spores during the first 20 h of incubation.

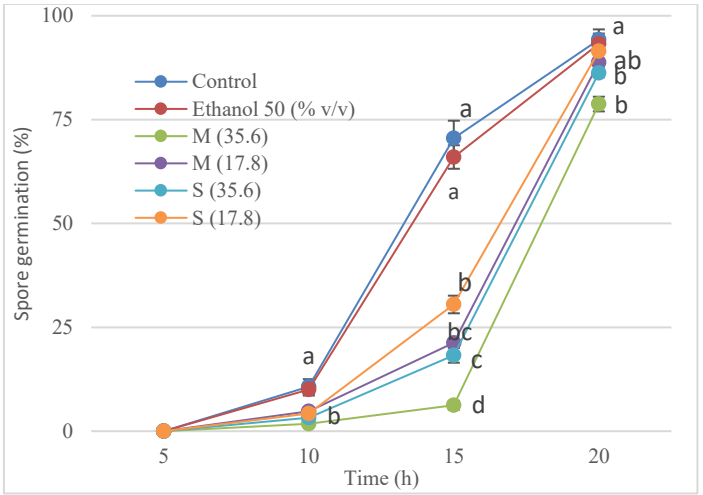

Figure 3 Effect of tomatillo calyx extracts obtained by different methods: maceration (M) and sonication (S) at different concentrations (35.6 mg/mL); (17.8 mg/mL) on the germination of *A. niger* spores at 30 °C in Czapek liquid medium, expressed as Spore germination (%).

Morphometric analysis

Table 2 presents the morphometric analysis of the spores and hyphae of *A. niger* after 10 hours of incubation in Czapek liquid medium. In terms of spore diameter, there were no significant differences ($p > 0.05$) between the Control and Solvent, suggesting that 50% ethanol (v/v) did not influence the development of the test. However, both were significantly different ($p < 0.05$) with respect to the two treatments. The spores exposed to the extracts showed a smaller diameter than the controls. The effect caused by each of the treatments was significantly equal ($p > 0.05$) on the diameter of *A. niger* spores.

Table 2 presents the morphometric analysis of *A. niger* spores and hyphae at 10 hours of incubation in Czapek liquid medium.

Morphometric parameters (10h)	Controls		Macerated extract (mg/mL)		Sonicated extract (mg/mL)	
	Growth	$Ethanol_{50}$ (v/v)	$M_{35.6}$	$M_{17.8}$	$S_{35.6}$	$S_{17.8}$
Hyphal length (µm)	428.73 ± 40.85[a]	365.29 ± 42.24[b]	28.77 ± 3.90[c]	50.19 ± 7.03[d]	73.19 ± 5.64[e]	95.57 ± 7.69[f]
Spore diameter (µm)	27.96 ± 3.66[a]	28.45 ± 4.03[a]	25.51 ± 2.71[b]	24.94 ± 3.45[b]	23.81 ± 2.98[b]	23.22 ± 2.81[b]

Values represent the mean of three replicates ± standard deviation. Means with a common letter are not significantly different ($p > 0.05$) for the same row. Macerated extract (M) at concentrations of 35.6 mg/mL and 17.8 mg/mL; Sonicated extract (S) at concentrations of 35.6 mg/mL and 17.8 mg/mL.

Regarding hyphal length, significant differences ($p < 0.05$) were obtained between each of the treatments observed. The longest hyphae were those germinated in the control medium, which were significantly longer than those present in the medium with 50% ethanol, which did have an effect in this test ($p < 0.05$). Among the treatments evaluated, the macerated extract inhibited hyphal growth to a greater extent, suggesting that it is more effective than its sonicated counterpart. In both treatments, a directly proportional relationship was observed between concentration and inhibition of apical growth, suggesting a dose-dependent effect. These results coincide with the radial growth test performed, where the macerated extract also showed better results.

Figure 4 shows the spores and hyphae *of A. niger* after 10 h of incubation, showing a higher degree of germination in the controls compared to the treatments. Moreover, showing a higher degree of germination in the controls compared to the treatments. Although the presence of the extracts slowed the germination process, no morphological changes were evident that would indicate damage to the fungal cell structures.

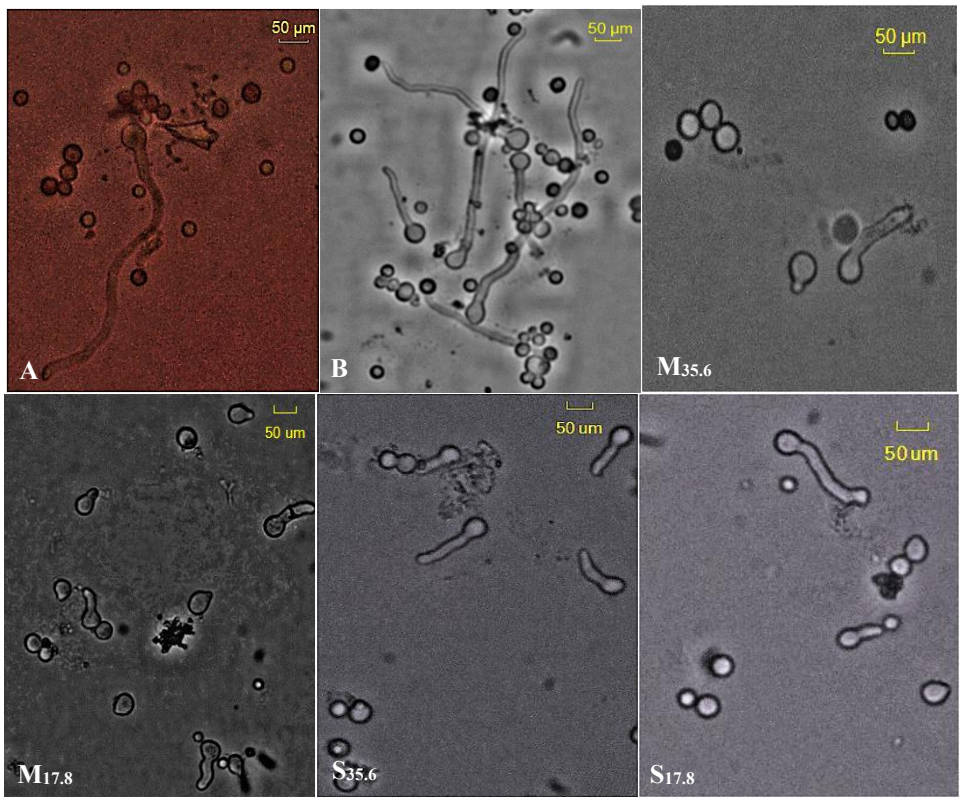

Figure 4 *Aspergillus niger* hyphae and spores observed after 10 h of inoculation in Czapek medium at 30 °C: (A) Distilled water growth control; (B) 50 (% v/v) ethanol solvent control; Macerated extract (M) at concentrations of 35.6 mg/mL and 17.8 mg/mL; Sonicated extract (S) at concentrations of 35.6 mg/mL and 17.8 mg/mL.

Acute toxicity of extracts to *Artemia salina* nauplii

Figure 5 shows the survival of *A. salina* nauplii after 24 hours in seawater combined with various concentrations of the tomatillo calyx extracts. The controls maintained 100% survival after this period. For treatments at a concentration of 10 µg/mL, 90 and 80.33% of the brine shrimp survived, for EM and ES respectively. By increasing the concentration to 25 µg/mL, for both cases the number of surviving nauplii decreased to about 50%. Similarly, after a further increase (50 µg/mL) the survival percentages dropped below 15 % in both cases, being slightly higher for the case of the macerated extract. For concentrations above 100 µg/mL, no survivors were observed for ES and only 3% of the nauplii for this concentration in the case of EM. For the two extracts, an inversely proportional effect on the survival of nauplii was observed in correspondence with increasing concentration, which is evidence that toxicity is associated with the present bioactive compounds. There were no significant differences between seawater control and the 50% ethanol control, however both controls were statistically ($p < 0.05$) significant compared to the treatments.

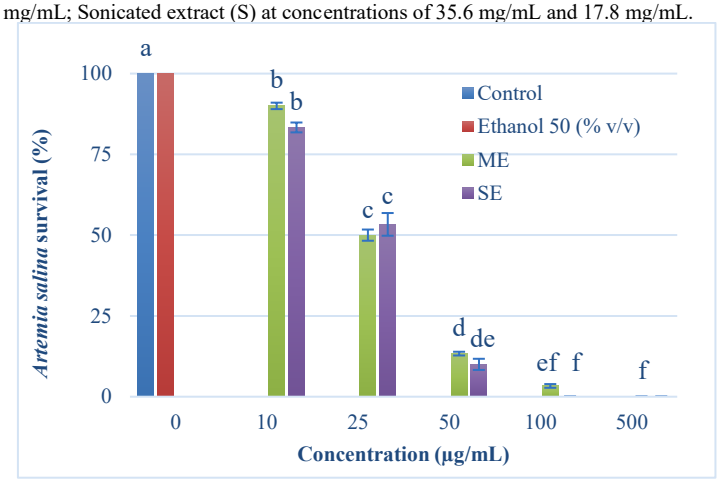

Figure 5 Percent survival of *A. salina* after 24 hours of exposure at several concentrations to tomatillo calyx extracts obtaining by maceration (ME) and sonication (SE).

From these data, the values of medium lethal dose (DL_{50}) were determined by Probit regression. These data represent the concentration of each extract that would cause the death of 50% of the nauplii contained in each sample. Statistical processing of the data yielded DL_{50} values of 17.81 µg/mL for sonicated extract and 30.36 µg/mL for macerated extract.

DISCUSSION

Phenolic and Flavonoid composition

Polyphenols or phenolic compounds (PCs) are the secondary metabolites most frequently found in plants cells. PCs are stress metabolites synthesized and accumulated in all plant parts to protect themselves against abiotic and biotic factors. Due to their natural proprieties as antioxidant, antimicrobial, hypoglycemic and anti-inflammatory agents, some authors consider them the most important bioactive phytochemicals **(Rathee *et al.*, 2023; Zagoskina *et al.*, 2023)**. Maceration and sonication techniques were used to extract the phenolic compounds present in the tomatillo calyx.

Maceration is a simple, economical, and widely used technique for extracting bioactive metabolites from plants. The technique is based on prolonged contact between the solvent and the plant matrix. However, it sometimes offers low yields due to the absence of external energy input, which results in an inability to break down cell walls and extract encapsulated compounds. On the other hand, ultrasound-assisted extraction uses high-frequency sound waves that create cavitation bubbles inside the solvent. The collapse of these bubbles causes high localized pressures and temperatures, which fracture the cell walls of plants and facilitate the release of bioactive compounds. This technique is advantageous for the food and pharmaceutical industries, where the preservation of bioactivity is essential. In addition, it increases yield values, shortens exposure times, and minimizes energy and solvent consumption **(Ma *et al.*, 2009; Kaur *et al.*, 2025)**. The results of present study correspond to those observed by several authors, who reported that the use of sonication allows excellent yields in terms of extraction of phenolic compounds and flavonoids present in plant matrices, being a more effective technique compared to several conventional methods **(Dzah *et al.*, 2020)**. On the other hand, **Ma *et al.*, 2009,** reported an increase in the phenolic compounds extracted from the peel of Satsuma mandarin (*Citrus unshiu* Marc) using sonication (caffeic: 97.5 ± 0.7, p-coumaric: 177.3 ± 1.4, ferulic: 2226.8 ± 27.1, sinapic: 219.8 ± 8.5) compared to maceration (caffeic: 31.7 ± 1.5, p-coumaric: 63.2 ± 1.9, ferulic: 763.5 ± 19.8, sinapic: 132.39 ± 0.3), all results are expressed in micrograms per gram of dry weight µg/g of DW. Kaur *et al.*, 2025, observed that using different extraction techniques altered the concentration of polyphenols extracted from mandarin (*Citrus reticulata*) pomace, reporting sonication (3.58 mg of GAE/g of pomace), maceration (TPC: 1.94 mg of GAE/g of pomace) and Soxhlet extraction (TPC: 2.39 mg of GAE/g of pomace).

Antioxidant activity of extracts

The highest in vitro antioxidant capacity in each of the tests performed was shown by the sonicated extract. This may be attributed to the higher concentration of phenolic compounds detected in the sonicated extract. Phenolic compounds can exert their antioxidant effect through hydrogen atom transfer (HAT) and single electron transfer (SET), their reducing power and ability to stabilize radicals will depend on their degree of hydroxylation, conjugation and the position of hydroxyl groups present in their structure **(Prior *et al.*, 2005; Shahidi and Ambigaipalan, 2015)**. The results of our study are in correspondence to those stated by several authors **(El-Otmani *et al.*, 2024; Righi *et al.*, 2025)** who state that plant extracts obtained by sonication method showed better in vitro antioxidant activity (DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and FRAP) compared to extracts obtained by maceration and infusion methods.

The high antioxidant activity of the extracts in the FRAP assay suggests that the most likely mechanism by which they exert their antioxidant activity is through electron sequestering (SET) **(González-Vega *et al.*, 2021)**. DPPH was the radical with the lowest antioxidant capacity; steric hindrance and high selectivity represent disadvantages when performing this assay, which can favor smaller antioxidant molecules and react slowly or be inert to antioxidants of larger molecular size **(Prior *et al.*, 2005)**. In the case of the ABTS$^{\bullet+}$ assay, a higher antioxidant activity was observed for both extracts in comparison with DPPH$^{\bullet}$. This may be due to the lower selectivity of ABTS$^{\bullet+}$ towards antioxidant agents and to its high reactivity with phenolic compounds (HAT) **(Ilyasov *et al.*, 2020)**, this reaction develops almost instantaneously, independently of pH and solvent used. Although it has been reported that both radicals (ABTS$^{\bullet+}$ and DPPH$^{\bullet}$) are mostly classified as SET, they can also be stabilized by HAT, which greatly complicates the elucidation of possible antiradical mechanisms and reaction patterns when the composition and structures of the evaluated antioxidants are not exactly known **(Prior *et al.*, 2005)**. The in vitro antioxidant activity of these extracts suggests the possibility of using them in foods to prevent degradation processes such as lipid peroxidation, a process that leads to the rancidity of lipid components and the loss of sensory and nutritional quality. According to a report by **(Martínez-Ávila *et al.*, 2011)**, the presence of peroxides in lipid samples should be minimal due to the presence of bioactive compounds with antioxidant activity, such as polyphenols, which can prevent auto-oxidation processes by stabilizing free radicals through mechanisms involving the transfer of electrons or hydrogen atoms. However, it is important to note that food matrices have their own pH, water activity, and may contain antimicrobial constituents and barriers that protect them against degradation processes (shell, skin, etc.). In addition, food matrices are complex systems composed of various macro (water, proteins, lipids, carbohydrates) and micro (vitamins, minerals) elements, which react with each other and with the external environment (oxygen, temperature, light), making this extrapolation even more complex **(Mafe *et al.*, 2024)**. Although these results are promising, it would be necessary to conduct shelf life or accelerated studies to ensure the effectiveness of the lipid peroxidation inhibition capacity of extracts obtained from this plant material after its application. In addition, it would be advisable to conduct further toxicity tests to ensure the safety of the extracts, since tests with *A. salina* indicated potential toxicity, which poses a problem given the intention to use the extracts as potential natural antioxidants.

Disk diffusion assay

The inhibition halos observed in the plates inoculated with the Gram (+) bacterium *S. aureus* can be attributed to the effect of bioactive compounds such as phenols and flavonoids present in the calyx of the tomatillo. It was reported that extracts obtained from tomatillo calyx inhibited most of the bacteria evaluated in their study, including *S. aureus*, *X. orayzae* and *B. subtillis* **(Khan *et al.*, 2016)**.

One study reported that the presence of quinic acid, chlorogenic acid and protocatechuic acid in extracts obtained from tomatillo calyx, which could be responsible for the antimicrobial effect **(Guerrero-Romero *et al.*, 2021)**. Quinic acid can alter the lipid conformation of the membrane of S. aureus, causing damage that affects its integrity and the release of cellular contents **(Bai *et al.*, 2017)**. Other authors report that the action of chlorogenic acid by binding to proteins involved in cell wall synthesis (amidase protein and penicillin transporter protein 4) affects the degree of cross-linking between the amino acid network that connects the peptidoglycan layers and reduces the rigidity of the cell wall **(Yu *et al.*, 2024)**. Protocatechuic acids are recognized as a potent antimicrobial agent utilizing as a mechanism of action the generation of ROS to exert prooxidant activity effects. This activity impacts various cellular macromolecules such as lipids, proteins and genetic material, causing DNA fragmentation and peroxidation of lipid components **(Ajiboye *et al.*, 2017)**.

The extracts did not inhibit the growth of the bacteria *E. coli* or the yeast *C. albicans*; in both cases it is known that there are morphological and structural differences with respect to their cell walls and that of *S. aureus*. According to **Nascimento *et al.*, (2025)**, Gram (+) bacteria shows a greater susceptibility to plant metabolites compared to Gram (-) bacteria, because the latter group present an external lipid bilayer consisting of its cytoplasmic membrane and an outer membrane of phospholipids that prevent the penetration of foreign substances, while Gram (+) bacteria only have a thick layer of peptidoglycan. The extracts showed no effectiveness against the fungal strain *C. albicans*. According to the research conducted by **Hasim and Coleman (2019)**, they explain that the cell wall of this microorganism is composed of polysaccharides such as glucans and chitins, along with glycoproteins. This composition confers protection against environmental and osmotic stress, thus preventing cell lysis and providing passive defense against the possible entry of harmful macromolecules, as in the case of the bioactive compounds present in the extract.

Radial growth

The morphological changes observed in the colonies that grew in the presence of the extracts and their smaller diameter coincide with that reported by **Kamilla *et al.*, (2009)** and **Sumathy *et al.*, (2013)**, who found alterations in morphogenesis, cell wall synthesis and mycelial growth of *A. niger* due to the activity of bioactive compounds present in extracts of *Clitoria ternatea* leaves and *Cassia surattensis* Burm. flowers, respectively. It has been reported that the presence of phenols and flavonoids is associated with antifungal activity **(Rathee *et al.*, 2023; Sun and Shahrajabian, 2023)**. The cellular targets and potential mechanisms of action through which these metabolites exert their effects could depend on their functionality, molecular size, and the presence and location of functional groups, such as hydroxyl groups. According to the literature, it is possible that the extracts could interact with the cell membrane of *A. niger*, potentially altering lipid dynamics or inducing the production of reactive oxygen species (ROS). Both factors have been described as potential causes of increased membrane permeability, leading to the leakage of cellular contents. Another mechanism is the action of reactive oxygen species (ROS) on nucleic acids, inducing the oxidation of fatty acids and causing mitochondrial dysfunction by reducing intracellular levels of adenosine triphosphate (ATP). It has also been reported that polyphenols can inhibit the synthesis of ergosterol or components that form the cell wall, making the microorganism more vulnerable **(Silva-Beltrán *et al.*, 2023)**.

Although the macerated extract exhibited lower polyphenol content and antioxidant activity, it stood out for its greater antifungal potency against *A. niger*. Direct correlations are generally reported between phenolic content, antioxidant activity, and antimicrobial activity. However, in the specific case of this study, the opposite behavior is observed; this can be explained by the fact that antimicrobial

or antifungal activity does not depend exclusively on the concentration of bioactive metabolites, but rather on the chemical nature and mechanism of action of the metabolites present. In the study by **(Ispiryan *et al.*, 2024)**, moderate positive correlation coefficients (r) were reported between phenolic content and the antimicrobial activity of various extracts from different parts of the raspberry plant (*Rubus idaeus* L.) against the bacteria *S. aureus* (0.487) and *B. subtilis* (0.475); a low correlation for *E. faecalis* (0.190), *L. monocytogenes* (0.222), *S. typhimurium* (0.303), and *P. aeruginosa* (0.278); and no correlation between *E. aerogenes* and *E. coli*. Furthermore, these authors concluded that neither antioxidant activity nor total polyphenol content showed the strongest correlation with antibacterial activity.

Spore germination

Spores are structures of vital importance for the survival and propagation of fungi. Utilizing agents capable of inhibiting their germination or causing the destruction of these structures, makes them potential candidates for controlling populations of filamentous fungi and their propagation in different substrates, the germination of these structures is considered as an indication of fungal adaptation to the presence of adverse factors **(Cota-Arriola *et al.*, 2013)**.

In agreement with the results shown in Figure 3., no significant differences were observed between the germination percentages of the growth and solvent (EtOH 50 % v/v) controls, indicating that 50% (v/v) ethanol did not affect the germination process. During the first 5 hours, no spore germination was observed, probably due to the longer physiological cycle. The low spore germination values observed in the treated media at 10 h are like the results reported by **(Rosas-Burgos *et al.*, 2011)**, who observed that the presence of the methanolic extract of *Baccharis glutinosa* caused 100% inhibition of spore germination of two members of the *Aspergillus* family, *A. flavus* and *A. parasiticus* at 9 h of incubation.

The results observed at 15 h coincide with the trend observed during the kinetic of the radial growth assay and confirm its validity, since both assays the macerated extracts were more effective in inhibiting the development of the physiological cycle of the filamentous fungus *A. niger*. At 20 h, there is a certain similarity to the findings of **Rosas-Burgos *et al.* (2011)**, who reported that at 24 h, the germination of the spores of *A. flavus* and *A. parasiticus* went from 0% previously reported (9 h) to 100%. This demonstrated that *Aspergillus* species can adapt to the presence of plant extracts and continue their physiological cycle. Additionally, it was observed that among the treatments evaluated, at the end of the kinetics only the macerated extract showed statistically different results from the controls and other treatments, managing to reduce the germination of *A. niger* spores during the first 20 h of incubation.

Morphometric analysis

The smaller diameter observed in the spores subjected to the treatments with respect to the controls (Table 2), indicates that the extracts constituted a factor that affected the germination development of the fungus and therefore delayed its reproductive cycle; this effect suggests a fungistatic effect, since under physiological conditions, most fungal spores swell due to water intake and the synthesis of new cell wall material, a process prior to the emergence of the germ tube **(Alonso *et al.*, 2023)**.

Figure 4 shows photographs of apical growth and spores contained in each of the media combined with the treatments and in the controls. Although the presence of the extracts caused a decrease in hyphal length, no increase in the number of branches, aggregation or deformation of spores, or sudden changes in the microscopic morphology of the fungus was observed. There were also no signs of damage to the cytoplasmic membrane or malformations in the cell wall. This could be an indicator that the extracts did not represent factors that morphologically affected *A. niger* during the first 10 hours of its germination process. Although the bioactive compounds in the extract managed to slow the growth of the fungus, they could not generate a fungicidal effect.

Acute toxicity of extracts to *Artemia salina* nauplii

The observed differences in survival rates between the controls and treatments suggest that the toxicity was caused by the presence of bioactive compounds that were extracted from the tomatillo calyx using sonication and maceration techniques **(Ma *et al.*, 2009; Dzah *et al.*, 2020)**. Additionally, it was verified that there were no significant differences in toxicity between the same concentrations of extracts, leading to the conclusion that the extraction method did not influence the toxicity of the extracts.

According to toxicity classification system for plant extracts based on the medium lethal dose (LD_{50}) reported by **Karchesy *et al.*, (2016)**, both extracts are categorized as strongly toxic. Furthermore, in this study the toxicity of 211 methanolic extracts from 128 plant species from the Pacific Northwest was examined using the *Artemia salina* method. In line with the high toxicity of tomatillo calyx extracts, these authors reported that 17 extracts obtained from 13 species were classified as highly toxic, representing approximately 8.0% of the total extracts evaluated. The highest toxicity was reported in the root extract of *Angelica arguta* (LD_{50} <10 µg/ml), as well as in the heartwood extract of *Cedrus deodara* (LD_{50} =15 µg/ml) and the aerial parts extract of *Leucanthemum vulgare* (known as oxeye daisy) aerial parts extract (LD_{50} =15 µg/ml); in addition, foliage extract (LD_{50} =42 µg/ml) and inner bark extract (LD_{50} =15 µg/ml) from *Callitropsis nootkatensis* (LD_{50} =15 µg/ml), values close to those reported in the present study. These results demonstrate that obtaining strongly toxic extracts is not an isolated or uncommon occurrence. Strong toxic extracts are a promising source of bioactive compounds, being an indicator of possible antitumor, insecticidal, fungicidal activity and combat pathogenic microorganisms affecting animals and plants. One strategy that could help mitigate the toxicity issues associated with these extracts is nanoencapsulation. According to **Makimori *et al.*, 2026**, this technique has been used to improve the stability of essential oils, prolong their antimicrobial activity, enhance their dispersibility in aqueous media, and reduce their toxicity. This could be an alternative applicable to the field of natural extracts, allowing for the controlled release of extracts and reducing their toxic effect on *A. salina* nauplii; it would also permit the incorporation of the extracts into polymeric matrices used for food packaging and preservation.

To date, no studies have been found that examine the toxicity of tomatillo calyx extracts for *A. salina*, making it difficult to compare our results. However, in the study of **Olivares-Bañuelos *et al.*, (2019)**, it is mentioned that high toxicity confirms the bioactive potential and cytotoxicity of plant extracts rich in bioactive phytocompounds; these are responsible for the antimicrobial activity and antioxidant activity of the extracts studied as part of this research. Previous studies consider that this toxicity assessment study has the limitation of not providing information on the possible mechanism that generates toxicity. Nevertheless, it is classified as a tool that shows good correlation with other mammalian models, and its results can be extrapolated to acute toxicity tests with mice **(Olivares-Bañuelos *et al.*, 2016; Ntungwe *et al.*, 2020)**. However, the results of studies in crustaceans do not always correlate with those in mice. According to reports (**Martins *et al.*, 2014**), essential oils from the leaves and fruits of *Schinus molle* L. showed LD_{50} values of 47.4±3.3 and 66.5±8.2 µg/mL in *A. salina* toxicity assay, respectively, indicating high toxicity; these values are slightly higher than those reported for tomatillo calyx extracts in the present study (Sonicated: 17.81 µg/mL), (Macerated: 30.36 µg/mL). However, in murine models using Windstar rats, these authors observed no clinical signs of toxicity, weight loss, or apparent damage to the animals' organs. The low toxicity observed is due to the mice's ability to metabolize and eliminate drugs, as is the case with essential oils. These findings highlight the importance of conducting more in-depth studies on the toxicity of tomatillo calyx extracts, since the brine shrimp assay is only a preliminary test; therefore, it would be advisable to evaluate these extracts in more complex organisms before reaching a verdict on their safety.

CONCLUSIONS

The tomatillo calyx extracts show promising bioactive potential indicated by their effective *in vitro* antioxidant activity and antimicrobial capacity against the Gram-positive bacterium *S. aureus* and the filamentous fungus *A. niger* associated with foodborne diseases and food spoilage, respectively. However, future studies are needed to evaluate their activity against a broader range of microorganisms. Despite the bioactivity, the high toxicity observed represented an important limitation, further research is required to confirm their safety, particularly in food/related applications. Additionally, the extracts may represent a viable alternative to traditionally synthetic additives and antioxidants, due to the presence of important phytochemicals such as polyphenols, which are partially responsible for their bioactivity. Future research on this plant material should focus on evaluating its toxicity using other techniques and on its application in food matrices that simulate real-world storage conditions. Although the results are encouraging, its bioactivity must be tested in real, complex matrices such as foods, which contain components (lipids, carbohydrates, proteins, water) that will interact directly with the phytochemicals present in the tomatillo calyx and could attenuate their bioactivity, in addition to serving as a substrate for various microorganisms. Additionally, collaborations with environmental scientists could further explore the ecological benefits of repurposing agricultural waste, thereby enhancing the inter-disciplinary impact of this study.

Conflict of interest: All authors declare that no conflicts of interest were identified during the conduct of the research and preparation of this article.

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Abbreviations:

ABTS: 2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)
ANOVA: analysis of variance
ATCC: American type culture collection
ATP: adenosine triphosphate
BHA: butylated hydroxyanisole
BHT: butylated hydroxytoluene

CFU Colony-forming unit
LD_{50}: medium lethal dose
DPPH: 2,2-Diphenyl-1-picrylhydrazyl
FAO: Food and Agriculture Organization
FRAP: ferric reducing antioxidant power
GAE: gallic acid equivalent
HAT: hydrogen atom transfer
ME: macerated extract
QE: Quercetin equivalent
ROS: Reactive Oxygen Species
SE: sonicated extract
SET: single electron transfer
TBHQ: tert –butylhydroquinone
TE: Trolox equivalen

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