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Plant extracts for food safety applications

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Abstract

The aim of this study was to investigate the chemical composition, antimicrobial, and antioxidant activities of *Curcuma longa* and *Bixa orellana* ethanolic extracts in the search for alternatives to synthetic additives used in the food industry. Mass spectrometry (GC-MS) identified bisdemethoxycurcumin, desmethoxycurcumin, and curcumin in *C. longa* extract and prunin and naringenin in *B. orellana* extract. *C. longa* extract showed antimicrobial activity against *Clostridium sporogenes* and *Staphylococcus aureus*. None of the extracts showed bactericidal activity against *Escherichia coli* and *Salmonella Typhimurium*. The antioxidant activity of the extracts was evidenced by ferric reducing antioxidant power and oxygen radical absorbance capacity assays. *B. orellana* extract had higher antioxidant activity by ferric reducing antioxidant power and oxygen radical absorbance capacity than *C. longa* extract. The biological effects of *C. longa* and *B. orellana* ethanolic extracts showed their potential use as an alternative to synthetic additives for the food industry.

Keywords: antioxidant activity; antibacterial activity; flavanones; food additives; anaerobic bacteria; aerobic bacteria.

Practical Application: Tumeric and annatto extracts can avoid lipid oxidation and inhibit the growth of bacteria in food.

1 INTRODUCTION

Despite significant advances in food science and technology and consumer knowledge regarding food manipulation, food-borne diseases (FBD) continue to grow, bringing harmful consequences to people's health and to the economy of developed and developing countries (Byrd-Bredbenner et al., 2015). The World Health Organization (WHO) estimates that food unsafety is linked to deaths of about 420 thousand people annually (WHO, 2024). From 2014 to 2023, Brazil recorded 6,874 FBD outbreaks, resulting in approximately 110,614 illnesses, 12,346 hospitalizations, and 121 confirmed deaths (Brasil, 2024). In the United States, around 1.4 million cases are reported per year, resulting in 700 deaths (Centers for Disease Control and Prevention, 2025).

Some microorganisms are directly related to FBD, such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, and *Clostridium spp.* (Centers for Disease Control and Prevention, n.d.). These bacteria can cause serious damage to human health, and the inspection service requires their control in foods (Viana, et al., 2025). In the United States, from 2017 to 2020, most of the etiological agents of FBD were *E. coli*, *Salmonella spp.*, and *Listeria spp*., and variants of these bacteria have developed resistance to important antimicrobial agents (Centers for Disease Control and Prevention, n.d.).

Consumer knowledge on the risk of synthetic food additives is steadily increasing (Tarvainen et al., 2015), resulting in the demand for the food industry to look for natural additives that can replace them. The WHO has already reported the risk of excessive consumption of nitrite-added processed meat, warning of the carcinogenic potential of this additive (Bouvard et al., 2015; WHO, 2015) and the need to replace synthetic preservatives in food manufacturing. Nevertheless, nitrite has a notorious antimicrobial activity, mainly against *C. botulinum* (Majou & Christieans, 2018), and substitutes for this additive might present a similar effect.

The progress of phytochemical and phytopharmacological sciences allows the discovery of the composition of plants with antimicrobial and antioxidant properties, which may find industrial applications (Albuquerque et al., 2007). Phytotherapy has been used for the treatment of diseases and as a dietary supplement due to the presence of phytonutrients such as vitamins, minerals, and bioactive compounds (Sharma et al., 2017; Tandon & Yadav, 2017).

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Natural products such as turmeric and annatto can improve the oxidative stability of food products and increase their shelf life, as they have antioxidant characteristics (Redondo-cuevas et al., 2017). Lipid oxidation is a major cause of chemical food spoilage because it causes protein, DNA, and lipid damage, resulting in loss of nutritional quality, changes in sensory properties (color, taste, and rancid odor), and occasionally leading to the production of toxic compounds (Gioti et al., 2007). The present in vitro study evaluated the antimicrobial and antioxidant activities of *C. longa* rhizome (turmeric) and *B. orellana* (annatto) fruit extracts, searching for natural alternatives to replace synthetic additives in the food industry.

1.1 Relevance of the work

This manuscript evaluates plant extracts from *Curcuma longa* and *Bixa orellana* against foodborne bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, and *Clostridium spp.*, which can harm human health. Antioxidants and antimicrobials are used in the food industry to prevent oxidation, preserve products, and extend shelf life. However, synthetic additives may cause toxicity and are linked to autoimmune diseases, creating the need for natural alternatives. Extracts from *C. longa* and *B. orellana* show antimicrobial and antioxidant properties. In this study, both inhibited *Clostridium sporogenes*, while *B. orellana* exhibited high antioxidant activity, associated with its phenolic compound content.

2 MATERIAL AND METHODS

2.1 Plants

The rhizome of *C. longa* (turmeric) and the fruit of *B. orellana* (annatto) were collected, dried in a circulation incubator for 72 h at 40 ± 1°C and crushed. The leaves, branches, fruits, and flowers were botanically authenticated at the Botany Department, Institute of Biosciences at Botucatu/SP (UNESP).

2.2 Production and chemical characterization of extracts

Initially, 1% dehydrated plant and 99% ethanol at 99% v/v were mixed and homogenized for 48 h at room temperature (27 ± 3°C). After this stage, filtration was performed, and the content was concentrated in a rotary evaporator at 50°C to obtain the extracts.

The chemical analyses of the extracts were performed in an Acquity UPLC system (Waters Corporation, MA, USA), coupled to a Quadrupole/TOF system (Waters Corporation, MA, USA) containing an Acquity UPLC BEH column (Waters Corporation, MA, USA) with the temperature set at 40°C. The mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), with a gradient ranging from 0–15 min (2–95%) of B; 15.1–17 min (100%) of B; 17.1–19.1 min (2%) of B, a flow of 0.4 mL/min, and a sample injection volume of 5 µL. The mass spectrometer was operated in negative and positive ionization (ESI) modes. The instrument was controlled by Masslynx 4.1 software (Waters Corporation, MA, USA).

2.3 Determination of antimicrobial activity for aerobic microorganisms

Reference strains of methicillin-resistant *Staph. aureus* (MRSA) (ATCC 33591), *Salmonella Typhimurium* (ATCC 14028), and *E. coli* (ATCC 35218) were reactivated by surface plating on tryptone soy agar (TSA) and cultivated in brain heart infusion broth (BHIb). To obtain the bacterial suspensions, saline solution was used, and the inoculum was standardized at 10^8 CFU/mL, by checking the optical density at 600 nm.

The minimum bactericidal concentration (MBC) of the extracts, i.e., the lowest concentration of extract capable of killing the inoculum, was determined by the serial microplate dilution technique, according to Clinical and Laboratory Standards Institute (CLSI, 2014). The extracts were diluted in ethanol at 2.5% (stock solution). In 96-well microtiter plates, 200 µL of Mueller–Hinton (MH) broth was distributed and then 200 µL of the stock solution of the extracts were added to the first well and, after homogenization, were transferred to the second, and so on, totaling eight dilutions.

The bacterial suspensions (100 µL) were prepared in MH broth (9.9 mL) and 10 µL of them were inoculated into microplate wells containing the extract dilutions. The microplates were incubated at 37 ± 1°C for 24 h, under aerobic conditions.

Positive controls were prepared with 200 µL of MH broth and 10 µL of each inoculated saline solution, negative controls were prepared with MH medium only, and the extractor control was prepared with 200 µL of MH medium, 200 µL of the extractor (absolute ethyl alcohol), and 10 µL of each inoculated saline solution.

Aliquots from the microplate wells were transferred to the plate surfaces containing MH agar using a replicator and incubated for 24 h at 37 ± 1°C. Assays were performed in triplicate and repeated three times.

2.4 Determination of antimicrobial activity for anaerobic microorganisms

Unlike *Clostridium botulinum*, *Clostridium sporogenes* is not pathogenic and is commonly related to food deterioration (Brown et al., 2012). Due to its genetic and physiological similarity to the species of *Cl. botulinum* Group I (proteolytic), *Cl. sporogenes* is often used as a substitute for that microorganism in laboratory tests (Ocio et al., 1994), as in the present study.

The lyophilized culture of *Cl. sporogenes* (ATCC 3584) was reactivated in differential reinforced Clostridium broth base (DRCBB) supplemented with 0.5% sodium sulfate solution (4%) and ferric citrate (7%), with incubation in anaerobic conditions at 37 ± 1°C for 48 h. Subsequently, the culture was centrifuged at 860 g for 5 minutes, the supernatant was discarded, and the freezing medium (30 mL glycerol; 0.5 g bacteriological peptone; 0.3 g yeast extract; 0.5 g NaCl; distilled water 100 mL) was added to the pellet, constituting the stock cultures, which were kept frozen during the experiment.

For reactivation of the stock cultures, DRCBB medium supplemented with 0.5% sodium sulfate and ferric citrate solution

was used, with incubation at 37 ± 1°C for 48 h. Standardization of the inoculum at 10^5 CFU/g was performed by determining the optical density at 600 nm in DRCBB medium, followed by plate count using Clostridium isolation agar base (40 g of casein; 5 g of yeast extract; 2 g dextrose; 0.02 g bromocresol purple; 5 g sodium phosphate 2.2 g sodium chloride; 0.01 g magnesium sulfate; 20 g agar). The plates were incubated at 37 ± 1°C for 48 h in anaerobic conditions.

The MBC determination was performed using the broth dilution method according to CLSI (2014) with modifications. The extracts were diluted in reinforced Clostridium broth base (RCBB) plus 0.5% (v/v) of Tween 80®, and evaluated at concentrations of 2.5; 5.0; 7.5; and 10.0% in triplicate, with three repetitions. Aliquots of 5 µL of standardized culture were transferred to screw tubes containing 5 mL of broth, plus extracts.

Positive control tubes were prepared with RCBB added with 0.5% (v/v) of Tween 80®, and the negative ones, with the culture medium only. After homogenization, the tubes were incubated at 37 ± 1°C for 24 h under anaerobic conditions, accomplished by the addition of sterile mineral oil. Aliquots of 1 mL of the medium were transferred to Petri dishes and added with Clostridium agar base, employing the overlay, and incubated at 37 ± 1°C for 48 h in anaerobiosis. MBC of the extracts was those where plate growth was not observed.

2.5 Determination of antioxidant activity

2.5.1 Oxygen radical absorption capacity

The determination of the total antioxidant activity by oxygen radical absorption capacity (ORAC) was performed using the AOAC hydrophilic method (Ou et al., 2013). In this assay, 25 µL of the sample was mixed with 150 µL of a fluorescein solution diluted in phosphate buffer (pH 7.4) in black microplates and incubated at a constant temperature of 37°C for 15 min. For that analysis, the reagents were prepared in 75 mM phosphate buffer at pH 7.4. Subsequently, 25 µL of 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH) solution was added, which starts the reaction. Fluorescence intensity (485 nm excitation/520 nm emission) was checked every 10 min for 80 min. Calculation of loss of fluorescence, or area under the curve (AUC), was performed using the following formula: AUC = 1 + f0 /f1 + f0/f2 + ⋯ + f0/fn, where f0 represents the fluorescence obtained at time 0 and fi represents the fluorescence obtained between 0 and 80 minutes. The area of fluorescence loss of a sample was calculated by subtracting the area corresponding to the control. Fluorescence determination was performed using a fluorescence spectrophotometer. Assays (three replications) were performed in triplicate and values were expressed as mM TE (Trolox equivalent)/g of sample, on a dry basis.

2.5.2 Ferric reducing antioxidant power

The ferric reducing capacity of the extracts was determined by the ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996) with modifications. The FRAP reagent was prepared in the dark with 300 mM/L acetate buffer (pH 3.6), 10 mM of 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) in a solution of 40 mM/L HCl and 20 mM/L $FeCl_3$. Samples and standard solutions were mixed with deionized water and FRAP reagent, and placed in a water bath for 30 min at 37°C. After cooling at room temperature, the absorbances of the samples and the standard solution were read at 595 nm. A Trolox standard curve was prepared using concentrations from 10 to 800 µM TE/L. Results were expressed as mM TE/g.

2.6 Data analysis

The results of the antioxidant activity analyses were statistically analyzed by ANOVA and Tukey's test at 5% probability, using the open statistics software Jamovi (https://www.jamovi.org/).

3 RESULTS

3.1 Chemical composition of Curcuma longa and Bixa orellana extracts

The qualitative analysis of the chemical composition of the extracts of *C. longa* and *B. orellana* revealed the presence of several bioactive compounds. In the *C. longa* extract, bisdemethoxycurcumin, demethoxycurcumin, and curcumin were the dominant components, in addition to amino acids (Figure 1). In the *B. orellana* extract, the main compounds identified were prunin and naringenin (Figure 2).

Based on the literature (Farag et al., 2014), peaks 1 and 2 in the negative chromatogram (Figure 1A) were identified as sucrose, confirmed in the positive ionization mode (Figure 1B), by characteristic fragmentations. Peak 3 in the negative ionization mode (Figure 1A) identified the amino acid phenylalanine, and peak 4 in the positive and negative ionization modes (Figures 1A and 1B) identified the amino acid tryptophan (Wrona et al., 2016).

According to Wiley (Herebian, et al., 2009) peaks 11, 12, 13, 14, 19, 20, 21, and 22 in the negative ionization mode (Figure 1A) are characteristic of compounds in the curcuminoids class, including bisdemethoxycurcumin (peaks 11, 19, and 20), curcumin (peaks 13, 14, and 22), and desmethoxycurcumin (peaks 12 and 21). Peaks 11, 20, and 22 were confirmed in positive ionization mode (Figure 1B), exhibiting characteristic fragments for bisdemethoxycurcumin (peak 20) and for curcumin (peaks 11 and 22). Peaks 5–10, 15–18, and 23 refer to substances whose molecular formulas are not reported in the literature (Figures 1A and 1B).

Peak 9 (Figure 2 A) in the negative ionization mode was identified as prunin, as already reported in the literature (Li et al., 2016). Peak 10 in positive ionization mode (Figure 2B) and peak 11 in negative ionization mode (Figure 2A) were identified as naringenin, also previously reported (Li et al., 2016). Peaks 1 and 2 in the negative chromatogram (Figure 2B) indicate the presence of sucrose (Farag et al., 2014). Peaks 3–7, 8, and 12 were not identified after an intense search in the literature (Figures 2A e 2B).

3.2 Antimicrobial activity of Curcuma longa and Bixa orellana extracts

The antimicrobial activity of the extracts was evaluated against Gram-positive, Gram-negative, aerobic, and anaerobic microorganisms. There was no antibacterial activity in the extract dilutions of the tested plants for Gram-negative bacteria (*E. coli* and *Sa. Typhimurium*). For MRSA, the MBC was 156 µg/mL for *C. longa* and 625 µg/mL for *B. orellana*. For *Cl. sporogenes*, MBC was 25 mg/mL for *C. longa* and 50 mg/mL for *B. orellana*. The negative control performed with ethanol did not show any inhibitory effect on the tested bacteria, and the positive controls allowed the growth of all of them.

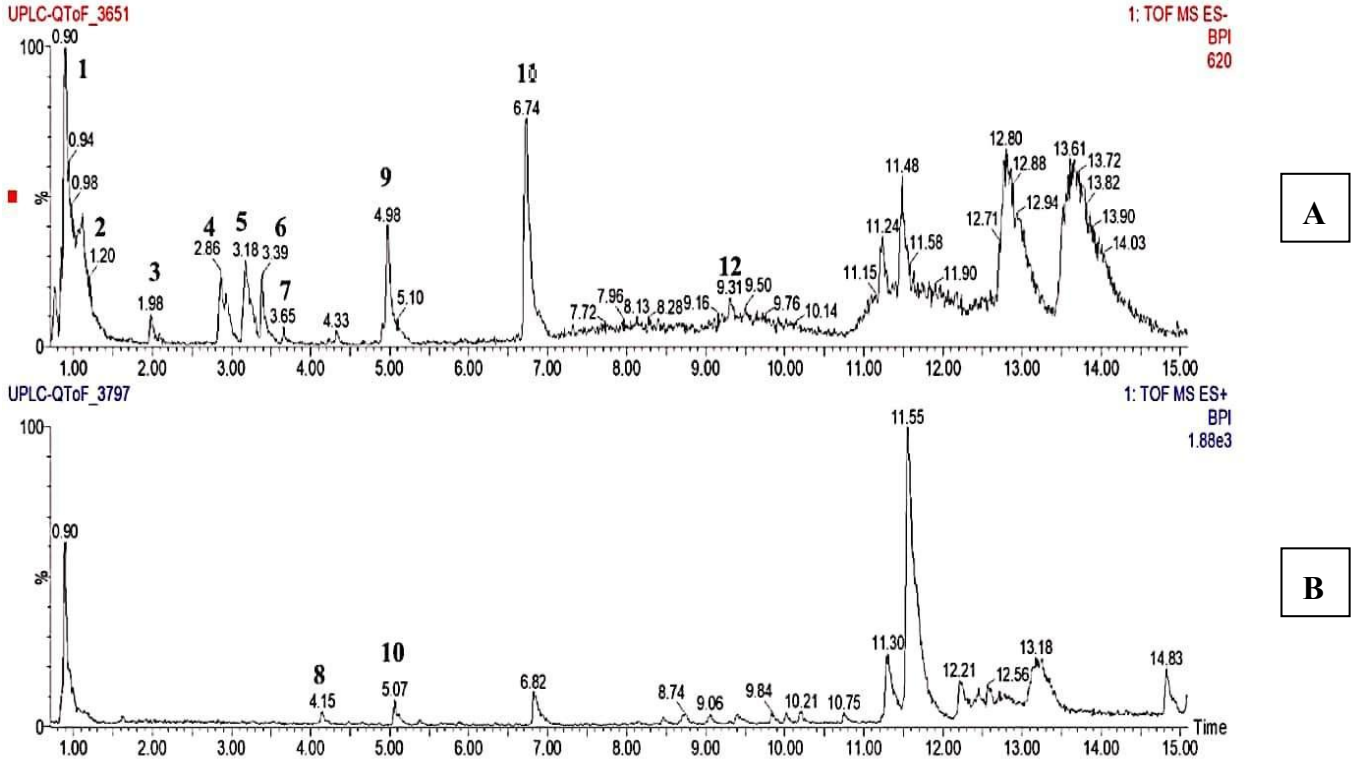

Figure 1. Chromatogram of raw ethanolic extract of *Curcuma longa* rhizome in negative (A) and positive (B) ionization modes.

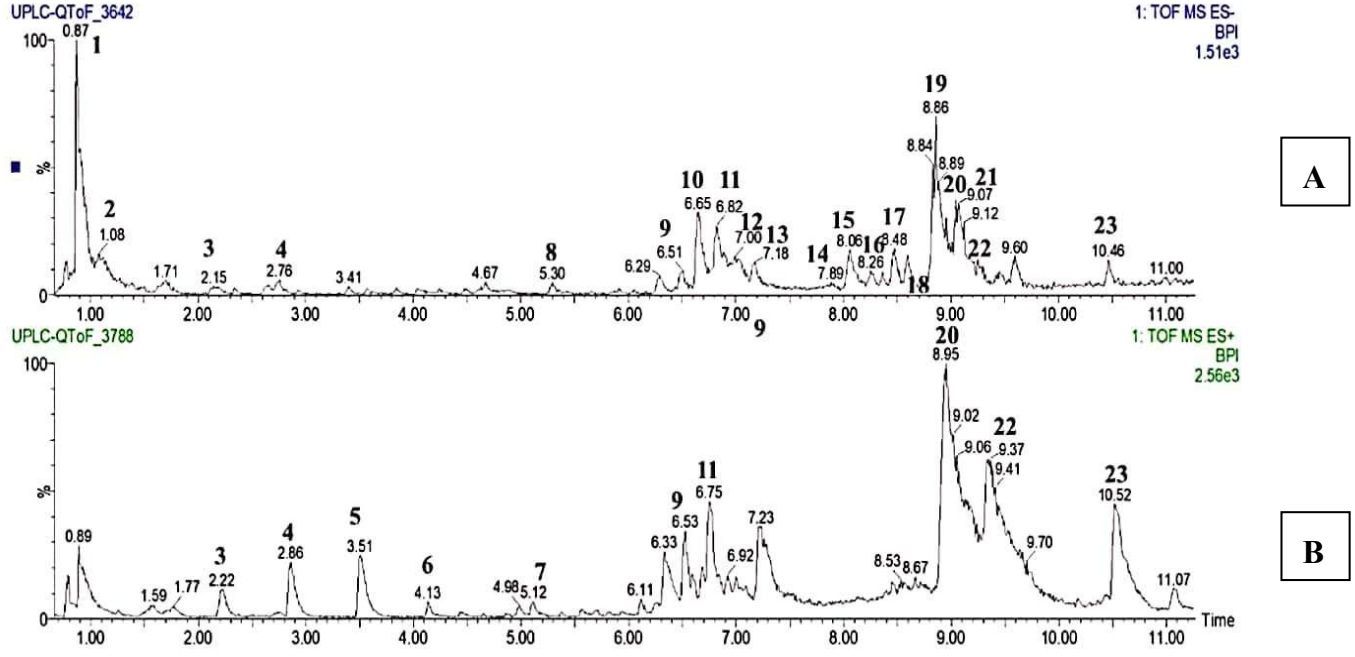

Figure 2. Chromatogram of raw ethanolic extract of the fruit of *Bixa orellana* in (A) negative and positive (B) ionization modes.

3.3. Antioxidant activity of Curcuma longa and Bixa orellana extracts

The antioxidant activity profile was analyzed at concentrations capable of inhibiting the growth of *Cl. sporogenes*, as shown in Table 1. In both techniques (FRAP and ORAC), the *B. orellana* extract at 5% had higher antioxidant activity than the *C. longa* extract at 2.5%. The coefficients of variation were considered satisfactory.

4 DISCUSSION

4.1 Chemical composition of Curcuma longa and Bixa orellana extracts

C. longa presented curcuminoids as dominant phenolic compounds, including curcumin and its derivatives, desmethoxycurcumin and bisdemethoxycurcumin (Jayaprakasha et al., 2006). Curcuminoids are important bioactive compounds with medicinal potential, which can be very useful in the food industry (Acosta-Estrada et al., 2014).

The chemical profile of *C. longa* from Korea was determined, and the flavonoids were found as dominant, in addition to glycosides and steroids (Kim et al., 2005). In another study, the composition and bioactivity of *C. longa* rhizomes collected from 20 different habitats were analyzed, and the main compounds found were ar-turmerone (0.92–42.85%), β-turmerone (5.13–42.54%), α-zingiberene (0.25–25.05%), ar-curcumene (1.21–15.70%), and β-sesquiphelandrene (0.05–14.88%) (Zhang et al., 2017). These results demonstrate that the chemical composition of *C. longa* rhizomes cultivated in different locations varies, providing distinct biological activities. The difference in composition is related to factors that directly affect the quality and quantity of secondary metabolites present in the final extract, such as climatic and seasonal conditions, precipitation, soil nutrient concentration, or collection time, among others (Jaberian et al., 2013).

The dominant compounds found in *C. longa* extracts prepared with different solvents were alkaloids, tannins, flavonoids, glycosides, and carbohydrates (Gupta et al., 2015). In the present study, the extraction with ethanol allowed the identification of flavonoids, carbohydrates, and amino acids. These differences can be explained by the phytochemical profile of the plants and by the different extractors in each experiment (Zhang et al., 2017). We found some qualitative differences from other GC-MS analysis reports of *C. longa* (Gupta et al., 2015; Kim et al., 2015; Zhang et al., 2017). However, all the previously mentioned studies identified compounds with biotechnological prospection capability, showing the importance of the *C. longa* rhizome as a source of bioactivity.

B. orellana presented prunin and naringenin as dominant compounds. Naringenin is a flavonoid that occurs in the form of a glycoside, with a wide spectrum of biological activities, including antibacterial, antifungal, antioxidant, and anticancer (Kozłowska et al., 2017). Naringenin derivatives were also observed in plant extracts of the *Asteraceae* family, plants with medicinal properties (Bigović et al., 2017). Phenolic compounds represent the main chemical constituents of different parts of *B. orellana*. These compounds exert toxic effects at the cell membrane level, with variable intensity, depending on their hydrophobicity (Sierra-Alvarez & Lettinga, 1991). Phenol exerts its toxic effect by influencing protein–lipid interaction and inducing the efflux of potassium ions, in addition to causing lysis of the bacterial cell wall (Heipieper et al., 1991; Keweloh et al., 1990).

4.2 Antimicrobial activity of Curcuma longa and Bixa orellana extracts

The extract of *C. long*a rhizome showed antimicrobial activity against the Gram-positive bacteria *Staph. aureus* (aerobic) and *Cl. sporogenes* (anaerobic), but not against the Gram-negative bacteria evaluated. The presence of the outer membrane in Gram-positive bacteria plays several roles, and its strong negative charge is an important factor in avoiding phagocytosis and cell lysis (Nikaido, 2003; Smith-Palmer et al., 1998). Similar results to those observed in the present study were reported for the most diverse plant extracts and essential oils (Fisher & Phillips, 2006; Smith-Palmer et al., 1998). Gul et al. (2004) analyzed the antimicrobial activity of *C. longa* against *Staph. aureus* and *E. coli*, and the result was similar to that found in this study (Gul et al., 2004). The antimicrobial activity of *C. longa* extract against *Staph. aureus* (156 μg/mL) and *Cl. sporogenes* (25 mg/mL) was attributed to the presence of curcuminoids identified in the GC-MS (Gul & Bakht, 2015). MRSA has been used for the evaluation of the antimicrobial properties of plant extracts, as it has a resistance profile to most antimicrobials on the market, demanding the search for alternative compounds with preservative properties to maintain food safety (Sepahpour et al., 2018).

Several parts of the *C. longa* plant have antimicrobial activity, but the rhizome presents the best activity (Gupta et al., 2015), justifying our choice for this study. Its bioactivities are mainly anti-inflammatory, antioxidant, antimicrobial, anticancer, and antiviral (Chaithra et al., 2015). However, the specific action mechanism for the antimicrobial activity of plants with

Table 1. Antioxidant activity* of raw ethanolic extracts of *Curcuma longa* and *Bixa orellana*.

Assay	Extract				*p*-value
	Curcuma longa (2.5%)		*Bixa orellana* (5%)		
	$\overline{X} \pm s$	CV	$\overline{X} \pm s$	CV	
FRAP	$130^{b} \pm 1.39$	0.826	$278^{a} \pm 3.63$	1.307	< .001
ORAC	$218^{b} \pm 2.91$	1.991	$455^{a} \pm 38.7$	10.412	< .001

*mM Trolox equivalent; X ± s: ± standard deviation; CV: coefficient of variation; FRAP: ferric reducing antioxidant power; ORAC: oxygen radical absorbance capacity. Means in the same row followed by different letters are significantly different ($p < .05$).

antimicrobial properties is not well-defined yet. Nevertheless, its effectiveness may occur in multiple ways in the microbial cell (Burt, 2004), such as cell wall degradation, damage to the cytoplasmic membrane and membrane proteins, cell content extravasation, cytoplasm coagulation, and depletion of driving force (Burt, 2004; Tiwari et al., 2009).

Lee et al. (2014) evaluated the antibacterial activity of the aqueous extract of Valeton domestic turmeric roots against *Clostridium perfringens* and found a minimum inhibitory concentration (MIC) of 183 μg/mL, evidencing the antimicrobial potential of plants of the Zingiberaceae family (Lee et al., 2014). Gul and Bakht (2015) tracked the antimicrobial activity of various *C. longa* extracts against common food bacteria, such as *Staph. aureus*, *E. coli*, *S. Typhimurium,* and *Candida albicans,* using the disc diffusion method, and all extracts (water, n-hexane, methanol, and chloroform) showed significant antibacterial and antifungal properties (Gul et al., 2004). These data demonstrate that the antimicrobial nature of the phytochemical is determined by its chemical properties, such as solubility (Meneses et al., 2013) and polarity, which, in turn, are influenced by the extractor (Sepahpour et al., 2018).

The antimicrobial activity of *B. orellana* extract for *Staph. aureus* (MBC 625 μg/mL) and for *Cl. sporogenes* (MBC 50 mg/mL) can be attributed to the dominant components identified by GC-MS, prunin and naringenin. Some studies have shown that naringenin increases cell membrane permeability and inhibits bacterial motility, exerting an antimicrobial effect (Bigović et al., 2017; Mirzoeva et al., 1997). However, there was no antimicrobial activity of the *B. orellana* extract against *E. coli* and *Sa. Typhimurium*. The inefficiency of *B. orellana* against Gram-negative bacteria was attributed to the high proportion of lipids they contain in their outer cell membrane, which was demonstrated in our experiment by the presence of lipids in the plant extract (Owen & Palombo, 2007).

However, the efficiency of the antibacterial activity of the ethanol extract from the leaf of *B. orellana* has been observed for other bacterial genera such as *Bacillus cereus*, *Pseudomonas aeruginosa*, *Shigella sonnei*, and *Listeria monocytogenes*, with MICs ranging from 256 μg/mL to 1024 μg/mL (Ciro Gómez et al., 2012). Muthukumar et al. (2014) conducted a study with collagen microspheres prepared with *B. orellana* leaf extract at a concentration of 1 mg/mL and observed antimicrobial properties against *Staph. aureus* and *E. coli* using the disk diffusion method (Muthukumar et al., 2014). This previous finding shows that the antimicrobial activity is dependent on the concentration of the plant extract, an effect that can be reinforced by increasing the extract dose (Jalayer-Naderi et al., 2016).

Selvi et al. (2011) tested the inhibitory activity of the methanol leaf and seed extracts of *B. orellana* against bacterial and fungal strains and observed that the leaf extract was more effective against the tested microorganisms. The authors attributed the findings to the ability of phenolic compounds present in the leaf extract to complex with proteins and bacterial cell walls (Selvi et al., 2011). The results obtained in our study differ from those observed by Selvi et al. (2011) because they come from different parts of the plant, where there are different bioactive compounds (Selvi et al., 2011). The lack of reproducibility of the antimicrobial activity of plants is one of the main obstacles to their industrial use, since qualitative and quantitative variations in the bioactive content of phytochemicals result in variable efficacy (Negi, 2012).

The action of the antimicrobial compound is dependent on the genus, species, and lineage of the target microorganism and on environmental factors such as pH, water activity, temperature, atmospheric composition, and microbial load of the food substrate (Negi, 2012). In this study, we used the broth dilution methodology recommended by CLSI (2014). Muthukumar et al. (2014) highlighted that this methodology stands out in studies like this one due to the total exposure of bacteria to compounds with possible antimicrobial activity, unlike when using methodologies such as disk diffusion, where the diffusion of extracts through agar is limited (Muthukumar et al., 2014).

4.3 Antioxidant activity of Curcuma longa and Bixa orellana extracts

Phenolic compounds present antioxidant activity, acting in the neutralization of free radicals and contributing to the control of oxidative stress (Jaakola & Hohtola, 2010; Kumazawa et al., 2004). The results found with the ORAC method showed that the ethanol extract of *C. longa* has strong antioxidant activity (217, 97 mM TE/g), suggesting high amounts of phenols. Some factors such as location, seasonality, and collection season can influence the concentration of bioactive compounds in plants and the biosynthesis of secondary metabolites (flavonoids), influencing their antioxidant potential and biological effects (Jaakola & Hohtola, 2010).

The higher antioxidant activity observed in *B. orellana* extract compared to *C. longa* extract may be due to the presence of the amino acids such as phenylalanine and tryptophan in *C. longa*, since the presence of these components can interfere with the quantification of this parameter in foods (Siddhuraju & Becker, 2003).

Tilak et al. (2004) studied the antioxidant capacity of processed and raw turmeric rhizome extracts by the FRAP and ORAC assays with different solvents (water/ethanol) and found differences between the assays and between the solvents used (Tilak et al., 2004). The authors found that ORAC showed greater affinity for ethanol, with higher antioxidant activity (25 μmol TE/g) in relation to the aqueous extract (10.27 μmol TE/g). In the FRAP assay, there was an effect dependent on the extract concentration (tested concentrations: 0.1 mM; 0.5 mM; 1 mM). Divergences between methods are due to peculiar characteristics of each one, such as pH, temperature, and type of radical, as well as to the interaction of the solvents used, the chemical structure, and the polarity of the solvent (Acquaviva et al., 2013). Jang et al. (2007) evaluated the antioxidant capacity of *C. longa* extract obtained in hot water, methanol, and acetone by the FRAP method and found an activity of 11 μmol/L (Jang et al., 2007). These results differ from those found in our study, probably due to differences in the extraction process, such as the type and number of solvents used.

5 CONCLUSIONS

The results of this study showed that the antimicrobial and antioxidant activities of *C. longa* and *B. orellana* extracts are related to their phenolic compounds, thus allowing a wider use of these plant extracts in the food industry.

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