Revista Ciência Agronômica, v. 51, n. 3, e20196619, 2020
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Bioaccessibility of the bioactive compounds and antimicrobial activity of aqueous extracts of *Physalis angulata* L.[1]

Bioacessibilidade de compostos bioativos e atividade antimicrobiana de extratos aquosos de *Physalis angulata* L.

Flayanna Gouveia Braga Dias[2*], Maria Jaiana Gomes Ferreira[2], Larissa Morais Ribeiro da Silva[2], Rayanne Clécia de Sousa Menezes[2] and Evânia Altina Teixeira de Figueiredo[2]

ABSTRACT - Plant diversity around the world, as well as the chemical variation, involves a large amount of bioactive substances. The use of plant extracts with biological properties appears as a viable and healthy alternative when compared to synthetic substances. In this context, the objective of this study was to evaluate the aqueous extracts of native and cultivated leaves of *Physalis angulata* L., as well as the influence of the extraction method on the bioaccessibility of bioactive compounds, antioxidant activity and antimicrobial activity. The extracts were obtained from the native and cultivated leaves of *P. angulata* using three different extraction methods: decoction, maceration and an ultrasound-assisted method. The analysis of variance showed significant differences between the extraction methods and the type of plant material. The extracts obtained by decoction showed the highest levels of phenolic compounds and higher antioxidant potential for both analytical methods (ABTS and DPPH), differing significantly from the other extraction methods. The bioaccessibility indices of the phenolic compounds obtained from the extracts were considered reduced after the simulated gastrointestinal digestion, and consequently, showed low antioxidant potential. The antimicrobial potential of the extracts was observed against the Gram-positive bacteria *Staphylococcus aureus* and *Listeria monocytogenes.*

Key words: Antimicrobial potential. Antioxidant potential. Bioactive compounds.

RESUMO - A diversidade de plantas em todo o mundo, bem como a variação química implica em uma grande quantidade de substâncias bioativas. O uso de extratos de plantas com propriedades biológicas surge como uma alternativa viável e saudável quando comparada a substâncias sintéticas. Neste contexto, objetivou-se esta pesquisa avaliar os extratos aquosos das folhas de *Physalis angulata* L., nativas e cultivadas, quanto a influência do método de extração na bioacessibilidade de compostos bioativos, atividade antioxidante e atividade antimicrobiana. Os extratos foram obtidos a partir das folhas nativas e cultivadas de *P. angulata* e três diferentes métodos extrativos, decocção, maceração e assistidos por ultrassom. A análise de variância mostrou diferenças significativas entre os métodos extrativos e o tipo de material vegetal. Os extratos obtidos por decocção apresentaram os maiores teores de compostos fenólicos e maior potencial antioxidante para ambos os métodos analíticos (ABTS e DPPH), diferindo significativamente dos demais métodos extrativos. Os índices de bioacessibilidade de compostos fenólicos dos extratos foram considerados reduzidos após a digestão gastrointestinal simulada, e consequentemente, apresentaram baixo potencial antioxidante. O potencial antimicrobiano dos extratos foi observado frente as bactérias Gram-positivas, *Staphylococcus aureus* e *Listeria monocytogenes.*

Palavras-chave: Potencial antimicrobiano. Potencial antioxidante. Compostos bioativos.

DOI: 10.5935/1806-6690.20200053
*Author for correspondence
Received for publication 21/02/2019; approved on 15/04/2020
[1]Parte da Dissertação do primeiro autor, apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Universidade Federal do Ceará/UFC
[2]Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Universidade Federal do Ceará/UFC, Fortaleza-CE, Brasil, flayanna@alu.ufc.br (ORCID ID 0000-0002-8647-5757), mjaiana@hotmail.com (ORCID ID 0000-0001-9981-5119), larissamrs@yahoo.com.br (ORCID ID 0000-0001-7302-401X), rayannecle@gmail.com (ORCID ID 0000-0001-5569-8221), evanialtina@gmail.com (ORCID ID 0000-0001-9209-0477)

INTRODUCTION

Physalis angulata L. (*Solanaceae*) is an annual herb, abundantly distributed in the North and Northeast regions of Brazil, where it is popularly known as "*camapú*". In these regions, the fruit is consumed *in natura* and its juice is used as sedative, diuretic, depurative and anti-rheumatic medicines. The leaves are used for the treatment of bladder inflammation, malaria, hepatitis and dermatitis (RENGIFO-SALGADO; VARGAS-ARANA, 2013).

Studies with extracts of *P. angulata* have shown important biological properties, such as anti-cancer and anti-inflammatory (HSEU *et al*., 2011), antibacterial (DONKOR; ODURO-MENSAH; FIAZORLI, 2016), analgesic and antioxidant activity (KUSUMANINGTYAS *et al*., 2015). These activities have been associated to the diversity of phytochemicals found in this species as simple or glycosylated flavonoids, linear chain fatty acids, hydroxylates, epoxylates, ascorbic acid, carotenoids, alkaloids and vitasteroids (RENGIFO-SALGADO; VARGAS-ARANA, 2013).

The antioxidant potential of plant extracts depends on the concentration of the phenolic compounds and the accumulation profile in plant tissues (BARRIADA-BERNAL *et al*., 2014). The concentration is influenced by environmental conditions, age and phenological stage, while the phenolic profile is more stable and varies between different groups of plants, following a specific tendency for each species (MEDINA-MEDRANO *et al*., 2015).

Bioaccessibility is defined as the amount of each ingested compound that is available for absorption in the intestine after digestion. Studies on the bioaccessibility of phenolic compounds and other antioxidants from solid matrices are important, since only compounds released from the food matrix and / or absorbed in the small intestine are bioavailable and able to be used for body functions, thus exerting their beneficial effects (PALAFOX-CARLOS; AYALA-ZAVALA; GONZÁLEZ-AGUILAR, 2011).

In vitro gastrointestinal digestion methods are widely used, as they are fast, safe, reliable and do not involve the ethical issues of *in vivo* methods. These assays consist in simulating the digestion and absorption processes (bioassimilation) or only the digestion process (bioaccessibility), thus allowing to study of changes that occur in the dietary components during gastric and intestinal digestion (BRIONES-LABARCA *et al*., 2011). In this context, the aim of this study was to evaluate the aqueous extracts of the *Physalis angulata* L. leaves, regarding the influence of the extraction method and plant material on the bioaccessibility of its bioactive compounds, antioxidant and antimicrobial activity.

MATERIAL AND METHODS

Plant material

The aerial parts of *P. angulata* were collected at Fazenda Sagitários, located in the municipality of Maranguape, state of Ceará, Brazil (Lat.: -4.095439; Long.: -38.854927) from May to August 2018. The exsiccates were identified at *Herbário Prisco Bezerra* at the Federal University of Ceará, under number 61269 (native plant) and 61298 (cultivated plant).

Obtaining the extracts

Initially, the leaves were selected, cleaned in running water, dried at 40 °C for 48 hours in a forced air circulation oven and powdered. The extracts were prepared at the 1:20 (maceration), 1:30 (ultrasound) and 1:40 (decoction) proportions of plant material and distilled water, respectively. The decoction extraction was carried out by immersing the plant material in water and heating it at 100 °C for 5 minutes (SUSANTI *et al*., 2015). The maceration was performed by immersing the plant material in distilled water at room temperature for 18 hours in the dark (SUSANTI *et al*., 2015). The ultrasound-assisted extraction was performed through direct sonication of the plant material and distilled water in an ultrasound equipment (Unique DES500, São Paulo, Brazil) with a 1.3 cm diameter tip for 8 minutes and power of 154 W (SCHINEIDER *et al*., 2015). All extracts were filtered, lyophilized and stored at -18 °C until the time of the analysis.

Determining the Total Phenolic Compounds (TPCs)

The quantification of TPCs was determined using the spectrophotometric method with the Folin-Ciocalteu reagent (LARRAURI; RUPÉREZ; SAURACALIXTO, 1997). The absorbance readings were performed on a spectrophotometer at 700 nm and the quantification was based on the standard curve of gallic acid (10-120 μg/ mL). The results were expressed in gallic acid equivalent milligrams per 100 g of *P. angulata* dry extract (mg GAE/100 g DE).

Antioxidant potential

2,2-Diphenyl-1-Picrylhydrazyl, DPPH radical assay

The antioxidant potential of the extracts was quantified through the capacity to capture the free radical DPPH (RUFINO *et al*., 2007). The method consists in monitoring the consumption of the DPPH radical through the decrease in absorbance when submitted to different concentrations (500-10,000 ppm) of the extracts. The absorbance readings were performed on

a spectrophotometer at 515 nm and the results were expressed as EC_{50} (Effective Concentration 50%) which corresponds to the necessary extract concentration (mg/mL) capable of reducing the initial concentration of the DPPH radical by 50%.

2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) radical assay

The antioxidant potential of the extracts was also determined by capturing the $ABTS^{\bullet+}$ free radical obtained by mixing ABTS and potassium persulfate as described by Rufino *et al*. (2007). In the dark, different concentrations (500-10,000 ppm) of the extracts were submitted to the $ABTS^{\bullet+}$ radical and, after 6 minutes, the absorbance readings were performed on a spectrophotometer at 734 nm. The antioxidant potential was quantified using a standard curve of the Trolox antioxidant (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the result was expressed in micromolar equivalent of Trolox per g of the *P. angulata* dry extract (µM Trolox/g DE).

Bioaccessibility of phenolic compounds *in vitro*

Simulated gastrointestinal digestion

The digestion was performed using simulated gastric and intestinal fluids, according to the methodology described by Helal *et al*. (2014). In the gastric phase, pepsin solubilized in 0.1 M HCl was used and in the intestinal phase, bile salts and pancreatin solubilized in 0.1 M $NaHCO_3$. The extracts (2 g), separately, were added to 100 mL of 0.01 M HCl and adjusted with a 0.1 M HCl solution to pH 2. Then, 3.2 mL of pepsin were added, followed by incubation in a water bath at 37 °C for 2 hours under agitation, simulating gastric digestion. The intestinal simulation was performed after titration with 0.5 M NaOH up to pH 7.5 (intestinal pH) and the samples were added to the dialysis membranes (33 × 21 mm, molecular weight: 12,000-16,000, porosity: 25 angstroms - INLAB , Brazil) containing 0.1 M $NaHCO_3$ aliquots equivalent to titratable acidity. After incubation in a water bath at 37 °C for 30 minutes, 5.0 mL of a bile and pancreatin solution were added and incubated again under the same conditions. After this stage, the membrane content (dialysate) was collected and the quantification analyses of TPCs and the antioxidant potential after simulated gastrointestinal digestion were carried out through the assays using the Folin-Ciocalteu reagent and the DPPH and ABTS free radicals, respectively.

Indexes of Bioaccessibility

The indexes bioaccessibility of phenolic compounds (BPC) and bioaccessible antioxidant activity (BAA) were determined according to the following equations:

$$BPC\ (\%) = (PCa / PCb)\ x\ 100 \quad (1)$$

BPC: Bioaccessible fraction of phenolic compounds in the samples;

PCa: Concentration of phenolic compounds after *in vitro* gastrointestinal digestion;

PCb: Concentration of phenolic compounds before *in vitro* gastrointestinal digestion;

$$BAA\ (\%) - (AA\alpha/AAb) \times 100 \quad (2)$$

BAA: Bioaccessible antioxidant activity of the samples;

AAa: Antioxidant activity after *in vitro* gastrointestinal digestion;

AAb: Antioxidant activity before *in vitro* gastrointestinal digestion.

Antimicrobial potential

Inoculum preparation

The antimicrobial potential of the aqueous extracts of *P. angulata* was determined on the bacterial strains of *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Escherichia coli* ATCC 25955 and *Salmonella Enteritidis* IAL 1132. The strains were grown using tryptone soybean agar (TSA-Sparks, USA) at 35 ºC for 24 hours in BOD (Biochemical Oxygen Demand, Quimis/ Model Q316-M26). After incubation, the isolated colonies were transferred to 5 mL of Soy Tryptone broth and incubated at 35 ˚C for 24 hours, thus yielding a bacterial suspension of 10^8 CFU/mL (CLINICAL AND LABORATORY STANDARDS INSTITUTE, 2017).

Agar diffusion method

Bacterial susceptibility was evaluated using the qualitative agar diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute, 2017. Petri dishes containing Mueller-Hinton agar were inoculated with bacterial suspensions of 10^8 CFU/ mL of the study microorganisms. After 10 minutes, wells measuring 6 mm in diameter and 4 mm deep were created, to which aliquots of 50 µL of the extract solutions (50 mg/mL) were added. The bacterial growth inhibition halos were measured after incubation at 35 ˚C for 24 hours. The extracts that showed inhibition halos were quantitatively analyzed using the broth microdilution method.

Minimum inhibitory concentration (MIC)

The MIC of the aqueous extracts of *P. angulata* was determined using the broth microdilution method in 96-well microplates, according to the methodology

described by Branen and Davidson (2004) and Brandt *et al*. (2010). Bacterial suspensions of 10^6 CFU/mL were obtained through dilutions in sterile distilled water and TSB broth. The absorbance readings were performed in an Elx 80 reader (Bio Tek instruments) at 630 nm, right after the plate distribution (T0) and after 24 hours of incubation at 35 ºC (T_{24}). The results were obtained by the difference in readings (T24-T0) $\leq$ 0.05, thus indicating the lowest extract concentration that inhibited bacterial growth.

Minimum bactericidal concentration (MBC)

The wells (microdilution) that showed inhibitory action, (T_{24}-T_0) $\leq$ 0.05, were inoculated (100 µL) by spread plate in TSA medium. After incubation at 37 ºC for 24 hours, the MBC was identified as the lowest extract concentration capable of reducing 3log $_{10}$ CFU/mL and / or the extract concentration that showed no bacterial growth (BRANDT *et al*., 2010).

Statistical analysis

The experiment was carried out using a completely randomized design, each analysis was performed in triplicate. The results were evaluated statistically through Analysis of Variance (ANOVA) and comparisons of means were performed using Tukey's test ($p<0.05$). All analyses were performed using the GENES software (CRUZ, 2006).

RESULT AND DISCUSSION

Antioxidant potential and content of phenolic compounds before and after *in vitro* digestion

The results of the analysis of variance of *Physalis angulata* aqueous extracts obtained by three different extraction methods for the five studied variables are shown in Table 1.

It can be observed that there are no significant differences between the variables for the isolated factors, except for the variables antioxidant activity (ABTS) and phenolic compounds after simulated digestion, corresponding to the plant material. Statistically significant results were observed for interaction between plant material and extraction methods in all analyzed variables, with the exception of antioxidant activity (ABTS) after digestion. This significant interaction indicates that one factor interferes with the behavior of the other to obtain the response, which can be justified by the differential behavior of the *Physalis* varieties (native and cultivated) considering the assessed extraction methods, as shown in Table 2.

Evaluating the content of phenolic compounds in the aqueous extracts of native and cultivated leaves of *Physalis angulata*, it was observed that these showed significant differences between them and regarding the extraction method employed, highlighting the native leaf extract obtained by decoction with 15310.79 mg GAE/100 g DE.

The technological potential of the aqueous extract (decoction) of *Physalis angulata* leaves was evaluated by Kusumaningtyas *et al*. (2015), and that showed contents of phenolic compounds of 49000 mg GAE/100 g of dry extract. Susanti *et al*. (2015) in their studies with aqueous extracts of *P. angulata* leaves obtained by subcritical water extraction and conventional methods, maceration and decoction, reported levels of phenolic compounds of 80620 mg GAE/100 g DE (100 °C), 58940 mg GAE/100 g DE and 63320 mg GAE/100 g DE, respectively. These authors observed that the temperature of extraction had a significant effect on the phenolic compound content, corroborating the findings of the present study, since the highest levels of phenolic compounds were obtained using the decoction extraction method. The use of high temperatures can facilitate extraction by promoting the permeability of plant cells, increasing solubility and, consequently, facilitating the diffusion of the substances to be extracted (SILVA; GARCIA; FRANCISCATO, 2016).

The lowest levels of phenolic compounds were observed for extracts of native and cultivated leaves obtained by the ultrasound-assisted method (Table 2). Carniel *et al*. (2018), in their studies with hydroalcoholic extracts of *P. angulata* obtained by the ultrasound-assisted method observed an association between the phenolic compound content and high temperatures, reporting polyphenol contents that ranged from 11.7 to 103.9 mg of GAE/100 g of extract obtained at 40 °C. Studies aiming at evaluating the optimal conditions of ultrasound-assisted extraction parameters have been carried out to optimize the yield of bioactive substances and, consequently, the biological activity of these substances. However, the structural complexity and rigidity of plant cell walls have proved to be obstacles, and further discussions on the variables involved in the extraction process are necessary (GIL-CHÁVEZ *et al*., 2013).

The antioxidant potential of the aqueous extracts of *P. angulata* was evaluated by two methods based on the capacity of the extracts to capture the ABTS and DPPH free radicals. These results showed statistically significant differences between the applied extraction methods and the studied plant materials, except for the extracts of native leaves obtained by the methods of decoction and maceration (DPPH) and the ultrasound-assisted method (ABTS).

Table 1 - Summary of the analysis of variance of total phenolic compounds (TPCs), antioxidant activity (ABTS and DPPH) and bioaccessibility of the aqueous extracts of *Physalis angulata* leaves before and after simulated digestion

Sources of variation	DF	Mean squares				
		TPCs before digestion	TPCs after digestion	ABTS before digestion	ABTS after digestion	DPPH before digestion
PM	1	220609996.64^{ns}	995.16*	57357.32*	184.91^{ns}	1085822.92^{ns}
EM	2	3247836.44^{ns}	65.98^{ns}	4504.46^{ns}	105.94^{ns}	295744.58^{ns}
PM x EM	2	49083053.55**	17.56**	1294.26*	17.04^{ns}	64470.39**
Residue	12	2028351.91	1.71	198.76	53.95	6424.92
Total	17	-	-	-	-	-
Mean		8181.64	22.15	184.69	45.37	476.62
CV (%)		17.40	5.21	7.63	16.18	16.81

TPCs: Total phenolic compounds; ABTS: 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; ND: Not determined; PM: Plant material (leaves from native and cultivated plants); ME: Extraction methods (Decoction, maceration and ultrasound-assisted method); DF: degree of freedom * significant at 5% of probability; ** significant at 1% probability by the F test

Table 2 - Comparisons between the means of phenolic compounds, antioxidant activity (ABTS and DPPH) and bioaccessibility of the aqueous extracts of *Physalis angulata*

Extraction methods	TPCs (mg GEA/100 g DE)					
	Before digestion		After digestion			
	Native	Cultivated	Native	BPC (%)	Cultivated	BPC (%)
Decoction	15310.79 Aa	8115.95 Bb	36.43 Aa	0.23	43.34 Aa	0.56
Maceration	9695.50 Bb	13607.62 Aa	17.28 Ab	0.17	21.71 Ab	0.16
Ultrasound	812.95 Bc	1547.01 Ac	15.99 Ab	1.96	16.14 Ac	1.04
	ABTS (µM Trolox /g DE)					
Extraction methods	Before digestion		After digestion			
	Native	Cultivated	Native	BAA (%)	Cultivated	BAA (%)
Decoction	317.06 Aa	254.74 Ba	38.74 Aa	12.2	47.19 Aa	18.5
Maceration	191.80 Ab	163.00 Bb	39.25 Aa	20.4	43.61 Aa	26.7
Ultrasound	92.66 Ac	88.88 Ac	50.84 Aa	54.8	52.60 Aa	59.1
	DPPH EC50 (mg/mL)					
Extraction methods	Before digestion		After digestion			
	Native	Cultivated	Native	BAA (%)	Cultivated	BAA (%)
Decoction	1.65 Bb	2.11 Ac	ND	ND	ND	ND
Maceration	2.63 Bb	6.95 Ab	ND	ND	ND	ND
Ultrasound	13.63 Ba	20.94 Aa	ND	ND	ND	ND

TPCs: Total phenolic compounds; ABTS: 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; EC_{50}: Effective concentration of the extract capable of reducing the initial concentration of the radical DPPH by 50%; BPC: Bioaccessibility of phenolic compounds; BAA: Bioaccessible antioxidant activity; DE: Dry extract; ND: Not determined. Means followed by the same uppercase letters horizontally do not differ statistically from each other and the means followed by the same lowercase letters vertically do not differ statistically from each other by Tuckey's test

The extracts obtained by decoction showed greater antioxidant potential through the method of the ABTS radical capture, with values of 317.06 µM Trolox/g DE (native) and 254.74 µM Trolox/g DE (cultivated), differing significantly between the plant materials. As for the assays with the DPPH free radical, it was observed that the higher the consumption of the radical, the lower the effective concentration of the extracts capable of neutralizing the action of the radical (CE_{50}) by 50% and the greater the sample antioxidant potential, with a highlight, in the following order, of the extracts obtained through the methods of decoction, maceration and the ultrasound-assisted method.

Cobaleda-Velasco *et al*. (2017), evaluating extracts from several parts of the cultivated *P. angulata* plant and at different stages of development, observed CE_{50} values that ranged from 0.15-0.25 mg/mL for the extracts obtained by maceration. These authors also found a significant correlation between the phenolic compounds and antioxidant properties for all extracts being assessed. Studies carried out with the extracts of *P. angulata* leaves and fruit found a greater potential for inhibiting the DPPH radical when using the ethanolic leaf extract obtained by maceration and aqueous extract obtained by decoction. These authors reported higher concentrations of phenolic compounds for these extracts and attributed the antioxidant activity to these substances (KUSUMANINGTYAS *et al*., 2015). These results are similar to those found in the present study, where the data obtained allow us to associate the phenolic composition with the antioxidant potential of the extracts obtained through different extraction methods.

The extraction of compounds with biological properties is a critical step in the research of natural products, as their efficiency depends on several factors, such as the substances to be extracted, part of the plant material used, extraction method and temperature, as well as the polarity of the extraction solvent (TIWARI *et al*., 2011; XYNOS *et al*., 2012). Moreover, the content of phenolic compounds in plants varies with species, variety, physiological conditions, soil, climate, and agricultural practices, among other factors (MEDINA-MEDRANO *et al*., 2015), therefore justifying the significant statistical differences found in plant materials regarding the quantification of these substances, as well as the antioxidant activity to which they are related.

After the gastrointestinal digestion, the bioaccessibility indexes for phenolic compounds were 0.23 and 0.53% for extracts obtained through decoction, 0.17 and 0.16 for extracts obtained through maceration and 1.96 and 1.04 for extracts obtained through the ultrasound-assisted method, for native and cultivated leaves, respectively. These indexes did not show any significant differences between the different plant materials, and there were no data in the literature regarding the bioaccessibility of phenolic compounds and antioxidant activity for *P. angulata* extracts. Some authors have reported values between 0 and 14% for the bioaccessibility of phenolic compounds of *Opuntia albicarpa* cv. Reyna and *Opuntia ficus-indica* cv. seeds (RAMÍREZ-MORENO *et al*., 2011). The bioaccessibility of phenolic compounds can vary from 30 to 100% for solid plant matrices. However, the action of digestive enzymes can interfere with the integrity and stability of these compounds (GARBETTA *et al*., 2014).

The bioaccessible antioxidant activity of the extracts was determined only for the ABTS analytical method, showing a reduction after gastrointestinal digestion. This result is related to the low levels of phenolic compounds quantified after digestion. During the gastrointestinal digestion, phenolic compounds can undergo structural changes caused by drastic changes in pH (alkaline conditions), action of enzymes used in digestion, with consequent molecule alteration, changes in bioactive groups such as the loss of hydrogen, resulting in a significant loss of antioxidant activity, and also the presence of phenolic acids linked to other molecules or to the food matrix, which hinders absorption through the dialysis membrane (RAMÍREZ-MORENO *et al*., 2011).

Antimicrobial potential

Agar diffusion

The aqueous extracts of *Physalis angulata* obtained through different extraction methods showed antimicrobial activity against Gram-positive bacteria *S. aureus*. The bacterium *L. monocytogenes* showed susceptibility to the aqueous extract obtained by decoction (CLEDEC). This and the other extracts did not show any inhibitory action against Gram-negative microorganisms *E. coli* and *S. Enteritidis*, as shown in Table 3.

The bacterial species *Staphylococcus aureus* is known for its capacity to produce toxins that can cause staphylococcal food poisoning, toxic shock syndrome and scalded skin syndrome. Additionally, these strains are resistant to conventional antibiotics, a fact that fosters research for more effective therapeutic alternatives. Donkor, Oduro-Mensah and Fiazorli (2016), when assessing the antimicrobial activity of the aqueous and ethanol extracts from the aerial parts of *P. angulata* obtained by maceration, reported a greater antimicrobial potential of ethanol extracts against *S. aureus* (Gram-positive) and *P. aeruginosa* (Gram-negative), showing inhibition halos measuring 21.00 mm and 23.00 mm, respectively, at the highest tested concentration (250 µg/mL). The aqueous extract, on the other hand, showed a 13-mm inhibition zone against *P. aeruginosa*, and no inhibitory activity against *S. aureus*.

These results are in disagreement with those found in the present study, as there was no evidence of antimicrobial activity against Gram-negative microorganisms (*E. coli* and *S. Enteritidis*). It is noteworthy that Gram-positive microorganisms are more susceptible to the antimicrobial action of plant extracts (FERREIRA *et al*., 2010), especially the bacterium *S. aureus*, for which the inhibitory effect of all assessed extracts can be observed (Table 3). These results can be justified by the fact that Gram-positive bacteria have a less complex cell structure compared to Gram-negative ones. The latter have a double layer, consisting of an outer membrane (lipopolysaccharides and proteins) that surrounds the

Table 3 - Values of the inhibition zone (mm) of aqueous extracts of *Physalis angulata* determined by agar diffusion

Extracts (50 mg/mL)	Inhibition zone (mm)			
	S. aureus	L .monocytogenes	E. coli	S. Enteritidis
CLEDEC	13	18	NI	NI
NLEDEC	8	14	NI	NI
CLEMAC	11	NI	NI	NI
NLEMAC	10	NI	NI	NI
CLEUS	16	NI	NI	NI
NLEUS	9	NI	NI	NI

CLEDEC: Cultivated leaf extract obtained by decoction; NLEDEC: Native leaf extract obtained by decoction; CLEMAC: Cultivated leaf extract obtained by maceration; NLEMAC: Native leaf extract obtained by maceration; CLEUS: Cultivated leaf extract obtained by ultrasound; NLEUS: Native leaf extract obtained by ultrasound; NI: Not identified

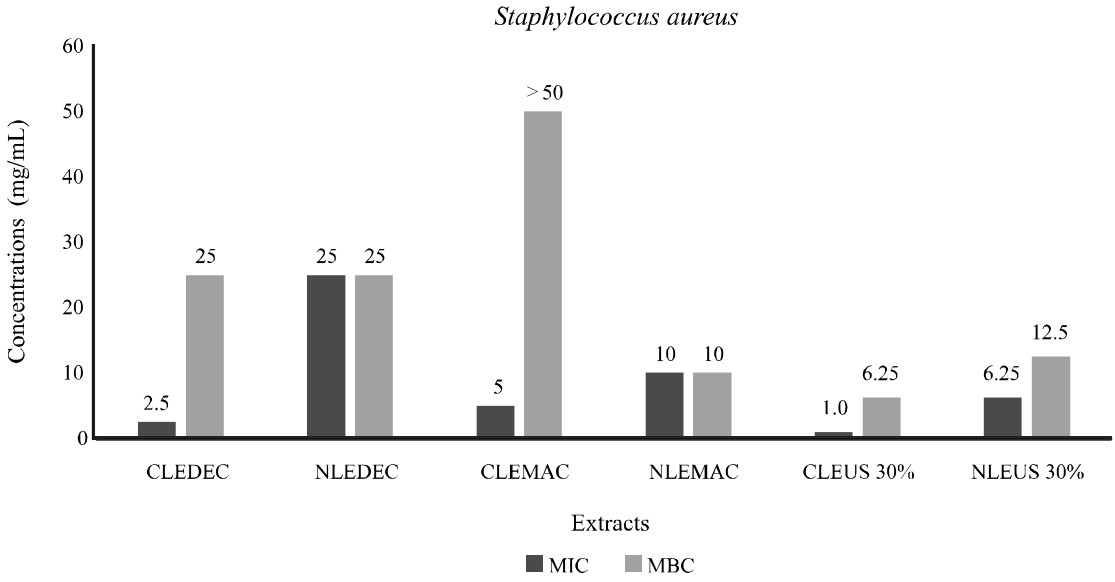
cell wall and an inner layer of lipopolysaccharides that give them a permeability barrier to substances, especially hydrophilic ones (SIMONETTI *et al*., 2016).

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The aqueous extracts of *Physalis angulata* that showed antimicrobial activity in the agar diffusion qualitative analysis were evaluated for antimicrobial potential through the quantitative broth microdilution method. Figures 1 and 2 show the results of the antimicrobial activity parameters, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the microorganisms that showed susceptibility in the qualitative assay. It can be observed that different MICs were obtained, with the lowest being 1.0 mg/mL for the cultivated leaf extract obtained by the ultrasound-assisted method (CLEUS 30%) against *S. aureus* and 2.5 mg/mL for the cultivated leaf extract obtained by decoction (CLEDEC) against the microorganisms *Staphylococcus aureus* and *L. monocytogenes*.

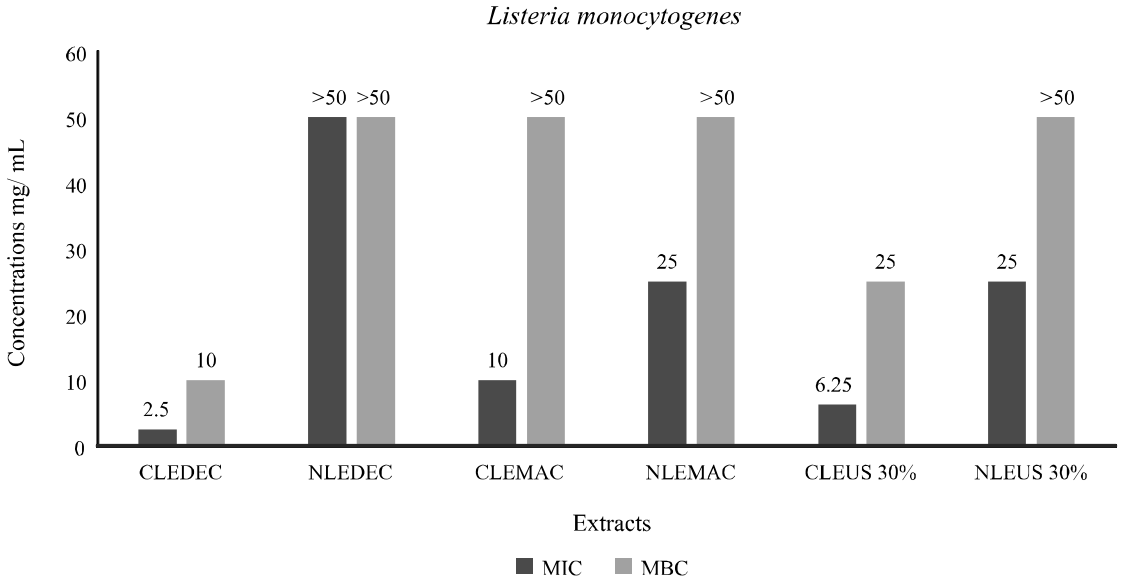
Bactericidal activity was observed against Gram-positive microorganisms, with the lowest values of

Figure 1 - Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous extracts of *Physalis angulata* leaves obtained through different extraction methods against *Staphylococcus aureus*



CLEDEC: Cultivated leaf extract obtained by decoction; NLEDEC: Native leaf extract obtained by decoction; CLEMAC: Cultivated leaf extract obtained by maceration; NLEMAC: Native leaf extract obtained by maceration; CLEUS: Cultivated leaf extract obtained by ultrasound (30% power); NLEUS: Native leaf extract obtained by ultrasound (30% power); Source: created by the author

Figure 2 - Minimum inhibitory concentration and minimum bactericidal concentration of aqueous extracts of *Physalis angulata* leaves obtained through different extraction methods against *L. monocytogenes*



CLEDEC: Cultivated leaf extract obtained by decoction; NLEDEC: Native leaf extract obtained by decoction; CLEMAC: Cultivated leaf extract obtained by maceration; NLEMAC: Native leaf extract obtained by maceration; CLEUS: Cultivated leaf extract obtained by ultrasound (30% power); NLEUS: Native leaf extract obtained by ultrasound (30% power); Source: created by the author

MBC of 6.25 mg/mL for the extract obtained through ultrasound against *S. aureus* and 10 mg/mL for the extract obtained by decoction against *L. monocytogenes*, both extracts obtained from the cultivated leaves of *P. angulata*. These results can be attributed to the action of phenolic compounds present in the plant extracts. These substances have an effect on the bacterial cell wall, breaking membrane structures, with consequent cell leakage. Moreover, the hydroxyl ions of these compounds are complexed with metal ions, decreasing the availability of essential ions for microbial metabolism (GYAWALI; IBRAHIM, 2014).

These studies reinforce the importance of plant extracts and the extraction methods used to obtain aqueous extracts with antimicrobial potential for use in food and drugs, thus avoiding the use of organic solvents and corroborating with folk medicine in the search for alternatives to achieve high added-value products.

CONCLUSIONS

1. The aqueous extracts of *Physalis angulata* L. leaves, obtained through different extraction methods showed an antioxidant potential before and after simulated gastrointestinal digestion;
2. The antimicrobial potential of *P. angulata* extracts was determined against the Gram-positive microorganisms *S. aureus* and *L. monocytogenes*. The greatest antimicrobial potential was observed for the extracts of the cultivated leaves obtained through the ultrasound-assisted method, followed by decoction;
3. The decoction of native leaves yields higher levels of phenolic compounds and, consequently, a greater antioxidant potential;
4. The assessed extracts showed reduced levels of phenolic compounds and less antioxidant potential after simulated gastrointestinal digestion.

REFERENCES

BARRIADA-BERNAL, L. G. *et al*. Flavonoid composition and antioxidant capacity of the edible flowers of Agave durangensis (Agavaceae). **CyTA - Journal of Food**, v. 12, n. 2, p. 105-114, 2014.

BRANDT, A. L. *et al.* Inhibition of *L. monocytogenes* by food antimicrobials applied singly and in combination. **Journal Joof Food Science**, v. 75, n. 9, p. 57-63, 2010.

BRANEN, J. K.; DAVIDSON, P. M. Enhancementofnisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. **International Journal of Food Microbiology**, v. 90, p. 63-74, 2004.

BRIONES-LABARCA, V. *et al*. Effects of high hydrostatic pressure (HHP) on bioaccessibility, as well as antioxidant activity, mineral and starch contents in Granny Smith apple. **Food Chemistry**, v. 128, n. 2, p. 520-529, 2011.

CARNIEL, N. *et al.* The effects of ultrasound-assisted extraction on polyphenolics compounds obtained from Physalis angulata using response surface approach. **Science Food Technology and Food Engineering**, v. 40, p. 3-6, 2018.

CLINICAL AND LABORATORY STANDARDS INSTITUTE. **Performance standards for antimicrobial susceptibility testing**. 27 th ed. Wayne, PA: Clinical and Laboratory Institute, 2017. CLSI supplement M100.

COBALEDA-VELASCO, M. *et al.* Phenolic profiles and antioxidant properties of *Physalis angulata* L. as quality indicators. **Journal of Pharmacy & Pharmacognosy Research**, v. 5, n. 2, p. 114-128, 2017.

CRUZ, C. D. **Programa Genes**: estatística experimental e matrizes. 1. ed. Viçosa: Editora UFV, 2006. v. 1, 285 p.

DONKOR, A.; ODURO-MENSAH, D.; FIAZORLI, M. Extracts of *euphobia hirta linn* and *P. angulata* and their amalgamation demonstrate potency against *S. aureus* and *P. aeruginosa.* **International Journal of Pharmacy and Pharmaceutical Sciences**, v. 8, n. 4, p. 322-326, 2016.

FERREIRA, S. B. *et al.* Avaliação da atividade antimicrobiana in vitro do extrato hidroalcoolico de *Stryphnodendron adstringens* (Mart.) Coville sobre isolados ambulatoriais de Staphylococcus aureus. **Revista Brasileira de Análise Clínica**, v. 42, n. 1, p. 7-31, 2010.

GARBETTA, A. *et al.* Antioxidant activity induced by main polyphenols present in edible artichoke heads: influence of in vitro gastro-intestinal digestion. **Journal of Functional Foods**, v. 10, p. 456-464, 2014.

GIL-CHÁVEZ, G. j. *et al.* Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. **Comprehensive Reviews in Food Science and Food**, v. 12, n. 1, p. 5-23, 2013.

GYAWALI, R.; IBRAHIM, S. A. Natural products as antimicrobial agents. **Food Control**, v. 46, p. 412-429, 2014.

HAYEK, S. A. *et al*. Antimicrobial natural products. Microbial pathogens and atrategies for combating them. **Science, Technology and Education**, v. 2, p. 910-921, 2013.

HELAL, A. *et al.* Bioaccessibility of polyphenols and cinnamaldehyde in cinnamon beverages subjected to in vitro gastropancreatic digestion. **Journal of Functional Foods**, v. 7, p. 506-516, 2014.

HSEU, C. Y. *et al*. Inhibitory effects of physalis angulata on tumor metastasis and angioneses. **Journal of Ethnopharmacology**, v. 135, n. 3, p. 762-771, 2011.

KUSUMANINGTYAS, R. W. *et al.* Potential of Ciplukan (*Physalis angulata* L.) as source of functional ingredient. **Procedia Chemistry**, v. 14, n. 1, p. 367-372, 2015.

LARRAURI, J. A.; RUPÉREZ, P.; SAURACALIXTO, F. Effect of drying temperatura on the stabilitity of polyphenols and antioxidant activityof red grape pomacepeels. **Journal of Agricultural and Food Chemistry**, v. 45, n. 4, p. 1390-1393, 1997.

MEDINA-MEDRANO, J. R. *et al.* Phenolic constituents and antioxidant properties of five wild species of *Physalis* (Solanaceae). **Botanical Studies**, v. 56, n. 1, p. 1-13, 2015.

PALAFOX-CARLOS, H.; AYALA-ZAVALA, J. F.; GONZÁLEZ-AGUILAR, G. A. The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. **Journal of Food Science**, v. 76, n. 17, p. 1750-3841, 2011.

RAMÍREZ-MORENO, E. *et al.* Intestinal bioaccessibility of polyphenols and antioxidant capacity of pulp and seeds of cactus pear. **International Journal of Food Sciences and Nutrition**, v. 62, p. 839-843, 2011.

RENGIFO-SALGADO, E.; VARGAS-ARANA, G. *Physalis angulata* L. (Bolsa Mullaca): a review of its traditional uses, chemistry and pharmacology. **Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas**, v. 12, n. 5, p. 431-445, 2013.

RUFINO, M. S. M. *et al.* Determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS. **Embrapa Agroindústria Tropical**, n. 128, p. 1-4, 2007.

SCHNEIDER, A. L. S. *et al.* Caracterização química e atividade biológica de extratos aquosos de *Brunfelsia cuneifolia* (Solanaceae). **Revista Brasileira de Plantas Medicinais**, v. 17, n. 4, p.1103-1111, 2015.

SILVA, C.; GARCIA, V. A. S.; FRANCISCATO, L. M. S. S. Extracção assistida por ultrassom de compostos bioativos das cascas de lichia (*Litchi Chinensis Sonn*.). **Revista Ciências Exatas e Naturais**, v. 37, n. 3, p. 81-96, 2016.

SIMONETTI, E. *et al*. Avaliação da atividade antimicrobiana de extratos de *Eugenia anomala* e *Psidium salutare* (Myrtaceae) frente à *Escherichia coli e Listeria monocytogenes*. **Revista Ciências Exatas e Naturais**, v. 18, n. 1, p. 9-18, 2016.

SUSANTI, R. F. *et al.* Phenol, flavanoid and antioxidant activity of *Physalis angulata* leaves extract by subcritical water extraction. **Modern Applied Science**, v. 9, n. 7, p. 190-198, 2015.

TIWARI, P. *et al*. Phytochemical screening and extraction: a review. **International and Pharmaceutica Science**, v. 1, n. 1, p. 98-106, 2011.

XYNOS, N. *et al*. Development of a green extraction procedure with super/subcritical fluids to produce extracts enriched in oleuropein from olive leaves. **The Journal of Supercritical Fluids**, v. 67, p. 89-93, 2012.