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Phytochemical screening of extracts from *Spiranthera odoratissima* A. St.-Hil. (Rutaceae) leaves and their *in vitro* antioxidant and anti-*Listeria monocytogenes* activities

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ABSTRACT. *Spiranthera odoratissima* A. St.-Hil (Rutaceae), a shrub whose common name is *manacá do Cerrado* in Brazilian Portuguese, is about 1-m high and has been used by folk medicine to treat stomachache, kidney and liver infections, headache, rheumatism and as a blood purifier. This study aimed at preparing hexane, ethyl acetate, methanolic, hydroethanolic and aqueous extracts from *S. odoratissima* leaves, at carrying out preliminary phytochemical screening and at evaluating their *in vitro* antioxidant and anti-*Listeria monocytogenes* activities. Antioxidant activity was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azinobis-3-ethybenzothiazoline-6-sulfonate) and FRAP (ferric reducing antioxidant power) methods. Antibacterial activity was investigated against *L. monocytogenes* and Minimum Inhibitory Concentration (MIC) values of plant extracts were calculated by the broth microdilution method with the use of 96-well plates. In aqueous, methanolic, hydroethanolic, ethyl acetate and hexane extracts from *S. odoratissima* leaves, the following classes of compounds were investigated: organic acids, reducing sugars, flavonoids, saponin compounds, coumarin compounds, phenolics, tannins, purine compounds, catechins, flavonol derivatives, sesquiterpene lactones and anthraquinones. All plant extracts, except the hexane one, exhibited high antioxidant activity. Regarding antibacterial activity, the most polar extracts showed high activity against *L. monocytogenes*; their MIC values ranged between 12.5 and 62.5 μg mL^{-1}, while the hexane one exhibited low activity (MIC = 1000 μg mL^{-1}). In short, extracts from *S. odoratissima* leaves may be considered promising sources of secondary metabolites with relevant antioxidant and antibacterial activities.

Keywords: Cerrado; manacá; medicinal plant; antibacterial activity; natural antioxidants.

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Introduction

The Brazilian *Cerrado*, which is the second largest biome in the country, has high potential for the development of research into new plant-based compounds (Oliveira, Moreira, Melo Júnior, & Pimenta, 2006). Biodiversity found in the *Cerrado* is vast and diverse, since its species comprise herbaceous plants, shrubs, trees and vines (Lima, Scariot, Medeiros, & Sevilha, 2012). Great variation in plants leads to an immense potential to use and apply their functional properties, which are attributed to bioactive compounds (Morais et al., 2019).

Phytochemical screening has stood out among investigation methods because it enables chemical constituents to be identified and/or evaluated in species under study. In case there are no studies of certain species and their secondary metabolites, preliminary phytochemical screening acts as an identification method of several classes of primary and secondary metabolites. This process may be carried out by distinct methods and solvents, which lead to consequent high diversity of results (Costa & Hoscheid, 2018).

More specifically, phytochemical screening enables preliminary assays to be conducted in order to identify chemical compounds in certain plant species. Classes of compounds comprise alkaloids, which have antitumoral and antiviral properties, flavonoids, which exhibit several anti-inflammatory, antioxidant, antiallergic and anticarcinogenic characteristics, tannins, that contribute to treatments for arterial

hypertension, fungi, bacteria and burns, saponins, which exhibit antiviral activity and act on cell membranes, steroids/triterpenes, which are natural anti-inflammatories, and other classes, such as coumarins, lignans, terpenes, flavonoids and limonoids (Bessa et al., 2013).

Thus, this study aimed at preparing hexane, ethyl acetate, methanolic, hydroethanolic and aqueous extracts from *Spiranthera odoratissima* leaves (Figure 1) and at submitting them to preliminary phytochemical screening, so as to identify classes of secondary metabolites found in this species. In addition, all plant extracts had their antioxidant (by the DPPH, ABTS and FRAP methods) and anti-*Listeria monocytogenes* activities evaluated.

Figure 1. *Spiranthera odoratissima* A. St.- Hil. (Rutaceae) leaves. Source: the authors/2019

Material and methods

Biological material

Spiranthera odoratissima A. St.- Hil. (Rutaceae) leaves were collected in May, when the *Cerrado* region, located in Goiás state (GO), undergoes its dry season. Collection was carried out in Iporá, GO (16°24'11.2"S and 51°06'41.4"W), whose altitude is 707 m (Figure 2). *Spiranthera odoratissima* leaves were stored in paper bags, identified and preserved. Plant material was identified by botanist Erika Amaral and deposited in the herbarium that belongs to the *Instituto Federal Goiano*, Campus Rio Verde, GO, exsiccate no.1039.

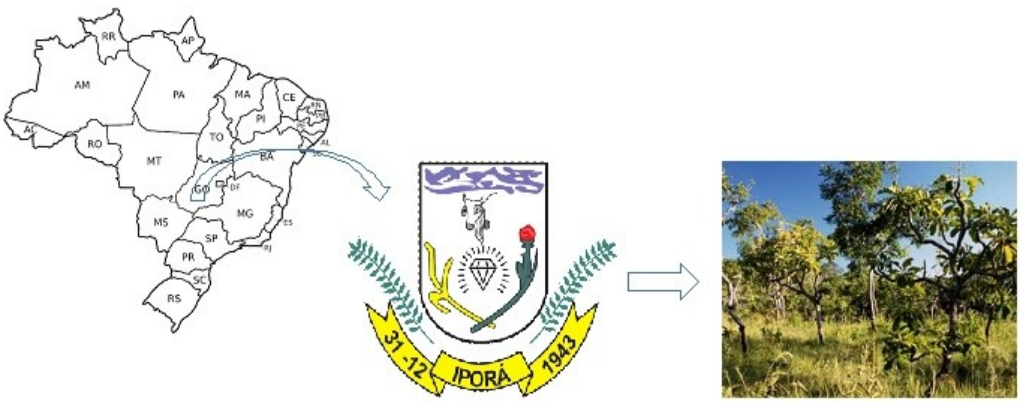

Figure 2. The *Cerrado* region in Iporá, GO, Brazil.

Solvents

Solvents used in the experiments were hexane (NEON, Suzano, SP, Brazil), ethyl alcohol (Ls Chemicals, Ribeirão Preto, SP, Brazil), methyl alcohol (Ls Chemicals, Ribeirão Preto, SP, Brazil) and ethyl acetate (Dinâmica, Indaiatuba, SP, Brazil). Deionized water was produced by a deionizer (LUCADEMA/LUCA-315).

Extract preparation

The methodology used for preparing extracts was described by Rodrigues, Souza Filho, and Ferreira, (2009), with modifications. The material was dried by a forced air oven at 50°C up to constant mass for about 24 hours. Afterwards, the material was ground by a Willey Star FT 50 knife mill and passed through a 5-mm sieve up to homogeneous powder.

Extraction with solvents

To yield crude extracts, 100 g homogeneous powder (ground leaves) was added to 1000 mL solvent (hexane, ethyl acetate, methanol, mixtures of ethanol and water (7:3) and water (100%). All extracts were stored in closed glass bottles. In this phase, extracts were kept in a dark place. After 24 hours, solutions were filtered through qualitative filter paper (80 g m^{-2}). Resulting liquids were submitted to a rotoevaporator at 70ºC, so that solvents could evaporate and the following extracts could be obtained: hexane (7.60 g), ethyl acetate (10.3 g), methanolic (5.80 g), hydroethanolic (12.2 g) and aqueous (3.30 g) ones. At the end, all extracts had the consistency of syrup.

Phytochemical screening

In preliminary phytochemical screening, classical procedures were carried out to identify the main classes of secondary metabolites. Identification of compounds found in plant extracts may be based on certain criteria, such as color, precipitation reactions and foam formation (Mariño et al., 2019).

The following classes of metabolites were analyzed: organic acids, reducing sugars, alkaloids, flavonoids, saponin compounds, coumarin compounds, cardiac glycosides, phenolics, proteins and amino acids, purines, tannins, polysaccharides, purine compounds, catechins, benzoquinones, naphthoquinones and phenanthraquinones, flavanols, flavanones, flavononols, xanthones, anthraquinones, sesquiterpene lactones and other lactones.

Organic acids

The methodology described by Henriques and Almeida (2013), was applied, with modifications. In a test tube, 5 mL distilled water was added to 2 mL extract. Then, 2 mL Pascová reagent was added to it. The reaction is considered positive when the reagent loses its color.

Reducing sugars

Reducing sugars were determined by the methodology proposed by Silva, Souza, Silva, Marques, and Graebner (2019), with modifications. In a test tube, 5 mL distilled water was added to 2 mL extract. Then, 2 mL Fehling's reagent and 2 mL Fehling's B were added to it. The tube was transferred to a vortex shaker to homogenize the solution and then kept in a water bath for 5 minutes. Positive results show brick-red precipitate.

Non-reducing sugars

Non-reducing sugars were also determined by the methodology proposed by Silva, Souza, Silva, Marques, and Graebner (2019), with modifications. In a test tube, 5 mL distilled water was added to 2 mL extract. Afterwards, homogenization was carried out in a vortex shaker and 1 mL concentrated hydrochloric acid (HCl) was added to the test tube.

The solution was heated in a water bath for about 5 minutes and then cooled. Neutralization was carried out by a sodium hydroxide solution (NaOH) 20% (m v^{-1}) and 2 mL Fehling's reagent and 2 mL Fehling's B were added. The solution underwent a water bath again for 5 minutes. After cooling, it was analyzed. The reaction is considered positive when it shows red precipitate.

Alkaloids

The methodology described by Henriques and Almeida (2013), with modifications, was used for determining the group of alkaloids. In a test tube, hydrochloric acid (HCl) at 5% (m v^{-1}) was added to 4 mL extract. Afterwards, 1 mL solution was placed in 4 test tubes in the following order:

Test tube 1 – extract solution + hydrochloric acid at 5% (m v^{-1}) + 500 µL Mayer's reagent. The reaction is considered positive when there is either white precipitate or a slightly turbid white solution.

Test tube 2 - extract solution + hydrochloric acid at 5% (m v^{-1}) + 500 µL Wagner's reagent. A positive reaction shows orangish precipitate.

Test tube 3 - extract solution + hydrochloric acid at 5% (m v^{-1}) + 500 µL Liebermann-Bouchard's reagent. A positive reaction shows orangish or reddish precipitate.

Test tube 4 - extract solution + hydrochloric acid at 5% (m v^{-1}) + 500 µL Bertrand's reagent. A positive reaction shows white precipitate.

Flavonoids

The Shinoda test (concentrated HCl and magnesium) was carried out in agreement with the methodology described by Henriques and Almeida (2013), with modifications. In a test tube, a little fraction of magnesium ribbon (0.5 cm in diameter) and 1 mL concentrated HCl were added to 3 mL extract. The solution exhibited total effervescence at the end of the reaction. A positive reaction shows colors that range from blackish red to red.

Saponin compounds

The method used for determining saponin compounds was described by Silva et al. (2019), with modifications. In a test tube, 10 mL deionized water was added to 5 mL extract. It was vigorously agitated by a vortex shaker for 2 minutes to form foam. The reaction is positive if foam persists in test tubes after resting for 15 minutes.

Coumarin compounds

The methodology described by Henriques and Almeida (2013), with modifications, was used for determining coumarin compounds. A test tube with 3 mL extract was covered with filter paper (80 g m^{-2}) and 1 mL aqueous solution of sodium hydroxide at 10% (m v^{-1}) was applied to the paper surface. The tube was heated in a water bath for 10 minutes. After having cooled at room temperature, the test tube was placed in an ultraviolet chamber and the paper was evaluated under UV light at wavelengths of 254 and 365 nm. Result is considered positive when fluorescence on the paper is either green or yellow.

Cardiac glycosides

The methodology described by Kloss, Albino, Souza, and Lima (2016), with modifications, was used for carrying out this assay. In a test tube, 3 mL extract was mixed with 2 mL KEEDE B reagent. The reaction is considered positive when precipitate is formed and/or its color becomes either blue or violet.

Phenolics

Phenolic compounds were evaluated by the methodology proposed by Henriques and Almeida (2013), with modifications. In a test tube, 5 mL distilled water was added to 3 mL extract. Then, 1 mL ferric chloride solution, $FeCl_3$, at 1% (m v^{-1}) was also added. The reaction is considered positive when colors vary between blue and red, an evidence of simple phenolics.

Proteins and amino acids

Proteins and amino acids were evaluated by the methodology proposed by Henriques and Almeida (2013), with modifications. In a test tube, 3 mL extract was mixed with 500 µL aqueous vanillin solution at 1%. Then, it was kept in a water bath for 10 minutes, up to ebullition. There was change in color after the solution rested. The result is positive when the solution becomes persistently violet.

Purines

Purine compounds were determined in agreement with the methodology proposed by Henriques and Almeida (2013), with modifications. In a test tube, 500 µL aqueous solution of hydrochloric acid at 32% and

500 µL aqueous solution of hydrogen peroxide 35% were added to 2 mL extract. The solution was then heated in a water bath for 10 minutes, up to total evaporation of the liquid. Reddish residue formed at the end of the water bath. Afterwards, 500 µL aqueous solution of ammonium hydroxide 6 N was added to the solution in the test tube, which was then homogenized by agitation for 1 minute. The reaction is considered positive when it becomes reddish and/or violet.

Tannins

Tannins were determined by the methodology proposed by Henriques and Almeida (2013), with modifications. In a test tube, 500 µL ferric chloride solution, $FeCl_3$, at 10%, was added to 2 mL extract and attention was focused on changes in color. Blue precipitate shows that there are hydrolysable tannins, while the green one shows condensed tannins.

Polysaccharides

The analysis of polysaccharide compounds was carried out in agreement with Henriques and Almeida (2013), with modifications. In a test tube, 5 mL distilled water was added to 2 mL extract. The solution was manually homogenized for 30 seconds and then 500 µL Lugol's iodine was added to it. Results are considered positive when the solution is blue.

Catechins

Catechins were evaluated in agreement with the methodology described by Silva et al. (2019), with modifications. In a test tube, 2 mL vanillin solution at 1% and 1 mL concentrated hydrochloric acid at 32% (P.A. – ACS) were added to 2 mL extract. The result is positive when the solution is reddish.

Benzoquinones, naphthoquinones and phenanthraquinones

Derivatives of benzoquinones, naphthoquinones and phenanthraquinones were detected by the methodology described by Barbosa et al. (2004), with modifications. In the test tube, 500 µL aqueous solution of anhydrous sodium carbonate (Na_2CO_3) at 15%, 500 µL formaldehyde solution at 4% and 500 µL 3,5-dinitrobenzoic solution at 5% were added to about 3 mL extract. The solution was then heated in a water bath for 5 minutes. The reaction is considered positive when it becomes red.

Flavonols, flavanones and xanthones

These compounds were analyzed in agreement with the methodology described by Barbosa et al. (2004), with modifications. In a test tube, a little fraction of magnesium ribbon (0.5 cm in diameter) and 1 mL concentrated HCl were added to 3 mL extract. After total effervescence of the magnesium ribbon, colors changed. The reaction is considered positive when it becomes reddish.

Anthraquinones

Anthraquinones were evaluated by the methodology proposed by Barbosa et al. (2004), with modifications. In a test tube, 2 mL aqueous solution of ammonium hydroxide at 10% was added to 3 mL extract. The solution was manually homogenized for 30 seconds. The positive reaction is pinkish, red or violet.

Sesquiterpene lactones and other lactones

The group of sesquiterpene lactones and lactones was evaluated in agreement with the methodology described by Barbosa et al. (2004), with modifications. In a test tube, 1 mL alcoholic hydroxylamine hydrochloride solution at 10% and 500 µL methanolic KOH solution at 10% were added to 2 mL extract. The solution was kept in a water bath for 10 minutes and, after cooling, it was acidified by a hydrochloric acid (HCl) solution at 1 N. Then, 500 µL aqueous solution of iron chloride III ($FeCl_3$) was added to it. The positive reaction is violet.

Antioxidant activity

Free radical scavenging activities of 2,2-diphenyl-1-picrylhydrazyl (DPPH), azino-bis (ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) were determined by the spectrophotometric method described by Lima et al. (2019), with modifications. In the

DPPH assay, different concentrations of extracts in methanol (10–100 μg mL^{-1}) were added to 2 mL 0.1 mM solution of DPPH that had been previously prepared and incubated in the dark for 30 min. Absorbance was recorded at 517 nm by a UV spectrophotometer. In the ABTS assay, 1980 mL diluted ABTS solution was added to 20 μL extract that had previously been diluted in ethanol. Absorbance at 734 nm was measured 6 min. after initial mixing. BHT was used as positive control. Assays were carried out in triplicate. Inhibition percentage was calculated as (I %) = (A0 − A/A0) × 100, where A0 is the absorbance of the control and A is the absorbance of samples. Finally, total antioxidant capacity of different plant extracts was evaluated by the FRAP method. In this colorimetric assay, reduction of ferric-tripyridyltriazine complex to ferrous form is expressed as antioxidant capacity. The FRAP reagent was added to different concentrations of plant extracts (10-1000 μg mL^{-1}). Absorbance was read at 570 nm by a microplate reader/spectrophotometer. The IC_{50} value was calculated as the concentration of a sample required to scavenge 50% of free radicals by graphing the I % versus extract concentration.

Antibacterial activity

Listeria monocytogenes (ATCC 15313) from the American Type Culture Collection (ATCC) was employed. Minimum inhibitory concentration (MIC) is defined as the lowest concentration of a sample that can inhibit bacterial growth. MIC was determined by microdilution on 96-well microplates, in triplicate. Extract samples were tested at concentrations ranging from 0.195 to 400 μg mL^{-1}. Positive control was penicillin (from Sigma-Aldrich, St. Louis, MO) for Gram-positive bacteria, at concentration of 5.9 μg mL^{-1}. Final dimethylsulfoxide (DMSO) content in the sample was 5% (v v^{-1}); the same concentration was employed as the negative control (without extracts). Inoculated wells containing the microorganism were only included to control bacterial growth. Non-inoculated wells (without any microorganisms) were also employed to ensure broth sterility. Inoculum cell concentration was adjusted to 5 X 10^5 colony-forming unit (CFUs) mL^{-1} based on the absorbance read at 625 nm by the spectrophotometer Nanodrop from Thermo Scientific. The complete methodology used for evaluating antilisterial activity of extracts was the one described by Fernández et al. (2018).

Results and discussion

Phytochemical screening

Results of the phytochemical screening of extracts from *Spiranthera odoratissima* (Rutaceae) leaves are shown in Table 1.

Table 1. Phytochemical screening of extracts from *S. odoratissima* (Rutaceae) leaves

Classes of Compounds	Aqueous extract	Methanolic extract	Hydroethanolic extract	Ethyl acetate extract	Hexane extract
Organic acids	+	+	+	+	+
Reducing sugars	-	+	+	+	+
Non-reducing sugars	+	-	-	-	-
Alkaloids:					
Mayer's reagent	-	-	-	-	-
Wagner's reagent	-	-	-	-	-
Libermann-Burchard's reagent	-	-	-	-	-
Flavonoids	+	+	+	+	-
Saponin compounds	+	+	+	-	-
Coumarin compounds	+	+	-	-	-
Cardiac glycosides	+	+	+	-	-
Phenolics	+	+	+	+	-
Proteins and amino acids	-	-	-	-	-
Tannins	GR+	GR+	GR+	GR+	-
Polysaccharides	-	+	+	-	-
Purine compounds	+	+	+	+	-
Catechins	+	+	+	-	-
Benzoquinones, naphthoquinones and phenanthraquinones	-	-	-	-	-
Flavonols, Flavanones and xanthones	+	+	+	+	-
Anthraquinones	+	+	+	+	-
Sesquiterpene lactones and other lactones	+	+	+	-	+

Results: (+) positive and (-) negative. GR = green for condensed tannins

Table 1 shows organic acids in aqueous, methanolic, hydroethanolic, ethyl acetate and hexane extracts from *S. odoratissima* leaves.

Reducing sugars were identified in methanolic, hydroethanolic, ethyl acetate and hexane extracts, but not in the aqueous one. Soares, Santos, Vieira, Pimenta, and Araújo (2016), carried out phytochemical screening and reported reducing sugars in extracts from *S. odoratissima* leaves, while non-reducing sugars were only found in the aqueous extract. Alkaloids were not detected in any extract under study.

Flavonoids, which are known for exhibiting antimicrobial and antioxidant activities (Machado, Nagem, Peters, Fonseca, & Oliveira, 2008), were found in aqueous, methanolic, hydroethanolic and ethyl acetate extracts from *S. odoratissima* leaves. Soares et al. (2016), also reported this class of compounds in *S. odoratissima* leaves.

Saponin compounds were only found in aqueous, methanolic and hydroethanolic extracts. They have several biological activities; the ones that should be highlighted are related to increase in immune response and rupture of erythrocyte membranes, such as immunologic adjuvant and hemolytic activities (Kaiser, Pavei, & Ortega, 2010). Soares et al. (2016) identified saponin groups in aqueous extract from *S. odoratissima* leaves.

Coumarin compounds were qualitatively identified in aqueous and methanolic extracts. Their biological activities include antiprotozoal, antimalarial, memory stimulating, antitumoral, antifungal, anti-inflammatory and antioxidant ones (Jalhan, Singh, Saini, Sethi, & Jain, 2017). Terezan et al. (2010), reported this group of secondary compounds in crude extract from *S. odoratissima* leaves. Soares et al. (2016) also described this type of compound in *S. odoratissima* leaves.

Cardiac glycosides were found in aqueous, methanolic and hydroethanolic extracts from *S. odoratissima* leaves, but neither ethyl acetate nor hexane extracts were detected. These compounds are capable of increasing the rate of contraction in cardiac muscle fibers. Thus, they have been specially used for treating congestive heart failure (CHF) (Palacios, Thompson, & Gorostiaga, 2019).

Phenolic compounds were detected in aqueous, methanolic, hydroethanolic and ethyl acetate extracts from *S. odoratissima* leaves. They act as the main sources of antioxidant activity in fruits and plants, since they protect species from environmental aggressions and contribute to improve human health not only as agents that decrease blood sugar levels and body mass, but also as anticarcinogens (Soares, 2002). Proteins and amino acids were not identified in any extract from *S. odoratissima* leaves under investigation.

Regarding tannins, this class was identified in all extracts under study, except in the hexane one. Condensed tannins have quite complex structures and astringent properties, besides exhibiting antidiarrheal, antiseptic, antimicrobial and antifungal attributes. They also help to cure burns, wounds and inflammations by developing a protective layer on which healing processes occur naturally (Monteiro, Albuquerque, Araújo, & Amorim, 2005). Soares et al. (2016), also found these compounds in *S. odoratissima* leaves.

Polysaccharide compounds were only identified in methanolic and hydroethanolic extracts. The class of purine compounds was qualitatively identified in all extracts under investigation, except the hexane one.

Catechins were only found in aqueous, methanolic and hydroethanolic extracts. This group of secondary metabolites has a broad number of biological activities, such as antioxidant, anti-inflammatory, anticarcinogenic and chemoprotective ones. It is also considered an agent against skin aging (Isemura, 2019). Derivatives of benzoquinones, naphthoquinones and phenanthraquinones were not found in any extract from *S. odoratissima* leaves under investigation.

Flavonoids and their derivatives – flavonols, flavanones and xanthones – were qualitatively identified in aqueous, methanolic, hydroethanolic and ethyl acetate extracts, but not in the hexane one, from *S. odoratissima* leaves. Soares et al. (2016) also found this class of metabolite in *S. odoratissima* leaves.

Even though anthraquinones are chemically characterized by the fact that they exhibit antibacterial, antifungal and antiviral activities, they may lead to diarrhea, vomiting and nausea when they are badly employed. In addition, overdoses may provoke severe crises of acute nephritis (Malik & Muller, 2016). These compounds were found in aqueous, methanolic, hydroethanolic and ethyl acetate extracts from *S. odoratissima* leaves. Classes of sesquiterpene lactones and other lactones were found in aqueous, methanolic, hexane and hydroethanolic extracts, but not in the ethyl acetate one.

Antioxidant and anti-*Listeria monocytogenes* activities

Antioxidant activity of plant extracts was evaluated by both DPPH, ABTS and FRAP methods, which have been commonly used for analyzing free radical scavenging capacity in several natural products (Alves, David, David, Bahia, & Aguiar, 2010).

Table 2 shows results of hexane, ethyl acetate, methanolic, hydroethanolic and aqueous extracts in terms of IC_{50} values (μg mL^{-1}). Positive control was BHT (IC_{50} = 18.00 μg mL^{-1}). The lowest antioxidant activity was exhibited by the hexane extract (IC_{50} = 1550.80 μg mL^{-1} by DPPH, IC_{50} = 1358.50 μg mL^{-1} by ABTS and IC_{50} = 1450.75 μg mL^{-1} by FRAP), while the highest antioxidant activity was attributed to the aqueous extract (IC_{50} = 3.18 μg mL^{-1} by DPPH, IC_{50} = 3.81 μg mL^{-1} by ABTS and IC_{50} = 3.50 μg mL^{-1} by FRAP). High antioxidant activity was also identified in ethyl acetate, methanolic and hydroethanolic extracts, whose IC_{50} values were close to the ones of the positive control BHT (IC_{50} = 18.00 μg mL^{-1}).

Silva, Costa, Santana, and Koblitz (2010), stated that the high free radical scavenging activity may be explained by the fact that phenolic compounds and flavonoids can be found at higher concentrations, by comparison with the hexane extract, which did not exhibit classes of compounds with antioxidant potential by phytochemical screening. In general, phenolic compounds prevent free radicals from acting in the body and, since they protect molecules, such as DNA, they may interrupt some carcinogenic processes. According to Youn et al. (2019), methodologies which use scavenging of radicals DPPH, ABTS and FRAP measure the activity of compounds of hydrophilic nature, i. e., compounds with high polarity. Therefore, antioxidant activities observed by this study may be directly connected with flavonoids in extracts under investigation.

Concerning anti-*Listeria monocytogenes* activity, ethyl acetate, methanolic, hydroethanolic and aqueous extracts exhibited high antibacterial activity, whose MIC values ranged between 12.5 and 62.5 μg mL^{-1} (Table 2). However, the hexane extract was the only one that had low activity against bacteria whose MIC value was 1000 μg mL^{-1} (Table 2).

Lemes et al. (2018) have recently reported that MIC values below 100 μg mL^{-1}, between 100 and 500 μg mL^{-1} and between 500 and 1000 μg mL^{-1} correspond to promising, moderate and weak activities, respectively, whereas MIC values above 1000 μg mL^{-1} denote inactivity. Thus, it confirms the antibacterial potential of the most polar extracts from *S. odoratissima* leaves.

A large amount of antimicrobial effects of plant extracts is believed to result mainly from flavonoids in their composition (Andrade et al., 2005). Throughout the extraction process, based on the polarity of its constituents, the hexane extract kept poor in flavonoids and rich in terpenoids. As a result, the hexane extract from leaves exhibited weak or no antimicrobial activity at all.

Due to the high susceptibility of Gram-positive microorganisms, the mechanism of antimicrobial activity of the extract may result from its interaction with the peptidoglycan found in the bacterial cell wall, which characterizes a more fragile barrier than cell walls of Gram-negative bacteria (Oliveira, Oliveira, Batalini, Rosalem, & Ribeiro, 2012).

Table 2. Antioxidant (IC_{50} = μg mL^{-1}) and anti-*Listeria monocytogenes* (MIC = μg mL^{-1}) activities of plant extracts from *S. odoratissima* leaves

Extracts	DPPH (IC_{50})	ABTS (IC_{50})	FRAP (IC_{50})	Bacteria (MIC)
Hexane	1550.80	1358.50	1450.75	1000
Ethyl acetate	28.84	30.50	32.44	50
Methanolic	19.16	15.95	16.74	62.5
Hydroethanolic	21.52	27.55	23.46	62.5
Aqueous	3.18	3.81	3.50	12.5
BHT (positive control)	18.00	18.00	18.00	-
Penicillin (positive control)	-	-	-	5.9

Conclusion

Results of this study of *S. odoratissima* leaves show that ethyl acetate, methanolic, hydroethanolic and aqueous extracts have promising antioxidant and anti-*Listeria monocytogenes* activities, which may be related to phenolic compounds found in them, mainly flavonoids. This study also revealed the potential of extracts from the species *S. odoratissima* as sources of natural compounds that may be used for producing active packaging to control *L. monocytogenes* in the food industry. In addition, antioxidant assays showed

that the most polar extracts from *S. odoratissima* leaves may act as free radical scavengers or reductants and as inhibitors of lipid peroxidation, a fact that may contribute to prevent and mitigate the development of pathologies associated with oxidative stress. In short, further studies are needed to isolate chemical constituents of this species so that they can be evaluated by experimental models and, thus, enable compounds in charge of certain biological activities to be defined.

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