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ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL AND EXTRACTS FROM LEAVES OF PSIDIUM MYRSINITES DC (MYRTACEAE) AGAINST BACTERIA ISOLATED FROM BROILER LIVER

Atividade antibacteriana do óleo essencial e extratos das folhas de Psidium myrsinites DC (Myrtaceae) contra bactérias isoladas do fígado de frangos de corte

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ABSTRACT

Antimicrobial resistance is one of the most relevant public health problems. The use of natural products with inhibitory activity is a promising alternative to combat pathogenic microorganisms. The objective of this study was to analyze the phytochemical composition and check for antibacterial activity in extracts, fractions, and essential oil from leaves of *Psidium myrsinites* DC (Myrtaceae) against bacteria isolated from chicken livers. The aqueous, ethyl acetate and chloroform fractions of the ethanolic extract crude ethanol extract, the crude acetone extract and the essential oil were tested on *E. coli*, *Pseudomonas aeruginosa* and *Streptococcus* spp. isolated from chicken livers. The antimicrobial activity was tested by the minimum inhibitory concentration technique. The susceptibility profile of the isolated microorganisms showed resistance to at least four tested antibiotics. Phytochemical screening identified flavonoids, tannins, anthraquinones, coumarins, triterpenoids, resins, chalcones and saponins. The extracts and essential oil of *P. myrsinites* showed moderate toxicity, except for the aqueous fraction of the ethanolic extract, which exhibited low toxicity. Despite not showing biocidal activity, the extracts and oil showed good antibacterial activity, with minimum inhibitory concentrations ranging between 125 and 500 $\mu g.mL^{-1}$. Our findings demonstrate the antibacterial activity of *P. myrsinites*, revealing its potential to combat pathogenic bacteria.

Keywords: Bacterial resistance; Phytochemical screening; Poultry farming.

RESUMO

A resistência antimicrobiana é um dos problemas de saúde pública mais relevantes. O uso de produtos naturais com atividade inibitória é uma alternativa promissora para combater microorganismos patogênicos. O objetivo deste estudo foi analisar a composição fitoquímica e verificar a atividade antibacteriana em extratos, frações e óleo essencial das folhas de *Psidium myrsinites* DC (Myrtaceae) contra bactérias isoladas de fígados de frango. As frações aquosas, acetato de etila e clorofórmio do extrato etanólico bruto, o extrato acetônico bruto e o óleo essencial foram testados em *E. coli*, *Pseudomonas aeruginosa* e *Streptococcus* spp. isolados de fígados de frango. A atividade antimicrobiana foi testada pela técnica de concentração inibitória mínima. O perfil de susceptibilidade dos microorganismos isolados mostrou resistência a pelo menos quatro antibióticos testados. A triagem fitoquímica identificou flavonoides, taninos, antraquinonas, cumarinas, triterpenoides, resinas, chalconas e saponinas. Os extratos e óleo essencial de *P. myrsinites* mostraram toxicidade moderada, exceto pela fração aquosa do extrato etanólico, que apresentou baixa toxicidade. Apesar de não mostrar atividade biocida, os extratos e óleo mostraram boa atividade antibacteriana, com concentrações inibitórias mínimas variando entre 125 e 500 µg/mL. Nossos achados demonstram a atividade antibacteriana de *P. myrsinites*, revelando seu potencial para combater bactérias patogênicas.

Palavras-chave: Resistência bacteriana; Triagem fitoquímica; Avicultura.

INTRODUCTION

The export, production and consumption of chicken meat are important sectors for the Brazilian economy, with exponential growth in recent years. In 2020, the country exported 6.097 million tons of chicken meat, generating revenue of US$4.241 million (ABPA, 2022), thus placing Brazil in the world rankings of chicken meat export, production, and consumption.

Antimicrobial resistance refers to the ability of the microorganism to multiply, even in the presence of high doses of antimicrobials (Wannmacher, 2004; Who 2018). It is a natural biological phenomenon that has been accelerated due to the continuous use of these drugs in hospital environments, animal husbandry and disease control, self-medication and non-adherence to the therapy prescribed by physicians (Silva *et al.*, 2012; Costa and Júnior, 2017).

Currently, it is one of the most relevant public health problems, since some microorganisms are no longer susceptible to the antimicrobials usually used (Who, 2005). As antibiotics become ineffective, diseases become more difficult to treat, which can lead to increased morbidity and mortality (Who, 2017). British economist Jim O'Neill stated that by 2050, antimicrobial resistance will be responsible for ten million deaths annually, in addition to causing a loss of approximately one hundred trillion dollars to public coffers (Estrela, 2018).

Some barriers in the fight against antimicrobial resistance are the lack of innovation (the introduction of the last class of antibiotics occurred more than three decades ago) and the lack of investment by pharmaceutical companies, resulting from the low financial return (Estrela, 2018). This problem justifies the need to search for alternatives to the use of traditional antimicrobials susceptible to the selection and emergence of microbial resistance mechanisms. The use of natural products with inhibitory activity is a promising alternative to combat pathogenic microorganisms, increasing treatment effectiveness and reducing the economic damage caused (Silva *et al.,* 2018; Prasad *et al.*, 2019). In this context, several plants have proven efficacy and low levels of toxicity (Askari *et al.,* 2012).

Psidium myrsinites DC (Myrtaceae) is an endemic species to Brazil, occurring in the north, southeast, central-west, and northeast regions (Sobral *et al.,* 2015). Research on *P. myrsinites* is still scarce, focusing on anatomical characteristics (Villarroel and Gomes-Bezerra, 2015; Bezerra *et al.,* 2013; Durães *et al.*, 2017), oil composition and yield (Castelo *et al.*, 2010; Medeiros *et al.*, 2015), antioxidant, carcinogenic (Silva *et al.*, 2013) and larvicidal (Dias *et al.,* 2015) activity. However, studies demonstrate the antibacterial activity of plants belonging to the genus *Psidium*, which indicate the potential of *P. myrsinites* to control microorganisms (Vieira *et al.,* 2012; Pío-León, 2013; Afonso *et al.*, 2018).

Therefore, it is necessary to develop new investigations on bioactive compounds of this plant species, with potential for the development of new antibiotics. In this scenario, the objective of this study is to carry out the phytochemical characterization and check for antibacterial activity in extracts and essential oil of *P. myrsinites* leaves in bacteria isolated from chicken livers, as well as to investigate the susceptibility of these microorganisms to the main antibiotics used in human and veterinary medicine.

MATERIAL AND METHODS

Ethics

This study was approved by the Animal Ethics Committee of the State University of Goiás (CEUA-UEG), under protocol 011/2019 and is in accordance with the Ethical Principles in Animal Experimentation, adopted by the Brazilian Society of Science in Laboratory Animals (SBCAL).

Microorganisms

The microorganisms used were isolated from apparently healthy broiler livers, collected from 50 livers during evisceration in slaughter lines at a slaughterhouse with an inspection system in the state of Goiás (Agrodefesa). The organs were collected using clean gloves, which were changed for each organ, and stored in individual plastic bags to avoid cross-contamination. The samples were placed in thermal boxes with ice for transport to the laboratory, where they were kept refrigerated at a temperature of 2 to 8°C during the processing of the analyses.

Approximately 1.0 g of each organ was macerated and placed in test tubes containing 9 mL 0.1% buffered saline, which was incubated at 37 °C for 18-24 h. Later, a 1 mL fraction of peptone water was transferred to 9 mL selenite-cystine broth (Kasvi®) and 9 mL Rappaport Vassiliadis broth (Kasvi®), which were oven-incubated at 37 °C for 18-24 h.

Subsequently, 0.1 mL each tube was transferred to Petri dishes containing methylene blue eosin agar (EMB), xylose lysine tergitol 4 (XLT4) (Kasvi®) and bright green agar (VB) (Kasvi®) for surface plating, and dishes were incubated at 37 °C for 18-24 h. With the aid of a nickel chromium loop, aliquots of Rappaport Vassiliadis and selenite-cystine broths were taken and streaked out on the agars.

After this period, three colony forming units (CFU) were selected from each medium used, with morphological, morphotintural and microscopic characteristics compatible with *E. coli*, *Pseudomonas* spp. and *Streptococcus* spp. and transferred to triple sugar iron agar (TSI), which were incubated at 37 °C for 18-24 h.

TSI tubes with characteristic growth of *E. coli* and *Pseudomonas* were subjected to of biochemical tests: production of urease, indole, H_2S, methyl red test, motility test, Simmons citrate, malonate. The use of glucose, lactose was added for *E. coli*.

For the identification of *Streptococcus*, staining tests were performed using the Gram method, catalase test, oxidation and fermentation (OF) test, and coagulase tests.

Antimicrobial susceptibility test

The test described by BAUER et al. (1966) and Clinical and Laboratory Standard Institute (CLSI, 2016), which consists of applying, with a sterilized swab, the microorganisms isolated from chicken livers to the entire surface of a 90 mm Petri dish containing commercially available Muller Hinton agar (Kasvi®). The experiment was conducted in triplicate. After a period of up to 15 minutes, discs impregnated with antimicrobials were used: amoxicillin (20 mg), cephalexin (30 mg), ciprofloxaxin (5 mg), enrofloxacin (5 mg), gentamicin (10 mg), trimethoprim-sulfamethoxazole (25 mg), sulfonamide (250 mg) and tetracycline (30 mg), on the seeded plate.

Dishes were incubated for 18-24 h in a bacteriological oven at 37 °C. After incubation, the diameters of the zones of inhibition of bacterial growth around each disc were measured in millimeters to determine the sensitivity of the bacterial sample. Zones were classified into the sensitive, intermediate or resistant categories according to CLSI (2016). These antibiotics were chosen for bacterial susceptibility tests because of their importance in veterinary medicine and public health.

Extraction of essential oil

The essential oil was extracted by hydrodistillation, in a Clevenger device. Dry plant material (100 gram) was weighed, placed in a round-bottomed flask containing 500 mL distilled water (Figure 4), which was later coupled to the Clevenger. This was then subjected to temperature of approximately 100 °C, where it remained for 3 hours after boiling (Siqueira *et al.*, 2011).

Phytochemical screening

Phytochemical screening was performed in duplicate according to Matos e Matos (1989), Costa (2001), Matos (2009), Farmacopeia Brasileira (Brasil, 2010) and Simões et al. (2017), with adaptations to optimize reagent use and improve detection sensitivity. These adaptations included adjustments to reagent concentrations, reaction times, and visualization techniques. The screening aimed to preliminarily identify the presence of resins, anthraquinones, tannins, coumarins, and flavonoids.

Analysis of essential oil constituents

The essential oil was analyzed by gas chromatography coupled to mass spectrometry (GC/MS) in a Shimadzu QP2010 Ultra equipment from the Center for Analysis, Innovation and Technology in Natural and Applied Sciences – CaiTec, in Goiás State University. A fused silica capillary column (CBP - 5; 30m x 0.25mm x 0.25µm) was used, maintaining a flow rate of 1 mL/min helium as carrier gas, heating at a programmed temperature (60 °C/2min; 3 °C min^{-1}/240 °C, 10 °C min^{-1}/280 °C, 280 °C/10min), and ionization energy of 70 eV. The injection volume was 1µL sample diluted in CH_2Cl_2 in a proportion of 1:5.

Chemical components of essential oils were identified by comparing their mass spectra and retention indexes with the literature (Adams, 2017). Co-injection of a series of alkanes from 9 to 32 carbons was performed to calculate the retention indexes, according to the equation of Van der Dool and Kratz (1963).

Dilution of plant extracts and oil

Initially, the stock solution was prepared at a concentration of 4,000 µg.mL^{-1} and from there, serial dilutions were made, according to the CLSI (2016), obtaining concentrations of 2,000 µg.mL^{-1}; 1,000 µg.mL^{-1}; 500 µg.mL^{-1}; 250 µg.mL^{-1} and 125 µg.mL^{-1}.

The extraction of essential oil from leaves of *P. myrsinites* was carried out by hydrodistillation in a Clevenger apparatus. The essential oil (EO) obtained was dried in anhydrous sodium sulfate and stored in N_2 atmosphere, in a hermetically sealed amber glass bottle, at -20 ºC.

The Crude Acetone Extract (CAE) was extracted from leaves of *P. myrsinites* by maceration with a 50% acetone/water mixture in an ultrasonic bath. Then, the obtained extract was filtered by simple filtration with cotton and acetone.

The Aqueous Fraction of the Ethanol Extract (AFEE) was divided into portions and extracted with ethyl acetate in a separatory funnel. Ethyl acetate fractions were pooled and rotoevaporated to recover the solvent and the aqueous fractions were also pooled and lyophilized.

The Crude Ethanol Extract (ECE) was obtained by extraction with ethanol 95% (P.A.) of the powder from *P. myrsinites* leaves in a percolator until solvent saturation. The ethanol was evaporated in a rotary evaporator at 40 °C.

The ECE was solubilized in methanol: water (7:3) solution and then fractionated with solvents with increasing polarity, hexane, chloroform, and ethyl acetate, respectively. The aqueous fraction obtained from this extraction was stored in a freezer and the other fractions were concentrated in rotatory evaporator until they were completely dry.

Artemia salina toxicity assay

Toxicity tests on *Artemia salina* followed the methodology described by Rehman *et al*. (2005) with adaptations. Initially, a saline solution was prepared at a concentration of 30 $g.L^{-1}$, supplemented with yeast extract (6 $\mu g.L^{-1}$) and pH adjusted between 7.0 and 8.0. Part of this solution was reserved for dilutions, while another part was transferred to the separatory funnel, where *A. salina* cysts were incubated for 36 h at room temperature, natural light and constant aeration.

After hatching, 10 nauplii were transferred to each well of a 96-well polystyrene plate, containing 100 µL saline and 100 µL extract or oil at concentrations 1,000; 500; 250; 125; 62.5; and 31.25 $\mu g.mL^{-1}$. The following controls were included in the assay: lethality control (using different concentrations of Potassium Dichromate - $K_2Cr_2O_7$), solvent control (Dimethyl sulfoxide (DMSO) for extracts and Tween 80 in the test with essential oil) and negative control (with nauplii and saline solution, to rule out any external changes that could interfere with the test result).

After 24 hours, the live and dead larvae were counted, determining the lethal concentration at 50% (LC50) of each extract, using the Probit regression test, in the statistical software IBM SPSS Statistics 20, with a confidence interval of 95%. The median lethal concentration (LC50) is the concentration required of a given compound or substance to kill 50% population used in a test (Bueno and Piovezan 2015). The experiment was carried out in triplicate for each extract and for the oil, according to Molina-Salinas and Said-Fernández (2006).

Antibacterial activity

To test the antibacterial activity, we used three bacteria isolated from the liver of broilers (*E. coli*, *Pseudomonas* spp. and *Streptococcus* spp.) and strains of *E. coli* ATCC 25312 and *S. aureus* ATCC 25923. The broth microdilution test followed the protocol established by CLSI for antimicrobial susceptibility testing by dilution of antimicrobial agents in broth (CLSI, 2016), from which the minimum inhibitory concentration (MIC) was determined for each extract and for the oil.

Microorganisms were seeded in Mueller Hinton medium, using the depletion technique, and incubated for 24 h at 37 ºC. After, 3 to 5 colonies were removed from the Petri dish and transferred to a tube containing saline solution, thus equating its turbidity, corresponding to 0.5 on the McFarland scale. Then, a 1:10 dilution was prepared in saline solution, to obtain a concentration of 10^7 $CFU.mL^{-1}$.

Subsequently, in a sterile 96-well polystyrene plate with a lid, 10 µL broth with the microorganism was pipetted into all wells, except

for those referring to the control of the culture medium, 100 µL Muller Hinton broth and 100 µL the diluted compound. Line H was used for controls: culture medium + microorganism, Tween, DMSO and pure culture medium. The plate was incubated in an oven at 37 °C for 24 h.

Bacterial growth was visually analyzed observing the formation of bacterial growth halos at the bottom of each well.

Minimum Biocidal Concentration (MBC)

To determine the MBC, 100 µL was taken from each well in which there was no perceptible bacterial growth to the naked eye and inoculated onto three Petri dishes containing Muller Hinton agar. Subsequently, plates were taken to the oven, where they remained at 37 ºC for 24 h and then analyzed for the presence or absence of bacterial growth (Santurio *et al.,* 2007).

RESULTS AND DISCUSSION

Sensitivity to antimicrobials

Out of the 50 analyzed chicken livers, a total of 17 *E. coli*, 2 *Streptococcus* spp. and 1 *P. aeruginosa* were isolated. The susceptibility profile of microorganisms isolated from chicken liver showed that the three microorganisms isolated are resistant to at least four antibiotics tested (amoxicillin, cephalexin, gentamicin, and tetracycline). Results for *E. coli* showed that 50% strains showed resistance to six of the eight antibiotics tested, as seen in TABLE 1.

Table 1 - Results of the absolute and relative frequency of the antimicrobial resistance test of samples of *E. coli*, *Streptococcus* spp. and *P. aeruginosa* isolated from broiler liver

Variables	*E. coli* N (%)	*Streptococcus* spp. N (%)	*P. aeruginosa* N (%)
Amoxicillin	11/17 (64.71)	1/2 (50.00)	1/1 (100.00)
Cephalexin	10/17 (58.82)	1/2 (50.00)	1/1 (100.00)
Ciprofloxacin	5/17 (29.41)	0/2 (0.00)	0/1 (0.00)
Enrofloxacin	10/17 (58.82)	0/2 (0.00)	0/1 (0.00)
Gentamicin	10/17 (64.71)	2/2 (100.00)	0/1 (0.00)
Sulfa Trimethoprim	8/17 (47.06)	0/2 (0.00)	0/1 (0.00)
Sulfonamides	11/17 (64.71)	0/2 (0.00)	1/1 (100.00)
Tetracycline	14/17 (82.35)	2/2 (100.00)	1/1 (100.00)

Probabiity	0.089	0.217	1.000

Source: own author (2024).

The pathogens in question can readily develop a mechanism of resistance to the most common antimicrobials used and the broiler is a relevant source of propagation of antimicrobial resistance to samples of *E. coli*, *Streptococcus* spp. and *P. aeruginosa*. The careless use of these drugs leads to the natural selection of more resistant pathogens, with a future loss of effectiveness of the drug in the occurrence of future infections by this pathogen, given that this resistance can be transferred to other microorganisms, and thus impair the treatment of bacterial infections (Wang *et al.*, 2016), not only in animals, but also in humans, causing public health concern.

Evidently, due to the presence of microorganisms resistant to antimicrobials used in the poultry farming routine, such drugs should be used with greater discretion, only in the treatment of infections and not as growth promoters. These precautions would thus avoid triggering allergies, resistance, and toxicity to humans, in addition to demonstrating the need to study new compounds with antibiotic potential.

Phytochemical screening

Reactions were positive for the following constituents: flavonoids, tannins, anthraquinones, coumarins, triterpenoids, resins and saponins. This result corroborates what has been described by other authors, such as Uboh *et al.* (2010), Dhiman *et al.* (2011), Castilho *et al.* (2014), Cruz *et al.* (2019), Pérez-Balladares *et al.* (2019) when studying plants of the genus *Psidium*, and Durães *et al.* (2017), who used *P. myrsinites* in their studies.

Dosages

The content of phenolic compounds, tannins and flavonoids of the acetone and ethanol extracts are listed in Table 2.

Table 2 - Mean content of phenolic compounds, tannins and flavonoids in the acetone and ethanol extracts of *Psidium myrsinites* leaves ($mg.g^{-1}$).

Samples	**Phenolic compounds** ($mg.g^{-1}$)	**Tannins** ($mg.g^{-1}$)	**Flavonoids** ($mg.g^{-1}$)
Acetone	11.17 ± 2.66	11.51 ± 0.39	20.03 ± 2.93
Ethanol	10.50 ± 1.66	16.98 ± 0.89	17.01 ± 1.18

Source: own author (2024).

Essential oil constituents

The analysis of the chemical composition of the essential oil indicated the presence of 19 substances, which are described in Table 3, according to their percentage present in the essential oil.

Table 3 - Constituents of the essential oil of *Psidium myrsinites*, percentage in the sample, and retention indexes

Constituent	%	RI lit	RI exp
NI	1.11	-	815
Myrcene	0.76	988	993
E-caryophyllene	28.03	1417	1421
α-humulene	22.11	1452	1455
β-selinene	3.54	1489	1487
Cis-β-guaiene	1.37	1492	1488
α-selinene	3.70	1498	1496
γ-cadinene	1.60	1513	1515
δ-cadinene	2.68	1522	1525
Caryophyllene oxide	5.50	1582	1585
Viridiflorol	11.28	1592	1595
Humulene epoxide II	5.66	1608	1612
Allo-aromadendrene epoxide	2.91	1639	1637
Caryophylla-4(12), 8(13)-dien-5α-ol	1.61	1639	1641
α-muurolol	3.24	1644	1645
α-cadinol	4.02	1652	1659
NI	0.88	-	-
Total identification	98.01		
Class			
Hydrocarbon monoterpenes	0.76		
Oxygenated monoterpenes	-		
Hydrocarbon sesquiterpenes	63.03		
Oxygenated sesquiterpenes	34.22		

NI – Not identified; RI lit – Retention Index according to [31]; RI exp – Retention Index expressed in GC-MS analysis.

Source: own author (2024).

The method used for extracting the essential oil provided a yield of 1.6% and the major compounds are the hydrocarbon sesquiterpenes *E*-caryophyllene and α-humulene, with 28.03 and 22.11%, respectively.

Artemia salina toxicity assay

Using the Probit test, it was not possible to obtain the LC50 of the crude ethanol extract (EBE) and of the essential oil (EO), because the data distribution was not linear. For this reason, data were linearized using the equation of the line to predict the lethal concentration at 50% of the two compounds.

The analyses indicated that the AFEE showed low toxicity, with an LC50 of 717 $\mu g.mL^{-1}$. The ECE, EEFEA, CFEE, CAE extracts and the OE showed moderate toxicity, with LC50 of 475, 312, 194, 230 and 143 $\mu g.mL^{-1}$, respectively, as described in Table 4.

Table 4 - LC50 and degree of toxicity of the oil and extracts of *Psidium myrsinites*

Samples	Toxicity	LC50 ($\mu g.mL^{-1}$)
AFEE	Low	717
ECE	Moderate	475
EEFEA	Moderate	312
CFEE	Moderate	194
CAE	Moderate	230
EO	Moderate	143
P	__	0.001

AFEE: Aqueous Fraction of the Ethanol Extract; ECE: Ethanol Crude Extract; EEFEA: Ethanol Extract Fraction of Ethyl Acetate; CFEE: Chloroform Fraction of the Ethanol Extract; CAE: Crude Acetone Extract; EO: essential oil.

Source: own author (2024).

Toxicity was measured according to Amarante *et al*. (2011), where compounds with LC50 greater than 1,000 $\mu g.mL^{-1}$ are considered non-toxic, LC50 above 500 $\mu g.mL^{-1}$ indicates low toxicity, LC50 between 100 and 500 $\mu g.mL^{-1}$ suggests moderate toxicity and LC50 below 100 $\mu g.mL^{-1}$ indicates that the compound is very toxic.

Antibacterial activity

The results of the minimum inhibitory concentration test allow a good classification of the antibacterial activity, as shown in Table 5.

Table 5 - Minimum inhibitory concentration (μg.mL^{-1}) of compounds against the microorganisms evaluated.

Samples	*E. coli*	*Pseudomonas aeruginosa*	*Streptococcus spp.*	*E. coli ATCC 25312*	*S. aureus ATCC 25923*
AFEE	500	500	500	>2,000	500
ECE	250	500	125	>2,000	125
EEFEA	125	500	125	>2,000	250
CFEE	500	500	125	>2,000	250
CAE	500	500	125	>2,000	250
EO	500	500	125	>2,000	500

Source: own author (2024).

The MIC results showed no significant difference between the different treatments, as the *p* value was greater than 0.05 (p= 0.594 for *E. coli*, p= 0.245 for *P. aeruginosa*, p= 0.091 for *Streptococcus* spp., p=1.021 for *E. coli* ATCC 25312 and p=0.365 for *S. aureus* ATCC 25923).

According to the classification by Holett *et al.* (2002), the extracts and essential oil, tested in this experiment, showed moderate antibacterial activity against the three microorganisms isolated from broilers, while in ATCC strains used as controls, the antibacterial activity for *E. coli* was nil and for *S. aureus*, moderate. These results corroborate Dhiman *et al.* (2011), Alvarenda *et al.* (2015) and Afonso *et al.*, (2018), which confirmed the antibacterial activity of the extract of plants belonging to the genus *Psidium*, the genus of the plant investigated herein.

With respect to the essential oil of *P. myrsinites*, it showed moderate antibacterial activity against microorganisms isolated from broilers and against *S. aureus* ATTC. Similar results were observed by Silva *et al.* (2019) when identifying the same major compounds in Psidium guajava L. and when observing moderate antibacterial activity against *Streptococcus*. According to Moreira *et al.* (2014), this activity can be explained by the chemical constituents of known antibacterial activity, such as E-caryophyllene and α-humulene, with 28.03 and 22.11% present in the oil. Several mechanisms are proposed to explain the antimicrobial activity of essential oils. It is believed that microbial growth inhibition by essential oils is due to the direct damage to cell membrane integrity caused by their lipophilic components, which affects cell pH maintenance and inorganic ion balance. According to the literature, the inhibitory effects of essential oils are consistent with the action of monoterpene and sesquiterpene constituents on the cell membrane, and the damage to membrane produces different effects on microorganisms (Oliveira *et al.*, 2016).

CONCLUSION

Despite the moderate antibacterial activity observed for the studied extracts, none showed bactericidal activity at the concentrations tested, which indicates that the MIC was greater than 1,000 µg.mL^{-1}. However, the results found for the strains isolated from broilers show the potential of using *P. myrsinites* extracts or oil in animal production, aiming at reducing or delaying bacterial resistance.

The susceptibility profile of microorganisms isolated from broiler liver showed that the three microorganisms (*E. coli*, *Streptococcus* spp. and *P. aeruginosa*) are resistant to at least four tested antibiotics (amoxicillin, cephalexin, gentamicin, and tetracycline). The results showed that out of the 17 strains of *E. coli*, 50% showed resistance to six out of the eight antibiotics tested.

The aqueous fraction of the ethanol extract showed low toxicity, while the other extracts and *P. myrsinites* essential oil showed moderate toxicity. Despite not showing biocidal activity, the extracts and oil show good antibacterial activity for microorganisms isolated from broilers, with minimum inhibitory concentrations ranging between 125 and 500 µg.mL^{-1}.

Our findings demonstrate the antibacterial activity of *P. myrsinites* leaves, indicating their potential in combating pathogenic bacteria, highlighting the need for further studies aimed at elucidating and isolating the substances responsible for this activity.

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