DOI: 10.5433/1679-0359.2021v42n1p179

Antibiotic resistance profile of gram-negative bacteria isolated from dog nasal swab samples, and antibacterial and antioxidant activities of aqueous extracts of *Alpinia purpurta* (Vieill.) K. Schum (Zingiberaceae)

Perfil de resistência a antibióticos de bactérias gram-negativas isoladas de amostras de swab nasal de cães e atividades antibacteriana e antioxidante de extratos aquosos de Alpinia purpurta (Vieill.) K. Schum. (Zingiberaceae)

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Highlights

An extract of *A. purpurata* rhizomes showed higher antioxidant capacity than *A. purpurata* leaf extract.

A high rate of resistance to β-lactams was found for gram-negative bacteria isolated from nasal swabs of dogs.

Aqueous leaf extracts have high levels of total phenolic compounds.

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Received: Mar. 22, 2020 - Approved: July 02, 2020

Abstract

The indiscriminate use of antibiotics in veterinary medicine and their negligent use among dog owners have contributed to the rise of antibiotic resistance in microorganisms found in pets. In addition, the search for medicinal plants with antibacterial properties has made the evaluation of aqueous extracts of *Alpinia purpurata* (Vieill.) K. Schum an important issue. Thus, the aim of this work was to determine the antibiotic resistance profile of gram-negative bacteria isolated from nasal swab samples of dogs and assess the antibacterial activity of the aqueous extracts of leaves and rhizomes of *A. purpurata*. The bacteria identified were tested using the agar disc diffusion assay for the evaluation of antibiotic resistance. A total of 16 isolates were obtained from the 19 samples collected, with a high prevalence of *Escherichia coli* (n=5). There was a high rate of resistance to β-lactams, where the highest percentage was seen for amoxicillin (72.5%). Aqueous leaf extracts had high levels of total phenolic compounds (637.47 µg GAE mg^{-1}), differing significantly ($p < 0.05$) from aqueous rhizome extracts (228.64 µg GAE mg^{-1}). There was no significant difference in EC50 of DPPH values between the aqueous extracts; however, the antioxidant capacity of rhizome extracts had higher values than leaf extracts. The minimum inhibitory concentration (MIC) of leaves and rhizomes for the evaluated bacteria ranged from 9000 to 32,000 µg mL^{-1}. For the minimum bactericidal concentration (MBC), most bacteria showed an MBC over 38,400 µg mL^{-1} for the rhizome. In conclusion, the bacteria isolated from dog nasal swabs showed a high resistance profile for the antibiotics of the penicillin class. Additionally, the results from the analysis of the aqueous extracts of rhizomes and leaves of *A. purpurata* showed an antimicrobial effect possibly associated with a high content of total phenolic compounds; these results can create a scope for using these extracts together with conventional antibiotics to control the emergence of antibiotic resistance among microbial species.

Key words: Antimicrobial. *Escherichia coli.* Ginger. *Serratia liquefaciens*. *Hafnia alvei. Pantoea agglomerans*.

Resumo

O uso indiscriminado de antibióticos na Medicina Veterinária e negligência de tutores de cães no uso adequado contribuíram no aumento de resistência em animais de companhia. Além disso, a busca por plantas medicinais que tenham efeito antibacteriano tornou importante a avaliação de extratos aquosos de *Alpinia purpurata* (Vieill.) K. Schum. Dessa forma, o objetivo do trabalho foi determinar o perfil de resistência aos antibióticos de bactérias Gram-negativas isoladas de amostras de swabs nasais de cães e verificar a atividade antibacteriana dos extratos aquosos das folhas e dos rizomas de *A. purpurata*. As bactérias identificadas foram submetidas à técnica de disco difusão em ágar para avaliação da resistência aos antibióticos. Das 19 amostras colhidas, foram isoladas 16 bactérias, com maior prevalência para *Escherichia coli* (n=5). Verificou-se um alto índice de resistência aos β-lactâmicos testados, com maior percentual para amoxicilina (72,5%). Os extratos aquosos das folhas apresentam elevados teores de compostos fenólicos totais (637,47 µg GAE mg^{-1} extrato), sendo diferente ($p < 0,05$) dos extratos aquosos dos rizomas (228,64 µg GAE mg^{-1} extrato). Não houve diferença significativa nos valores de EC_{50} _ DPPH para os extratos aquosos, entretanto, para a capacidade antioxidante FRAP, os extratos dos rizomas de *A. purpurata* demonstraram maiores valores quando comparados com os extratos aquosos das folhas. A Concentração Inibitória Mínima (CIM) das folhas e rizomas para as bactérias avaliadas variaram entre 9.000 a 32.000 µg mL^{-1}. Para a Concentração Bactericida Mínima (CBM), a maioria das bactérias apresentaram CBM acima de 38.400 µg

mL^{-1} para o rizoma. Conclui-se que as bactérias isoladas de *swab* nasal de cães apresentam alto perfil de resistência aos antibióticos da classe das penicilinas, no entanto, os resultados dos extratos aquosos dos rizomas e das folhas de *A. purpurata* sinalizam efeito antimicrobiano possivelmente associado ao elevado teor de compostos fenólicos totais, podendo ser utilizado em conjunto aos antibióticos convencionais, reduzindo assim sua resistência.

Palavras-chave: Antimicrobianos. *Escherichia coli*. Gengibre. *Serratia liquefaciens*. *Hafnia alvei*. *Pantoea agglomerans*.

Introduction

Antibiotics are drugs that have revolutionized the treatment of infectious diseases caused by bacteria and have tremendously reduced morbidity and mortality rates associated with bacterial infections worldwide (Costa & Silva, 2017). However, their indiscriminate use has led to the emergence of antibiotic resistance (Kadwalia et al., 2019; Macedo, 2019), reducing the options of effective drugs for the treatment of several infections, thus increasing the risk of clinical complications in patients (Costa & Silva, 2017).

Studies have reported the transmission of several multiresistant bacteria between humans and animals (Drougka et al., 2016; Leite-Martins et al., 2015; Wendlandt et al., 2015), and dogs and cats represent a potential source for the spread of antibiotic resistance, due to the wide use of these agents in veterinary routine and the close contact between them and humans (Leite-Martins et al., 2014; Wieler, Ewers, Guenther, Walther, & Lubke-Becker, 2011).

Bacterial resistance occurs when a bacterium acquires or alters its genes, thus managing to interfere with the mechanism of action of the antibiotic. It may occur by the spontaneous mutation of DNA or by transformation and transfer of plasmids (Bozdogan et al., 1999).

There are many examples of increased antimicrobial resistance among microorganisms found in various animal species. This is worrisome because many of these microorganisms have become resistant to antimicrobials used in human medicine (Bahr Arias & Carrilho, 2012), and because bacteria serve as reservoirs of resistant genes and could transfer this resistance to human-adapted pathogens or to the human gut microbiota via direct contact, food or the environment (Argudín et al., 2017).

Natural antimicrobials found in medicinal plants can act against infections caused by microorganisms. In recent years, several medicinal plants and spices have attracted research interest due to their medicinal potential (Manandhar, Luitel, & Dahal, 2019). Bioactive compounds isolated from various parts of plant species are showing a boost in new scientific research in the fields of biochemistry, pharmacology, and medicine for the development of new alternative therapeutic agents (Ghosh & Rangan, 2013).

Plants of the family Zingiberaceae have garnered special attention because of their ability to produce many bioactive compounds used in the pharmaceutical and cosmetic industries as antioxidants and antimicrobials (Chan & Wong, 2015).

Among the species of this family, the medicinal potential of *Alpinia purpurata* (Vieill.) K. Schum, popularly known as red ginger (used as an ornament), has been investigated. Different parts of this plant produce bioactive compounds (phenolic compounds) with therapeutic properties (Chan & Wong, 2015; Sumabranian & Suja, 2011) and they have been recognized as safe by the American Food and Drug administration (FDA) (Azizi et al., 2015).

Al-Enazi (2018) evaluated the acute toxicity (LD_{50}) of the aqueous extract of *A. purpurata* and demonstrated that up to 5000 mg kg^{-1} did not produce any symptoms of acute toxicity in rats. Thus, this species can be used in the treatment of infections.

Different extracts of red ginger leaves, rhizomes, and roots have demonstrated antioxidant and antimicrobial activities associated with their bioactive compounds (Ghosh & Rangan, 2013; Kona, Thofeeq, & Venkata, 2015; Sahoo, Singhi, & Nayak, 2014; Soares et al., 2018; Villaflores et al., 2011).

Thus, the aim of this work was to evaluate the antimicrobial resistance profile of gram-negative bacteria found in dog nasal swabs, and to determine the antibacterial and antioxidant activities of the aqueous extracts of the leaves and rhizomes of *A. purpurata* obtained from the Medicinal Garden of the Paranaense University, Umuarama-PR.

Materials and Methods

This project was approved by the Research Ethics Committee Involving Animal Experimentation (Comitê de Ética em Pesquisa Envolvendo Experimentação Animal [CEPEEA]) of the Paranaense University under protocol 32876/2018.

Nineteen nasal swab samples were obtained from dogs belonging to students of the veterinary medicine course of the Paranaense University, UNIPAR. To determine the number of samples, the total number of veterinary medicine students enrolled (N = 181) in the year of 2017 was taken into account, which was 10% of the students.

Isolation and identification of gram-negative bacterial strains

The 19 nasal swabs were collected according to the following procedure: the swab was first moistened with Stuart's transport medium and then introduced into the animal's nasal orifice, pushing it with rotating movements, and the swab was then removed and stored in the transport medium and sent to the Laboratory of Preventive Veterinary Medicine and Public Health of the Post-Graduate Program in Animal Science of the Paranaense University.

Tubes containing 3.0 mL of brain heart infusion (BHI) medium were inoculated with swabs and kept in a microbiological incubator for 24 h 37ºC. The cultures obtained streaked on MacConkey agar and incubated at 37ºC for 24 h for isolation of single colonies. The colonies with higher prevalence were isolated and then submitted to macroscopic observation (colony characteristics) and microscopic characteristics according to the methodology described by Quinn, Carter, Markey and Carter (1994).

The biochemical identification of bacteria belonging to the order Enterobacteriales was made using the following tests: sulfide indol motility - SIM, triple sugar iron agar – TSI, lysine iron agar –

LIA, Simmons citrate agar and urea broth. For storage, all the selected single colonies were grown in BHI medium and then stored in 80% glycerol at -20ºC.

Phenotypic antibiotic sensitivity tests

The agar disc diffusion assay was used according to the recommendations of the Clinical and Laboratory Standards Institute [CLSI] (2018).

Twelve antibiotics of the following classes were tested: β-lactams: ampicillin (10 µg), amoxicillin (10 µg) and amoxicillin + clavulanate (20/10 µg); cefoxitin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg) and ceftazidime (30 µg); aminoglycosides: gentamicin (10 µg) and amikacin (30 µg); fluorquinolones: ciprofloxacin (5 µg) and enrofloxacin (10 µg); and sulfonamide: sulfazotrim (25 µg).

Botanical material

Botanical material was collected from Horto Medicinal – UNIPAR campus, Umuarama-PR, (geographical coordinates 23°45"44.9" S and 53°16"17.5" W) located in the city of Umuarama, northwestern region of PR, and was deposited in the Horto Medicinal Herbarium of Campus II of UNIPAR, under registration No. 344.

The rhizomes and leaves of *A. purpurata* were collected during April 2017. The leaves and rhizomes were washed under running water, dried with paper towels and immediately dried further in a forced circulation oven at 35 °C for 20 days (leaves) or 40 °C for 20 days (rhizomes). The rhizomes were then cut into slices and the leaves left whole. The dried leaves and rhizomes were ground in an industrial blender (bench) until powder was obtained (Otunola, Oloyede, Oladiji, & Afolayan, 2014).

The powder samples of rhizomes and leaves were subjected to aqueous extraction according to the method described by Otunola et al. (2014) with some modifications. A total of 40 g of each powder sample was added to 800 mL (5% w v^{-1}) of boiling distilled water (95 - 100 °C) under stirring for 10 min. The aqueous extract was filtered and freeze-dried for 48 h, and the dried extract was stored in airtight glass bottles in the freezer (- 20 °C) for further analysis.

Determination of total phenolic compounds

The concentration of total phenolic compounds in aqueous extracts (leaves and rhizomes) was determined by the *Folin-Ciocalteu* method (Singleton & Rossi, 1965), with gallic acid (GA) used as reference, and the results were expressed in mg mL^{-1} of GA equivalents (mg GAE mL^{-1}).

Antioxidant activity DPPH test

The sequestrating activity of the free radical 1.1 diphenyl 2 picrylhydrazil (DPPH) was performed according to Pyrzynska and Pekal (2013). The sequestrating activity was expressed as % of free radical sequestration efficiency, i.e., % = (1 - Sample/Control) x 100.

The synthetic butylhydroxytoluene antioxidant BHT (0.2 mg mL^{-1}) was used as the positive control. The results were expressed as EC50 (concentration of extract expressing 50% antioxidant activity – mg mL^{-1}), which was obtained by interpolation of linear regression analysis.

Total antioxidant capacity FRAP test

The ferric reducing antioxidant power (FRAP) assay was performed according to Lim & Lim (2013). The standard used was Trolox® (Sigma) and results were expressed in nmol Trolox equivalents mg^{-1} extract.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The determination of the minimum inhibitory concentration (MIC) was performed in accordance with the microdilution assay (CLSI, 2018).

Different concentrations (600, 1200, 2400, 4800, 9600, 19,200, 38,400 and 64,000 µg mL^{-1}) of aqueous extracts of rhizomes and leaves of *A. purpurata* were prepared following the methods of Kuete (2010) for the determination of MIC. The MIC assays were performed in triplicate. After incubation at 37 ºC 24 h, 10 µL of 10% triphenyltetrazolium 2.3.5-chloride (TTC) developer were added, and the microplates were incubated again for 30 min. Samples with any pink tint (occurrence of bacterial growth) were considered positive. MIC was defined as the lowest concentration of the extract, in µg mL^{-1}, capable of preventing bacterial growth (Barbosa et al., 2014; Bona, Pinto, Fruet, Jorge, & Moura, 2014).

Minimum bactericidal concentration (MBC) was determined using the microdilution broth method in 96-well polystyrene microplates (CLSI, 2013). A sample of the content of each of the 96 wells was streaked on a plate containing Müeller Hinton agar, with the aid of a replicator, and the plates incubated at 36 ºC ± 0.5 for 24 h. MBC was determined by the absence of visible bacterial growth after the incubation period.

Statistical analysis

Graphs were prepared to express the absolute (n) and relative (%) frequency of isolated bacteria and resistance to the main antibiotics evaluated. The results of the determination of total phenolic compounds and antioxidant activity (DPPH and FRAP) of rhizome and leaves of *A. purpurata* were expressed as mean ± standard error. The results of the MIC of rhizome and leaves the bacteria tested were expressed as the mean. The differences (rhizome and leaves) were compared by the Student t-test for independent samples at a 5% significance level using the Bioestat 5.0 statistical program (Ayres, Ayres, Ayres, & Santos, 2007). The results of MBC were expressed as the absolute frequency (n) for each single species for concentrations above 38,400 and below 64,000 µg mL^{-1}.

Results and Discussion

From the 19 nasal swab samples obtained from dogs belonging to veterinary medicine students, 16 isolates showed growth on MacConkey agar. The 16 isolated bacteria were identified as belonging to four species, with a higher prevalence of *Escherichia coli* (*E. coli*) at 31.25% (5/16) (Figure 1).

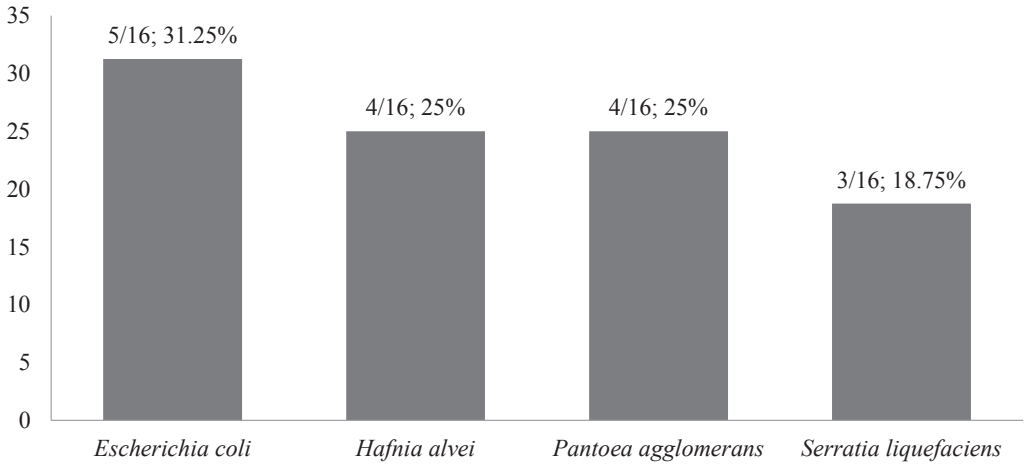

Figure 1. Number and percentage of the 16 enterobacterial isolates from dog nasal swabs taken by students of the veterinary medicine course of the Paranaense University, UNIPAR, 2018.

Among the antibiotics studied, 11/16 (68.75%) of the isolates were resistant to amoxicillin, 10/16 (62.5%) resistant to ampicillin and 10/16 (62.5%) resistant to amoxicillin combined with clavulanic acid. The other antibiotics tested showed 100% sensitivity.

When the level of resistance of enterobacterial isolates was evaluated, it was evident that *Serratia liquefaciens* was 100% resistant to the three antibiotics: amoxicillin, ampicillin and amoxicillin + clavulanate (Figure 2).

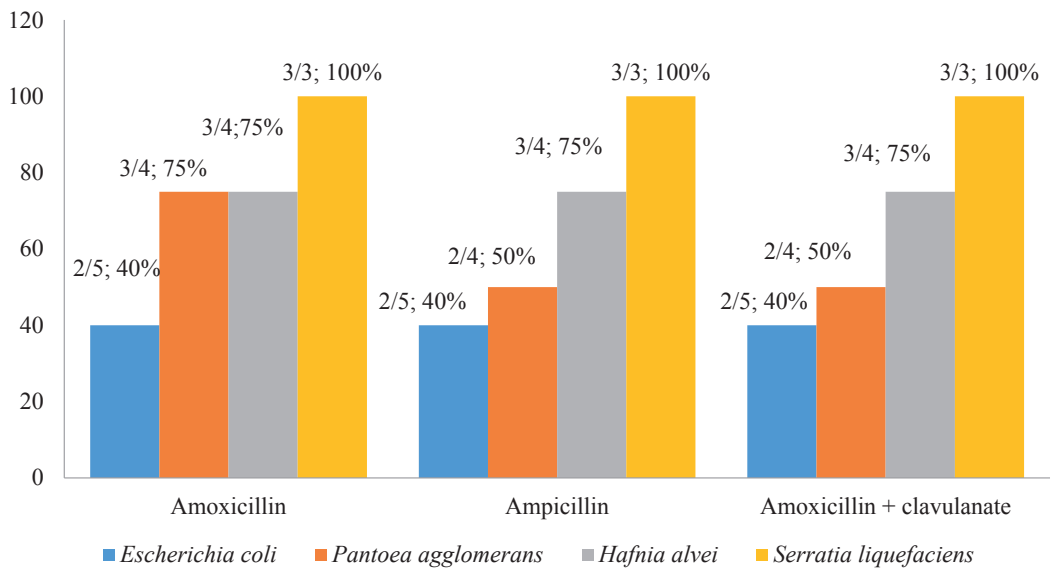

Figure 2. Number and percentage of resistant strains among 16 enterobacterial isolates from dog nasal swab samples taken by students of the veterinary medicine course at the Universidade Paranaense - UNIPAR.

Among the four bacterial species isolated, three are classified as having intrinsic resistance to antibiotics of the penicillin class. They have the characteristics and genes for resistance to the antibiotics used in research, since according to Del Fio, Matos and Groppo (2000), intrinsic resistance is part of the natural/phenotypic characteristics of the microorganism; i.e., it is part of its genetic inheritance, which is transmitted vertically to the offspring without loss of the characteristic and without any risk to therapeutic treatment (Del Fio et al., 2000). Moreover, it has been reported that there is a high percentage of beta-lactam-resistant Enterobacteriaceae in feces of dogs (Dupouy et al., 2019) and canine rectal swabs (Urumova, 2019).

A study evaluating a community pharmacy in the state of Paraíba (Paula, 2014) and a study by Lima, Gallani, Toledo and Lopes (2008) in a company that used a pharmaceutical benefit management system in the region of Campinas, SP, showed that amoxicillin was the most prescribed drug. This suggests that the high resistance profile may be associated with its use without criteria.

The concentration of total phenolic compounds in the aqueous extracts of the leaves and rhizomes of *A. purpurata*, determined using the *Folin-Ciocalteu* reagent, is shown in Table 1. The results showed that the aqueous leaf extracts have high contents of these compounds (637.47 ± 6.80 µg GAE mg^{-1} extract), being statistically different ($p < 0.05$) from the aqueous extracts of rhizomes (228.64 ± 10.17 µg GAE mg^{-1} extract), which could explain the greater antioxidant activity for the aqueous extract of leaves, since several studies have indicated that antioxidant activity is directly related to the total content of phenolic compounds produced by plants (Cutrim, Teles, Mouchrek, Mouchrek, & Everton, 2019), which act mainly in the sequestration of free radicals (Sahoo et al., 2014).

Table 1
Mean ± standard error of mean of concentrations of total phenolic compounds in aqueous extracts of rhizomes and leaves of *A. purpurata*

A. purpurata	Total phenolic compounds (µg GAE/mg extract)
Rhizomes	228.64 ± 10.17[a]
Leaves	637.47 ± 6.80[b]

GAE: gallic acid equivalents (Sigma standard). Data expressed as mean ± SEM (n = 4). Means followed by different letters in the column differ according to the t-test for independent samples at the 5% significance level.

According to Shareef, Muhammed, Hussein and Hammed (2016), plants produce different chemically heterogeneous groups of these phenolic compounds, which, according to their chemical diversity, have various functions.

Regarding the results of the antioxidant activity measured by the DPPH and FRAP methods, there was no significant difference in the EC50 of DPPH values for the aqueous extracts of the rhizomes and leaves of *A. purpurata* (Table 2). However, for the total antioxidant capacity (FRAP), rhizome extracts showed a higher mean ($p<0.05$) compared to leaf extracts *(*Table 2).

Table 2
EC50–antioxidant activity (DPPH) and total antioxidant capacity (FRAP) of aqueous extracts of rhizomes and leaves of *A. purpurata*

A. purpurata	DPPH EC50 (mg/mL)*	FRAP nmols eq. Trolox/mg extract
Rhizomes	0.062± 0.05	45.40 ± 0.64a
Leaves	0.063± 0.16	38.60 ± 0.42b

The results of DPPH are expressed as EC50 (lowest concentration of extract expressing 50% antioxidant activity). FRAP results are expressed as Trolox equivalents/mg extract in iron reduction. Data expressed as mean ± SEM (n = 4). * not significant according to Student's t-test for independent samples at the 5% significance level. Means followed by different letters in the column differ according to Student's t-test for independent samples at the 5% significance level.

The MIC of leaf extracts of *A. purpurata* varied from 18,400 µg mL^{-1} to 32,000 µg mL^{-1} and the MIC of rhizome extracts varied from 9,000 to 21,333 µg mL^{-1} against the isolated bacteria (Table 3). Only for *Hafnia alvei* was the MIC of rhizome extract significantly lower (9,000 µg mL^{-1}) than that of leaf extract (32,000 µg mL^{-1}) (Table 3).

Table 3
Mean minimum inhibitory concentrations (MICs) (µg mL^{-1}) of aqueous extracts of *A. purpurata* leaves and rhizomes against bacteria isolated from dog nasal swabs

Isolated bacteria	Leaves µg/mL	Rhizomes µg/mL
Enterobacter agglomerans	18,400	21,333
Escherichia coli	21,120	10,666
Hafnia alvei	32,000[a]	9000[b]
Serratia liquefaciens	21,333	9600

Means followed by different letters in the row differ according to Student's t-test (P<0.05).

A. purpurata has been investigated for its medicinal potential, and it has been found that various parts of the plant (rhizomes, leaves, flowers and roots) produce bioactive compounds with therapeutic efficacy. The bioactive compounds produced can act as antimicrobial agents and antioxidants (Soares et al., 2018), and show anticancer, anti-inflammatory and neuroprotective effects (Ghosh & Rangan, 2013), demonstrating the importance of their evaluation against microorganisms isolated from dogs.

The difference in MIC between *A. purpurata* rhizome extract (9000 µg mL^{-1}) and leaf extract (32,000 in µg mL^{-1}) (Table 3) against *Hafnia alvei* suggests a mechanism of antimicrobial action (interaction with the bacteria cell wall) (Romaniuk & Cegelski, 2015; Soares et al., 2018). Although the content of total phenolic compounds was higher in leaf

extract, MIC was significantly lower in the rhizome extract. The bioactive compounds present are biochemically diverse (Shareef et al., 2016), so it is possible that in this study, antibacterial activity was related to other bioactive compounds not only phenolic compounds.

For *A. purpurata* rhizome, it was not possible to determine MIC for some bacteria, since the result was above the highest concentration assessed, i.e., above 64,000 µg mL^{-1}. These bacteria included a strain of *Enterobacter agglomerans*, two strains of *E. coli* and a strain of *Serratia liquefaciens*. This variation in the MIC may be related to the difference in the isolated bacterial strain itself, even whether they belong to the same species.

The results of the present work corroborated Kona et al. (2015). These authors evaluated the antibacterial activity of the ethanolic extracts of leaves, rhizomes and roots of *A. purpurata* by the disc diffusion method against *E. coli*. The results showed that leaf and root extracts were more effective at inhibiting bacterial growth, showing that, most likely, the solvent used in the extraction (ethanol) enabled a high content of phenolic compounds (Kona et al., 2015), which in turn, enabled this greater inhibition.

However, the methanolic extracts from the leaves of several *Alpinia* species showed no antibacterial activity (disc diffusion method) against *E. coli* and *Staphylococcus aureus* in the study conducted by Wong, Lim and Omar (2009). In that study, there was a high content of total phenolic compounds (1189 mg GAE $100g^{-1}$) and consequent antioxidant activity, where DPPH was EC50: 0.3–0.4 mg mL^{-1} (values above those found in this study -Table 2), but without inhibition of *E. coli* growth. These results might have been due to the distinct characteristics of the cell wall of gram-negative bacteria, which hinder the interaction of bioactive compounds (phenolic compounds) in inhibiting bacterial growth (Wong et al., 2009) and the method of evaluation (disc-diffusion) that was used in that study.

With regard to MBC, there was great variation among the isolated strains. However, for the rhizome, most strains had MBC above 38,400 µg mL^{-1}, which differed from the leaves, in which most strains had MBC above 64,000 µg mL^{-1}, although the content of total phenolic compounds was higher in the aqueous extracts of *A. purpurata* leaves than rhizomes. According to Shareef et al. (2016), the bioactive compounds present in *Gingiber officinale* Roscoe are biochemically diverse, which could explain the variations in bactericidal activity.

According to Chan and Wong (2015), *A. purpurata* was not sufficiently studied, where there are just two compounds identified in the rhizome, five in leaves and none in flowers. To establish and understand the efficacy of antimicrobial agents (bioactive compounds) of A. *purpurata*, studies to identify and quantify bioactive compounds are necessary to agree with the understanding of the mechanisms of biological actions. However, Al-Enazi (2018) found that the extract of *A. purpurata* showed a low degree of acute toxicity (LD_{50}) in rats and that it could be used in the treatment of microbial infections.

The results of this work demonstrate the importance of evaluating the antibacterial activity of plants, such as the aqueous extracts of rhizomes and leaves of *A. purpurata* against gram-negative bacteria isolated from dogs

that showed resistance to antibiotics of the β-lactam class, which according to Negi (2012), may become a good alternative to conventional antibiotics due to the ability of the bioactive compounds studied here, which are deemed safe by the FDA, to inhibit the growth of pathogenic microorganisms (Azizi et al., 2015).

Conclusion

The isolated bacteria had a high antibiotic resistance profile against compounds belonging to the penicillin class; however, it should be realized that among the four bacterial species found, three showed intrinsic resistance. The results of this study indicate a potential antibacterial effect of aqueous extracts of rhizomes and leaves of *A. purpurata* (Vieill). K. Schum (Zingiberaceae). This potential is related to high levels of bioactive compounds (total phenolic compounds), and these extracts could be used together with conventional antibiotics, which could decrease the need for repeat therapy and also contribute to reducing the emergence of bacterial resistance to antibiotics.

Acknowledgment

This work was carried out with the support of the Executive Board of Research and Post-Graduate Management - DEGPP UNIPAR, and the Coordination for the Improvement of Higher Level Personnel (CAPES) and Brazilian National Council for Scientific and Technological Development (CNPq). Dr. A. Leyva (USA) provided English editing of the manuscript.

Compliance with ethical standards

This project was approved by the Research Ethics Committee Involving Animal Experimentation (CEPEEA) of Paranaense University under protocol 32876/2018. Nineteen samples of nasal swab were collected from dogs from students of the Veterinary Medicine course of the Paranaense University, UNIPAR, after their authorization.

Conflicts of interest

The authors declare no conflicts of interest.

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