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Composition and Antimicrobial Activity of Ginger (*Zingiber officinale* Roscoe)

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Abstract

Zingiber officinale Roscoe has been widely used for hundreds of years, whether for its analgesic, antipyretic, or antimicrobial potential. The aim of this study was to evaluate the composition and the ranges of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous rhizome and leaf extracts of *Z. officinale* and synergism of these extracts with antibiotics against six isolates of *Staphylococcus* spp. The chemical composition of the crude extract from the rhizome of *Z. officinale* cultivated in the region of Umuarama (Brazil) was analyzed by gas chromatography coupled with a mass spectrometer and detected Zingerone (47.65%), α-Zingiberene (14.92%), β-Sesquiphellandrene- (6.16%), α-Curcumene (4.49%), Methyl 10-trans, 12-cis-octadecadienoate (4.42) and Gingerol (4.37). Six samples of *Staphylococcus* spp. from Veterinary Students' nasal swabs were collected for antimicrobial susceptibility tests. The antibacterial activities of the aqueous extracts of ginger rhizome and leaves were evaluated with broth microdilution, and then synergism of the rhizome extract with the antimicrobials was verified to calculate the fractional inhibitory concentration index (FICI). All the *Staphylococcus* spp. samples showed resistance to ampicillin and penicillin, 66.67% to erythromycin, and 33.33% to tetracycline. The $MIC_{90\%}$ of each extract was estimated to be 235 mg/mL and 13.27 mg/mL for the leaf and rhizome, respectively. With the aqueous leaf extract, none of the samples presented MBC within the studied concentration range. On the other hand, the MBC obtained by the aqueous rhizome extract was 7.81 mg/mL for 50% of the samples. Calculation of the FICI showed that ½ MIC yielded the best result, with two synergistic relationships when combined with ampicillin. This research shows a potential use for the aqueous extract of ginger as an alternative or auxiliary therapy against resistant microorganisms.

Keywords: Aqueous extract; E-test; Ginger; *Staphylococcus.*
Abbreviations: FICI_Fractional Inhibitory Concentration Index, MBC_Minimum Bactericidal Concentration, MIC_Minimum Inhibitory Concentration**.**

Introduction

Humans, animals, and plants are large reservoirs of bacteria, which are found lining the skin, mucous membranes, and surface of the gastrointestinal tract in men and animals (Rajapaksha et al., 2019). The *Staphylococcus* spp. is one of the most commonly encountered bacteria in clinical practice as it colonizes the skin of up to 15% of humans and is easily found in the nasal cavities, besides causing infectious diseases ranging from a simple infection (pimples, boils, and cellulitis) to serious infections such as pneumonia, meningitis, endocarditis, toxic shock syndrome, and septicemia (Becker et al., 2014; Saber et al., 2017). This microorganism can also be found in animals, whether production or pet, there are also reports in the literature of their transmission between animals and humans (Kuroda et al., 2016; Van Duijkeren et al., 2011).

From 1950, when antibiotics became widely used, the phenomenon of bacterial resistance began. Since then, the problem of resistance to antibiotics has come to represent considerable importance in public health (Singer et al., 2016) and a major global problem (WHO, 2018).

The misuse of antimicrobials, the wrong prescription by the health professional, the non-compliance with the stipulated therapeutic doses, the abandonment of treatment, among other causes, are cited in the literature as causing bacterial resistance; and it is known that staphylococcal infections are no longer confined to intensive care units, acute care hospitals or any health institution (ANVISA, 2004).

According to WHO (2018) currently, the main public health problem in the world is antimicrobial resistance, and it is caused by the misuse of antimicrobials, faulty prescriptions by health professionals, non-compliance with the prescribed therapeutic doses, and abandonment of the treatment, among others. Additionally, staphylococcus infections are no longer confined to intensive care units, acute care hospitals, or any healthcare facility (ANVISA, 2008).

Medicinal plants are spread all over the world, and they are known to be a rich source of molecules that can therapeutically be exploited. They can be classified according to their medical values: those directly used in therapy, followed by those that constitute the raw material for compounding, and, lastly, those used in the industry to obtain bioactive ingredients or as precursors in semi syntheses (Hasenclever et al., 2017).

Standing out because of its bioactive compounds, the Zingiberaceae family has long been used in diets as seasonings in addition to the pharmaceutical and cosmetic industries as antioxidant and antimicrobial agents. The bioactive compounds of this plant family are of fundamental importance for maintaining "One Health" and can prevent or ameliorate various types of diseases (Chan and Wong, 2015; Soares et al., 2018).

The *Zingiber officinale* Roscoe, popularly known as ginger, is an aromatic plant used by Asians as a condiment and medicinal herb since ancient times. It has been distributed across the continents via spice trade and during the Age of Discovery. It is currently marketed fresh, preserved, crystallized, dried, or powdered (Soares et al., 2018). The ginger rhizome has a slightly flat, elongated body with irregularly fragmented branches, which are 3–16 cm long, 3–4 cm wide, and 2 cm thick. Externally, its coloration ranges from yellow leather to bright brown; it is longitudinally striated, sometimes fibrous, and has endings that obliquely arise from the rhizomes. The endings, also known as "fingers", are flat, obovate, and short extensions of 1–3 cm in length. Starch is the main constituent of the cortex and central cylinder. Internally, it has a yellowish-brown color with fibrovascular bundles scattered over the entire surface. Additionally, there are numerous gray spots and oleoresin cells with yellow contents; a yellow endodermis separates the narrow cortex from the broad stele (Soares et al., 2018).

The rhizome is the most exploited part of the plant. The essential oil of rhizomes is used in the food industry as a flavoring oil and condiment; in the cosmetic industry, it is used as a fragrance and antioxidant, and in the pharmaceutical industry, as an anti-inflammatory, -bacterial, and -tumor agent (Shahrajabian et al., 2019). Tea-making is another very popular use of this part of ginger, and ginger tea is known for its antimicrobial, analgesic, and anti-inflammatory potential, among other benefits (Shahrajabian et al., 2019).

Gingers are recognized as safe by the American Food and Drug Administration (FDA) and have no side effects when consumed in moderate amounts (Azizi et al., 2015). Research shows that in addition to rhizomes, the leaves and flowers of some ginger species have medicinal potential (Chan et al., 2011; Soares et al., 2018).

The aim of this study was to evaluate the composition and the antimicrobial activity using the minimum inhibitory/bactericidal concentrations (MICs/MBCs) of aqueous extracts of *Zingiber officinale* Roscoe as well as the synergistic potential of these extracts with antimicrobials against a standard strain and isolates of *Staphylococcus* spp.

Results

Disk Diffusion Test

According to the disk diffusion test (Table 1), all the samples (100%) showed resistance to ampicillin and penicillin, four (66.67%) were resistant to erythromycin, two (33.33%) were resistant to tetracycline, and one (16.67%) was resistant to gentamicin. However, none of the samples showed resistance to cephalothin, clindamycin, cefotaxime, enrofloxacin, oxacillin, or vancomycin (Table 2).

Extract analysis by gas chromatography and mass spectroscopy

The chemical composition of the crude extract extracted from the rhizomes of *Z. officinale* cultivated in the region of Umuarama (Paraná-Brazil) was analyzed by gas chromatography coupled to a mass spectrometer (GC-MS). In total, 17 compounds were identified, representing 97.45% of the sample's volatile components (Table 3). The major substances identified were Zingerone (47.65%), α-Zingiberene (14.92%), β-Sesquiphellandrene- (6.16%), α-Curcumene (4.49%), Methyl 10-trans, 12 -cis-octadecadienoate (4.42), and Gingerol (4.37).

Evaluation of the antibacterial activity of the extract

Among the *Staphylococcus* spp. Samples tested against the aqueous extracts of *Zingiber officinale Roscoe* leaves, five (83.33%) exhibited MIC of 250 mg/mL, and one (16.67%) had a MIC equal to 125 mg/mL, but none of the six samples presented MBC within the concentration range studied (Table 4).

The results obtained by the aqueous extract of the *Zingiber officinale* Roscoe rhizome were lower, in which four (66.67%) of the samples presented a MIC equal to 7.81 mg/mL, and two (33.33%) samples presented 15.62 mg/mL. As for the MBC, three (50.00%) samples yielded 7.81 mg/mL, two (33.33%) samples had the values of 15.62 mg/mL and 31.25 mg/mL, and another sample's MBC was not lower than 250 mg/mL (Table 5).

The $MIC_{90\%}$ of each extract was calculated, and the values obtained were 235 mg/mL and 13.27 mg/mL for leaf and rhizome, respectively.

Verification of the synergism between the aqueous extract of Z. officinale rhizome and conventional antimicrobials

The samples were classified according to the E-test using the FICI calculation, whereby two synergistic relationships and two additives were obtained from the ratio of ½ of the extract's MIC with ampicillin, whereas there was one synergistic and two additive relationships with oxacillin, and there were three additive relationships with vancomycin (Table 6).

Regarding the results for the tests using ¼ MIC in combination with ampicillin, there were two synergistic and two additive relationships, while the combination with oxacillin had four additive relationships, and the combination with vancomycin had one additive relationship (Table 7).

Discussion

The antimicrobial resistance is a problem involving One Health and tackling this problem is of worldwide interest. Antimicrobial resistance can naturally be caused by environmental selective pressure, presence of resistance genes, self-medication, or faulty prescription of antimicrobials by health professionals (WHO, 2018; Moraes et al., 2016; Tavares, 2000).

Popularly ginger tea made from chunks of the freshly boiled rhizome is used to treat colds, coughs, and colds as it is used in juices and salads. Ginger leaf tea also has good results for antibacterial action, but the concentration of phenolic compounds in rhizome aqueous extract is higher than those found in leaves according to the literature. This study used the aqueous extract of ginger in order to get as close as possible to its popular use, which is in the form of teas, and thus verify its effectiveness as a possible inhibitor of microorganisms as well as the hydroalcoholic extract of the same plant (Shahrajabian et al*.,* 2019; Azizi et al*.,* 2015).

Among the isolates tested, 100% (six) were resistant to two of the β-lactams tested— ampicillin and penicillin. Penicillin resistance was first described in 1944, a few years after its discovery, and it is today well known that *Staphylococcus* spp. are resistant to penicillin and ampicillin, explaining the prevalence of resistance to these antimicrobials among the isolates evaluated in the present study. However, these same isolates were not found resistant to oxacillin and cephalothin, showing that these two antimicrobials of the same class can be used against infections caused by such bacteria (Tavares, 2000).

The high prevalence of penicillin resistance was also observed by Coelho et al. (2007) among the *Staphylococcus* spp. samples he evaluated. He observed that 80.9% of the samples were resistant to ampicillin and penicillin, but 100% of these samples were resistant to oxacillin, contrary to what was found in the present study.

One sample (16.66%) was found resistant to gentamicin in this study. This observation also differs from those of Coelho et al. (2007), who observed that 52.3% of human isolates of *Staphylococcus* spp. were gentamicin-resistant.

Two samples (33.33%) were resistant to tetracycline, a result similar to that found by Lozano et al. (2017), where, in their research with humans who had contact with dogs, 75% of their samples were tetracycline resistant. This result demonstrates a possible transmission of microorganisms and their resistance between both species as already stated by Van Duijkeren et al. (2011).

Menegotto and Picoli (2007) observed 45% erythromycin-resistance among the 40 isolates they studied. This observation is similar to the results of the present study, in which 66.66% of the samples were resistant to erythromycin. Argudín et al. (2015) also observed a high prevalence of erythromycin-resistance, with 43% of the samples showing resistance. Even though erythromycin-resistance was found in human *Staphylococcus* spp. isolates, there are no records showing that this resistance is as prevalent as that observed among the isolates found in animal samples. Argudin et al. (2015), raising the possibility of a microorganism sharing between animals and man.

Although there are several studies that showed a high frequency of bacterial resistance (Coelho et al*.,* 2007; Argudin et al*.*, 2015), in this study, two of six (33.33%) samples are considered multi-resistant. This result reveals the worrisome selection microorganisms have undergone in many ways, besides highlighting the presence of multiresistance in isolates from apparently healthy individuals.

The problem of antimicrobial resistance is of worldwide interest, and the search for new drugs, changes in the strategy to fight these microorganisms, and also the search for medicinal plants that have antimicrobial properties are fundamental for the establishment of alternative therapies with high expectations of success (WHO, 2018; Moraes et al., 2016; Tavares, 2000).

The major compound was found in the extract used for this study, zingerone is a product of the heating of gingerols, and its high presence can be explained by the form of extraction used in this study, in addition to elucidating the antibacterial effects found that may be caused by the presence of gingerols (Azizi et al., 2015; Jardim et al., 2019). Azizi et al. (2015), in his study, found a MIC of 0.02 mg/mL of the ethanolic extract of Z. officinale compared to isolates of *Streptococcus mutans*, a lower result than the present study, such concentration can be explained by the chosen form of extraction, because the ethanolic extraction tends to release more phenolic opposites than aqueous.

Sebiomo et al. (2011) found in their work, using the ginger rhizome, a MIC of 200 mg/mL against an isolate of *Staphylococcus aureus*, which is a value similar to the one found in the present study, considering that the MIC_{90} of these samples was 235 mg/mL. However, another factor that should be noted is the bacterial resistance in the tested microorganisms, which showed an average of three antimicrobial resistances per isolate. Saad et al. (2014) obtained a MIC equal to 100 mg/mL, a value that is lower than the one found in this study, but the tested sample did not show multiresistance either, or the presence of a resistance-conferring gene.

The need for a high concentration of ginger leaf aqueous extract to obtain an antimicrobial activity may be linked to the phytochemical characteristics of the leaves as previously reported. Therefore, for medicinal use, the priority should be given to the parts of the plant that comprise the components with antimicrobial properties (Chand, 2019).

Sebiomo et al. (2011) also verified the MIC of the aqueous extract of ginger rhizome, obtaining a MIC value of 200 mg/mL. Likewise, Saad et al. (2014) obtained a MIC of 100 mg/mL. Both these values are higher than the one found in the present study, which obtained a MIC_{90} of 13.27 mg/mL. This discrepancy may be due to the amount of the phenolic components that may be present in the extracts used in the different studies. However, studies performed with the aqueous extract of this plant are lacking, and thus further studies should be performed to find the action mechanism of this plant against microorganisms.

Many studies have shown that ginger bioactive compounds can be an excellent antimicrobial against several pathogens, especially for Gram-negative and Gram-positive bacteria (Soares et al., 2018; Voravuthikunchai, 2007; Suhad, 2012).

Infectious diseases represent a major cause of morbidity and mortality in the general population. Pharmaceutical companies have been motivated to develop new

Table 1. Resistance profiles of *Staphylococcus* spp. negative coagulase isolated from six nasal swabs from veterinary students at the University of Paraná (UNIPAR), Paraná, Brazil, 2018.

	Antibiotics and Resistance										
	AMP	CFL	CLI	CTX	ENO	ERI	GEN	OXA	PEN	TET	VAN
1	R	S	S	S	S	R	S	S	R	R	S
2	R	S	S	S	S	R	S	S	R	S	S
3	R	S	S	S	S	R	S	S	R	R	S
4	R	S	S	S	S	I	S	S	R	S	S
5	R	S	S	S	S	S	R	S	R	S	S
6	R	S	S	S	S	R	S	S	R	S	S

Legend: AMP - Ampicillin, CFL - Cephalothin, CLI - Clindamycin, CTX - Cefotaxime, ENO - Enrofloxacin, ERI - Erythromycin, GEN - Gentamicin, OXA - Oxacillin, PEN - Penicillin, TET - Tetracycline, VAN - Vancomycin, R - Resistant, S - Sensitive, I- Intermediate.

Table 2. Resistance profiles and multiresistance indexes of the antibiotics used against the *Staphylococcus* spp. negative coagulase isolated from six nasal swabs from veterinary students at the University of Paraná (UNIPAR), Paraná, Brazil, 2018.

Bacterial Resistance			
Antibiotics			Multiresistance Index
	R	%	
Ampicillin	06	100	1
Cephalothin	0	0	0
Clindamycin	0	0	0
Cefotaxime	0	0	0
Enrofloxacin	0	0	0
Erythromycin	04	66.67	0.667
Gentamicin	01	16.67	0.167
Oxacillin	0	0	0
Penicillin	06	100	1
Tetracycline	02	33.33	0.333
Vancomycin	0	0	0

Table 3. Chemical composition of the crude extract extracted from the rhizomes of *Z. officinale* cultivated in the region of Umuarama), Paraná, Brazil, 2018.

Peak	Retention time	Compounds	Relative Area (%)	Calculated RI	Identification methods
1	11.258	Decanal	2.62	1205	a,b,c
2	25.464	α-Curcumene	4.49	1478	a,b,c
3	26.177	α-Zingiberene	14.92	1490	a,b,c
4	26.898	β-Bisabolene	2.76	1502	a,b,c
5	26.981	α-Farnesene	3.27	1504	a,b,c
6	27.695	β-Sesquiphellandrene-	6.16	1518	a,b,c
7	34.398	Zingerone	47.65	1641	a,b,c
8	48.779	Methylhexadecanoate	4.42	1922	a,b,c
9	56.185	Methyl 10-trans,12-cis-octadecadienoate	1.88	2090	a,b,c
10	56.405	10-Octadecenoic acid, methyl ester	1.16	2095	a,b,c
11	59.531	Gingerol	4.37	2237	a,b,c
14	63.227	Ethyl iso-allocholate	2.28	2521	a,b,c
16	64.610	n.i	0.82		
17	68.361	n.i	0.64		
		Total identified	97.45		

[a]Compounds listed according to the HP-5MS elution order; [b]retention index (RI) calculate dosing C_7 to C_{40}*n*-alkanes in capillary column (HP-5MS); [c]Identification based on the comparison of mass spectra of NIST libraries; Relative Area (%): Percentage of the area (%) that the compound occupies within the chromatogram; RI: Retention Index; n.i: not identified.

Table 4. Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *Zingiber officinale* Roscoe, the leaf aqueous extract against the *Staphylococcus* spp. negative coagulase isolated from six nasal swabs from veterinary students at the University of Paraná (UNIPAR), Paraná, Brazil, 2018.

Inhibitory Concentration – Leaves		
Bacterial Samples	Leaves	
	MIC	MBC
01	250mg/mL	>250mg/mL
02	250mg/mL	>250mg/mL
03	250mg/mL	>250mg/mL
04	250mg/mL	>250mg/mL
05	125mg/mL	>250mg/mL
06	250mg/mL	>250mg/mL

Legend: MIC - Minimum Inhibitory Concentration, MBC - Minimum Bactericidal Concentration.

Table 5. Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *Zingiber officinale* Roscoe the rhizome aqueous extract against the *Staphylococcus* spp. negative coagulase isolated from six nasal swabs from veterinary students at the University of Paraná (UNIPAR), Paraná, Brazil, 2018.

Inhibitory Concentration – Rhizomes		
Bacterial Samples	Rhizomes	
	MIC	MBC
01	7.81mg/mL	7.81mg/mL
02	7.81mg/mL	7.81mg/mL
03	7.81mg/mL	15.62mg/mL
04	7.81mg/mL	7.81mg/mL
05	15.62mg/mL	>250mg/mL
06	15.62mg/mL	31.25mg/mL

Legend: MIC - Minimum Inhibitory Concentration, MBC - Minimum Bactericidal Concentration

Table 6. Classification according to the fractional inhibitory concentration index (FICI) for the combinations between the drugs and ½ MIC of the *Zingiber officinale* Roscoe the rhizome aqueous extract against the *Staphylococcus* spp. negative coagulase isolated from six nasal swabs from veterinary students at the University of Paraná (UNIPAR), Paraná, Brazil, 2018.

FICI – ½ MIC			
Bacterial Samples	AMP	OXA	VAN
1	Synergistic	Synergistic	Additive
2	Additive	Additive	Indifferent
3	Additive	Additive	Additive
4	Indifferent	Antagonist	Antagonist
5	Indifferent	Indifferent	Indifferent
6	Synergistic	Indifferent	Additive

Legend: AMP - Ampicillin, OXA - Oxacillin, VAN – Vancomycin

Table 7. Classification according to the fractional inhibitory concentration index (FICI) for the combinations between the drugs and ¼ MIC of the *Zingiber officinale* Roscoe rhizome aqueous extract against the *Staphylococcus* spp. negative coagulase isolated from six nasal swabs from veterinary students at the University of Paraná (UNIPAR), Paraná, Brazil, 2018.

FICI – ¼ MIC			
Bacterial Sample	AMP	OXA	VAN
1	Indifferent	Additive	Indifferent
2	Additive	Additive	Indifferent
3	Synergistic	Additive	Additive
4	Additive	Antagonist	Antagonist
5	Indifferent	Indifferent	Indifferent
6	Synergistic	Additive	Indifferent

Legend: AMP - Ampicillin, OXA - Oxacillin, VAN – Vancomycin.

pharmaceutical drugs (plant origin), especially due to the constant emergence of microorganisms resistant to conventional antimicrobials (Silva and Fernandes-Júnior, 2010).

According to the study of Jardim et al. (2019), the results showed that the aqueous extracts of the leaves presented high levels of these compounds (160.86 ± 0.17 µg eq. AG/mg extract), statistically different ($p < 0.05$) from the aqueous extract of the rhizomes (132.018 ± 3.31µg eq. AG/mg extract). Several studies indicate that antioxidant activity is directly related to the content of total phenolic compounds, which act mainly on free radical sequestration (Beal, 2006; Sahoo et al., 2014).

The antioxidant capacity of *Z. officinale* rhizomes is known to be attributed especially by the presence of phenolic compounds that are chemical structures that have hydroxyls and aromatic rings, in single or polymer forms (Angelo and Jorge, 2007). Its antioxidant capacity is due to the presence of hydroxyls in its molecules that eliminate free radicals by the formation of phenoxyl (Gupta and Sharma, 2014).

The extracts tested showed inhibitory activity against the *Staphylococcus* spp., with the aqueous extract of the rhizome standing out. The aqueous extract of the leaves also showed good results as an antibacterial, but only in higher concentrations. In this work, the higher potential of the rhizome extract relative to that of the leaf extract may be a consequence of its phytochemical compositions since the leaves do not have as many bioactive compounds as the rhizome. Besides flavonoids, tannins, and saponins that are also present in the leaves, the rhizome also has alkaloids and glycosides that confer on them greater antioxidant and antimicrobial capacities, among others (Jardim et al., 2019; Saad et al., 2014).

Research using ginger to verify its antimicrobial activity and synergistic potential has previously been carried out (Baljeet et al., 2015; Betoni et al., 2006); however, studies using the E-test approach as a way of verification is scarce in the literature. The present work sought some specificity to clarify this potential since the E-test is a safe alternative to other tests, such as broth microdilution (Manfredini et al., 2011) and has the advantage of being easy to use and interpret, besides allowing tests involving microorganisms that are difficult to manipulate (ANVISA, 2008).

Betoni et al. (2006) tested the synergism of the ginger extract by Kirby and Bauer's disc diffusion method, concluding that there was synergism with only two drugs, netilmicin, and tetracycline. This result is in line with the results of the present study, in which synergism with ampicillin was observed at the two concentrations tested. In this work, the choice of the E-test strips to find the new MIC of the ginger rhizome extract combined with the selected drugs is due to the fact that this is an easily interpreted method and that produces accurate MIC results, thus showing a more reliable result.

Shahrajabian et al. (2019) has reported that ginger, among other spices, has been used in traditional Chinese medicine for nearly 200 years for its anti-inflammatory, antimicrobial, antipyretic, antioxidant, and analgesic properties, among others. The authors have also pointed out the great commercial potential of this medicinal plant's extract, either as a dietary supplement or in the pharmaceutical industry.

Considering the potential of the aqueous extracts of *Zingiber officinale* Roscoe found in this study either as auxiliary agents to synthetic drugs already in the market or as single products, the therapeutic use of this plant cannot be ignored, especially considering the effectiveness of the low concentration used against the multiresistant samples.

Materials and methods

Origin and number of samples

The bacterial collection was carried out in the municipality of Umuarama, located at latitude 23º45'59 "south and longitude 53º19'30" west, northwestern region of Paraná state, Brazil. Nasal swab samples were collected from six dog tutors, who were students of the Veterinary Medicine course at the University of Paraná (UNIPAR).

This research was approved by the Research Ethics Committee Involving Human Beings (CEPEH) under number CAAE 71715417.5.0000.0109.

Along with the isolates, a standard strain of *Staphylococcus aureus* from the American Type Culture Collection (ATCC25923) was used.

For the collection of the nasal swab samples of dog's owners, it was first moistened in the transport medium itself. This moistened swab was introduced into the tutor's nasal orifice, compressing it with rotational movements, then introduced into the transport medium for further processing at the Preventive Veterinary Medicine and Public Health Laboratory of the Animal Science Graduate Program with Emphasis Bioactive Products.

Culture and isolation

Each swab was inserted into Brain Heart Infusion (BHI), and incubated at 37ºC for 24 hours, then seeded in Mannitol Salt Agar medium and incubated at 37ºC for up to 48 hours for isolation of gram-positive bacteria. Each colony was subjected to analysis of macroscopic, microscopic, and biochemical characteristics, allowing the identification of these microorganisms as *Staphylococcus* spp. negative coagulase (Quinn et al., 1994; Winn et al., 2008). In isolation, a standard strain of *Staphylococcus aureus* from the American Type Culture Collection (ATCC 25923) was used.

Disk Diffusion Test

The antimicrobial susceptibility testing on the nasal swab samples was performed according to the Clinical and Laboratory Standards Institute (CLSI, 2018). The disk diffusion test was performed after standardization of the inoculums in brain heart infusion (BHI) broth according to the 0.5 McFarland scale using antimicrobial discs. The results were recorded according to the CLSI (2018) guidelines for bacteria isolated from humans and the size of the inhibition halos (in millimeters). The antimicrobials evaluated were ampicillin (10 µg), cephalothin (30 µg), cefotaxime (30 µg), clindamycin (2 µg), enrofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), oxacillin (1 µg), penicillin (10 U), tetracycline (30 µg), and vancomycin (30 µg).

Preparation of the Extract

Biological Material

The species *Zingiber officinale* Roscoe (rhizomes and leaves) was cultivated in the experimental beds of the Medicinal Garden of Campus II of the UNIPAR, Umuarama (23º45'44.9" S and 53º16'17.5" W), and the soil has the Caiuá sandstone formation, which belongs to class LVd19 (Merlin et al., 2016) and is deposited in the Herbarium of the Medicinal Garden of UNIPAR campus, under number 163. Afterward, the rhizomes and leaves were collected in the morning period in April 2017 and dehydrated in a forced circulation oven at 35 °C for 20 days.

Extraction of bioactive compounds - raw extract

The powdered samples of rhizomes and leaves were subjected to aqueous extraction according to Otunola et al. (2014) and Jardim et al. (2019). Briefly, 40 g of each powder sample was mixed with 800 mL (5%, p/v) of boiling distilled water (95–100°C) by stirring for 10 min. The filtrates were lyophilized with a Lyophilizer VirTis K (VirTis, Gardiner, New York, USA) for 48 h. The lyophilizates of each sample was stored at -20 °C in an airtight glass vial protected from light for further analysis.

Extract analysis by gas chromatography and mass spectroscopy

The chemical identification of the essential oil was performed by GC-MS (Agilent 7890B - 5977A MSD). The capillary column was HP-5MS IU 5% (30 m x 0.25 mm x 0.25 µm), with an initial temperature of 40 °C for 2 minutes, followed by heating of 5 °C/min until reaching a temperature of 250 °C remaining for 10 min and ending with heating from 40 °C/min to 300 °C remaining for 1 min. Helium was used as carrier gas at a linear velocity of 1 mL min^{-1} up to 300 °C and a pressure release of 8.23 psi. The injector temperature was 280 °C; the injection volume was 1 µL; the injection occurred in Split mode (2: 1). The transfer line was maintained at 280 °C, the ionization source, and quadrupole at 230 °C and 150 °C, respectively. The EM detection system was used in "scan" mode, at a mass/charge rate (m/z) of 40-600, with a "solvent delay" of 3 min. The compounds were identified by comparing the mass spectra found in NIST 11.0 libraries and by comparing the retention indices (RI) obtained by a homologous series of standard (C7-C28) (Adams, 2012).

Evaluation of the antibacterial activity of the extract

The antibacterial activities of the *Zingiber officinale* Roscoe plant extracts were evaluated by the Broth Microdilution method on a 96-well U-bottom microplate layout with the lid used to identify the plant parts to which the extracts corresponded (rhizomes or leaves) (CLSI, 2018).

A bacterial suspension of the isolates was prepared using the 0.5 McFarland scale with the BHI medium. Then, a dilution was performed to obtain a final concentration of 10^6 CFU/mL. Subsequently, a stock solution containing 500 mg/mL of the extract (leaves and rhizomes) in a Mueller Hinton Broth medium was prepared to obtain serial dilutions ranging from 1.95 mg/mL to 250 mg/mL. The control assays of the culture medium, extract, and bacterial inoculum were included in the 96-well microplate. After the dilution on the plate, 5 µL of the standardized bacterial suspension was added into each well, and the plates were subsequently incubated at 37°C for 24 h. After the incubation period, the growth was evidenced by the appearance of pink color in the wells upon the addition of 10 µL of 2,3,5-Triphenyltetrazolium chloride indicator at 10%. The MIC, defined as the lowest concentration that inhibits the bacterial growth, and $MIC_{90\%}$ of each extract was calculated.

A loopful of the culture was collected from each well that had no bacterial growth and inoculated onto a Mueller-Hinton Agar (MHA) plate. The plates were incubated at 37°C for 24 h, after which the MBC, defined as the lowest concentration that kills the inoculum, was assessed. All the assays were performed in triplicate.

Verification of the synergism between the aqueous extract of Z. officinale rhizome and conventional antimicrobials

Synergism was observed against the six isolates of *Staphylococcus* spp. *in vitro* by the disk diffusion test (CLSI, 2018) using E-test® strips (bioMérieux, Marcy-l'Étoile, France). Susceptibility tests were performed for each isolate on the control MHA plates alongside experimental MHA plates containing the diluted ginger extract in MHA (% v/v) to obtain the ½ MIC and ¼ MIC according to the values previously obtained in the broth microdilution assays (Mahon and Manuselis, 1995). Synergism of the ginger extract with the following three drugs was evaluated: ampicillin (AMP), to which all the isolates showed resistance in the disc diffusion assay; oxacillin (OXA) and vancomycin (VAN), because of their relevance for the treatment of staphylococcus infections. The rhizome extract was used because it exhibited the highest antibacterial activity at the previous stage. The MIC values of the E-test method (µg/mL) were recorded by observing the elliptic inhibitory areas after incubation at 37°C for 24h, and the results were obtained by comparing the values of the control and experimental samples (Zago et al., 2009).

The combined actions of the antimicrobials with the ginger rhizome extract were classified by calculating the fractional inhibitory concentration index (FICI) through the following formula: $FIC_A + FIC_B$.

FIC_A is the MIC_A of the drug and extracts combination divided by the MIC_A of the extract alone, and FIC_B is the MIC_B of the drug and extract combination divided by the MIC_B of the extract alone. The results were then separated into four groups: synergistic (FICI < 0.5), additive (0.5 ≤ FICI ≤ 1), indifferent (1 <FICI ≤ 4), and antagonist (FICI > 4), the additive being the mixture with the result equal to the sum of the results alone, and the synergistic the mixture where the result is greater than the sum of the results alone (Gutierrez et al., 2009).

Conclusion

This study shows the use of aqueous ginger extract (*Zingiber officinale* Roscoe) from the rhizomes as an antimicrobial or auxiliary agent to antimicrobials, as well as a good therapeutic alternative for the treatment of *Staphylococcus* spp., even for multiresistant ones, revealing the need for further studies on the mechanisms of action of bioactive compounds and viability of extracts of this species *Zingiber officinale* Roscoe. Both parts of the *Zingiber officinale* Roscoe plant (rhizome and leaves) showed evidence as an alternative therapy for antibacterial control. The aqueous extracts of *Zingiber officinale* Roscoe, under the conditions of this study, showed high antioxidant activity by DPPH (free radical 1,1 diphenyl 2 picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) techniques. The results of this research demonstrated the bacteriostatic effect of aqueous extracts of *Zingiber officinale* Roscoe rhizomes and leaves against *E. coli* isolates from human feces samples.To understand the effectiveness of aqueous extracts of *Zingiber officinale* Roscoe (rhizomes and leaves) in their role in modulating biological and molecular pathways, thus enabling new therapeutic strategies, more detailed research in vitro and in vivo will be necessary.

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