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MICROBIOLOGY

Essential oils isolated from popular medicinal plants and spices as alternative antimicrobial and antibiofilm compounds against the pig pathogen *Actinobacillus pleuropneumoniae*

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ABSTRACT: *Actinobacillus pleuropneumoniae is the causative agent of swine pleuropneumonia, a contagious respiratory disease associated with high morbidity and economic losses. While antibiotic therapy helps to control the spreading of the pathogen on the farm, resistance to several classes of antibiotics were reported, and treatment can be impaired by the bacterial ability to form biofilms. This increases the need for alternative therapy approaches, including the use of natural compounds with antimicrobial and/or antibiofilm activities. In this research we analyzed, by the broth microdilution method, the inhibitory and bactericidal activities of the essential oils obtained from eighteen Brazilian popular medicinal plants or spices against clinical isolates of Actinobacillus pleuropneumoniae. After that, sub-inhibitory concentrations of active oils were tested for their antibiofilm effects, analyzed by the crystal violet method. Among the eighteen oils tested, eight (extracted from cinnamon, coriander, peppermint, spearmint, thyme, marjoram, eucalyptus, and laurel) presented bacteriostatic and bactericidal activities against all isolates, and subinhibitory concentrations of five of them disrupted up to 80% preformed biofilms, and significantly inhibited biofilm formation. The chemical composition of such oils was assessed by gas chromatography/mass spectrometry (GC/MS) and is presented, indicating that their bactericidal and antibiofilm properties were mostly associated with the presence of monoterpenes and phenylpropanoids. To our knowledge, this is the first report of essential oils with potential to control environmental contamination and animal infection with A. pleuropneumoniae, representing an alternative to increasing levels of antibiotic resistance.*
Key words: *natural medicine, natural compounds,* Pasteurellaceae, *phytotherapy, porcine pleuropneumonia.*

Óleos essenciais isolados de plantas medicinais e temperos populares como compostos antimicrobianos e antibiofilme alternativos contra o patógeno de suínos *Actinobacillus pleuropneumoniae*

RESUMO: *Actinobacillus pleuropneumoniae é o agente causador da pleuropneumonia suína, uma doença respiratória contagiosa associada à alta morbidade e perdas econômicas. Embora a antibioticoterapia ajude a controlar a disseminação do patógeno na fazenda, a resistência a várias classes de antibióticos tem sido relatada e o tratamento pode ser prejudicado pela capacidade bacteriana de formar biofilmes. Isso aumenta a necessidade de abordagens terapêuticas alternativas, que incluem o uso de compostos naturais com atividades antimicrobianas e/ou antibiofilme. Neste trabalho, analisamos, pelo método de microdiluição em caldo nutriente, os efeitos inibitórios e bactericidas dos óleos essenciais obtidos de dezoito plantas medicinais brasileiras populares e/ou temperos contra isolados clínicos de Actinobacillus pleuropneumoniae. Então, concentrações subinibitórias dos óleos ativos foram testados quanto a suas atividades antibiofilme, analisados pelo método do cristal violeta. Dentre os dezoito óleos testados, oito (extraídos da canela, coentro, hortelã-pimenta, hortelã, tomilho, manjerona, eucalipto e louro) apresentaram atividade bacteriostática e bactericida contra todos os isolados, e concentrações subinibitórias de cinco deles romperam biofilmes pré-formados em até 80%, além de inibirem fortemente a formação de biofilmes. A composição química desses óleos foi avaliada por cromatografia gasosa/espectrometria de massa (CG/MS) e é apresentada, indicando que suas propriedades bactericidas e antibiofilme estavam principalmente associadas à presença de monoterpenos e fenilpropanóides. Este é o primeiro relato de óleos essenciais com potencial para controlar a contaminação ambiental e infecção animal por A. pleuropneumoniae, representando uma alternativa contra o crescente aumento de resistência bacteriana aos antibióticos.*
Palavras-chave: *medicina natural, compostos naturais,* Pasteurellaceae, *fitoterapia, pleuropneumonia suína.*

INTRODUCTION

Actinobacillus pleuropneumoniae is a Gram-negative bacterium, responsible for swine pleuropneumonia, a highly contagious disease leading to significant economic losses worldwide (SASSU et al., 2018). There are currently 19 serotypes of *A. pleuropneumoniae* recognized, with

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different geographic distribution and pathogenicity, being serotype 8, associated with high morbidity, the most widespread in Brazil (ROSSI et al., 2013; STRINGER et al., 2021).

Pigs get contaminated by direct contact with infected animals, airborne transmission, or by the presence of the pathogen in the farm environment, as *A. pleuropneumoniae* is likely to survive and persist in abiotic surfaces and in drinking water (ASSAVACHEEP & RYCROFT, 2013; LOERA-MURO et al., 2013). Improving sanitary conditions of the environment, guaranteeing better ventilation and animal well-being, are known to decrease the occurrence of respiratory diseases, but antibiotic therapy is one of the most used strategies to prevent and treat such infections. The use of several antimicrobials for prophylactic purposes and as swine growth promoters is allowed in Brazil, which contributes to the selection and spreading of drug-resistant bacteria (CARDOSO, 2019). In fact, we have previously detected several serotype 8 *A. pleuropneumoniae* clinical strains from Brazilian farms with resistance to multiple classes of antibiotics, narrowing the possibility of animal treatment (PEREIRA et al., 2018).

Antimicrobial resistance is positively correlated with bacterial growth in the form of biofilms, dense populations embedded in a self-produced matrix, enriched with exopolysaccharides, that allow bacteria to survive outside the pig, trade genetic information for resistance, as well as it protects them from host immune responses and decreases the accessibility of antibiotics (LOERA-MURO et al., 2013; HATHROUBI et al., 2018; PEREIRA et al., 2018).

Because of the increasing levels of antimicrobial resistance, there is an urge for the search of alternative ways of treating bacterial infections. Among them, essential oils, complex volatile compounds naturally synthesized by different parts of plants in secondary metabolic pathways, stand out given their potential to treat infections caused by bacteria resistant to multiple drugs, combat biofilms, sanitizing environments and improving zootechnical indexes (EVANGELISTA et al., 2021; NUTA et al., 2021).

This study evaluated the antibacterial and antibiofilm activities of essential oils isolated from popular Brazilian medicinal plants and/or spices against clinical strains of *A. pleuropneumoniae* and determined the composition of those with promising activities.

MATERIALS AND METHODS

Microorganisms and culture conditions

The tests were performed with three Brazilian multidrug-resistant, biofilm-forming, and virulent clinical strains of serotype 8 *A. pleuropneumoniae*, namely MV518, MV780 and MV1022 (PEREIRA et al., 2018), one serotype 8 strain isolated from the United Kigdom, MIDG2331 (BOSSÉ et al., 2016), and one serotype 1 Argentine strain, Shope 4074 (POHL et al., 1983). Before each test, bacteria from culture stocks kept at -80 ºC were inoculated in brain heart infusion broth (BHI, HiMedia, India) supplemented with 0.01% nicotinamide adenine dinucleotide - NAD (Sigma-Aldrich, USA) and incubated at 37 ºC under shaking at 180 rpm until reaching a concentration of 10^8 CFU/mL (McFarland 0.5).

Essential oils

Essential oils from eighteen different plants (Table 1), used for popular medicinal purposes, as spices or both, were either purchased from specialized manufacturers, or extracted from fresh plants at the Laboratório de Síntese de Agroquímicos (Universidade Federal de Viçosa, Viçosa, Brazil), by hydrodistillation with a Clevenger extractor, as suggested by JIMÉNEZ-CARMONA et al. (1999). Prior to each test, the crude oils were diluted with tween 80 solution.

Screening for the antimicrobial activity of the essential oils

Essential oils were screened for their antibacterial activity against *A. pleuropneumoniae* strains as suggested by ALIGIANNIS et al. (2001), with a few modifications. Briefly, tests were performed in microtiter polystyrene plates, containing $1x10^6$ CFU/mL of the strain tested and oil aliquots to the concentration of 5.0 mg/mL. After incubation at 37 °C for 24h in a 5% CO_2 atmosphere, 50 µL of 0.01% resazurin reagent (Sigma–Aldrich, USA) were added to each well, following another incubation at 37 °C for 2h. Live cells were detected by their capacity to reduce resazurin (blue) to resorufin (pink). The oils that were able to kill bacteria at 5.0 mg/mL were selected for further evaluation. Tests were performed in triplicate and the antibiotic florfenicol was used as positive control, and BHI + NAD with Tween 80 (0.33%), as a negative control.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of selected essential oils

Minimum inhibitory concentrations (MIC) of the oils selected above were determined by the

Table 1 - Essential oils used in this study.

Source	Plant species (popular name and part of the plant used)
Produced in this study by hydrodistillation	*Eucalyptus citriodora* (eucalyptus leaves), *Citrus aurantifolia* (key lime leaves), *Melaleuca alternifolia* (tea tree leaves), *Citrus reticulata* (mandarin leaves)
Purchased from Laszlo Aromaterapia e Aromatologia, Brazil[1]	*Rosmarinus officinalis* (rosemary leaves), *Cinnamomim zeylanucum* (cinnamon bark), *Coriandrum sativum* (coriander leaves), *Myristica fragrans* (nutmeg seed), *Citrus sinensis* (sweet orange bark), *Origanum majorana* (marjoram leaves), *Mentha spicata* (spearmint leaves), *Laurus nobilis* (laurel leaves), *Citrus limonum* (lemon leaves), *Murraya koenigii* (curry leaves), *Coffea arabica* (coffee bark)
Purchased from Ferquima Indústria e Comércio de Óleos Essenciais, Brazil[2]	*Mentha piperita* (peppermint leaves), *Zingiber officinale* (ginger rizoma), *Thymus vulgaris* (thyme leaves)

More details can be found at: [1]http://www.emporiolaszlo.com.br/, and [2]http://www.ferquima.com.br/.

broth microdilution method, as suggested by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, available at eucast.org (document "Broth microdilution – EUCAST reading guide v. 4.0"). Briefly, tests were performed in 96-well microtiter polystyrene plates, containing 1×10^6 CFU/mL of the strains in BHI+NAD and oil aliquots serially diluted to the concentration range of 0.009–5 mg/mL. Cell incubation and survival was evaluated as above. The MIC was considered as the lowest concentration of essential oil at which no resazurin reduction was observed. The MBC was determined by plating directly the content of the wells with concentrations higher than the MIC value, as suggested by MAH (2014). Tests were performed in triplicate, using the same controls as above.

Evaluation of biofilm disruption by essential oils

Bacterial biofilms were formed on 96-well microtiter polystyrene plates by inoculating 100 µL of BHI+NAD containing 1×10^6 CFU/mL of the strains, which were then incubated at 37 °C for 24h. After that period, the supernatant was removed, wells were gently washed twice with PSB and refilled with 200 µL of a BHI + NAD solution containing essential oils at concentrations ranging from 0.125-4×MIC. The microplates were then incubated at 37 °C for 6h. At the end of this period, the supernatant was carefully removed, and wells were washed twice with distilled water. The remaining biofilms were quantified by the crystal violet method (STEPANOVIĆ et al., 2007). Positive controls consisted of cells incubated with florfenicol at 2xMIC and negative controls, wells at each only BHI+NAD, with no oils was added. Experiments were carried out in biological and experimental triplicates. Percentage of biofilm disruption was calculated considering the optical density (OD) of each well stained with crystal violet at 570 nm of the negative control (100%) subtracted by the OD of the treatments.

Evaluation of essential oils in the prevention of biofilm formation

The activity of essential oils against biofilm formation was analyzed by cultivating the bacterial strains in subinhibitory concentrations (0.5×MIC, 0.25×MIC and 0.125×MIC) of the oils, at 37 ºC for 24h. Biofilm density was quantified by the crystal violet, as described above. BHI + NAD with Tween 80 was used as negative control. Experiments were performed in biological and experimental triplicate. Percentage of biofilm reduction was calculated considering the optical density (OD) of each well stained with crystal violet at 570 nm of the negative control (100%) subtracted by the OD of the treatments.

Chemical composition of active essential oils

Essential oils with promising antibacterial effects were diluted to 5 mg/mL in hexane. Gas chromatography/mass spectrometry (GC-MS) analysis was then carried out using a Shimadzu GCMS-QP5050A spectrometer, as suggested by ADAMS (2007). Chromatographic analysis was performed using the parameters described by NASCIMENTO et al. (2017). Compounds were identified by comparing spectral patterns with those stored in Wiley 7 and National Institute of Standards and Technology (NIST) library databases.

Statistical analysis

Results of the effects of the oils in biofilm disruption and prevention of biofilm formation were analyzed by analysis of variance (ANOVA). Differences between treatments and the control were assessed by Dunnett's test ($P \leq 0.05$), and differences among treatments were evaluated by Tukey's test ($P \leq 0.05$).

RESULTS

Antibacterial activity of the essential oils

Of the 18 essential oils analyzed, eight (44%) exhibited antibacterial activity: *Cinnamomum zeylanicum* (cinnamon), *Coriandrum sativum* (coriander), *Mentha piperita* (peppermint), *Mentha spicata* (spearmint), *Thymus vulgaris* (thyme), *Origanum majorana* (marjoram), *Eucalyptus citriodora* (eucalyptus), and *Laurus nobilis* (laurel). These oils were further evaluated for their MICs and MBCs against the five clinical strains of *A. pleuropneumoniae* (Table 2). MIC and MBC values were consistent in both experimental and biological triplicates. Their values were coincident, indicating that the oils are bactericidal, not bacteriostatic. In most situations, MIC/MBC values for a specific oil were the same for all strains. MIC/MBC concentrations ranged from 0.31 to 5 mg/mL, with coriander oil presenting the lowest MICs and MBCs (0.31 mg/mL), followed by cinnamon oil (0.62 mg/mL); while laurel oil was the least effective (5.0 mg/mL).

Disruption of biofilms by the essential oils

Because of their capacity of forming moderate to strong biofilms, as described by PEREIRA et al. (2018), the tests of biofilm disruption and formation in the presence of essential oils were only performed with the serotype 8 strains, i.e., MV780, MV518, MV1022, and MIDG2331. Figure 1A shows the percentage of biofilm disruption, for the oils that presented

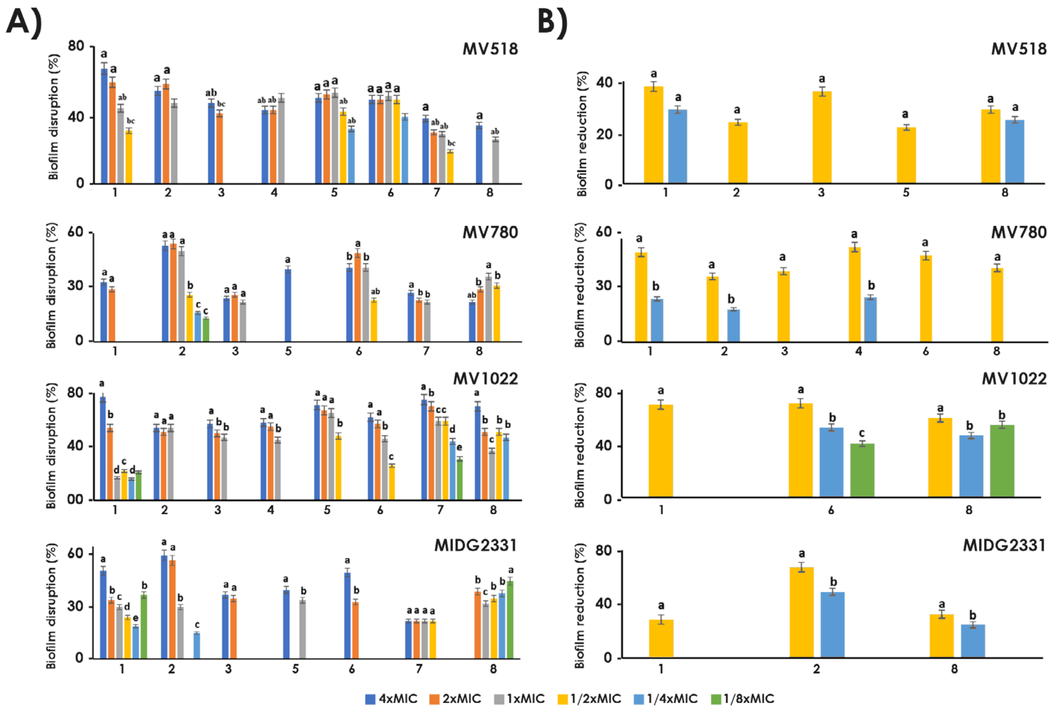

Figure 1 - Effects of different concentrations of the essential oils on preformed biofilm disruption (A) and on the reduction of biofilm formation (B) against serotype 8 *A. pleuropneumoniae* strains. Oils tested: cinnamon (1), coriander (2), peppermint (3), spearmint (4), thyme (5), marjoram (6), eucalyptus (7) and laurel (8). Means followed by the different letters for each oil and strain differ significantly by Tukey's test ($P < 0.05$).

Table 2 - Minimum inhibitory and bactericidal concentration - MIC and MBC (mg/mL), respectively, of essential oils against clinical strains of *Actinobacillus pleuropneumoniae.*

Essential oil	---------Strain---------									
	------MV518------		------MV780------		------MV1022-----		---MIDG2331---		---Shope 4074---	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Cinnamon	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
Coriander	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Peppermint	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Spearmint	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Thyme	1.25	1.25	1.25	1.25	1.25	1.25	0.62	0.62	1.25	1.25
Marjoram	2.5	2.5	2.5	2.5	5.0	5.0	1.25	1.25	2.5	2.5
Eucalyptus	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Laurel	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

positive results for each strain. Coriander and cinnamon oils presented the most prominent results: at 1×MIC, 2×MIC and 4×MIC (i.e., 0.62 mg/mL, 0.31 mg/mL and 0.16 mg/mL for coriander and 0.31 mg/mL, 0.16 mg/mL and 0.08 mg/mL for cinnamon), these oils were able to disrupt all preformed biofilms. Peppermint oil also disrupted the biofilms of all strains, by at least 30%, at 2×MIC, and 4×MIC (i.e., 2.5 mg/mL and 5.0 mg/mL). The capacity of the oils to disrupt biofilms varied among the strains: while some oils disrupted biofilms of the strains MV5178 and MV1022 by more than 50% and up to 80%, disruption of biofilms formed by MV780 and MIDG2331 was around 30-60%. Oils like cinnamon, eucalyptus, and laurel dispersed biofilms of some strains in concentrations as low as 1/8×MIC (i.e., 0.08 mg/mL, 0.16 mg/mL and 0.62 mg/mL, respectively).

Reduction of biofilm formation by subinhibitory concentrations of the essential oils

Sub-inhibitory concentrations of the oils, varying from 1/2×MIC to 1/8×MIC, inhibited biofilm formation by the strains (Figure 1B). However, not all oils displayed that capacity. All strains were able to form biofilms in the presence of subinhibitory concentrations of eucalyptus oil. Conversely, cinnamon and laurel oils inhibited biofilm formation by all strains, from 30 to 70%. The most susceptible strains were MV518 and MV780. Concentrations as low as 1/8×MIC of the marjoram and laurel oils, i.e., 0.62 mg/mL, inhibited biofilm formation by MV1022 by more than 40%

Chromatographic analysis of essential oils with antimicrobial activity

The eight oils with antimicrobial activity were submitted to GC-MS for identification of their major components. The oils contained from three (laurel oil) to ten (marjoram oil) different components, with varying importance (Table 3). Each oil consisted of a major compound, such as cinnamaldehyde in cinnamon oil, decenal in coriander oil, *d*-limonene in peppermint and eucalyptus oils, menthol in spearmint oil, carvacol and *p*-cymene in thyme oil, terpinem-4-ol in marjerom oil, and 1.8-cineol in laurel oil. These compounds varied from 12.5 to 69.6% of the composition identified for each oil. Some trace compounds are produced by more than one plant, such as α-pinene, identified in peppermint, spearmint, thyme, eucalyptus and laurel oils, and γ-terpinene, reported in peppermint, thyme, marjoram, and eucalyptus oils.

DISCUSSION

In this study, we have shown for the first time, as the authors are aware, the antimicrobial and antibiofilm activities of essential oils obtained by plants that are often used in Brazilian culture as natural medicines and/or spices, against the pig pathogen *Actinobacillus pleuropneumoniae*. This is especially important given the increasing levels of antimicrobial resistance observed for *A. pleuropneumoniae* (PEREIRA et al., 2018), together with the alarming global increase of bacterial resistance against traditional antibiotics, which calls for

Table 3 - Chemical composition of essential oils with antimicrobial activities against *A. pleuropneumoniae* clinical strains.

Compound (%)	---Essential oil---							
	Cinnamon	Coriander	Peppermint	Spearmint	Thyme	Marjoram	Eucalyptus	Laurel
Sabinene	-	-	-	-	-	11.0	-	-
α-Pinene	-	-	2.0	6.4	2.1	-	1.1	20.3
β-Pinene	-	-	11.3	6.9	-	-	10.4	15.0
d-Limonene	-	-	28.5	4.9	-	8.0	33.3	-
α-Terpinene	-	-	-	-	-	13.0	-	-
β-Myrcene	-	-	1.3	-	-	-	1.7	-
p-Cymene	-	-	-	-	28.8	-	3.9	-
1.8-Cineol	-	-	-	-	-	-	-	64.6
β-Cymene	-	-	4.2	-	-	4.6	-	-
γ-Terpinene	-	-	11.3	-	1.3	14.0	1.2	-
Linalool	5.8	10.1	-	-	3.8	-	-	-
Thymol	-	-	-	-	6.0	-	-	-
cis-Limonene oxide	-	-	-	-	-	-	0.7	-
trans-Limonene oxide	-	-	-	-	-	-	1.2	-
trans-Sabinene hydrate	-	-	-	-	-	5.5	-	-
Menthone	-	-	-	26.0	-	-	-	-
Neoisomenthone	-	-	-	9.3	-	-	-	-
Menthol	-	-	-	30.2	-	-	-	-
Terpinolene	-	-	-	-	-	3.0	-	-
cis-Sabinene hydrate	-	-	-	-	-	11.0	-	-
Decanal	-	7.5	-	-	-	-	-	-
Decenal	-	12.5	-	-	-	-	-	-
trans-Dodecanol	-	8.0	-	-	-	-	-	-
Cinnamaldehyde	69.6	-	-	-	-	-	-	-
Carvacrol	-	-	-	-	48.4	-	-	-
Caryophyllene oxide	-	-	-	-	2.8	-	-	-
Terpinen-4-ol	-	-	-	-	-	31.0	-	-
Eugenol	4.9	-	-	-	-	-	-	-
α-Copaene	2.6	-	-	-	-	-	-	-
β-Caryophyllene	2.2	-	-	-	-	3.0	-	-
Neryl acetate	-		1.1	-	-	-	-	-
Cinnamyl acetate	6.6	-	-	-	-	-	-	-
Decanol	-	5.3	-	-	-	-	-	-
β-Bisabolene	-	-	2.7	-	-	-	-	-
8-Hexadecenal	-	4.1	-	-	-	-	-	-

rapid discovery and development of alternative forms of treatment and prevention of infection, including the exploration of natural compounds (TACCONELLI et al., 2018; THEURETZBACHER et al., 2020).

Consistent with the literature, the oils that displayed the most prominent activities against *A. pleuropneumoniae* had been described before as alternative antimicrobial compounds against several

Gram-negative and Gram-positive pathogens, with promising applications in biotechnology, health sciences, pharmaceutical and food industries. Some examples can be observed in recent studies by ALBOOFETILEH (2018), SABO & KNEZEVIC (2019), AREZOO et al. (2020), CHENG et al. (2020), KAČÁNIOVÁ et al. (2020), EL-NAGGAR et al. (2021) and ÖZOGUL et al. (2020; 2022).

The fact that the MIC and MBC of the oils were the same indicated that these compounds may be bactericidal, not bacteriostatic. Since *A. pleuropneumoniae* is a Gram-negative pathogen, meaning that it has two layers of lipid membranes (SASSU et al., 2018), it is likely that the hydrophobic nature of the essential oils and their major constituents - which are mostly monoterpens - may interact with such membranes, provoking a disbalance leading to bacterial death. Several mechanisms of antimicrobial activitivies of monoterpens have been proposed; although, not all of them have been fully elucidated. Carvacol and thymol from thyme oil, for example, disturb the outer membrane of gram negative bacteria, releasing lipopolysaccharides, leading to leakage of ATP (BASSOLÉ et al., 2010). This mechanism of action differs from those of common commercial antibiotics used to control bacterial diseases in livestock, such as florfenicol, a protein synthesis inhibitor, to which the strains studied here are resistant.

Because these major compounds are present in the oil as a mixture of organic metabolites, MIC and MBC values, as well as their efficiency in killing bacteria, would be improved if they were purified, although their application as crude extracts is promising without purification, as exemplified by the studies mentioned above. Conversely, the multi-component nature of essential oils potentially reduces the chances of resistance, as different components might have different mechanisms of action against bacteria (YAP et al., 2014).

It is important to acknowledge that the production and composition of the essential oils may depend seasonal variations, environmental conditions and plant parts used; although, some components remain the main ones. For example, studies with *Laurus nobilis* conducted in different locations around the world have shown that 1.8-cineole is always the main compound in laurel essential oil, but its proportion can be as small as 30% or as high as 68%, the latter being close to the values observed in this work. This variation in oil composition is likely related to differences in antimicrobial activities observed against various Gram-negative and Gram-positive pathogens (ÖZCAN & CHALCHAT, 2005; SHOKOOHINIA et al., 2014; STEFANOVA et al., 2020), as 1, 8 -cineole alone is well characterized by its antimicrobial properties (FARHANGHI et al., 2022).

Nonetheless, our study showed that eight oils, with different compositions, can be potentially used to combat *A. pleuropneumoniae* strains capable of causing swine pleuropneumonia. The strains studied here have been shown to be genetically distant, resistant to multiple drugs, virulent, and capable of forming biofilms (PEREIRA et al., 2015, 2018). Here we observed that these differences are also reflected in their susceptibility to different oils and in different levels, making some oils, such as cinnamon, coriander and eucalyptus more likely to kill a greater variety of strains in a population. More important is the fact that these oils disrupt biofilms and avoid them from being formed, which reinforces their applications as sanitizers in the farm environment, besides their potential in the treatment of pleuropneumonia. Poly-N-acetyl-glucosamine (PGA) is the major component of the *A. pleuropneumoniae* matrix (HATHROUBI et al., 2018), and, as most biofilms, it is a barrier to antimicrobial drugs, to the host immune system, in addition to allowing the bacteria to persist in the environment (FLEMMING et al., 2016).

Previous studies have suggested that some phenolic components of essential oils, such as those described in here, might interfere with biofilms by interacting with the cell wall and the extracellular polymeric matrix (OUSSALAH et al., 2006), thereby destabilizing these structures and disrupting preformed biofilms. Regarding biofilm development, essential oils might also interfere with cell-cell communication (quorum-sensing), a process that is necessary for bacteria to form biofilms (O'BRYAN et al., 2015).

CONCLUSION

This study demonstrated that cinnamon, coriander, peppermint, spearmint, thyme, marjoram, eucalyptus, and laurel essential oils have the potential to be used as antibacterial and antibiofilm agents against *A. pleuropneumoniae*, with MIC and MBC values varying from 0.62 mg/mL for coriander oil to 5.0 mg/mL to laurel oil. Cinnamon and coriander oils were the most effective in disrupting preformed biofilms and in inhibiting biofilm formation by the four isolates analyzed. Although the values for antibiofilm activities varied according to the bacterial strain tested, biofilms formed by all strains were significantly avoided when treated with at least 1/2×MIC of cinnamon or coriander oils (0.31 mg/mL and 0.16 mg/mL, respectively) and were disrupted by at least 2×MIC (1.25 mg/mL) of cinnamon oil or 1×MIC (0.31 mg/mL) of coriander oil, thereby presenting great potential in the control and prevention of *A. pleuropneumoniae* infections.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization: FAFR, GCS, DMSB, MAND; Methodology: FAFR, GCS; Formal analysis and investigation: FAFR, GCS, MFS, DMSB, CCR, MAND; Writing: FAFR, GCS, MFS, DMSB, CCR, MAND; Funding acquisition: MAND, DMSB; Supervision: MAND.

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