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Antimicrobial and modulating activity of essential oils against bacteria isolated from goat dairy products in northeastern Brazil

Atividade antimicrobiana e moduladora de óleos essenciais contra bactérias isoladas de leite de cabra no Nordeste do Brasil

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Highlights

Dairy goat farming plays a key role in the economy of northeastern Brazil.
Pathogenic microorganisms were identified in the goat milk processing plants.
Synergy between essential oils and antimicrobials against pathogenic microorganisms.

Abstract

In recent years, novel strategies to combat (multi-) drug-resistant microorganisms have been investigated. Essential oils (EOs) with bactericidal, bacteriostatic, and fungicidal activity have been used to treat infections and in food sanitation. This study aimed to determine the antimicrobial and modulating activity of *Cinnamomum cassia* (cinnamon) and *Eugenia caryophyllus* (clove) essential oils against microorganisms isolated from goat milk processing plants in northeastern Brazil, and their synergistic effect when combined with antimicrobial agents. The microdilution technique was used to obtain the minimum inhibitory (MIC) and bactericidal concentrations (MBC) and the antibiotics studied were ampicillin, amoxicillin/clavulanic acid, cephalothin, ceftazidime, chloramphenicol, gentamicin, meropenem, norfloxacin, sulfamethoxazole/trimethoprim, and tetracycline. *Klebsiella pneumoniae* (MIC50) and *Escherichia coli* (MIC90) were sensitive to cinnamon EO. Clove EO did not inhibit the growth of either microorganism. In regard to MBC, cinnamon EO had a bactericidal effect against six *K. pneumoniae* and six *E. coli* samples. For the antibiotics evaluated, a greater synergistic effect was

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observed for cinnamon EO associated with gentamicin and meropenem, and antagonistic effect with ampicillin, sulfamethoxazole/trimethoprim, and tetracycline. As such, EOs may be an alternative for the control of pathogenic microorganisms.
Key words: Dairy products. Microbiology. Phytotherapy. Synergism.

Resumo

Nos últimos anos, novas estratégias têm sido pesquisadas para combater microrganismos que apresentam resistência a multidrogas. Óleos essenciais com atividade bactericida, bacteriostática e fungicida vêm sendo utilizados no tratamento de infecções e na higienização em indústrias de alimentos. Este trabalho objetivou determinar a atividade antimicrobiana e moduladora de óleos essenciais de *Cinnamomum cassia* (canela) e de *Eugenia caryophyllus* (cravo) através da técnica de microdiluição para Concentração Inibitória Mínima (CIM) e Concentração Bactericida Mínima (CBM) contra isolados da indústria processadora de leite de cabra do Cariri paraibano em associação com os antimicrobianos ampicilina, amoxicilina/ácido clavulânico, cefalotina, ceftazidina, cloranfenicol, gentamicina, meropenem, norfloxacina, sulfametoxazol, trimetoprim e tetraciclina. *Klebsiella pneumoniae* e *Escherichia coli* foram sensíveis ao óleo essencial de canela, CIM50 e CIM90, respectivamente. O óleo essencial de cravo não apresentou resultados frente ao crescimento das duas espécies. Quanto à CBM, o óleo essencial de canela apresentou efeito bactericida para seis amostras de *K. pneumoniae* e seis de *E. coli.* Entre os antibióticos avaliados, houve um maior efeito sinérgico do óleo essencial de canela com a gentamicina, meropenem e amoxicilina/ácido clavulânico, e um maior efeito antagônico com a ampicilina e a tetraciclina. Diante disso, observa-se que os óleos essenciais podem se tornar uma alternativa no controle de microrganismos patogênicos.
Palavras-chave: Fitoterapia. Laticínios. Microbiologia. Sinergismos.

Introduction

Food safety is critical to health. Despite advances in the field of microbiology regarding foodborne diseases (FBD) and their treatment, Brazil is still largely dependent on control within the food supply chain. Hospitals and health care units in the country remain a focal point for treating patients with FBD (Coelho et al., 2019).

Waterborne diseases (WBD) and FBD, especially those caused by bacteria, remain a serious public health issue. The prevalence of these illnesses and food spoilage has heightened awareness of the urgent need to ensure food quality and safety (Marques et al., 2023).

Among FBD-inducing microorganisms, *Escherichia coli* is the most well-known. Belonging to the family *Enterobacteriaceae,* these coliform bacteria are generally found in human and animal intestines and capable of growth at 45°C. They can be isolated from animal-based food and are considered an indicator of fecal contamination (Cruz et al., 2019). In addition to *E. coli,* microorganisms such as *Klebsiella pneumoniae,* gram-negative bacteria from the same family, have also been reported at milk processing facilities. The genus *Klebsiella* includes medically important species responsible for frequent cases of resistance (Dong et al., 2022). Found in contaminated water, these

microorganisms can cause mastitis (M. V. Santos & Fonseca, 2019a).

Milk and its derivatives can carry pathogenic microorganisms, resulting in economic losses, especially when combined with antimicrobial resistance, which has increased over the years. The expansion of antibiotic resistance has made treating bacterial diseases a challenge for modern medicine. Several infections are caused by bacterial species be present in people without causing disease. These strains are generally non-pathogenic, but can become pathogenic if they are found in an atypical location or if their numbers increase significantly due to immunosuppression (Neagu et al., 2024). This has prompted an increase in research on essential oils (EOs) as a natural alternative to inhibit microorganism growth in the food supply chain.

Defined as secondary metabolites produced by plants, EOs have several biological properties, including antimicrobial activity. These concentrated natural extracts are proven sources of bioactive compounds with antioxidant and antimicrobial properties (Man et al., 2019).

Oils have shown promise in different fields, including aromatherapy, personal care products, natural medicine, and even as alternatives to traditional cleaning products or pest control solutions (Loukili et al., 2023). They may also be a viable alternative for the development of new drugs to control microorganisms resistant to traditional antibiotics (S. G. Silva et al., 2021). Given the aforementioned characteristics and the need for new alternatives to control microorganisms in the dairy industry, this study aimed to determine the antimicrobial and modulating activity of *Cinnamomum cassia* (cinnamon) and *Eugenia caryophyllus* (clove), via their minimum inhibitory (MIC) and bactericidal concentrations (MBC), and assess the synergistic effect of these oils when combined with antimicrobial agents, against microorganisms isolated from goat milk processing plants in northeastern Brazil.

Material and Methods

Essential oils

Clove and cinnamon EOs obtained via steam distillation were purchased from specialist companies. The clove (*Eugenia caryophyllus*) EO (*Bio Essência*®; ANVISA No. 25351.223983/2014-21, Jaú, São Paulo, Brazil) was extracted from buds and its main component is eugenol, while the cinnamon (*Cinnamomum cassia*) EO (QUINARÍ®; ANVISA No. 25351.280763/2018-11, Ponta Grossa, Paraná, Brazil) was extracted from bark, with cinnamic aldehyde as its main component. For the pharmacological assays, the substances were dissolved in 10% v/v DMSO (dimethyl sulfoxide) and 1% v/v Tween 80, and diluted in distilled water.

Bacterial isolates

Four goat milk processing plants from the Cariri region of Paraíba state that supply milk to the Food Acquisition Program (PAA) were selected, and identified as A, B, C, and D. Samples were collected from different locations at each plant, including cooling and pasteurization tanks, bottling machines, packaging, employees' hands, and walls. To assess milk quality, two milk samples were taken from each plant: one from the raw milk in the cooling tank (bulk milk), and the other from milk after pasteurization, totaling 32 samples.

Samples taken from equipment were collected after cleaning to assess the effectiveness of sanitation practices in the plants. For surface samples, a sterile swab with a 12 cm long handle, moistened with 0.1% peptone water, was used. A sterilized template was used to delimit a 100 cm² surface area. The swab was applied in a rotating motion at constant pressure and an approximate angle of 30°, initially moving from left to right and then right to left. Next, the swab's handle was broken off inside a 10 ml peptone water bottle containing the dilution solution, and aliquots were subsequently plated onto appropriate culture media (Andrade, 2008).

Samples were collected from employees' hands using a sterile swab with a 12 cm long handle, moistened with 0.1% peptone water. It was rubbed three times from the wrist toward each finger, and then from the wrist, between the fingers and back. The microorganisms collected were transferred to a tube containing 10 ml of peptone water (Andrade, 2008), followed by dilutions for specific media.

Raw milk samples were collected from cooling tanks with a sterile ladle and placed in sterile tubes, while pasteurized samples were taken directly from the packaging, stored in thermal boxes with recyclable ice, then transported to the microbiology laboratory. Both raw and pasteurized milk were diluted as needed and plated onto culture media specific to the desired microorganisms.

Twenty *Klebsiella pneumoniae* and 22 *Escherichia coli* isolates were collected from raw and pasteurized goat milk, equipment, utensils, and employees at four goat milk processing plants in Paraíba, northeastern Brazil.

Klebsiella and *Escherichia coli* were isolated using MacConkey (Merck) and Methylene Blue Eosin agar (EMB), respectively, at 37°C for 24 hours (American Public Health Association [APHA], 2001). Mass spectrometry was performed to identify typical colonies (MALDI-TOF MS) (Barcelos et al., 2019).

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the broth microdilution technique (Cleeland & Squires, 1991; Hadacek & Greger, 2000), in sterile 96-well plates with lids. In each well, 100 µL of double strength BHI (brain-heart infusion) broth was added. Next, 100 µL of EO emulsion at an initial concentration of 2048 µg/mL was added to the wells in the first row. Using two-fold serial dilution, concentrations of 1024, 512, 256, 128, 64, 32 µg/mL were obtained, with the highest concentration recorded in the first row and the lowest in the last. Finally, 10 µL of the inoculum with approximately 1.5 x 10^8 CFU/mL of the bacterial species was added to the wells, with each plate column allocated to a specific bacterial strain.

A microorganism control was prepared by placing 100 µL of the same double strength BHI broth and 10 µL of each species' inoculum into the wells. A control was also used to assess medium sterility, consisting of 100 µL of the double strength BHI broth in a well without bacteria.

The plates were aseptically sealed and incubated at 35°C for 24 hours before being read. The MIC for the EOs was defined as the lowest concentration that visibly inhibited bacterial growth when compared to the control. The experiments were performed in duplicate.

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the EOs was also determined for each bacterial strain. After the 24-hour MIC reading, 10 µL aliquots were removed from each well of the microdilution plate that showed no bacterial growth and transferred to wells in a new microtiter plate containing 100 µL of double strength BHI broth with no antimicrobials. The inoculated plate was aseptically sealed and incubated at 35°C for 24 hours before being read. The MBC was defined as the lowest EO concentration that visibly inhibited microorganism growth (Ncube et al., 2008; Guerra et al., 2012).

Antimicrobial drugs

The antimicrobial resistance profiles of the isolates were determined using the CLSI disk diffusion method (Clinical and Laboratory Standards Institute [CLSI], 2020). The antibiotics tested were ampicillin (10 µg), amoxicillin/clavulanic acid (30 µg), cephalothin (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), meropenem (10 µg), norfloxacin (10 µg), sulfamethoxazole/trimethoprim (25 µg), and tetracycline (30 µg) (CLSI, 2020).

Analysis of EO interference in antibiotic drug function

EO interference in antibiotic effectiveness was investigated using agar diffusion assays and filter paper discs. The EOs were evaluated based on the MIC determined here.

The discs containing the antibiotics at their respective concentrations were soaked with 10µL of EOs at MIC, and then placed in sterile Petri dishes containing Muller-Hinton agar inoculated with swabs of the bacterial inoculum. After incubating the plates at 37°C for 24 hours, interference of the essential oil MIC in the effect of antibiotics against the bacterial strains tested was determined. A synergistic effect was considered when the essential oil- antibiotic combination resulted in a microbial growth inhibition halo ≥ 2mm in diameter when compared to the inhibition zone obtained with the antibiotic drug alone, an antagonistic effect for halos ≤ 2mm, and an indifferent effect when the inhibition halo of the essential oil-antibiotic combination was the same diameter as that resulting from use of the antibiotic alone (Cleeland & Squires, 1991).

Results and Discussion

Table 1 shows the MIC and MBC results for clove and cinnamon EOs. Antimicrobial activity was observed at concentrations of 1024 to 256 µg/mL and 1024 to 512 µg/mL for cinnamon and clove oil respectively. For both EOs, 512 µg/mL inhibited bacterial growth in 50% of the tested strains. Cinnamon EO exhibited antimicrobial activity in 23 (100%) *E. coli* and 17 (80.95%) *Klebsiella pneumoniae* isolates, whereas clove EO showed antimicrobial activity against only two (9.52%) *Klebsiella pneumoniae* and six (26.08%) Escherichia coli samples, as shown in Table 1.

Table 1
MIC and MBC of *Cinnamomum cassia* (cinnamon) and *Eugenia caryophyllus* (clove) essential oils against strains of *K. pneumoniae* and *E. coli* isolated from goat milk processing plants in the Cariri region of Paraiba state, Brazil

Isolates	Niche	CINNAMON		CLOVE	
		MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
K. pneumoniae	Pasteurized milk	256	-	-	-
K. pneumoniae	Employee's hand	1024	-	-	-
K. pneumoniae	Packaging	1024	1024	-	-
K. pneumoniae	Pasteurized milk	512	1024	-	-
K. pneumoniae	Raw milk	1024	1024	-	-
K. pneumoniae	Pasteurized milk	256	512	-	-
K. pneumoniae	Raw milk in natura	1024	1024	-	-
K. pneumoniae	Pasteurization tank	-	-	-	-
K. pneumoniae	Raw milk	512	1024	-	-
K. pneumoniae	Employee's hand	512	-	-	-
K. pneumoniae	Raw milk	512	512	-	-
K. pneumoniae	Pasteurized milk	512	1024	512	-
K. pneumoniae	Pasteurized milk	512	512	1024	1024
K. pneumoniae	Reception tank	256	1024	-	-
K. pneumoniae	Reception tank	-	-	-	-
K. pneumoniae	Packing machine	-	-	-	-
K. pneumoniae	Raw milk	-	-	-	-
K. pneumoniae	Packaging	512	512	-	-
K. pneumoniae	Pasteurization tank	256	1024	-	-
K. pneumoniae	Pasteurized milk	512	512	-	-
E. coli	Raw milk	256	-	-	-
E. coli	Raw milk	256	-	-	-
E. coli	Raw milk	256	1024	-	-
E. coli	Raw milk	256	-	-	-
E. coli	Raw milk	512	-	-	-
E. coli	Raw milk	512	-	-	-
E. coli	Raw milk	256	512	-	-
E. coli	Raw milk	512	-	-	-
E. coli	Raw milk	512	-	-	-
E. coli	Raw milk	512	1024	-	-
E. coli	Raw milk	512	1024	1024	-
E. coli	Raw milk	256	-	1024	-
E. coli	Raw milk	512	512	1024	-
E. coli	Pasteurized milk	512	512	-	-

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E. coli	Raw milk	64	512	1024	1024
E. coli	Raw milk	512	1024	-	-
E. coli	Pasteurized milk	512	-	-	-
E. coli	Raw milk	256	512	-	-
E. coli	Raw milk	512	512	-	-
E. coli	Raw milk	512	512	-	-
E. coli	Raw milk	512	1024	512	1024
E. coli	Pasteurized milk	512	-	1024	-
E. coli	Raw milk	512	-	-	-

MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration.
- (feature): no activity.

At the MBC of the species studied, cinnamon EO displayed antimicrobial activity against 13 (61.90%) Klebsiella pneumoniae isolates and 12 (52.17%) Escherichia coli isolates, and clove EO against one (4.76%) Klebsiella pneumoniae and two (8.69%) Escherichia coli samples (Table 1).

Table 2 shows the resistance profile and modulating effect of the EOs. Resistance percentages for the antimicrobials investigated were 59.18% for ampicillin, 21.95% for amoxicillin and clavulanic acid, 53.65% for ceftazidime, 9.75% chloramphenicol, 14.63% for gentamicin, 19.51% for meropenem, 26.83% for norfloxacin, 21.95% for sulfamethoxazole/trimethoprim, and 56.09% for tetracycline. Microorganisms resistant to at least one of the antimicrobials tested were identified in samples from processing plants B and D, while 69.23 and 90.90% of resistant isolates were obtained from plants A and C, respectively.

With respect to synergism between cinnamon EO and the antimicrobials, the effects observed varied, with an indifferent effect predominating. However, analysis of only synergism and antagonism between cinnamon EO and antimicrobials identified a predominantly synergistic effect, meaning that cinnamon EO may be used as an adjuvant in combating microorganisms resistant to different drugs, thus helping to control infections.

The antimicrobial drugs with the highest synergistic indices in the four processing plants studied were gentamicin and meropenem, while the lowest values were obtained for ampicillin, sulfamethoxazole/trimethoprim, and tetracycline.

Considered an indicator of fecal contamination, Escherichia coli, from the family Enterobacteriaceae, can be isolated from animal-based food (Cruz et al., 2019) and is typically found in human and animal intestines. In Europe, the family Enterobacteriaceae has been used as an indicator of dairy quality and microbial hygiene processes (Hervet et al., 2016). Its presence in the in the plant, on the equipment, and in processed milk indicate quality control failures and cross-contamination at these facilities.

Table 2
Modulatory effect of *Cinnamomum cassia* (cinnamon) essential oil on microorganisms isolated from goat milk processing plants in the Cariri region of Paraíba state, Brazil.

ISOLATES	NICHE	LOCATION (processing plant)	AMP	AMC	CFL	CAZ	CLO	GEN	MPM	NOR	SUT	TET
K. pneumoniae	Pasteurized milk		R*	R*	S↓	I↑	I*	S*	S*	S*	R*	R*
E. coli	Raw milk		R↓	S*	I↑	I*	S↓	S*	I↓	S*	S↓	S*
K. pneumoniae	Employee's hand		R↑	S↑	S↑	R↑	S↑	S↑	S↑	R*	S↑	S↑
K. pneumoniae	Packaging		R*	S↑	R↓	R↓	S*	I↑	I↑	S↑	S*	S↑
K. pneumoniae	Raw milk		R↓	S↑	R↑	I↑	S*	S↑	I↑	S↑	S*	S↑
K. pneumoniae	Pasteurized milk		R↓	R↓	R↓	S↑	S↑	S↑	S↑	S↑	S↑	R↓
K. pneumoniae	Raw milk		R↓	S↑	S↑	S↑	S↑	S↑	S*	S↓	S*	S↑
E. coli	Raw milk		R*	S↓	R↓	I↓	S*	S*	S*	R*	R*	R*
E. coli	Raw milk		S↓	S*	R↑	S↓	S↓	S*	S*	S↑	S↑	S*
E. coli	Raw milk		R*	S*	R*	S*	S*	S*	S*	S*	S*	R*
E. coli	Raw milk		S*	S*	R*	S↓	S↓	S*	S*	S*	S*	S
E. coli	Raw milk		S↑	S↑	R*	S↑	S*	S↑	S*	S*	S*	S*
E. coli	Raw milk		S↓	S↓	I ↓	S*	S*	S↑	S↑	S↑	S↑	S↑
K. pneumoniae	Pasteurized milk		S↑	S*	R↑	S↑	S↑	S↑	S↑	S↑	S↑	I↑
K. pneumoniae	Pasteurized milk		R*	I*	R*	R*	S*	R*	R*	R↓	R↓	S↓
K. pneumoniae	Raw milk		R*	I↓	I*	I↓	S↓	S*	S↑	R*	R*	R*
E. coli	Raw milk		I↓	I↓	R↑	R↑	S↑	R*	I*	R↓	S↓	R*
K. pneumoniae	Reception tank		R*	I*	I↑	I↑	R↓	R*	I*	R↓	R*	S↓
E. coli	Pasteurized milk		R*	S*	R↓	I*	I*	I↑	I↑	I↓	R*	R*
K. pneumoniae	Packaging		R*	R*	I↑	R↑	I↑	R*	R↑	S*	I*	R*
K. pneumoniae	Reception tank		R*	S↑	S↓	R↓	I*	I↑	R↑	I*	S↑	S*
K. pneumoniae	Raw milk		R*	I↑	S↑	R↑	S↑	S↑	R↑	R*	S↓	R*
E. coli	Raw milk		S↓	S↓	S↓	↓S	S*	S*	S↑	S*	S*	R↓

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E. coli	Raw milk	S↓	S↑	I*	S*	S↓	S*	S*	S↓	S*	S↑
K. pneumoniae	Raw milk	R*	S↑	I*	S*	S↑	S↑	S*	S↑	S*	R*
E. coli	Pasteurized milk	S↓	S↓	R↓	S↓	S↓	S↑	S↑	S↑	S↑	R*
K. pneumoniae	Employee's hand	R↑	R*	R*	I↑	S↑	S↑	S↑	S↑	S*	S↓
K. pneumoniae	Pasteurized milk	R*	R*	R*	R↓	R*	S↑	R↑	R↑	R*	R*
K. pneumoniae	Raw milk	R*	S↓	R↓	S*	S*	S*	S*	S↓	R↑	R*
E. coli	Raw milk	S*	S*	S*	S*	S*	R↓	S↓	S↓	S*	S*
E. coli	Raw milk	R*	R↓	R*	S↓	S*	S*	S*	S↓	S*	R↑
E. coli	Raw milk	R*	S*	R*	I*	S*	S↑	R↓	S↑	S*	R*
E. coli	Raw milk	R*	I*	I↑	S*	S*	S*	S*	S↓	S*	R*
E. coli	Raw milk	R*	S*	I↑	I↓	S*	S*	S↓	S↑	S↑	R*
K. pneumoniae	Packaging	S*	S↑	R↓	S*	S*	S↓	S*	S↓	S↑	S↑
K. pneumoniae	Pasteurization Tank	R*	R*	R*	S*	R↓	S*	S*	S*	R*	R↓
K. pneumoniae	Pasteurized milk	R*	S↑	S↑	S*	S*	S↑	S↑	S*	S↓	S↑
E. coli	Pasteurized milk	S↑	S↑	R↑	R↑	S↑	S↓	R↓	R↓	S*	R↑
E. coli	Raw milk	S↑	S↑	R↑	I↑	S↑	R↑	I↓	I↓	S↓	R↑
E. coli	Raw milk	R↑	R↑	R*	I↑	S↑	S↑	S*	S↑	S↑	R*
E. coli	Raw milk	S↓	S↓	S*	S*	S↓	S*	M↑	R*	S↑	S↑
E. coli	Raw milk	R↓	S↓	S*	S*	S↓	S*	S↑	S*	S↑	R↑
E. coli	Raw milk	R↑	R↑	S*	R↓	R↓	I↓	R*	R*	I*	R*

AMP: Ampicillin; AMC: Amoxicillin/clavulanic acid; CFL: Cephalothin; CAZ: Ceftazidime; CLO: Chloramphenicol; GEN: Gentamicin; MPM: Meropenem; NOR: Norfloxacin; SUT: Sulfamethoxazole/trimethoprim; TET: Tetracycline; R (Resistant); S (Sensitive); I (Intermediate); TET: Tetracycline: Norfloxacin; SUT: Sulfamethoxazole/trimethoprim; TET: Tetracycline; R (Resistant); S (Sensitive); I (Intermediate); ↑Synergism; ↓Antagonism; *Indifferent.

Escherichia coli and *Klebsiella pneumoniae* were found in several locations at different goat milk processing plants in Paraíba state, Brazil, demonstrating the need for adjustments in hygiene and sanitation practices at these of establishments. Several investigations have reported the presence of *Escherichia coli* in milk (Machado et al., 2018) and equipment (Pedro et al., 2024; Zegarra et al., 2009), and in the present study, *Klebsiella pneumoniae* was isolated from raw and pasteurized milk, and from samples collected at different locations in the plants, including packaging, employees' hands, the pasteurization and receiving tanks, and packaging machine. *Klebsiella* spp. is a mastitis-causing bacteria commonly found in the environment, bedding and soil, the most frequent being *Klebsiella pneumoniae* and *Klebsiella oxytoca* found in milk (D. D. A. Santos et al., 2019b; M. V. Santos & Fonseca, 2019a).

The development of new resistance mechanisms in recent years has prompted an increase in research on *Enterobacteriaceae* species and alternatives to help combat resistance.

Cinnamomun cassia EO showed significant activity against the bacterial strains tested. The predominant volatile compound in *Cinnamomum cassia* is cinnamaldehyde, a fragrant flavonoid considered the representative component of this plant species (C. Zhang et al., 2019). It is important to note that this oil is insoluble in water and can be extracted from plants through steam distillation (S. Liu et al., 2021). A review on the antibacterial mechanisms of cinnamon components found that the plant extract inhibits cell division, blocks membrane porins, and suppresses bacterial motility and biofilm formation (Vasconcelos et al., 2018).

Clove oil showed no antimicrobial activity against most of the isolates evaluated. This contrasts with the findings of Condò et al. (2020), who reported that clove oil was effective against different microorganisms, including E. coli, corroborating Mohamed et al. (2018), who also observed the effectiveness of clove oil against *Klebsiella pneumoniae* (a multidrug-resistant biofilm-forming organism).

Clove EO contains eugenol (Biondo et al., 2017; Radünz et al., 2019), known for its lipophilic properties, which allow the oil to interact with lipids in the bacterial membrane and alter its permeability (Radünz et al., 2019), consequently exhibiting bactericidal effects (Rabêlo et al., 2024). Clove EO has been widely studied as an antimicrobial agent against pathogenic microorganisms in food. For example, Guimarães et al. (2017) observed the antibacterial activity of clove EO against some *Staphylococcus aureus* and *E. coli* isolates.

Isolates of *E. coli* and *Klebsiella pneumoniae* were susceptible to the synergism between the cinnamon EO and antimicrobials tested. According to Q. Liu et al. (2015), combining antibiotics and cinnamon EOs can inhibit quorum sensing (QS). This occurs when the cinnamon oil penetrates the biofilm, helping the antibiotics reach the bacterial cells. Several studies have demonstrated the synergistic effect of cinnamon oil against microorganisms such as Carbapenase-resistant *Klebsiella pneumoniae* (KPC) (Qian et al., 2020), *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* (Hemaiswarya

& Doble, 2010). This was also reported by Neagu et al. (2024), who observed the synergistic effect of clove oil against both Gram-positive and Gram-negative bacteria. Synergism has also been reported between the cinnamaldehyde component of cinnamon oil and ampicillin, tetracycline (Palaniappan & Holley, 2010), meropenem (Yang et al., 2017), and piperacillin (Yap et al., 2013). Neagu et al. (2024) noted the synergistic effect of clove oil against Staphylococcus aureus and Escherichia coli, whereas Zago et al. (2009) found no synergy for cinnamon against *Staphylococcus aureus* and *Escherichia coli.* This interaction was attributed to cinnamon's ability to interfere with bacterial membrane stability, increasing its external permeability and ultimately facilitating the influx of meropenem, as reported by Yap et al. (2015) and Y. Zhang et al. (2016).

Yang et al. (2017) investigated interaction between meropenem and different types of oils against *Klebsiella pneumoniae* and observed a synergistic effect for cinnamon oil, corroborating the findings of Yasir et al. (2022). In their study with microorganisms isolated from milk, Yasir et al. (2022) observed the synergistic effect of essential oils against both Gram-positive and Gram-negative bacteria, including Escherichia coli and *Klebsiella pneumoniae.*

Synergism between antimicrobial agents is based on the principle that combining them can increase efficacy, bioavailability, and toxicity, mitigate adverse effects, and reduce the required dose and bacterial resistance (Cottarel & Wierzbowski, 2007). Synergistic interaction is interpreted as a decrease of more than 2 log.CFU/mL in the combination treatment group when compared to any treatment group of an isolated agent. An increase of more than 2 log.CFU/mL is considered an antagonistic effect (Kang et al., 2023).

The antimicrobial activity of essential oils has been described for a wide variety of Gram-positive and Gram-negative microorganisms, which respond differently depending on the chemical composition of the oils (Diniz et al., 2024; A. R. M. Silva et al., 2023; Kafa et al., 2022; Yasir et al., 2022; Quendera et al., 2018). This may be related to difficulty penetrating the outer layer due to the hydrophilic barrier, which, though not totally impermeable, hinders the passage of macromolecules and hydrophobic components.

Combining compounds is a promising strategy to combat microbial resistance, which is less likely to develop with combined compounds than individual active constituents. Mixtures of natural products are a valuable resource for drug development. Synergy can occur through various mechanisms, including pharmacodynamic and pharmacokinetic, the reduction of adverse effects, and overcoming resistance mechanisms (Caesar et al., 2019). Ju et al. (2022) identified the main factors that affect the antimicrobial activity of essential oils. However, there is a need for more effective combinations of essential oils to optimize their efficacy against foodborne pathogens.

Conclusion

Cinnamon essential oil showed antimicrobial action against the isolates studied, and a synergistic effect with gentamicin, meropenem and amoxicillin/clavulanic acid. However, further research is

needed to evaluate the role of these essential oils as an adjuvant in medical therapies, and as alternatives in sanitation procedures in milk processing plants, with a view to improving sanitation efficiency, combatting biofilm formation, and preventing microbial contamination.

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Conflict of interest

There are no conflicts of interest.

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