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Evaluation of the Antibacterial Activity of *Eucalyptus radiata* Essential Oil against *Escherichia coli* Strains from Urinary Tract Infections in Dogs and Cats

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ABSTRACT

Urinary tract infections (UTIs) are common in the clinical practice of companion animals such as dogs and cats, with *Escherichia coli* bacteria being the main cause of these infections. Therefore, this study sought to evaluate the antibacterial activity of *Eucalyptus radiata* essential oil against *Escherichia coli* strains from urinary tract infections in dogs and cats. The study was conducted using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). In addition, the disc diffusion technique was used to evaluate *E. radiata* essential oil associated with synthetic antimicrobials. The Minimum Inhibitory Adhesion Concentration (MICA) was also performed. The results demonstrated that *E. radiata* exhibited antimicrobial activity against the *E. coli* strains tested, obtaining MIC values ranging from 500µg/mL to 1000µg/mL and MBC values greater than 1000µg/mL. According to the results of the associations, it was observed that *E. radiata* oil exhibited synergistic interference with the antibiotic amikacin in most strains. It is also effective in inhibiting the adhesion of *E. coli* bacterial strains, showing an antibiofilm effect as efficient as 0.12% chlorhexidine digluconate, an antibacterial agent. Therefore, *E. radiata* essential oil showed antimicrobial potential against *E. coli* strains from urinary tract infections in dogs and cats. It may be a viable and promising alternative to prevent or combat the spread of infections caused by this bacterium, subject to further complementary research.

Keywords: Canines, Felines, Microbiology, Bacterial resistance.

INTRODUCTION

Urinary tract infections (UTIs) are common bacterial conditions in the clinical practice of companion animals, such as dogs and cats, and are classified according to the location where the infection occurs, characterized by the colonization of bacteria in organs of the urinary tract that are physiologically sterile. UTIs are often caused by gram-negative bacteria, especially the genus *Escherichia. Escherichia coli* stands out as one of the main pathogens causing urinary tract infections, being the most commonly cited bacterial species as the etiological agent causing these infections, which can also cause bacteremia and sepsis (Rezatofighi et al. 2021; Rezende et al. 2023). In this context, the practice of prescribing antimicrobials for the treatment of urinary tract infections without bacterial characterization has resulted in increased resistance among uropathogens and reduced the effectiveness of oral therapies (Terlizzi et al. 2017).

In addition, domestic animals can become important reservoirs and transmitters of such bacteria to humans, including their owners. As a result, the concept of One Health has been commonly addressed, advocating perspectives on the control of antimicrobial resistance and highlighting joint action in the interconnection of human, animal, and environmental health (Brasil 2020).

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Therefore, given the existence of bacteria resistant to these drugs, studies suggest the use of natural components with phytotherapeutic principles such as essential oils, which are gaining notoriety mainly for their biological activities, such as antibacterial agents, and also recognized as bioactive substances that can be used to prevent or combat the spread of infections (Doghish et al. 2023; Khair et al. 2026). Thus, species of the genus *Eucalyptus*, such as *Eucalyptus radiata*, rich in essential oil, stand out. Its antimicrobial activity has already been reported against bacterial strains isolated from food, as in the study by Santos et al. (2024). However, there are no unpublished studies on animal strains.

For this reason, and in view of cases of resistance including *E. coli* bacteria, as well as frequent occurrences of urinary tract infections in companion animals caused by this pathogen, this study aims to evaluate the antibacterial activity of *Eucalyptus radiata* essential oil against *Escherichia coli* bacterial strains isolated from urinary tract infections in dogs and cats, proposing the possibility of finding a natural antibacterial agent with potential for use in veterinary medicine.

MATERIALS AND METHODS

Research location and test substance

The experimental research activities were carried out at the Biochemistry Laboratory of the Federal University of Campina Grande (UFCG) at the Center for Rural Health and Technology (CSTR). The 100% pure *Eucalyptus radiata* essential oil was acquired from Indústria Terra Flor (Ponta Grossa – Paraná) and has batch number 25027. For the pharmacological tests, the compound was solubilized in dimethyl sulfoxide (DMSO) and diluted in distilled water. The concentration of DMSO used was less than 0.1% v/v.

Culture media and bacterial strains

The culture media used in the tests to evaluate antibacterial activity were Mueller Hinton liquid medium and Mueller Hinton agar solid medium. *Escherichia coli* bacterial strains isolated from urinary tract infections (Ecg 13, Ecg 14, Ecc 29, Ecc 30, Ecc 42, and Ecc 46) were used. All clinical strains were kept in Mueller Hinton Agar (MHA) at 4°C. The inocula were obtained from overnight cultures in MHA at 37°C and diluted in sterile saline to obtain a final concentration of approximately 1.5 x 10^8 Colony Forming Units permL (CFU/mL), adjusted by turbidity compared to tube 0.5 on the McFarland scale (Bona et al. 2014).

Antimicrobials

Ampicillin (10µg), amikacin (30µg), gentamicin (10µg), ceftriaxone (30µg), and norfloxacin (10µg) were used according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2018).

Chromatographic analysis of *Eucalyptus radiata* essential oil

Gas chromatography analyses were performed by the Graduate Department of Chemistry (PGQUI) at the State University of Southwest Bahia (UESB). The chemical composition of *Eucalyptus radiata* essential oil was analyzed at PGQUI-UESB using gas chromatography coupled with quadrupole mass spectrometry (GC-MS), employing Shimadzu GC-QP2010 equipment with a DB-5 capillary column. The chromatographic conditions included injection at 220°C with a 1:10 split, helium carrier gas at 0.6mL/min, and interface and ionization source temperature at 250°C. The oven temperature program started at 40°C (2 min), with an increase of 3°C/min up to 240°C for 5 min. The identification of compounds was based on the comparison of mass spectra with the NIST 14 library and the use of linear retention indices (LRI), calculated in relation to a homologous series of hydrocarbons (C8 to C26). For identification, only spectral matches above 90% were considered (Van den Dool and Kratz 1963; Adams 2007).

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined by microdilution in a 96-well plate. For this purpose, 100µL of double-concentrated Mueller Hinton broth and 100µL of essential oil (*Eucalyptus radiata*) were added at concentrations ranging from 1000 to 31.2µg/mL. Next, 10µL of a microorganism suspension (approximately 1.5 x 10^8 CFU/mL) was added to each well. The assay included a sterility control (broth only) and a growth control (broth and microorganism) and was performed in duplicate. The plates were incubated at 35±2°C for 24 hours for the first reading. Subsequently, 20µL of sodium resazurin solution was added, followed by a new incubation for the final reading and determination of the MIC (Palomino et al. 2002; Ostrosky et al. 2008; CLSI 2012; Bona et al. 2014).

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of *Eucalyptus radiata* essential oil was also determined for the bacterial strains. After reading the MIC, inoculations (10µL) of dilutions from the MIC were made into Mueller Hinton broth (100µL/well) in a sterile microdilution plate, followed by incubation at 35±2°C for 24 hours. After this time, 20µL of resazurin was added, followed by a new incubation at 35±2ºC to read the MBC result (Ncube et al. 2008; Guerra et al. 2012).

Determination of Minimum Inhibitory Adhesion Concentration (MICA)

The Minimum Inhibitory Adhesion Concentration (MICA) of the essential oil was determined with modifications based on the method of Albuquerque et al. (2010), using 5% sucrose in the assay. The concentrations of essential oil tested ranged from the pure compound to a 1:128 dilution. Initially, the bacterial strain was cultured in Mueller Hinton broth at 35±2°C. Next, 0.9mL of the bacterial subculture was distributed into test tubes, to which 0.1mL of the solution corresponding to the oil dilutions was added. Incubation was performed for 24 hours at 35±2ºC with the tubes arranged at a 30º angle. The results were read by visual observation of the adhesion of the bacteria to the tube walls after shaking. The test was conducted in duplicate and used 0.12% chlorhexidine digluconate (Periogard®) as a positive control for comparison.

Study of the association of *Eucalyptus radiata* essential oil with synthetic antimicrobials

The study of the association of *Eucalyptus radiata* essential oil with antimicrobials was performed using the disk diffusion technique in solid medium using filter paper disks. In sterile smooth Petri dishes containing Muller Hinton agar medium previously inoculated with the bacterial suspension, discs containing antimicrobials were introduced and 20µL of the MIC of the test product was added. A negative control containing only Muller Hinton agar medium with the bacterial suspension and antimicrobial discs was also performed. The plates were then incubated at 35±2°C for 24-48 hours, followed by reading (Cleeland and Squires 1991). All tests were performed in duplicate.

RESULTS

Characterization of the chemical constituents of *Eucalyptus radiata* essential oil

Spectrometric chromatography data was used to identify and quantify the main chemical constituents present in *E. radiata* essential oil. Based on the analyses, it was possible to observe the time (TR) that the constituents took to move, described as retention time, as well as quantitative differences between the compounds (Table 1).

Table 1: Chemical constituents identified by GC-MS in the essential oil of *Eucalyptus radiata*

Compound	RT (min)	A%
Eucalyptol (1,8-cineole)	12.G	87.6
Alpha-terpineol	20.3	10.4
Limonene	27.6	1.9

Note: RT = Retention time; A% = Percentage of área.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *E. radiata* essential oil against *E. coli* strains are shown in Table 2. The inhibition of bacterial growth confirms the activity of the essential oil, which has an MIC 50 of 1000µg/mL.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) inµg/mL of *Eucalyptus radiata* against strains of *Escherichia coli* from urinary tract infections in dogs and cats

	Eucalyptus radiata					
	Bacterial Strains					
	Ecc 29	Ecc 30	Ecc 42	Ecc 46	Ecg 13	Ecg 14
MIC (µg/mL)	1000	1000	1000	500	1000	1000
MBC (µg/mL)	>1000	>1000	>1000	>1000	>1000	>1000

Note: MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration.

Minimum Inhibitory Adhesion Concentration (MICA)

The results of the Minimum Inhibitory Adhesion Concentration (MICA) of *E. radiata* essential oil compared to the positive control (0.12% chlorhexidine) against *Escherichia coli* strains (Ecc 46) and (Ecg 13) are shown below (Table 3). Given the results, it can be observed that E. radiata was able to inhibit biofilm adhesion at low in proportions of 1:4 and 1:128, thus presenting antibiofilm effects against the strains studied.

Table 3: Minimum inhibitory adhesion concentration (MICA) inµg/mL of *Eucalyptus radiata* essential oil and 0.12% chlorhexidine digluconate against *Escherichia coli* strains (Ecc 46 and Ecg 13)

	Ecc 46							
E. radiata	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
	-	-	-	+	+	+	+	+
0,12% Digluconato de Clorexidina	-	-	-	-	-	-	-	-
Ecg 13								
E. radiata	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
	-	-	-	-	-	-	-	-
0,12% Digluconato de Clorexidina	-	-	-	+	+	+	+	+

Note: (+) Adhesion to the tube wall (-) No adhesion to the tube wall.

Association of *Eucalyptus radiata* essential oil with synthetic antimicrobials

The inhibition halos (mm) resulting from the combinations of *E. radiata* essential oil with synthetic antimicrobial discs against *E. coli* strains are described in Table 4. It can be observed that *E. radiata* showed a synergistic effect in 66.6% of the strains when combined with the antibiotic amikacin.

Table 4: Interference of *Eucalyptus radiata* essential oil in combination with synthetic antimicrobials for *Escherichia coli* strains

		Eucalyptus radiata				
Strains		AMP	AMI	GEN	CRO	NOR
Ecc 29	AIH	0 mm	22 mm	10 mm	10 mm	0 mm
	AIH w/ ER	0 mm (*)	22 mm (*)	10 mm (*)	8 mm (↓)	0 mm (*)
Ecc 30	AIH	0 mm	22 mm	20 mm	10 mm 10 mm (*)	0 mm
	AIH w/ ER	0 mm (*)	22 mm (*)	20 mm (*)		0 mm (*)
Ecc 42	AIH	0 mm	20 mm	20 mm	10 mm	30 mm
	AIH w/ ER	0 mm (*)	22 mm (↑)	20 mm (*)	10 mm (*)	30 mm (*)
Ecc 46	AIH	0 mm	20 mm	20 mm	10 mm	34 mm
	AIH w/ ER	0 mm (*)	22 mm (↑)	20 mm (*)	10 mm (*)	34 mm (↑)
Ecg 13	AIH	0 mm	20 mm	8 mm	0 mm	0 mm
	AIH w/ ER	0 mm (*)	22 mm (↑)	8 mm (*)	0 mm (*)	0 mm (↑)
Ecg 14	AIH	0 mm	20 mm	8 mm	0 mm	0 mm
	AIH w/ ER	0 mm (*)	22 mm (↑)	0 mm (↓)	0 mm (*)	0 mm (↑)

Note: AIH: Antimicrobial Inhibition Halo. ER: *Eucalyptus radiata*. Synergistic effect (↑); antagonistic effect (↓); indifferent effect (*); AMP: ampicillin; AMI: amikacin; GEN: gentamicin; CRO: ceftriaxone; NOR: norfloxacin. Statistical analysis: performed using mean ±standard error of the mean.

DISCUSSION

Spectrometric and chromatography data (Table 1) indicate eucalyptol (1,8-cineole) as the major constituent of *Eucalyptus radiata* essential oil, with 87.6%. This is consistent with other studies found in the literature that highlight this monoterpene as the most abundant compound in Eucalyptus species, including *E. radiata*, as reported by (Luís et al. 2016).

Based on the Minimum Inhibitory Concentration (MIC) results (Table 2), it was possible to observe that *Eucalyptus radiata* essential oil presented MIC values ranging from 500 µg/mL to 1000 µg/mL against different strains of *Escherichia coli*. According to Sartoratto et al. (2004), antimicrobial activity is considered strong when it has a MIC of up to 500 µg/mL, MIC values ranging from 600 µg/mL to 1500 µg/mL are considered moderate, and MICs above 1500 µg/mL are considered weak. Thus, the Ecc46 strain showed strong antimicrobial activity and the other strains showed moderate antimicrobial activity. According to Hafidh et al. (2011), a compound is considered bactericidal or bacteriostatic. To determine this, the ratio of MBC to MIC is analyzed. When the ratio is between 1:1 and 2:1, the compound is considered bactericidal, and if the ratio is greater than 2:1, it is considered bacteriostatic. Thus, according to the results obtained for Minimum Bactericidal Concentration (MBC) (Table 2), it is observed that *E. radiata* oil presented values greater than 1000 µg/mL for all strains tested, demonstrating bacteriostatic activity.

Thus, the compound in question showed strong bacteriostatic activity against the strain (Ecc 46) and moderate bacteriostatic activity against the other strains. These results can be explained by the significant presence of the compound eucalyptol (1,8-cineole). Authors suggest that the antibacterial activity of *E. radiata* oil is related to the cooperation of this monoterpene, the major compound in this essential oil. Eucalyptol exhibits several biological functions that are highly relevant today. Its antimicrobial property can be attributed, above all, to its ability to permeate the cell membrane (Ojeda-sana et al. 2013; Hąc-Wydro and Szydlo 2016). In addition, it has antioxidant and anti-inflammatory action (Hoch et al. 2023).

Sequential studies evaluating the antibacterial properties of essential oils (Diniz et al. 2023; Diniz et al. 2024) observed significant results for the species *Thymus vulgaris* (thyme) and *Origanum vulgare* (oregano). For thyme essential oil, the authors reported Minimum Inhibitory Concentration (MIC) values ranging from 64 to 512µg/mL and Minimum Bactericidal Concentration (MBC) values ranging from 256 to 1.024µg/mL (Diniz et al. 2023).

Similarly, oregano essential oil demonstrated a bactericidal effect for most of the strains tested, presenting MIC values of 32 to 512µg/mL and MBC values ranging from 128 to 512µg/mL (Diniz et al. 2024).

The results obtained in the Minimum Inhibitory Adhesion Concentration (MICA) of *E. radiata* (Table 3) show that for strain (Ecc 46), the essential oil was as effective against the adhesion of *E. radiata* bacterial strains as 0.12% chlorhexidine digluconate. For strain (Ecg 13) *E. radiata*, it had a significant effect when compared to the antibacterial agent, since in the last concentrations there was no inhibition of the adhesion effect by digluconate. This is particularly significant given that *E. coli* bacteria have been showing significant resistance to various antimicrobials, such as the ability to quickly adhere to surfaces and form biofilms (Alghamdi and Shakir 2020).

The interference of *E. radiata* essential oil on the antibacterial action of synthetic antibiotics (Table 4) presented results that took into account the comparison of the diameters of bacterial growth inhibition halos with synthetic antibiotics alone and also in association with the compound under study. It is possible to note an interference of the essential oil on most strains for the antibiotic amikacin, with a synergistic action being observed. This synergistic association suggests a combination between the antibiotic and the natural compound under study. Laboratory tests such as antibiograms are indispensable because they monitor bacterial resistance profiles, helping to implement prophylactic measures against the spread of multidrug-resistant microorganisms (Brasil 2022). They determine the most effective antibiotics for controlling infection, assisting in the correct prescription according to the antimicrobial sensitivity test (Passos et al. 2024).

Conclusion

Eucalyptus radiata essential oil has demonstrated efficacy in inhibiting the growth of *Escherichia coli* strains isolated from urinary tract infections in dogs and cats. The oil showed a synergistic effect, potentiating the antibacterial activity of synthetic antimicrobials such as amikacin. Furthermore, it was effective in inhibiting the adhesion of *E. coli* strains, achieving an effect as significant as that obtained with 0.12% chlorhexidine digluconate. Therefore, *E. radiata* oil demonstrates promising antibacterial potential against the tested *E. coli* and may be a viable alternative for the prevention or treatment of infections caused by this bacterium. Subject to further research, such as toxicity and in vivo studies, in order to investigate its toxicological profile and thus ensure the safety and application of this product.

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability: The datasets generated and analysed during the current study are available from the corresponding author on reasonable request and may be provided for academic purposes.

Ethics Statement: This study was evaluated and approved by the Ethics Committee on the Use of Animals (CEUA) of the Center for Rural Health and Technology (CSTR) of the Federal University of Campina Grande, under protocol 022026.

Author's Contribution: BS conducted the study, analyzed the data, drafted the manuscript, CTP and FMCM edited the preliminary manuscript, JHAF and MAAM reviewed and edited the preliminary manuscript; MSA developed the research idea, MMS managed the project, ASS and BBNJ performed the chromatographic analyses, HSS and RMA reviewed the chromatographic analyses, AAOF reviewed the manuscript for submission.

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