

Original Article

Antibacterial potential of *Thymus vulgaris* (Thyme) oil against *Escherichia coli* strains isolated from food

Potencial antibacteriano do óleo de *Thymus vulgaris* (tomilho) contra cepas de *Escherichia coli* isoladas de alimentos

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Abstract

The search for meat-based foods, even if it allows to increase the shelf life of commercialized foods, can lead to contamination by pathogenic microorganisms. In this sense, the present study aimed to test the potential antibacterial effect of *Thymus vulgaris* (thyme) essential oil against food-borne strains of the bacterium *Escherichia coli*. Therefore, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) analyses were performed on microdilution plates. In addition, the study of the association of the product with synthetic antimicrobials, performed by the disk diffusion technique, as well as the antiadherent activity, which was determined in the presence of sucrose, in glass tubes. Therefore, thyme oil showed a strong inhibitory activity, as well as a bactericidal effect against *Escherichia coli* strains, with MIC and MBC values equal to 512 µg/mL for all strains analyzed. Furthermore, *T. vulgaris* oil exhibited interactions in the association, with an antagonistic effect for most antibacterials. Regarding antiadherence activity, the test product was effective in inhibiting the adhesion of the bacterial strains under study and presented a more significant effect than 0.12% chlorhexidine digluconate. Thus, thyme oil presented an antibacterial result against *E. coli*, being a natural product that may represent a promising alternative in the fight against foodborne diseases.

Keywords: foodborne diseases, phytotherapy, microbiology, pathogenic bacteria.

Resumo

A busca por alimentos derivados cárneos, mesmo que permita aumentar a vida útil dos alimentos comercializados, pode levar à contaminação por microrganismos patogênicos. Nesse sentido, o presente estudo teve como objetivo testar o potencial efeito antibacteriano do óleo essencial de *Thymus vulgaris* (tomilho) contra cepas da bactéria *Escherichia coli* de origem alimentar. Portanto, foram realizadas as análises de Concentração Inibitória Mínima (CIM) e Concentração Bactericida Mínima (CBM) em placas de microdiluição. Além do estudo de associação do produto com antimicrobianos sintéticos, realizado pela técnica de difusão em disco, bem como a atividade antiaderente, que foi determinada na presença de sacarose, em tubos de vidro. Logo, o óleo do tomilho apresentou uma forte atividade inibitória, bem como um efeito bactericida contra as cepas de *Escherichia coli*, com os valores de CIM e CBM igual a 512 µg/mL para todas as cepas analisadas. Ainda, o óleo de *T. vulgaris* exibiu interações na associação, com efeito antagonista para a maioria dos antibacterianos. Em relação a atividade antiaderente, o produto teste foi eficaz na inibição da aderência das cepas bacterianas em estudo e apresentou um efeito mais significativo que o digluconato de clorexidina a 0,12%. Assim, o óleo de tomilho apresentou um resultado antibacteriano contra *E. coli*, sendo um produto natural que pode representar uma alternativa promissora no combate às doenças de origem alimentar.

Palavras-chave: doenças alimentares, fitoterapia, microbiologia, bactérias patogênicas.

1. Introdução

The prospecting of meat derivatives, although it provides an increase in the shelf life of commercialized foods, can also lead to microbial contamination by pathogenic microorganisms since failures can occur during the handling of meat, cleaning of machines and utensils, and even with

the addition of contaminated ingredients and/or water (Menendez et al., 2018).

The transmission of pathogenic microorganisms in food is a concern for both retailers who lose products due to spoilage and health agencies, since the ingestion

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of contaminated food can lead to outbreaks of waterborne and foodborne diseases (DTHA) with a high number of morbidity and even mortality (Bhardwaj et al., 2021).

In Brazil, according to the Notifiable Diseases Information System (Sinan), the most prevalent pathogen in DTHA outbreaks was *Escherichia coli*, accounting for 29.6% of confirmed cases between 2010 and 2022 (Brasil, 2022). Abreham et al. (2019) point out that the ingestion of raw or undercooked meat and meat products is the most frequent vehicle for transmission of *E. coli* O157:H7 (Abreham et al., 2019).

Since it is a commensal species in the gastrointestinal tract of animals, finding *E. coli* in food is a warning of possible contamination by feces or excrement (Aijuka et al., 2018). Although commensal, *E. coli* can be pathogenic and cause severe and difficult-to-treat infections, since it can share resistance genes through plasmid mediated transmission with other bacteria (Headd; Bradford, 2020).

Another aggravating factor of *E. coli* contamination is the ability to form biofilm on various surfaces, since when in this form they present a high adaptive resistance to antimicrobials and sanitizers used in clinical and food processing areas. Antimicrobial resistance (AMR) is a global concern, not only with regard to human health, but also to animal and agricultural production and the environment, as it poses a risk to the sustainability of agri-food systems and, therefore, to food and nutrition safety (WHO, 2021).

Considering the various problems surrounding the ingestion of food contaminated by *E. coli*, such as the difficulty in eliminating biofilms of these pathogens in various settings, in addition to the occurrence of many infections and their resistance to antimicrobials, it is necessary to investigate new antimicrobial agents that can combat microbial growth and the formation of biofilms (Malveira et al., 2024).

In this perspective, essential oils (EOs) have gained notoriety as natural antibacterial agents, being recognized as natural bioactive substances that can be used to prevent or combat the spread of infections (Doghish et al., 2023). The species *Thymus vulgaris* L., an aromatic, condiment and medicinal plant, popularly known as thyme, is an herb of the Lamiaceae family, a family that is prominent in the production of essential oils, thus becoming an important species for studies that aim to investigate the antimicrobial activity of natural products (Escobar et al., 2020; Correia et al., 2022).

Therefore, in accordance with the need for new compounds to combat bacteria pathogenic to humans, the present study aimed to test the potential antibacterial effect of *Thymus vulgaris* essential oil against food-borne *Escherichia coli* strains.

2. Material and Methods

2.1. Study location

The study was carried out at the Microbiology Laboratory of the Federal University of Campina Grande (UFCG), at the Center for Rural Health and Technology (CSTR).

2.2. Test substances

Thymus vulgaris (thyme) essential oil was purchased from Quinare industry® (Ponta Grossa - Paraná) which presents in its chemical composition, thymol as the main secondary metabolite. To perform the pharmacological tests, the compound was solubilized in dimethyl sulfoxide (DMSO) and diluted in distilled water. The DMSO concentration used was less than 0.1% v/v.

2.3. Bacterial strains

Foodborne *Escherichia coli* bacterial strains (Ec 41, Ec 42, Ec 44, and Ec 45) were used in the study. The strains originated from meat derivatives, such as sausage and mortadela. All strains were maintained on Mueller Hinton agar (MHA) at 4 °C.

Inoculates were obtained from overnight cultures on MHA at 35 ± 2 °C and diluted in sterile saline to obtain final concentrations of approximately 1.5 x 10⁸ colony forming units per mL (CFU/mL), adjusted for turbidity, when compared with the McFarland 0.5 tube scale (Bona et al., 2014).

2.4. Culture media

The culture media used in the tests were liquid Mueller Hinton broth and solid Mueller Hinton Agar. The culture media were purchased from Difco® and prepared according to the manufacturer's instructions.

2.5. Determinação da concentração inibitória mínima (CIM)

The MIC was determined using the microdilution technique in a 96-well plate with a U-shaped bottom. Initially, in each well, 100 µL of double-concentrated Mueller Hinton broth and 100 µL of the studied compound (*Thymus vulgaris*) were added to the plate, performing a serial dilution (ratio of two), at concentrations of 1024 to 16 µg/mL. The MIC determination was performed using 10 µL of the microorganism suspension in each well, being approximately 1.5 x 10⁸ CFU/mL. In the penultimate well, the sterile control containing 200 µL of Muller Hinton broth alone was performed, and in the final well, the growth control was performed, containing only 10 µL of the microorganism suspension in 200 µL of broth. The assay was performed in duplicate. The plates were incubated at 35 °C for 24 hours and, after this adequate bacterial incubation time, the first reading of the results was performed. Then, 20 µL of sodium resazurin solution (SIGMA), previously solubilized in sterile distilled water, at a concentration of 0.01% (w/v) were added. Resazurin is recognized as a colorimetric indicator of oxidation-reduction for bacteria. Then, a new incubation was performed at 37 °C. The reading was done visually to verify the absence or presence of microorganisms. Growth was determined by the formation of a cell cluster (button), as well as by observing changes in the color of the solution, from blue to pink, to indicate growth. The MIC was determined as the lowest concentration of the compound that inhibits the visible growth of the microorganism, as verified by the change in color of the solution, from blue to pink, which

indicates the growth of the microorganism (Palomino et al., 2002; Ostrosky et al., 2008; CLSI, 2012; Bona et al., 2014).

2.6. Determination of minimum bactericidal concentration (MBC)

To perform CBM, inoculations (10 µL) of CIM dilutions were performed in Mueller Hinton broth medium (100 µL/well) in a sterile microdilution plate and, subsequently, incubation was carried out at 37 °C for 24 hours. After incubation, 20 µL of resazurin were added, and a new incubation was performed at 37 °C to confirm the concentration capable of completely inhibiting the growth of the bacterial species, which would be verified by the absence of color change of the indicator dye (Ncube et al., 2008; Guerra et al., 2012).

2.7. Associations of *Tymus vulgaris* (Thyme) with synthetic antimicrobials

Association studies of *Tymus vulgaris* using synthetic antimicrobials were performed by the disk diffusion technique on solid medium with filter paper disks of ampicillin, gentamicin, ciprofloxacin and ceftazidime (Bauer et al., 1966; Oliveira et al., 2006). Using a sterile swab, a volume of approximately 1 mL of each bacterial suspension was plated on the solid surface of Mueller Hinton agar (MHA) contained in flat sterile plates. Then, the paper disks (impregnated with the antimicrobials) were applied to the MHA with the bacterial suspension. Immediately after, an aliquot of 20 µL (MIC) of the tested compound was transferred to the disks containing the antimicrobials. In addition, a negative control containing only the bacterial suspension with antimicrobial disks was performed. The plates were incubated at 37 °C for 24–48 h, followed by reading. The effect was considered synergistic if the microbial growth inhibition zone formed by the combination (compound + antimicrobial) had a diameter ≥ 2 mm, when compared to the inhibition zone formed by the action of the antimicrobial alone. When the formation of the inhibition zone resulting from the combination. If the combined action (compound + antimicrobial) was smaller in diameter than that developed by the isolated action of the antimicrobial, an antagonistic effect was considered. The effect was considered indifferent when the inhibition zone resulting from the combined application (compound + antimicrobial) had the same diameter as that resulting from the isolated application of the antimicrobial (Cleeland and Squires, 1991; Oliveira et al., 2006). All tests were performed in duplicate.

2.8. Determination of minimum inhibitory concentration for adhesion (MICA)

The Minimum Inhibitory Concentration for Adhesion (MIC) of the compound was determined in the presence of sucrose (5%), according to Albuquerque et al. (2010) with modifications, using compound dilutions of up to 1:256. For bacterial growth, the bacterial strain was grown at 37 °C in Mueller Hinton broth (DIFCO, Michigan, United States). Then, 0.9 mL of the subculture was distributed into test tubes and 0.1 mL of the solution corresponding to the compound dilutions was added. Incubation was carried out at 37 °C for 24 hours with the tubes inclined

at 30°. The reading was performed by visual observation of the adhesion of the bacteria to the walls of the tube after shaking. The procedure was performed in duplicate. The same procedure was performed for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA). CIMA was considered the lowest concentration of the agent in contact with sucrose, which prevented adhesion to the glass tube.

3. Results

3.1. Minimum Inhibitory Concentration (MIC)

Data regarding the minimum inhibitory concentration of *Thymus vulgaris* essential oil against different strains of *E. coli* are detailed in Table 1. Efficacy was assessed by observing bacterial growth, which occurred with a MIC of 512 µg/mL.

3.2. Minimum Bactericidal Concentration (MBC)

The results regarding the Minimum Bactericidal Concentration of the essential oil extracted from *Thymus vulgaris* against *E. coli* are outlined in Table 2. An analysis of the data reveals that the MBC was recorded to be 512 µg/mL.

Table 1. Minimum inhibitory concentration (MIC) in µg/mL of *Thymus vulgaris* (Thyme) against *Escherichia coli* strains isolated from meat products.

	<i>Thymus vulgaris</i>			
	Ec 41	Ec 42	Ec 44	Ec 45
1024 µg/mL	+	+	+	+
512 µg/mL	+	+	+	+
256 µg/mL	-	-	-	-
128 µg/mL	-	-	-	-
64 µg/mL	-	-	-	-
32 µg/mL	-	-	-	-

Legend: (+) Inhibition of bacterial growth (-) Did not inhibit bacterial growth.

Table 2. Minimum Bactericidal Concentration (MBC) in µg/mL of *Thymus vulgaris* (Thyme) against *Escherichia coli* strains isolated from meat products.

	<i>Thymus vulgaris</i>			
	Ec 41	Ec 42	Ec 44	Ec 45
1024 µg/mL	+	+	+	+
512 µg/mL	-	+	+	+
256 µg/mL	-	-	-	-
128 µg/mL	-	-	-	-
64 µg/mL	-	-	-	-
32 µg/mL	-	-	-	-

Legend: (+) Inhibition of bacterial growth (-) Did not inhibit bacterial growth.

3.3. Association of *Thymus vulgaris* oil with synthetic antimicrobials

The diameters of the inhibition zones (mm) generated by the combination of *T. vulgaris* essential oil with synthetic antibacterials in *E. coli* strains are documented in Table 3. An analysis of the results indicates that, in most strains, *T. vulgaris* oil demonstrated an antagonistic effect when combined with the synthetic antibacterials.

3.4. Minimum Inhibitory Concentration for Adhesion (MICA)

The results of the Minimum Inhibitory Concentration of Adhesion (MIC) of *Thymus vulgaris* essential oil, as well as a comparison with the positive control (chlorhexidine 0.12%) against the *Escherichia coli* strain (Ec 41), are presented in Table 4.

4. Discussão

Parham et al. (2020) highlight that bioactive substances present in plants and their derivatives, such as essential oils, have pharmacological activity capable of inhibiting the pathogen, with herbal medicines being predominant in the treatment of infections in certain developing countries, and because they have low toxicity to human cells, natural products become valid resources for investigations of future antimicrobial drugs.

According to Sartoratto et al. (2004), the minimum inhibitory concentration for a natural product is strong, when the MIC is 50 to 500 µg/mL, moderate when it presents values of 600 to 1,500 µg/mL and weak when above 1,500 µg/mL, based on the MIC value of the essential oil of *T. vulgaris* showed strong inhibitory activity against *Escherichia coli* strains. Similar results to those of the present study were evidenced in the study by Cáceres et al. (2020) when testing the action of *T. vulgaris* essential oil against pathogenic strains of *E. coli*, including serotype O157:H7, obtaining MIC values in the range of 0.37 to 0.75 mg/mL, emphasizing a high inhibitory power, thus highlighting the potential of this essential oil as a promising alternative for the control of infections caused by *E. coli*.

Hafidh et al. (2011) determine that for a compound to be considered bactericidal or bacteriostatic, the MIC and MBC values must be analyzed. When they are in a ratio of 1:1 to 2:1, the compound is considered bactericidal; if it is in a ratio greater than 2:1, it is considered a bacteriostatic product. In this sense, it was possible to observe that the antibacterial activity of the *T. vulgaris* EO tested was bactericidal for all strains of *E. coli*.

Sateriale et al. (2022) also highlight the bactericidal activity of *T. vulgaris* essential oil against *Escherichia coli* isolated from ground chicken meat, with MBC values below 100 µL/mL –1, emphasizing that thyme EO can be a viable source for the development of natural antimicrobials against foodborne pathogens, acting as an alternative to

Table 3. Interference of *Thymus vulgare* essential oil in association with synthetic antimicrobials for *Escherichia coli* strains.

Antibiotics		Microorganisms			
		<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
		Ec 41	Ec 42	Ec 44	Ec 45
Ampicillin	HIA	18 mm	20 mm	32 mm	30 mm
	HIA w/ TV	20 mm ↑	12 mm ↓	8 mm ↓	10 mm ↓
Gentamicin	HIA	24 mm	26 mm	18 mm	18 mm
	HIA w/ TV	16 mm ↓	20 mm ↓	16 mm ↓	18 mm *
Ceftazidime	HIA	34 mm	34 mm	24 mm	24 mm
	HIA w/ TV	22 mm ↓	22 mm ↓	26 mm ↑	22 mm ↓
Ciprofloxacin	HIA	40 mm	38 mm	26 mm	24 mm
	HIA w/ TV	36 mm ↓	32 mm ↓	16 mm ↓	30 mm ↑

Legend: HIA: Antimicrobial inhibition zone. TV: *Thymos vulgaris*. Synergistic effect (↑); Antagonistic effect (↓); Indifferent effect (*).

Table 4. Minimum Inhibitory Concentration for Adhesion (MIC) of *Thymus vulgaris* essential oil and 0.12% chlorhexidine digluconate against the *Escherichia coli* strain (Ec 41).

<i>Thymus vulgaris</i>								
1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
-	-	-	-	-	+	+	+	+
0.12% Chlorhexidine digluconate								
1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
-	+	+	+	+	+	+	+	+

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

synthetic preservatives in order to ensure food safety, and consequently that of consumers.

The combination of the compound with ciprofloxacin and ampicillin for the Ec41 strain resulted in a synergistic effect, suggesting that the compound can potentiate the action of antimicrobials against *Escherichia coli*. Previous studies have also reported synergistic effects in disc diffusion tests involving essential oils and antimicrobials. Santos et al. (2024) observed an increase in inhibition halos when combining *Eucalyptus radiata* EO with ciprofloxacin against food-borne *E. coli*.

Similarly, Diniz et al. (2024) reported that the association of *Origanum vulgare* essential oil with ampicillin led to a significant increase in the inhibition halos of this antimicrobial, indicating a possible complementary action of the compounds on other strains isolated from meat products. In this context, the synergy observed suggests that these natural compounds can act as therapeutic adjuvants, contributing to the development of strategies to combat infections caused by these pathogens.

However, antagonistic effects were also observed when *T. vulgaris* EO was combined with the antimicrobials gentamicin and ceftazidime, differing from the results presented by Diniz et al. (2023) who, when testing the association of thyme EO with gentamicin and ceftazidime, observed synergism in at least one strain of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus*.

Thus making it evident that there are intrinsic differences in the structures and defense mechanisms within Gram-negative species that alter the action of these compounds, as occurs with synthetic antimicrobials, highlighting the importance of carefully evaluating antimicrobial activity in different strains and contexts to fully understand the efficacy of essential oils.

Vukić et al. (2023) emphasize that the association of bacteria in biofilm is one of the great difficulties for the industry, since the addition of chemical preservatives and processing to eliminate this biofilm diminishes the interest of consumers who prefer preservative-free and minimally processed products, making the search for a natural compound capable of inhibiting biofilm extremely necessary.

When investigating the anti-adherent action of *T. vulgaris* essential oil, it was noted that it was able to inhibit the adhesion of *E. coli* to the wall of the glass tube up to a ratio of 1:32, with a result superior to that presented by chlorhexidine digluconate at 0.12%. The result observed is relevant in combating biofilm since it affects an important stage in the formation of this structure, which is the irreversible attachment of bacteria to surfaces (Rather et al., 2021).

In research carried out by Santos et al. (2024) with *Eucalyptus radiata* EO against *E. coli* strains from meat derivatives, they showed that eucalyptus EO presented greater anti-adherent action than chlorhexidine, in line with the results obtained for thyme EO. These results demonstrate the significant potential of essential oils as effective alternatives in combating the formation of bacterial biofilms, highlighting their relevance for food safety and infection control.

5. Conclusion

Thymus vulgaris essential oil showed strong inhibitory activity against *Escherichia coli* strains. When combined with synthetic antimicrobials, thyme showed an antagonistic effect against most bacterial strains. It also showed anti-adherent action, preventing the formation of biofilm of the strains analyzed. Thus, these results show that *Thymus vulgaris* essential oil is a promising alternative for combating pathogenic bacteria. However, more in-depth research is needed to further explore its effect.

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