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Original Article

Antibacterial activity of *Thymus vulgaris* (thyme) essential oil against strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* isolated from meat product

Atividade antibacteriana do óleo essencial de *Thymus vulgaris* (tomilho) contra cepas de *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* e *Staphylococcus saprophyticus* isoladas de produtos cárneos

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Abstract

Meat products represent an important component of the human diet and are a good source of nutrients. Food-borne microorganisms are the main pathogens that cause human diseases as a result of food consumption, especially products of animal origin. The objective of the present research was to verify the antibacterial activity of the essential oil of *Thymus vulgaris* against strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus* isolated from meat products. For this, the analyses of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were performed in microdilution plates. The association of the product with antimicrobials was also studied using disk diffusion. And the anti-adherent activity, which was determined in the presence of sucrose, in glass tubes. Thyme oil showed a strong inhibitory activity against *K. pneumoniae*, *P. aeruginosa* and *S. saprophyticus*, with the MIC values ranging from 64 to 512 µg/mL, and bactericidal effect for most strains, with MBC values ranging from 256 to 1,024 µg/mL. *T. vulgaris* oil exhibited varied interactions in association with the antimicrobials, with synergistic (41.67%), indifferent (50%) and antagonistic (8.33%) effects. Regarding the anti-adherent activity, the test product was effective in inhibiting the adherence of all bacterial strains under study. Therefore, thyme oil presents itself as an antibacterial and anti-adherent agent against *K. pneumoniae*, *P. aeruginosa* and *S. saprophyticus*, being a natural product that can represent an interesting alternative in the efforts to combat foodborne diseases.

Keywords: biology, health science, microbiology, medicinal plants.

Resumo

Os produtos cárneos representam um importante componente da dieta humana e constituem uma boa fonte de nutrientes. Microrganismos de origem alimentar são os principais patógenos que causam doenças humanas como resultado do consumo de alimentos, principalmente, produtos de origem animal. O objetivo da presente pesquisa foi verificar a atividade antibacteriana do óleo essencial de *Thymus vulgaris* frente às cepas de *Klebsiella pneumoniae, Pseudomonas aeruginosa* e *Staphylococcus saprophyticus* isoladas de produtos cárneos. Para isso, foram realizadas as análises de Concentração Inibitória Mínima (CIM) e a Concentração Bactericida Mínima (CBM) em placas de microdiluição. Assim como, o estudo de associação do produto com antimicrobianos, realizado por difusão em disco. E a atividade antiaderente, que foi determinada na presença de sacarose, em tubos de vidro. O óleo de tomilho apresentou uma forte atividade inibitória contra *K. pneumoniae*, *P. aeruginosa* e *S. saprophyticus*, com os valores de CIM variando entre 64 a 512 µg/mL, e efeito bactericida para a maioria das cepas, com valores de CBM entre 256 a 1.024 µg/mL. O óleo de *T. vulgaris* exibiu interações variadas na associação com os antimicrobianos, com efeitos sinérgicos (41,67%), indiferente (50%) e antagonista (8,33%). Em relação a atividade antiaderente, o produto teste foi eficaz na inibição a aderência de todas cepas bacterianas em estudo. Portanto, o óleo de tomilho apresenta-se como agente antibacteriano e antiaderente frente a *K. pneumoniae*, a *P. aeruginosa* e a *S. saprophyticus*, sendo um produto natural que pode representar uma alternativa interessante nos esforços para combater doenças transmitidas por alimentos.

Palavras-chave: biologia, ciência em saúde, microbiologia, plantas medicinais.

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1. Introduction

Meat products represent an important component of the human diet, and their consumption has registered a global increase in recent years (Ursachi et al., 2020). These foods are a good source of essential nutrients, high quality protein, fat and minerals (Aminzare et al., 2019). Safety and quality assessment of animal source foods are necessary in order to reduce losses, mitigate health risks, and ensure consumer safety (Ahmed et al., 2018).

Foodborne microorganisms are major pathogens that affect food safety and cause human illness worldwide as a result of consumption of food, mainly, animal products, contaminated with vegetative pathogens or their toxins (Abebe et al., 2020).

Waterborne and foodborne diseases impede socioeconomic development, burdening health systems and damaging national economies, tourism, and trade (WHO, 2015). An estimated 600 million - nearly 1 in 10 people worldwide - fall ill after eating contaminated food and 420,000 die each year (WHO, 2015).

Of the biological hazards, bacterial pathogens are the most serious concern regarding meat safety issues for consumers (Zelalem et al., 2019). Among these pathogens, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus saprophyticus* are species that have been found in meat products (Calbo et al., 2011; Charmpi et al., 2020; Silva et al., 2022).

The *Klebsiella pneumoniae* species is Gram-negative bacillary, belonging to the Enterobacteriaceae family (Li et al. 2023), and is an opportunistic pathogen capable of causing a variety of infections, such as pneumonia, urinary tract infections, and bacteremia (Choby et al., 2020). *Pseudomonas aeruginosa* is a Gram-negative bacillary species belonging to the family Pseudomonadaceae and is an important human pathogen that is often associated with hospital infections, affecting mainly immunosuppressed patients (Urganci et al., 2022). And, *Staphylococcus saprophyticus* is a coccoid-shaped, Gram-positive bacterium, belonging to the Staphylococcaceae family, found in the human microbiome, which causes hospital infections, and is especially important in young women and those of reproductive age (Oliveira et al., 2023).

Synthetic antimicrobials consist of one of the main drugs used to treat bacterial infections, however the overuse of antibiotics in animals and humans, antibiotics sold without prescription, increased international travel, lack of sanitation/hygiene, and release of unmetabolized antibiotics or their residues into the environment through manure/feces, has caused the emergence of multidrug-resistant bacteria (Aslam et al., 2018). Currently, the introduction of a new antibiotic into the market is almost immediately followed by the emergence of resistant bacterial strains, which are responsible for infections that are more difficult to treat, requiring the use of more toxic and more expensive drugs (Serwecińska, 2020), making alternative routes in the treatment of bacterial diseases necessary, entering in this context the medicinal plants.

Plants are living chemical factories for the biosynthesis of a huge array of secondary metabolites and, in fact, it is these metabolites that form the basis of many commercial pharmaceutical drugs as well as herbal remedies derived from medicinal plants (Li et al., 2020).

Essential oils are composed 'of a mixture of terpenes, terpenoids, phenylpropanoids, and various low molecular weight compounds (Wińska et al., 2019). They are isolated from leaves, bark, flowers, shoots, seeds, roots, stems, and fruits of different aromatic plants (Maurya et al., 2021), having their bioactive components of volatile nature, which provide a well-built odor and have the ability to release aroma or flavor (Smith et al., 2005). They are isolated from different plants, including those in the families Asteraceae, Lamiaceae, Cyperaceae, Zingerberaceae, Piperaceae, Apiaceae, Myrtaceae, Solanaceae, Apocynaceae, and Lauraceae (Nuzhat and Vidyasagar, 2013).

Thymus vulgaris is one of the most important species of the Lamiaceae family, native to the Mediterranean region and used worldwide as a seasoning in cooking and in liquors, in addition to therapeutic use (Morales, 2002). The main active agent of thyme herb is the volatile oil, which has several constituents (such as thymol, γ-terpinene, p-cymene, carvacrol and linalool) (György et al., 2020).

Thus, due to the antibacterial activity verified in thyme spice against some species (Millezi et al., 2012; Fournomiti et al., 2015; Miladi et al., 2016; Ozogul et al., 2020), in addition to the increasing consumer demand for effective, safe and natural products (Hammer et al., 1999), quantitative data about the activity of the essential oil of this vegetable becomes urgent. Based on this, the present study tested the antibacterial and anti-adherent potential of *T. vulgaris* essential oil against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus* strains.

2. Material and Methods

2.1. Obtaining the essential oil

The *Thymus vulgaris* (thyme) essential oil used was from the brand QUINARÍ® (Ponta Grossa - Paraná). It was solubilized in the presence of Tween 80 and DMSO, and diluted in distilled water (Allegrini et al., 1973).

2.2. Microorganisms

We used strains of *Klebsiella pneumoniae* (Kp 42 and Kp 44), *Pseudomonas aeruginosa* (Pa 43 and Pa 44) and *Staphylococcus saprophyticus* (Sa 41 and Sa 45), isolated from meat products from the Microbiology Laboratory of the Central Laboratory of the UACB - UFCG.

These strains were preserved in Muller-Hinton Agar (MHAG) and glycerin at 4ºC.

The inocula were obtained from these overnight cultures in AMH at 37 ºC and diluted in sterile 0.9% saline solution to obtain a final concentration of approximately 1.5 x 108 colony forming units per mL (CFU/mL), adjusted by turbidity compared to a suspension of barium sulfate and sulfuric acid in tube # 0.5 of the McFarland scale (Bauer et al., 1966; Cleeland and Squires, 1991).

The laboratory tests were performed in the Microbiology Laboratory of the Central Laboratory of the Academic Unit

of Biological Sciences (UACB) of the Federal University of Campina Grande (UFCG) / Patos - PB.

2.3. Antimicrobial agents

Ampicillin (10 µg/mL), gentamicin (10 µg/mL), ceftadezime (30 µg/mL) and ciprofloxacin (5 µg/mL), were used as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018).

2.4. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the microdilution technique in a 96-well plate with a U-shaped bottom. In a 96-well plate, 100 µl Mueller Hinton broth, doubly concentrated, and 100 µl of thyme essential oil were added at concentrations of 1024 to 16 µg/ml. The determination of the MIC was conducted with 10 µl of the microorganism in each well, approximately 1.5 x 108 CFU/ml. The penultimate well containing 200 µl of the broth was inoculated with the microorganism suspension, being the positive growth control, and the last well received only 200 µl of the broth, being the negative control. The assay was performed in duplicate. The plates were incubated at 35 °C for 24 hours. After the proper incubation time of the assays with the bacteria, the first reading of the results was performed. Then, 20 µl of sodium resazurin solution (SIGMA) in sterile distilled water at a concentration of 0.01% (w/v), recognized as a colorimetric oxide-reduction indicator for bacteria, was added. The reading was done, visually, by the absence or presence of growth of the microorganism by the formation of a cluster of cells (button). And also by observing the change in color of the solution, from blue to pink, indicating growth. A new incubation was performed at 37 ºC. The MIC was determined as the lowest concentration of the essential oil that inhibited the visible growth of the microorganism and also by observing the change in the coloration of the solution, from blue to pink, indicating growth of the microorganism (Palomino et al., 2002; Ostrosky et al., 2008; CLSI, 2012; Bona et al., 2014).

2.5. Determination of the Minimum Bactericidal Concentration (MBC)

After reading the results, inoculations (10 µL) of three dilutions from the MIC were made into Mueller-Hinton broth medium (100 µL/cavity) in sterile microdilution plate for the determination of the MBC. After incubation at 37 °C for 24 hours, 20 µL of resazurin was added. The assays were incubated at 37 ºC for another 24 hours for confirmation of the concentration capable of inhibiting the total growth of the bacterial species, verified by a non-change in the coloration of the indicator dye (Ncube et al., 2008; Guerra et al., 2012).

2.6. Association study of the product with antimicrobials

To study the association of the product with antimicrobials, the solid medium disk diffusion technique using filter paper disks was used (Bauer et al., 1966; Oliveira et al., 2006). An aliquot of 20 µL of the MIC of the test product was transferred to the discs containing the antimicrobials in their respective concentrations, and then placed in smooth sterile Petri dishes (140 x 15) containing the AMH medium, which were previously inoculated with sterile swabs, an approximate volume of 1 mL of the bacterial suspensions. Subsequently, the plates were incubated at 37 °C for 24-48 h, followed by reading (Oliveira et al., 2006; Koneman et al., 2008; Ostrosky et al., 2008). The interfering effect of the combination of the product plus antimicrobials, was evaluated according to the methodology described by Cleeland and Squires (1991). It was considered as synergistic effect, when the occurrence of halo of inhibition of microbial growth formed by the combined application of the essential oil (EO) plus the antimicrobial (AB) with diameter ≥ than 2mm, when compared with the halo of inhibition formed by the action of the AB alone. When the formation of a halo of inhibition resulting from the combined action of AB and OE had a smaller diameter than the one developed by the action of AB alone, it was considered an antagonistic effect. It was considered an indifferent effect when a halo of inhibition was observed as a consequence of the combined application of AB and OE with a diameter equal to that of the application of AB alone.

2.7. Determination of the Minimum Inhibitory Adherence Concentration (MIAC)

The Minimum Inhibitory Adherence Concentration (MIAC) of the compound was determined in the presence of 5% sucrose, according to Albuquerque et al. (2010), using concentrations corresponding to the compound up to 1:1024 dilution. From the bacterial growth, the bacterial strain was grown at 37 ºC in Mueller Hinton broth, then 0.9 mL of the subculture was dispensed into test tubes and then 0.1 mL of the solution corresponding to the compound dilutions was added. Incubation was done at 37 ºC for 24 hours with tubes tilted at 30º. The reading was done by visual observation of the adherence of the bacteria to the walls of the tube after shaking it. The assay was performed in duplicate. The same procedure was performed for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA). The MIAC was considered the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube.

3. Results

The results of antibacterial activity of *T. vulgaris* essential oil with MIC and MBC values are shown in Table 1 and the activity was measured in terms of the presence of microorganism growth.

By analyzing Table 1, it is observed that thyme essential oil shows MIC values between 64 µg/mL and 512 µg/mL against the growth of different strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus*. Thus, the oil shows strong inhibitory activity. It also shows bactericidal activity for most of the strains, where the MBC ranges between 256 µg/mL and 1,024 µg/mL.

Table 2 shows the interference of thyme essential oil on the antibacterial action of clinical use antimicrobials. Considering the comparison of the diameters of the halos of inhibition of bacterial growth in the assays with the antimicrobials alone and in association with the essential oils, it can be observed, in some interactions, the occurrence of interference of the essential oils on the antibacterial power of the antimicrobials. This interference occurs with modulations for synergism, indifference and antagonism, being respectively 41.67%, 50% and 8.33%.

Gentamicin and ceftadizime were the antimicrobials that had the greatest interference of the essential oils, being observed synergism in the three bacterial species under study, in at least one type of strain in each species. Ampicillin and ciprofloxacin showed indifferent effect in most associations.

Regarding the results of the minimum adherence inhibitory concentration, the essential oil of *T. vulgaris* was effective in inhibiting the adherence of the bacterial strains of *K. pneumoniae*, *P. aeruginosa*, and *S. saprophyticus* in the presence of sucrose. The minimum adherence inhibitory concentrations of this herbal agent are shown in Table 3. The antibacterial agent chlorhexidine digluconate 0.12%, also shows a significant inhibition in the adhesion effect against *S. saprophyticus*, while for *K. pneumoniae* and *P. aeruginosa* there is no inhibition.

Table 1. Antibacterial activity of *Thymus vulgaris* essential oil against *K. pneumoniae*, *P. aeruginosa* and *S. saprophyticus* isolated from meat products.

Bacterial strain	MIC	MBC	Negative Control	Positive Control
Klebsiella pneumoniae Kp 42	512 µg/mL	512 µg/mL	-	+
Klebsiella pneumoniae Kp 44	512 µg/mL	512 µg/mL	-	+
Pseudomonas aeruginosa Pa 43	512 µg/mL	512 µg/mL	-	+
Pseudomonas aeruginosa Pa 44	512 µg/mL	512 µg/mL	-	+
Staphylococcus saprophyticus Sa 41	512 µg/mL	1.024 µg/mL	-	+
Staphylococcus saprophyticus Sa 45	64 µg/mL	256 µg/mL	-	+

Legend: (-) there was no bacterial growth; (+) there was bacterial growth.

Table 2. Interference of *Thymus vulgaris* essential oil in association with clinical antimicrobials against strains of *K. pneumoniae*, *P. aeruginosa* and *S. saprophyticus*. Average of duplicates.

Antimicrobials		Microorganisms					
		K. pneumoniae **Kp 42**	***K. pneumoniae*** **Kp 44**	***P. aeruginosa*** **Pa 43**	***P. aeruginosa*** **Pa 44**	***S. saprophytic*** **Sa 41**	***S. saprophytic*** ***Sa*** **45**
Ampicillin	HI	0 mm	0 mm	0 mm	14 mm	28 mm	28 mm
	HITHY	8 mm ↑	0 mm *	0 mm *	14 mm *	28 mm *	30 mm ↑
Gentamicin	HI	20 mm	18 mm	18 mm	20 mm	30 mm	28 mm
	HITHY	20 mm *	20 mm ↑	20 mm ↑	18 mm ↓	40 mm ↑	30 mm ↑
Ceftazidime	HI	24 mm	24 mm	24 mm	28 mm	20 mm	10 mm
	HITHY	26 mm ↑	24 mm *	26 mm ↑	28 mm *	18 mm ↓	14 mm ↑
Ciprofloxacin	HI	32 mm	30 mm	30 mm	34 mm	34 mm	34 mm
	HITHY	32 mm *	30 mm *	30 mm *	34 mm *	44 mm ↑	34 mm *

Legend: HI: diameter of the growth inhibition zone determined by the antimicrobial alone. HITHY: diameter of the growth inhibition zone determined by the association of the antimicrobial and *Thymus vulgaris* oil. ↑ = Synergistic effect. ↓ = Antagonistic effect. * = Indifferent effect.

Table 3. Minimum adhesion inhibitory concentration of *Thymus vulgaris* and chlorhexidine against *K. pneumoniae*, *P. aeruginosa* and *S. saprophyticus* bacteria.

Bacterial strain	***Thymus vulgaris***	Clorexidina
Klebsiella pneumoniae Kp 42	1:1	-
Pseudomonas aeruginosa Pa 43	1:32	-
Staphylococcus saprophyticus Sa 45	1:8	1:4

Legend: (-) showed biofilm formation.

4. Discussion

The results of the MIC indicate strong inhibitory activity of thyme essential oil, since, when the MIC is from 50 to 500 µg/mL it is considered that the test substance presents a strong activity; from 600 to 1,500 µg/mL a moderate activity, and, above 1,500 µg/mL a weak activity (Sartoratto et al., 2004).

According to Hafidh et al. (2011), a compound can be considered bactericidal or bacteriostatic and, for that, the ratio of MBC and MIC is analyzed, when this ratio is between 1:1 to 2:1, the compound is considered bactericidal and, to be considered as bacteriostatic the ratio should be higher than 2:1. In this case, the oil shows bactericidal activity for most strains and bacteriostatic for Sa 45.

These results of strong inhibitory activity and bactericidal/bacteriostatic effect of thyme essential oil against strains of *K. pneumoniae*, *P. aeruginosa* and *S. saprophyticus* may be related to the high content of the compound p-cymene, which alone has inhibitory effects on microorganisms (Van Zyl et al., 2006), promoting a decrease in the viability of biofilms (Silva et al., 2012), besides causing damage to cell membranes, suggesting that it is the main mechanism of action of this compound (Andrews et al., 1980). Phenolic compounds, such as thymol, present in this oil, confer strong antibacterial action against many pathogenic bacteria (Sienkiewicz et al., 2012), presenting a mode of action that involves interference with cytoplasmic membrane functions, including proton motive forces and active transport (Eklund, 1985).

Most studies on the action of essential oils against food spoilage organisms and foodborne pathogens have observed that essential oils are relatively more active against Gram-positive than Gram-negative bacteria (Lambert et al., 2001). In the present research, it was possible to verify this through the results of the *S. saprophyticus* Sa 45 strain, which has the minimum inhibitory concentration of 64 µg/mL and minimum bactericidal concentration of 256 µg/mL.

Research has revealed that thyme oil against *Klebsiella pneumoniae* also showed antibacterial activity. Using the disk diffusion technique, using standard bacteria against thyme essential oil, Hammer et al. (1999) observed a MIC of 0.25% (v/v) for *K. pneumoniae*. Ozogul and collaborators (2020), using standard strain (ATCC) of *K. pneumoniae*, through the agar disc diffusion method, obtained an average of the inhibition halos of 23.4 mm, and, through the minimum inhibitory concentration and the minimum bactericidal concentration, had a value of 3.13 mg/mL for both.

Through the well diffusion assay technique, Al-Dosary (2018), observed a zone of inhibition of 7 mm for standard *K. pneumoniae* (ATCC) and resistance for five strains of *K. pneumoniae* isolated from patients in a hospital in Saudi Arabia. Whereas, using the minimum inhibitory concentration method, a MIC of 0.75 µg/mL was observed for the standard strain (ATCC) and, again, resistance for all five strains of *K. pneumoniae* (Al-Dosary, 2018).

Fournomiti et al. (2015), in a research conducted with strains isolated from patients in a hospital in Greece and with standard strain (NCTC) of *K. pneumoniae*, using the broth microdilution method, observed the mean MIC value of 9.5 µg/mL for thyme oil. Using microwell dilution method, Al-Bayati (2008) observed that thyme essential oil proved active against *K. pneumoniae* obtained from Department of Biology, College of Science (University of Mosul, Iraq), with MIC value of 500 µg/mL. Damjanović-Vratnica et al. (2015), using *K. pneumoniae* strains isolated from clinically treated or hospitalized patients in a Medical Health Center (Podgorica), using the agar disc diffusion method, recorded a 38 mm diameter of the halo of inhibition at the lowest concentration of the oil (4.5 µg).

Other studies performed with thyme oil against *Pseudomonas aeruginosa* also showed effective antibacterial activity. Hammer and collaborators (1999), conducted a research using standard bacteria against thyme essential oil through the disk diffusion technique, and had as a result an MIC of > 2.0% (v/v). In another study, also with standard strains (ATCC), the minimum inhibitory concentration of the essential oil of *T. vulgaris* against *P. aeruginosa* was 5%, and at this concentration the formation of a halo of inhibition averaged 6.5 mm, and at the highest concentration (50%), the average halo was 8.83 mm (Millezi et al., 2012). Galovičová et al. (2021), using standard strain of *P. aeruginosa*, through the disk diffusion method, obtained moderate antibacterial activity (mean zone of inhibition of 10.67 mm), while for MIC and MBC, the values were 103.28 µL/mL and 169.19 µL/mL, respectively.

Only, Al-Bayati (2008), using microwell dilution method, observed that thyme essential oil was not active against *P. aeruginosa* obtained from Department of Biology, College of Science (University of Mosul, Iraq). Damjanović-Vratnica et al. (2015), using *P. aeruginosa* strains of reference (ATCC) and isolated from clinically treated or hospitalized patients in a Medical Health Center (Podgorica), using the disc diffusion method on agar, recorded a diameter of 18 mm and 14 mm of the halo of inhibition at the oil concentration of 9 µg, respectively, while at the lowest concentration (4.5 µg) of the oil, there was resistance of both strains.

Other bacterial species isolated from food were also tested against the essential oil of *T. vulgaris*, corroborating this research, demonstrating the test substance to have an antibacterial effect: *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella enterica*, *Salmonella Typhimurium*, *Serratia marcescens*, *Proteus mirabilis*, *Proteus vulgaris*, *Yersinia enterocolitica*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Micrococcus* spp, *Sarcina flava*, *Bacillus licheniformis*, *Bacillus thuringiensis*; *Listeria innocua*; *Pseudomonas luteola*, *Serratia liquefaciens*, *Vibrio vulnificus*, *Photobacterium damselae, Citrobacter* spp. and *Klebsiella* spp. (Marino et al., 1999; Miladi et al., 2016; Ozogul et al., 2020; Yasir et al., 2022).

The essential oil of *T. vulgaris* was tested against two standard *Staphylococcus aureus* strains in combination with the antimicrobial clindamycin and showed a potent inhibitory activity against both strains with MIC ranges between 125-500 µg ml -1. The synergistic activity was confirmed, suggesting that the use of an antimicrobial-plant combination may be a successful technique to reduce antimicrobial consumption, which would overcome antimicrobial resistance or delay its onset (Mayyas et al., 2021), corroborating the data of the present research

regarding gentamicin and ceftadizime, which showed synergistic effect when associated with thyme oil.

Regarding the data of the anti-adherence activity of the oil in the present research, it is observed that the thyme essential oil was effective in inhibiting the adherence of all strains, presenting a better result for *P. aeruginosa*. Al-Shuneigat et al. (2014), also obtained good results regarding this species using the essential oil of *T. vulgaris*, which was able to inhibit *P. aeruginosa* strains adhering to polystyrene surface at a subinhibitory level, where a very small amount was sufficient to eliminate biofilms and planktonic cells.

Previous research has suggested that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria (Snoussi et al., 2018; Bordoni et al., 2019). However, there were no differences between Gram-positive and Gram-negative bacteria with respect to the anti-adherent effect.

Other studies have also observed the anti-stick effect of thyme oil against various bacteria. Jafri et al. (2014), observed that the essential oil of *T. vulgaris* acts effectively inhibiting biofilm formation in Staphylococcus aureus strains. Miladi and collaborators (2016), reported that thyme essential oil acts against *Salmonella typhimurium*, through the XTT [2, 3-bis (2-methyloxy 4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] reduction assay method, in which they visualized inhibitory effect on biofilm formation of reference and food isolated strains. Kryvtsova et al. (2019) found that *T. vulgaris* essential oil exhibits high antibiofilm activity against clinical strains of *S. aureus*.

Alibi et al. (2020) demonstrated that thyme essential oil reduced biofilm formation on biomaterial surfaces against clinical strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Salmonella enteridis*, *Salmonella typhimurium*, *Salmonella zanzibar*, *Salmonella livingstone*, *Salmonella derby*, *Salmonella heidelberg*, *Corynebacterium striatum* and *Staphylococcus aureus*. Oliveira et al. (2021) also detected antibiofilm effect of *T. vulgaris* against the standard *Streptococcus mutans* strain in the initial biofilm formation of the salivary microcosm.

Bacteria in the biofilm are known to be much more resistant to antimicrobial agents than free-living cells (Lebert et al., 2007) and are medically important due to their implication in the pathogenesis of numerous bacterial infections that are difficult to eradicate with antimicrobials (Snoussi et al., 2018). In this regard, phytochemicals with antibiofilm properties may increase the efficacy of antimicrobials, allowing to reduce their use (Langeveld et al., 2014).

5. Conclusion

Thyme oil showed antibacterial potential, with a strong inhibitory activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus*, and bactericidal for most strains tested. It proved to be an alternative route in the search for compounds that potentiate the action of antimicrobial drugs, because it showed synergistic effect when combined with ampicillin, gentamicin, ceftadizime, and ciprofloxacin. In addition to presenting an anti-adherent effect against the tested strains. Therefore, the *T. vulgaris* oil presents itself as a natural product that can represent an interesting alternative in the efforts to combat foodborne diseases.

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