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Research Article

Essential Oils from *Thymus capitatus* and *Thymus algeriensis* as Antimicrobial Agents to Control Pathogenic and Spoilage Bacteria in Ground Meat

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The antibacterial effects of essential oils (EOs) extracted from *Thymus capitatus* and *Thymus algeriensis* were assessed and evaluated against four pathogenic bacteria (*Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 19118), *Staphylococcus aureus* (ATCC 25923), and *Salmonella typhimurium* (ATCC 1402)) and one spoilage bacterium (*Pseudomonas aeruginosa* (ATCC 27853)). Both investigated EOs presented significant antimicrobial activities against all tested bacteria with a greater antibacterial effect of *T. capitatus* EO. In fact, the results indicated that the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of *T. capitatus* EO are in the range of 0.006–0.012% and 0.012–0.025%, respectively, while those of *T. algeriensis* EO ranged between 0.012 and 0.025% and 0.05%, respectively. Furthermore, the inhibitory effects of both EOs were appraised against the spoilage bacterium *P. aeruginosa*, inoculated in minced beef meat, at two different loads (10^5 and 10^8 CFU) mixed with different concentrations of EOs (0.01, 0.05, 1, and 3%) and stored at 4°C for 15 days. The obtained data demonstrated that the antibacterial effect of tested EOs varies significantly in regard to the levels of meat contamination and the concentrations of EOs. In fact, in the presence of 0.01 and 0.05% of oils, a decrease in bacterial growth ($p < 0.01$) was observed; but, such an effect was more pronounced in the presence of higher concentrations of EOs (1 and 3%), regardless the level of meat contamination. Besides, at the low contamination level, both EOs exerted a rapid and a more pronounced antibacterial effect, as compared to the high contamination level. The results illustrated the efficacy of both EOs as preservatives in food against well-known pathogens of food-borne diseases and food spoilage, particularly in *P. aeruginosa* in beef meat. As regards sensory evaluation, the presence of *T. capitatus* EO proved to improve the sensory quality of minced beef meat.

1. Introduction

Meat and meat products represent one of the most perishable foodstuffs [1] due to their complex composition which consists of proteins, saturated and unsaturated lipids, carbohydrates, vitamins, pigments, high water content, and moderate pH [2, 3]. A large amount of spoiled meat has to be discarded engendering significant economic losses. According to the European Regulation (EC) No 178/2002 [4], spoiled meat is considered as unsafe, unsuitable food for human consumption and forbidden by the law.

The mechanisms responsible for meat spoilage are related to microbial growth, lipid oxidation, and enzymatic autolysis. The breakdown of fats, proteins, and carbohydrates in meat leads to the formation of off-odors, off-flavors, and slime formation, rendering meat unacceptable for

the consumer [5–7]. The presence of pathogenic bacteria such as *E. coli*, *Salmonella*, and *S. aureus* in minced meat and contact surface samples can cause serious health risks [8]. Microorganisms which are generally responsible for meat spoilage are *Pseudomonas* spp., Enterobacteriaceae, and *Brochothrix thermosphacta* [9]. *Pseudomonas* causes meat and meat product spoilage and develops repulsive characteristics as putrefaction of proteins and lipids with changes in pH continue [10, 11].

Additionally, grinding has several detrimental effects on meat by increasing the surface area exposed to air and bacterial contaminations [12]. It increases losses of intracellular reductants as well as polyunsaturated fat, leading to deterioration of meat and the warmed-over flavors [13, 14].

To extend the shelf life and decrease bacterial growth on meat, the common method used is refrigeration. However, lower temperatures might also modify the composition of the microbiota present on meat, such as psychrotrophic bacteria like *Pseudomonas* spp., which could grow at low temperature [1]. For this, it is an important challenge to find a solution to prevent bacterial contamination of meat.

Many strategies are being used to control the growth of pathogenic and spoilage bacteria and prolong the shelf life of meat. Since ancient times, plants and plant extracts have been used as flavoring agents in the food processing industry; they also exhibit some antibacterial, antifungal, and antioxidant properties [15]. Several aromatic plants, essentially rosemary, garlic, lavender, leek, olive leaf, onion, oregano, pepper, peppermint, sage, and *Satureja montana*, are being added to meat and meat products [16].

Thyme oil is one of the top 10 EOs used as a natural preservative in food [17]. The genus *Thymus* L. is a member of the Lamiaceae family and contains about 215 species, particularly prevalent in the Mediterranean area [18]. Thyme species are aromatic plants widely used in Tunisia and they are well known for their antispasmodic, antimicrobial, expectorant, and antioxidant activities.

Several *in vitro* studies have reported the efficiency of plant EOs against food-borne pathogens, whilst few published papers have studied the antibacterial effect of *Thymus* EOs on pathogen growth, in meat.

Thus, the purpose of the current work is to evaluate the antioxidant and the antimicrobial activities of *Thymus capitatus* (*T. capitatus*) and *Thymus algeriensis* (*T. algeriensis*) EOs against pathogenic bacteria and the impact of different concentrations of such EOs on the proliferation of spoilage bacterium *P. aeruginosa* at two contamination levels of 10^5 CFU/g and 10^8 CFU/g at 4°C.

2. Materials and Methods

2.1. Extraction of the Essential Oils. The aerial parts of *T. capitatus* and *T. algeriensis* were collected from Zaghouan region (north of Tunisia) in June 2018. The freshly cut plants were dried for two weeks, in the shade, at room temperature. They were grounded into powder, followed by hydrodistillation in a Clevenger-type apparatus for 3 hours. The EOs were extracted, dried over anhydrous sodium sulphate (Na_2SO_4), filtered, and then stored in the dark at 4°C.

2.2. Free Radical Scavenging Assay. The DPPH (2, 20-diphenyl-1-picryl hydrazyl) radical scavenging capacity was measured according to the method described by Boulanouar et al. [19]. One ml of each concentration of the EO extract (200, 300, 400, and 500 μg/mL) was mixed with 250 μl of 0.2 mM methanolic DPPH solution. A negative control was prepared by mixing the same amounts of methanol and DPPH solution. The mixture was shaken vigorously and incubated for 30 min, in the dark, at room temperature. The absorbance was then measured at 517 nm using a UV spectrophotometer, and the percentage of activity inhibition (I%) was calculated by the following formula: $(I\%) = [(A0 - At/A0) \times 100]$, where A0 is the absorbance of the control sample (without EO) and At is the absorbance of the EO with DPPH at 30 min.

The EO concentration providing an I% of 50 (IC_{50}) is calculated from the regression equation prepared from the concentrations of the EO and the inhibition percentages. The experiment was carried out in triplicate.

2.3. Microorganisms and Growth Conditions. The bacteria used in the present study were obtained from the culture collections of ATCC and Institute Pasteur of Tunis. The strains of *L. monocytogenes* (ATCC 19118) were cultivated in PALCAM *Listeria* agar (Biokar Diagnostics), *S. aureus* (ATCC 25923) in Baird-Parker (Biokar Diagnostics), *E. coli* (ATCC 25922) in Mac Conkey Sorbitol (Biolife), *S. typhimurium* (ATCC 1402) in Hektoen (Biolife), and *P. aeruginosa* (ATCC 27853) in *Pseudomonas* agar F (King's Medium B) (Biolife), at 37°C. Working cultures were prepared by adding a loopful of each test bacterium to 5 ml of Luria–Bertani Medium (LB) (Oxoid Ltd., UK) and then incubated at 37°C for 18 h [20].

2.4. Determination of MIC and MBC. The minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of *T. capitatus* and *T. algeriensis* were determined using the medium dilution method with minor modifications (NCCLS). The tested microorganisms were cultured at 37°C and diluted to approximately 10^6 CFU/ml, the negative control containing only the tested bacteria.

The inoculated plates were inverted and incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of EO at which no visible growth of bacteria is shown. The plates showing a concentration of EO greater than or equal to MIC were incubated at 37°C for further 24 h. The concentration at which no visible growth is noticed was defined as the MBC. The experiment was carried out in triplicate.

2.5. Inhibitory Effect of EO against Pseudomonas aeruginosa Inoculated in Minced Beef Meat. The procedure reported by Careaga et al. [21] was followed with some slight modifications to study the inhibitory effect of EOs.

2.6. Preparation of the Meat Model. Four kilos and 500 g of fresh beefsteaks were obtained from a local meat supermarket. Meat samples were collected and transported for analysis in an insulated cooler. Each piece of meat was plunged in boiling water for 5 min to reduce the number of microorganisms attached to the beef muscle surface, which was eliminated with a sterile knife under aseptic conditions.

2.7. Treatment of Minced Beef Meat. To evaluate the antimicrobial activity of *T. capitatus* EO against bacteria in meat samples, pieces of meat were minced in a sterile grinder with 19 cm in diameter, and portions of 22 ± 0.1 g were put into a high-density polyethylene bag.

Decimal dilutions were prepared from a fresh culture of 24 h. For each dilution, the optical density at 620 nm and the CFU were determined by subculturing on agar. The data obtained were used to make a calibration. Thus, the initial inocula (10^5 CFU *P. aeruginosa* and 10^8 CFU *P. aeruginosa*) were obtained based on a spectrophotometer reading.

Halves of the meat samples were inoculated with 10^5 CFU *P. aeruginosa*/g of beef and the remaining halves with 10^8 CFU *P. aeruginosa*/g of beef. Then, the samples were treated with different concentrations (0.01, 0.05, 1, and 3%) of *T. capitatus* or *T. algeriensis* EO, dissolved in 10% DMSO and homogenized in a stomacher for 5 min. For the control sample, the EO extract was replaced by DMSO. Finally, all bags containing the meat samples were stored at 4°C and examined every three days, during 15 days of storage [20]. The experiment was carried out in triplicate.

2.8. Bacterial Enumeration. *P. aeruginosa* count was done by adding 9 ml of BHI broth to 1 g meat sample placed in a polyethylene bag. Bacterial strain enumeration was determined by the plate colony count technique. For this, a series of dilutions was performed with physiological saline solution, and 100 μL of each sample dilution was spread onto the surface of *Pseudomonas agar* F "King's Medium B" plates, followed by incubation at 37°C for 24 hours. The obtained results were expressed as $\log_{10}$ CFU/g of meat.

2.9. Sensory Analysis. The sensory test was carried out at the Laboratory of Epidemiology and Veterinary Microbiology, Institute Pasteur of Tunis, Tunisia. The day before the event tasting. meat samples were thawed in a refrigerator at 4°C. Minced beef meat samples were cooked with no added salt and divided into samples of 10 g. The samples should be of uniform size. These were placed in aluminum trays covered with aluminum foil identified and put in a conventional oven. The beef meat samples were warmed before the evaluation, covered with aluminum foil. and presented to the panelists. Twelve trained panelists, comprised student and employees of the Laboratory of Epidemiology and Veterinary Microbiology, Institute Pasteur of Tunis, were served five meat samples: 1, control; 2, treated with 1% *T. capitatus*; 3, treated with 3% *T. capitatus*; 4, treated with 1% *T. algeriensis*; 5, treated with 3% *T. algeriensis*, with water and an unsalted snack in between to remove the remaining flavor. Coffee was also served to neutralize their noses between samples. The panel evaluated each sample in triplicate. Judges were requested to evaluate the cooked beef meat (offered in a randomized order) with a 3-digit code. Each attribute was scored on a scale of 10 cm for each characteristic: taste, color, tenderness, flavor, juiciness, and odor. The attributes were ranged from the lowest intensity of each trait to the highest. They measured overall acceptability in beef meat samples using the 9-point hedonic scale (1: dislike extremely, 2: dislike very much, 3: dislike moderately, 4: dislike slightly, 5: neither like nor dislike, 6: like slightly, 7: like moderately, 8: like very much, and 9: like extremely) [22].

2.10. Statistical Analysis. For each test, the results were presented as mean ± SD of three independent samples. The inhibitory concentration 50% (IC_{50} values) for antioxidant activities was calculated by a nonlinear regression analysis using GraphPad Prism, version 5.0. The *in situ* antibacterial activity was also performed using GraphPad Prism, version 5.0. The results were analyzed by two-way analysis of variance (ANOVA) to evaluate different antimicrobial treatment effects during the time of storage of 0, 3, 6, 9, 12, and 15 days. The statistical data analysis processed by ANOVA was calculated at a significance level of $p < 0.05$ using Bonferroni's multiple comparison tests.

CMI, CMB, and sensory data were analyzed by one-way ANOVA with the general linear model procedure of SAS (9.1). The residual mean square error was used as the error term. Means were separated using Duncan's test with a significance level of $p < 0.05$ (SAS, 9.1).

3. Results and Discussion

3.1. Antioxidant Activity: Free Radical Scavenging Assay. The EOs of herbs possess antioxidant properties that improve the shelf life of food. Thus, incorporation of EOs directly into food helps preserving it from oxidation phenomena [23]. In this context, it was shown that the antioxidant activity of EO of *T. capitatus* exhibits higher antiradical activity with an IC_{50} value of 213.53 μg/ml than that of *T. algeriensis* showing an IC_{50} value of 861.12 μg/ml, butylated hydroxytoluene (BHT) presenting an IC_{50} value of 30 ± 0.01 μg/ml (Table 1). These results agreed with those of Amarti et al. [26], who showed that *T. capitatus* EOs possess strong antioxidant activities with IC_{50} equal to 69.04 μg/ml. However, the used *T. algeriensis* EO demonstrated weaker antioxidant effect with IC_{50} equal to 745 μg/ml.

The antiradical activity of *T. capitatus* EO could be attributed to its high content of carvacrol (88.89%). On the contrary, *T. algeriensis* EO presented a weaker activity because of its poor content in phenolic compounds. In fact, a highly positive link between phenolic compounds and antioxidant activity was provided in this study, which is in agreement with other reported findings [27–29]. Based on these results, *T. capitatus* can be used as a natural antioxidant in food or for pharmaceutical applications.

Table 1: Antioxidant and antimicrobial activities of *Thymus capitatus* and *Thymus algeriensis* essential oils.

	Thymus capitatus essential oils		*Thymus algeriensis* essential oils	
Main essential oil compounds*	Carvacrol (88.98%), thymol (0.51%), *p*-cymene (1.14%), and α-terpinene (0.40%)		Linalool (17.62%), camphor (13.82%), terpinen-4-ol (6.80%), α-terpineol (6.41%), and α-terpenyl acetate (6.27%)	
Antioxidant activities IC_{50} (μg/ml)	213.53		861.12	
Antimicrobial activities (%)	MIC	MBC	MIC	MBC
E. coli	0.006^{Ba}	0.012^{Ba}	0.025^{Aa}	0.05^{Aa}
S. aureus	0.006^{Aa}	0.012^{Ba}	0.020^{Ba}	0.05^{Aa}
L. monocytogenes	0.012^{Bb}	0.025^{Bb}	0.025^{Aa}	0.05^{Aa}
P. aeruginosa	0.012^{Bb}	0.025^{Bb}	0.025^{Aa}	0.05^{Aa}
S. typhimurium	0.006^{Aa}	0.012^{Ba}	0.025^{Ba}	0.05^{Aa}

*The detailed results were reported by El Abed et al. and Ahmed et al. [24, 25]. IC_{50}: concentration of essential oil required for 50% of inhibition. MIC: minimal inhibitory concentration. MBC: minimal bactericidal concentration. A and B: different letters in the same row indicate significant differences ($p < 0.05$). a and b: different letters in the same column indicate significant differences ($p < 0.05$).

3.2. In Vitro Antibacterial Effect of Thyme Essential Oil. According to the results of MIC and MBC, illustrated in Table 1, both investigated EOs' activities presented an antimicrobial activity against all tested bacteria with a greater antibacterial effect of *T. capitatus* EO. As illustrated in a previous study of El Abed et al. [24], the EOs of *T. capitatus*, harvested from Zaghouan region, presented 19 compounds with the presence of several bioactive compounds, including carvacrol (88.98%), thymol (0.51%), *p*-cymene (1.14%), and α-terpinene (0.40%). As reported by Ben Hadj Ahmed et al. [25], the EOs of *T. algeriensis* from Zaghouan region presented 39 compounds, with linalool as a major compound (17.62%), followed by camphor (13.82%), terpinen-4-ol (6.80%), α-terpineol (6.41%), and α-terpinyl acetate (6.27%).

In addition, the results indicated that the MICs and MBCs of *T. capitatus* EO are in the range of 0.006–0.012% and 0.012–0.025%, respectively, while those of *T. algeriensis* EO ranged between 0.020 and 0.025% and 0.05%, respectively. These results are similar to those presented by Amarti et al. [30], which reported that *T. capitatus* EO from Morocco, mainly composed of carvacrol (70.92%), inhibits the growth of *E. coli* and *S. aureus* at a concentration of 1/2000 (v/v). A previous study, carried out in Tunisia by Aouadhi et al. [27], showed that the *T. capitatus* plant from Bizerte, containing thymol (81.49%), exhibits significantly higher antibacterial activity than *T. capitatus* from Sousse that contains thymol (69.95%), with MIC values ranging between 0.025 and 0.8%. Our results indicated that the used *T. capitatus* EO, collected from Zaghouan, exhibits the strongest antibacterial effect due to its high content of carvacrol (88.98%) [24, 31, 32].

Findings from the present study indicated that *T. capitatus* EO has a very significant ($p < 0.0001$) antibacterial action against *E. coli*, *S. typhimurium*, and *S. aureus,* whereas *L. monocytogenes* and *P. aeruginosa* seem to be the least sensitive. These results are in accordance with previous studies revealing that the weakest activity of *T. pectinatus* EO is observed against *P. aeruginosa* [31]. The EO extracted from the *T. algeriensis* plant exhibited a moderate antimicrobial effect against most tested bacteria, without any significant difference ($P > 0.05$) between them. Our results showed that both EOs did not have selective antibacterial activity on the basis of the cell wall differences of bacterial microorganisms. These findings are in agreement with previous works carried out with several *Thymus* species [33].

The mechanism of action of EOs and phenolic compounds on microorganisms has not been elucidated; it is generally proved that these not only attack the cytoplasmic membrane, thus destroying its permeability and releasing intracellular constituents, but also could cause membrane dysfunction with respect to electron transport, nutrient absorption, nucleic acid synthesis, and ATPase activity. This could be the result of the alteration of various enzymatic systems, including those involved in the production of energy and the synthesis of structural components [34].

Besides, our results showed that the antibacterial properties could be attributed to the high percentages of linalool (17.62%) and camphor (13.82%) of *T. algeriensis* EO [25, 35]. In this regard, the research of Liu et al. [36] showed a good antibacterial activity of linalool against *P. aeruginosa* with MIC and MBC values in the range of 431 and 832 μg/ml, respectively. Likewise, the study carried out by Rezzoug et al. [37] showed that the EO of *T. algeriensis* grown in the Atlas Algerian Sahara and composed of linalool (1.2%) inhibits *P. aeruginosa* growth with a MIC value of 512 μg/ml, while that of Moroccan *T. algeriensis*, composed of camphor (27,7%), showed a weak antibacterial effect against *E. coli* and *S. aureus*, with a MIC value of 1/100 [38]. It is worth noting that the chemical compositions of the EOs of *T. algeriensis* from Algeria and Morocco are completely distinct from that of Zaghouan, used in the present work.

Our study revealed that the antimicrobial activity of *P. aeruginosa* is significantly ($p < 0.0001$) more sensitive to EO of *T. capitatus* than that of *T. algeriensis*. These results are consistent with the previous report [39].

3.3. Antibacterial Efficacy of T. capitatus Essential Oil against Pseudomonas aeruginosa in Minced Beef Meat. To study the antibacterial effect of *T. capitatus* EO, depending on the inoculum concentrations, two different loads of *P. aeruginosa* (10^5 CFU/g and 10^8 CFU/g) were used before treating minced beef meat with EOs. As shown in Figure 1,

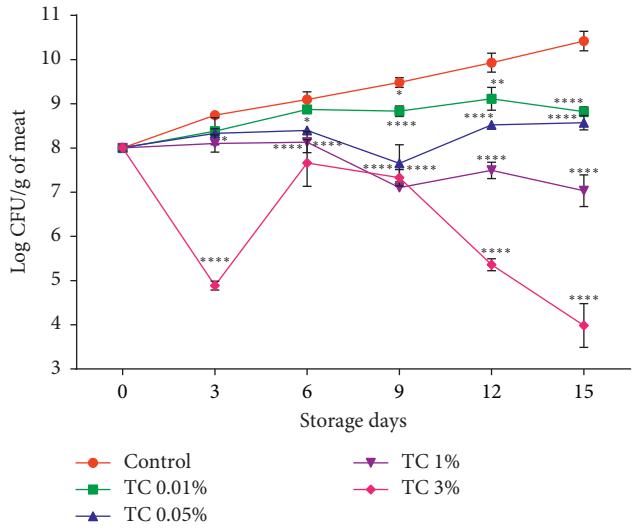


Figure 1: Time-related survival, at 4°C, of the high meat contamination level (10^8 CFU/g) of *P. aeruginosa*, following treatment with increasing concentrations of *T. capitatus* EO. The results represent the means of three replicate experiments, and error bars represent the standard error of the mean. Statistical significance differences: $^{*}p < 0.05$ (significant), $^{**}p < 0.01$ (very significant), and $^{***}p < 0.001$ and $^{****}p < 0.0001$ (extremely significant). CFU: colony-forming unit; TC: *Thymus capitatus*.

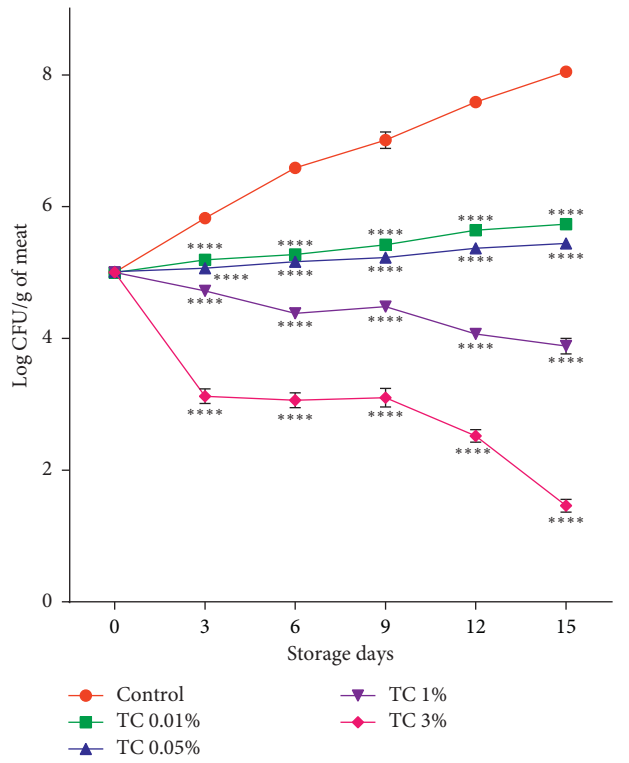


Figure 2: Time-related survival, at 4°C, of the low meat contamination level (10^5 CFU/g) of *P. aeruginosa*, following treatment with increasing concentrations of *T. capitatus* EO. The results represent the means of three replicate experiments, and error bars represent the standard error of the mean. Statistical significance differences: $^{*}p < 0.05$ (significant), $^{**}p < 0.01$ (very significant), and $^{***}p < 0.001$ and $^{****}p < 0.0001$ (extremely significant). CFU: colony-forming unit; TC: *Thymus capitatus*.

an increase in the *Pseudomonas* count was detected, from the first day of incubation, when a high inoculum (10^8 CFU/g) was used. *P. aeruginosa* count then increased by 2.41 $\log_{10}$ CFU/g and reached 10.42 $\log_{10}$ CFU/g, fifteen days later. In contrast and according to the results shown in Figure 2, lower inoculum (10^5 CFU/g) induced an exponential increase of *P. aeruginosa* growth by 3.04 $\log_{10}$, reaching 8.05 $\log_{10}$ CFU/g at the end of the incubation period. Thus, it can be assumed that, at high inoculum concentration, bacteria growth is limited due to a deficiency in nutrients, as described by Mytle et al. [40].

In this study, we have tested the antimicrobial effect of both *T. capitatus* and *T. algeriensis* EOs against one of meat food-borne pathogens (*P. aeruginosa*), inoculated in minced beef meat, at different concentrations (0.01, 0.05, 1, and 3%). As demonstrated in Figure 1 and Table 1, the concentrations employed for the *in situ* tests are higher than those used for the MIC and MBC tests. This could be due to intrinsic and extrinsic factors (proteins, fat, temperature, and oxygen limitation) which may influence the behavior of bacteria in food ecosystems and their interactions with the antimicrobial agents [21]. In fact, high protein and fat contents in meat are known to solubilize phenolic compounds, decreasing their sensitivity to the antimicrobial action. It is worth noting that the antimicrobial effects of the spices are lower in food systems than in microbiological media [41]. In addition, some studies have reported that many plant extracts and EOs are used to decrease food pathogens in meat products [42].

On the contrary, meat samples inoculated with a low bacterial contamination level (10^5 CFU/g), in the presence of low concentrations of *T. capitatus* EOs (0.01% and 0.05%), showed approximately 5.73 and 5.44 $\log_{10}$ CFU/g of *P. aeruginosa*, respectively, as compared to 8.05 $\log_{10}$ CFU/g shown for the control untreated samples, at the end of the storage period (15 days). Moreover, meat samples inoculated with a high level of bacterial contamination (10^8 CFU/g) presented the same trend of growth with a reduction of 2 $\log_{10}$ CFU/g. In fact, *Pseudomonas* growth titers achieved 8.82 and 8.57 $\log_{10}$ CFU/g in the presence of 0.01% and 0.05% of *T. capitatus* EO, respectively, as compared to 10.42 $\log_{10}$ CFU/g of the control untreated sample. Treatment based on low bacterial inoculum in the presence of a concentration of 1% of *T. capitatus* EO was able to significantly decrease *P. aeruginosa* growth by 4.17 $\log_{10}$ CFU/g, reaching 3.88 $\log_{10}$ CFU/g after 15 days of storage at 4°C.

When beef meat was inoculated with a high concentration of *P. aeruginosa*, in the presence of 1% of *T. capitatus* EO, a reduction in the bacterial load, from 7.03 to 3.39 $\log_{10}$ CFU/g, was obtained. Treatment with 3% of *T. capitatus* EO showed a greater antibacterial effect than in the presence of 1, 0.05, and 0.01%. Moreover, a 3% concentration of *T. capitatus* EO induced a bacteriostatic effect, leading to a very significant ($p < 0.0001$) reduction of bacterial growth, from 6.59 and 6.44 $\log_{10}$ CFU/g to 1.46 and 3.98 $\log_{10}$ CFU/g, for low and high inoculum loads, respectively, after a 15-day storage period.

3.4. Antibacterial Efficacy of T. algeriensis Essential Oil against Pseudomonas aeruginosa in Minced Beef Meat. As shown in Figures 3 and 4, meat samples contaminated with two different concentration levels of *P. aeruginosa* and then treated with EOs of *T. algeriensis*, at 0.01 and 0.05%, displayed a bacterial growth significantly lower than that of untreated meat samples ($p < 0.0001$), by the end of the storage period. Based on the data shown in Figure 4, applying an initial inoculum of 10^5 CFU/g and 0.01 and 0.05% of *T. algeriensis* EO induced a reduction of 2.22 and 2.46 $\log_{10}$ CFU/g in bacterial growth, respectively. As shown in Figure 3, practically, the same evolution was observed; using the higher inoculum of 10^8 CFU/g, a reduction of 1.56 and 1.68 $\log_{10}$ CFU/g, respectively, was obtained after 15 days of storage.

On the contrary, a concentration of 1% of *T. algeriensis* EO allowed a high bacteriostatic effect, leading to a significant decrease in bacterial titers ($p < 0.0001$) of 3.67 $\log_{10}$ CFU/g for the low contamination level as compared to 2.86 $\log_{10}$ CFU/g for the high contamination level, by the end of the storage days. An identical trend was observed after the addition of 3% of *T. algeriensis* EO, leading to a significant reduction in bacterial titers ($p < 0.0001$) of 4.75 $\log_{10}$ CFU/g for low initial inoculum and 3.76 $\log_{10}$ CFU/for high initial inoculum.

Therefore, it is important to note that both used EOs are effective and able to inhibit the growth of *P. aeruginosa*. Hence, increasing the concentrations of EOs to the treated samples allowed a gradual decrease in bacteria counts. This is in accordance with the findings of Emiroglu et al. [43], which revealed that *Pseudomonas* spp. was reduced in the ground beef patties when coated with thyme and oregano EOs. In contrast, the report of Ouattara et al. [44] did not show any significant effect of thyme oil on the growth of meat spoilage microorganisms such as *Pseudomonas fluorescens*.

The antibacterial effect of *T. capitatus* EO reported in the present study was significantly stronger than that of *T. algeriensis* EO, for both meat contamination levels.

We have thus examined the impact of the initial inoculum on the antibacterial effects of *T. capitatus* and *T. algeriensis* EOs on the growth of *P. aeruginosa*. It was shown that both *T. capitatus* and *T. algeriensis* EOs induce a rapid antibacterial activity against a low inoculum (10^5 CFU/g) of *P. aeruginosa*. In fact, our results demonstrated that the antibacterial effect of both EOs appears to be significantly weak when used at low concentrations but becomes more pronounced at higher EO concentrations, even in the presence of high inoculum. These findings are in accord with those reported by Udekwu et al. [45] who stated that bacteria may appear susceptible to bioactive molecules when the inoculum is of a standard level (10^5 CFU/ml) but resistant if the inoculum is increased. Accordingly, Bulitta et al. [46] proved that killing *P. aeruginosa* is 23-fold slower at a concentration of 10^9 CFU/ml and 6-fold slower at 10^8 CFU/ml than at 10^6 CFU/ml. Besides, our study did not show any immediate lethal (bactericidal) effect against the *Pseudomonas* population when *T. capitatus* and *T. algeriensis* EO are applied. In addition, *T. capitatus* EO did exhibit more pronounced antimicrobial activity than *T. algeriensis* EO.

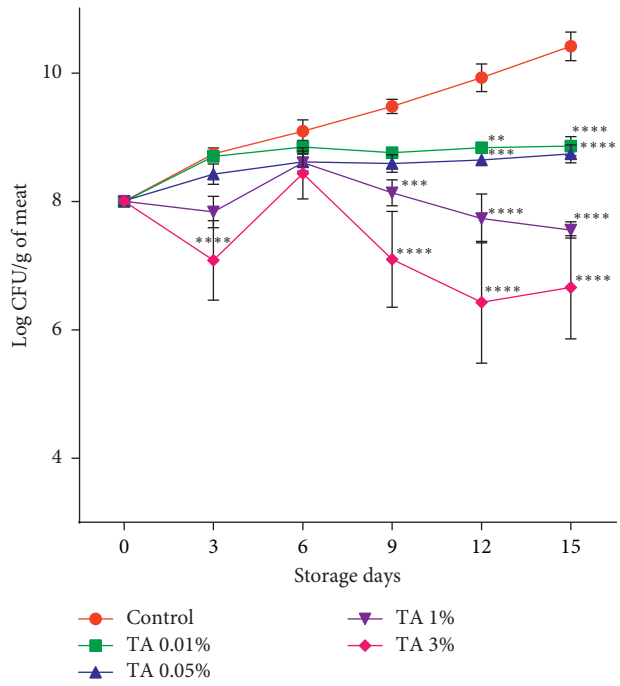


Figure 3: Time-related survival, at 4°C, of the high meat contamination level (10^8 CFU/g) of *P. aeruginosa*, following treatment with increasing concentrations of *T. algeriensis* EO. The results represent the means of three replicate experiments, and error bars represent the standard error of the mean. Statistical significance differences: $^{*}p < 0.05$ (significant), $^{**}p < 0.01$ (very significant), and $^{***}p < 0.001$ and $^{****}p < 0.0001$ (extremely significant). CFU: colony-forming unit; TC: *Thymus algeriensis*.

Both EOs showed rapid antibacterial activities against a low initial inoculum of 10^5 CFU/g of *P. aeruginosa* and weak and delayed antibacterial activities at a high concentration of initial inoculum of 10^8 CFU/g. This may be explained by the fact that the EO activities depend on the type, the composition, and the concentration of used EO, as well as the dose of targeted microorganisms present in meat.

3.5. Sensory Analysis. The sensory evaluation results are reported in Figures 5 and 6 and Table 2. Panelists ranked the samples treated with 3% of *T. capitatus* EO as superior ($p < 0.05$) to the other samples and to the control for smell. Notably, taste score showed that samples treated with 3% of *T. capitatus* EO were significantly higher ($p < 0.05$) compared to the samples treated with 1% of *T. capitatus* EO and the samples treated with 3 and 1% of *T. algeriensis* EO. Moreover, the samples treated with 1% of *T. capitatus* EO and the samples treated with 3 and 1% of *T. algeriensis* EO were scored significantly higher ($p < 0.05$) compared with the control. For the flavor, the treated samples with 3% of *T. capitatus* EO were ranked as superior ($p < 0.05$) to the treated samples with 1% of *T. capitatus* EO followed by treated samples with 1% of *T. algeriensis* EO and treated samples with 3% of *T. algeriensis* EO. For the tenderness attribute, samples treated with *T. capitatus* EO and *T. algeriensis* EO showed that the application of EO made

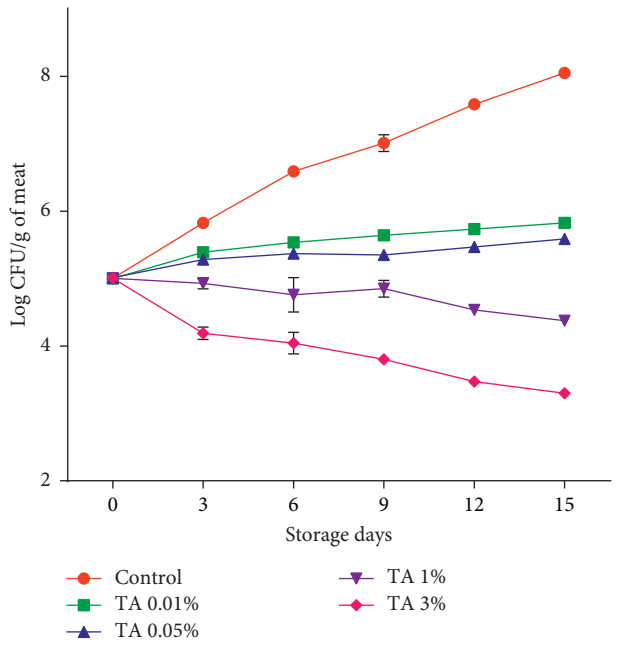


Figure 4: Time-related survival, at 4°C, of the low meat contamination level (10^5 CFU/g) of *P. aeruginosa*, following treatment with increasing concentrations of *T. algeriensis* EO. The results represent the means of three replicate experiments, and error bars represent the standard error of the mean. Statistical significance differences: $^{*}p < 0.05$ (significant), $^{**}p < 0.01$ (very significant), and $^{***}p < 0.001$ and $^{****}p < 0.0001$ (extremely significant). CFU: colony-forming unit; TC: *Thymus algeriensis*.

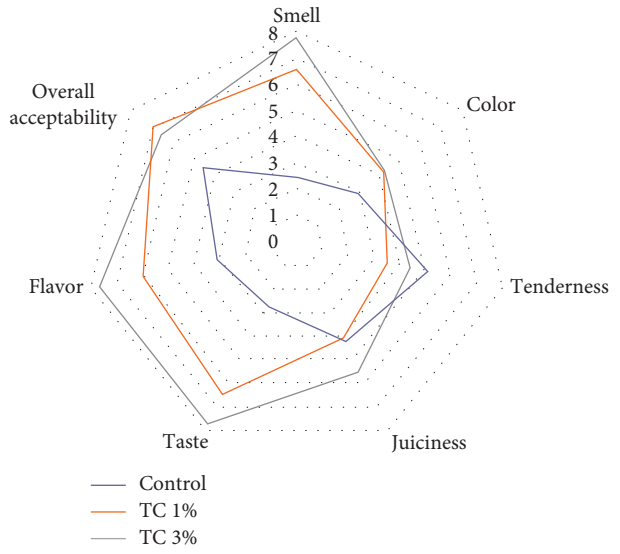


Figure 5: Sensory assay of meat samples treated with *T. capitatus* EO.

meat less tender. In terms of juiciness and color, there were no significant differences among samples treated with *T. capitatus* EO and samples treated with *T. algeriensis* EO and the control. The overall acceptability indicated that samples treated with *T. capitatus* EO were more acceptable ($p < 0.05$) than samples treated with *T. algeriensis* EO and

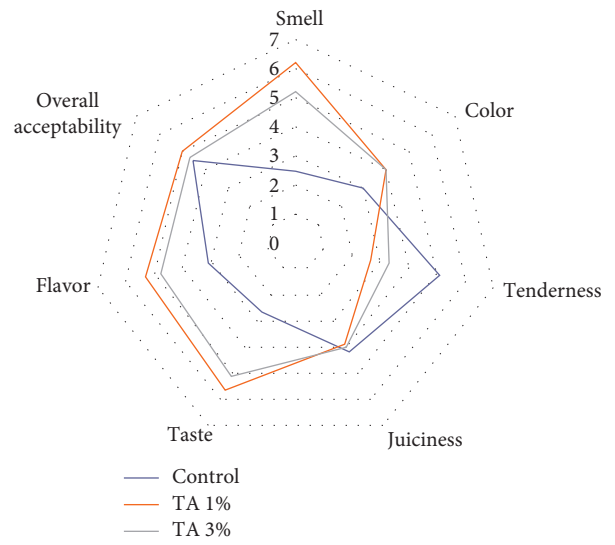


Figure 6: Sensory assay of meat samples treated with *T. algeriensis* EO.

Table 2: Mean (standard deviation) of sensory attributes of minced beef meat samples.

Sensory attribute	Control	TC1	TC3	TA1	TA3
Odor	2.44^{c}	6.55^{ab}	7.77^{a}	6.22^{ab}	5.22^{b}
Flavor	3.11^{c}	6^{ab}	7.66^{a}	5.33^{b}	4.77^{bc}
Taste	2.66^{c}	6.44^{ab}	7.66^{a}	5.66^{b}	5.11^{b}
Juiciness	4.22^{a}	4.11^{a}	5.44^{a}	3.88^{a}	4^{a}
Tenderness	5.11^{a}	3.55^{abc}	4.44^{ab}	2.66^{c}	3.33^{bc}
Color	3^{a}	4.22^{a}	4.33^{a}	4^{a}	4^{a}
Overall acceptability	4.55^{b}	7^{a}	6.55^{a}	5^{b}	4.66^{b}

Means in the same row followed by the same letter are not significantly different ($p > 0.05$). Acceptance was evaluated using a 9-point scale, where 1 = extremely dislike and 9 = extremely like.

the control. Generally, the present findings asserted that the application of *T. capitatus* EO has an important place in the improvement of the characteristic odor, taste, and flavor of minced beef meat. These results are in agreement with those obtained by Shalaby et al. [47], which reported that using olive leaf extracts as a natural preservative on minced beef improves the sensory attributes.

4. Conclusion

This study showed an interesting antioxidant effect using the DPPH assay and an interesting antimicrobial profile shown by MIC and MBC of *T. capitatus* EO. The results of "*in situ*" antibacterial activity confirmed those obtained by "*in vitro*" tests. It was also shown that *T. capitatus* EO is more effective than *T. algeriensis* EO, inhibiting *Pseudomonas* growth in inoculated minced beef meat at high concentrations (1% and 3%). In addition, at the low level of contamination, both EOs exerted a rapid and a more pronounced antibacterial effect, as compared to the high level of contamination. However, based on the sensory data, minced beef meat treated with *T. capitatus* EO was most acceptable to the panelists.

Therefore, *T. capitatus* EO could be used as a safe and a natural biopreservative for the improvement of microbiological and sensory quality of beef meat.

Data Availability

All the data used to support the findings of this study are approved and included within this article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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