

Evaluation of the efficacy of essential oils in inhibiting the growth of meat-spoilage bacteria

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Abstract:

Due to their high moisture content and protein composition, meat products generally spoil rapidly (within 24 hours) when they become contaminated with bacteria. The primary purpose of this experiment was to examine the ability of clove, thyme, oregano, and cinnamon essential oil to inhibit the growth of common spoilage organisms isolated from three categories of meat (fresh beef, chicken, and frozen). A total of 30 samples (10 each of 3 categories) were purchased from three different sources and tested. Microorganisms isolated from the various sample types were identified using the cultural, morphological, and biochemical characteristics. The agar well diffusion method was used to evaluate the antibacterial properties of the four essential oils, and the MIC of each oil was determined using the diluted broth method. Statistical analysis of variance (ANOVA), chi-squared tests, and Pearson correlational tests were performed. *E. coli*, *S. aureus*, *Pseudomonas* spp., *Salmonella* spp., and *Bacillus* spp. were the most dominant microorganisms isolated in this study. Fresh meat had significantly greater bacterial contamination than frozen meat samples ($p < 0.05$). The highest antibacterial activity was seen with both oregano and thyme oils, as there were greater inhibition zones and lower MIC values for these oils. There was an overall significant negative correlation between both CFU/g and inhibition zone ($r = -0.812$). Essential oils have strong antibacterial potential to inhibit spoilage microorganisms on meat and could be used as a natural alternative to synthetic preservatives to increase the safety and shelf life of meat.

Introduction:

Meat is one of the most perishable foods because of how rich in nutrients, as well as being susceptible to bacterial and chemical spoilage. Meat also serves as an important source of high biological value protein, essential amino acids, iron, zinc, and vitamin B12, all of which are extremely important for nutrition and health (1). The fact that meat has high water activity, near-neutral (pH) level, and an excellent source of nutrients means that the opportunity for microbial proliferation and enzymatic

degradation is very quickly realized. The quality and shelf life of meat are determined by intrinsic factors, including pH and water activity, as well as extrinsic factors, including storage temperature, packaging atmosphere, and handling (2). The spoilage of meat also creates significant economic loss on a global scale due to reduced shelf life, increased food waste, and financial burden along the meat supply chain (3). Meat spoilage occurs when it has lost quality, includes odor, quality of how it

looks, texture, or safety to eat through microbial growth and activity by microorganisms. Mostly during slaughtering, processing, transportation and storage animals along with their carcasses, cross-contamination occurs from various facilities, equipment, handlers, water and/or the environment. (4). The growth of microorganisms causes spoilage. Microbial activity is primarily linked to the growth of psychrotrophic microorganisms (Pseudomonas, Bacillus, Enterobacteriaceae) that produce proteolytic (protein) or lipolytic (lipid) enzymes creating rancid odours, slime and changes in colour (5). Additionally, bacteria contributing to spoilage of meat include Pseudomonas spp., Enterobacteriaceae (e.g. Escherichia coli or Salmonella spp.), Staphylococcus aureus and Bacillus spp.. These species also produce off-odours as a result of protein degradation (putrefaction) and lipid oxidation (rancid) at refrigerated temperatures and within packages. Pseudomonas spp. are the most common bacteria in the spoilage of meat due to their psychrophilic functionality and high levels of enzyme production (6; 7). Some traditional ways of controlling meat spoilage are refrigeration, freezing, modified atmosphere packaging, and chemical preservatives. These approaches decrease microbial growth and slow down biochemical degradation; however, these methods may not always work due to stress-adapted/psychrotrophic bacteria. MAP changes the levels of O₂ and CO₂ to reduce spoilage flora, while refrigeration slows metabolic activity. There is growing concern about the politics of using chemical preservatives, along with the rise in consumer demand for "clean label" products, which has resulted in an increased interest in the use of natural preservation strategies (8; 9). Essential oils (EOs) are

complex mixtures of volatile compounds made from aromatic plants. The majority of EOs are created using steam distillation or cold pressing. EOs contain compounds including terpenes, terpenoids, and phenolic compounds (example: carvacrol, thymol) (Thymol, Cinnamaldehyde) that provide EOs with antimicrobial properties (10). Recent studies show that bioactive compounds in EOs damage bacterial cell membranes, increase permeability, and cause cytoplasmic material to leak, resulting in bacterial death. In addition, EOs inhibit bacterial growth and biofilm development by disrupting bacterial energy metabolism, interfering with enzymatic activity, and disrupting quorum sensing (11). The antimicrobial efficacy of an EO is heavily dependent on the EO's chemical makeup and how concentrated the EO is at the time of application. Because of these attributes, EOs are quickly being recognized as viable natural food preservatives, starting with the preservation of meat products (12). There have been many studies on the spoilage of meat and natural agents that can stop meat from spoiling. However, only a few studies have looked at the potential for different essential oils to kill various types of bacteria associated with spoiling meat at the same time and in the same experiment. Most previous studies have only tested one type of essential oil on one type of bacteria or have looked at the classification of bacteria but have never looked at it from the perspective of all bacteria spoiling meat, all levels of spoilage, and all minimum inhibitory concentrations (MIC) of the various essential oils. Also, very few studies have compared the effectiveness of different essential oils when used on all types of meat and under all storage conditions. Thus, the goal of this study is to evaluate selected essential oils for their ability to kill the main types of bacteria associated with spoiling

meat, develop MICs for the oils, and compare the overall effectiveness of the oils

in order to determine which oil is the best potential natural preservative alternative.

Materials and Methods

Study Design and Sample Collection

This study was designed a cross-sectionally designed experimental experiment to measure the antimicrobial effect of certain essential oils toward common meat spoiling bacteria. The samples of meat taken for study came from three different sources; 30 total samples of meat were collected in amounts of ten each (n=10); from meat

shops, grocery stores and supermarkets (30 total samples). All samples had a weight of approximately 25 grams, were collected by qualified technicians using sterile equipment, placed into sterile test tubes under aseptic conditions, transported in a cool environment, and tested within 2 hours of being taken from their original source.

Microbiological Analysis

Each sample of meat was mixed with 100 ml of sterile peptone water to create a homogenized solution, the solutions were then diluted serially from 1 to 10,000 (dilution factor = 1:10) to obtain a dilution factor of 10^6 for microbiological testing purposes. A quantity of the diluted solutions was cultured using the appropriate agar plates (e.g., nutrient, selective). The agar

plates were then incubated for up to 48 hours at 37°C for growth of microorganisms. Colony Forming Units (CFU) per gram were calculated using standard plate count methods. The morphological characteristics of the colonies were recorded based on size, shape, color, elevation, and margin.

Isolation and Identification of Bacteria

olony characteristics were refined through subculture with purification methods and established by gram staining + standard biochemical methods; the Gram negative were *Escherichia coli*, *Salmonella* spp. + *Pseudomonas* spp. while the Gram positive included *Staphylococcus aureus* + *Bacillus*

spp. Identification was done biochemically by catalase test, oxidase test, indole test, methyl red (MR) test, Voges-Proskauer (VP) test, citrate utilization test, + coagulase test according to established microbiological protocols.

Preparation of Essential Oils

The essential oils tested included clove, thyme, oregano, and cinnamon. All essential oils were obtained from commercially available products as pure plant extracts +

stored at 4 °C in dark bottles until use. Working solutions were made at concentrations of 100 µL/disc, 50µL/disc + 25µL/disc for bacterial testing.

Antibacterial Activity Assay

Antibacterial capability assessment of essential oils was accomplished through the use of the agar with wells diffusion method. Each plate of Mueller-Hinton agar was inoculated with a standardized suspension of the target bacterium (0.5 MacFarland

standard). Each plate was tested with one or more concentrations of essential oil in a well that was emptied after adding the essential oil. Each plate was incubated for 24 hours at 37 °C and measured the size of the zones of inhibition in mm.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth dilution method. Essential oils were diluted serially, and target bacteria were added. Tubes were incubated for 24 hours at 37 °C,

and the MIC was the next lowest essential oil concentration in which no visible bacterial growth was observed.

Statistical Analysis

SPSS was used to conduct the statistical analyses (version 26). All data are presented as mean $\pm$ standard deviations (SD). ANOVA (with one-way) was used to compare means of bacterial load and size of zones of inhibition among the three meat types. Chi-square was used to assess

whether there were differences between the three meat types with respect to bacterial species distribution. Pearson's correlation was used to assess the relationship between CFU/g, MIC, and size of inhibition zone. The level of significance was $p < 0.05$.

Results

The bacterial occurrence and levels of contamination of the various types of meat and under the listed methods of storage evaluated in this study are shown in Table 1. Chicken meat had the greatest incidence of overall mean bacterial load at $(6.50 \pm 0.44 \times 10^5 \text{ CFU/g})$ followed by beef $(4.58 \pm 0.46 \times 10^5 \text{ CFU/g})$ while frozen meat had the lowest incidence of contamination $(2.81 \pm 0.19 \times 10^5 \text{ CFU/g})$. The most frequently isolated bacterial species from all samples were *E. coli* and *Staphylococcus aureus* with both isolates occurring in 30% of the cases,

while *Pseudomonas* species occurred in 20% of the cases. *Salmonella* species were isolated mostly from chicken and frozen, while beef samples did not yield any isolates. Statistical tests indicated that there was a significant association between the type of meat tested and the distribution of the bacteria ($\chi^2 = 8.91$, $p = 0.031$) which demonstrates that both meat type and the methods used for storage have a large effect on the levels of microbial contamination and subsequent risk of spoilage.

Table 1. Characteristics of Meat Samples, Bacterial Load, and Distribution of Bacterial

Meat Type	Storage Condition	No. of Samples	Mean CFU/g ($\times 10^5$) $\pm$ SD	E. coli n (%)	S. aureus n (%)	Pseudomonas spp. n (%)	Salmonella spp. n (%)	Bacillus spp. n (%)	χ^2	p-value
Beef	Fresh	10	4.58 $\pm$ 0.46	3 (30)	3 (30)	2 (20)	0 (0)	2 (20)	8.91	0.031*
Chicken	Fresh	10	6.50 $\pm$ 0.44	3 (30)	3 (30)	2 (20)	2 (20)	0 (0)		
Frozen Meat	Frozen	10	2.81 $\pm$ 0.19	3 (30)	3 (30)	2 (20)	2 (20)	2 (20)		
Total	—	30	4.63 $\pm$ 1.60	9 (30)	9 (30.0)	6 (20.0)	4 (13.3)	4 (13.3)		

Isolates Recovered from Different Meat Types (n = 30)

Table 2 provides a summary of the morphologic characteristics, microscopic characteristics, and biochemical characteristics of the bacterial isolates and it had differing morphologies of their colonies; E. coli and Salmonella spp. had circular, convex colonies, whereas Pseudomonas spp. and Bacillus spp. had colonies that displayed irregular forms. Gram staining was used to differentiate the Gram-negative organisms

(E. coli, Salmonella spp., Pseudomonas spp.) from the Gram-positive organisms (S. aureus, Bacillus spp.). The biochemical profile of the isolates confirmed the identification of those isolates; E. coli was indole positive and MR positive and citrate negative, while S. aureus was coagulase positive and Pseudomonas spp. were oxidase positive. Salmonella spp. showed MR–VP reaction.

Table 2. Morphological, Microscopic, and Biochemical Characteristics of Bacterial Isolates

Bacterial Species	Colony Morphology	Colony Color	Gram Reaction	Microscopic Appearance	Catalase	Oxidase	Indole	MR	VP	Citrate	Coagulase
E. coli	Circular, convex, entire	Cream	Negative	Short rods	+	–	+	+	–	–	NA
S. aureus	Circular, convex, entire	Golden yellow	Positive	Cocci in clusters	+	–	NA	NA	NA	NA	+
Pseudomonas spp.	Irregular, flat, undulate	Greenish	Negative	Rods	+	+	NA	NA	NA	NA	NA

Salmonella spp.	Circular, convex, entire	Gray	Negative	Rods	+	—	—	—	+	+	NA
Bacillus spp.	Irregular, raised, lobate	White	Positive	Large rods	+	+	NA	NA	NA	NA	NA

Gram-negative bacteria made up the majority (n = 56.7%) of the total bacterial isolates while Gram-positive bacteria represented a lesser number (n = 43.3%). This would imply that enteric and environmental organisms responsible for spoilage if they occur are likely the main types of bacteria present in meat. Approximately 86.7% of all of the isolates were catalase-positive, indicative of their

ability to survive during conditions of oxidative stress that are frequently present during storage of meat products. Conversely, oxidase-positive isolates occurred less frequently with only 36.7%. Additionally, coagulase-positive strains of Staphylococcus spp. were demonstrated from 30.0% of the bacterial isolates, as presented in table 3.

Table 3. Distribution of Phenotypic Characteristics Among Bacterial Isolates

Parameter	Positive n (%)	Negative n (%)
Gram-positive bacteria	13 (43.3)	—
Gram-negative bacteria	17 (56.7)	—
Catalase positive	26 (86.7)	4 (13.3)
Oxidase positive	11 (36.7)	19 (63.3)
Coagulase positive	9 (30.0)	21 (70.0)

Table 4 displays the antimicrobial effectiveness of four essential oils (clove, thyme, oregano, and cinnamon) against major meat spoilage pathogens, along with their MICs. Oregano had the most potent antimicrobial properties with the largest average inhibition zones (23.8 ± 2.1 mm) followed by thyme (22.6 ± 2.4 mm), clove (20.4 ± 2.0 mm), and cinnamon (18.7 ± 2.3

mm). There was increased susceptibility in the participants of the study (Salmonella spp.; S. aureus) while the Bacillus spp.; E. coli showed decreased susceptibility. The MICs of all the essential oils (excluding cinnamon) ranged from .39 to 3.12 µg/ml. The MIC of cinnamon ranged up to 6.25 µg/ml indicating less potency than the other three essential oils.

Table 4. Antibacterial Activity and MIC Values of Essential Oils Against Meat Spoilage Bacteria

Essential Oil	E. coli	S. aureus	Pseudomonas spp.	Salmonella spp.	Bacillus spp.	Overall Mean Zone (mm)	MIC Range (µg/mL)
Clove	16–19	17–24	18–23	20–25	14–18	20.4 ± 2.0	0.39–3.12
Thyme	18–	19–27	20–25	22–27	16–20	22.6 ± 2.4	0.39–3.12

	21						
Oregano	19–22	20–28	21–26	23–28	17–21	23.8 ± 2.1	0.39–3.12
Cinnamon	14–18	16–23	17–22	19–24	13–18	18.7 ± 2.3	0.39–6.25

In Table 5, A strong inverse correlation was found between the MIC and the diameter of the inhibition zone ($r = -0.689$, $p < 0.001$), suggesting that higher antibacterial activity is associated with lower MIC values. Similarly, there was a very strong negative correlation between the CFU/g and the inhibition zone ($r = -0.812$, $p < 0.001$),

indicating that higher bacterial loads are associated with decreased effectiveness of the antimicrobial agent. For individual essential oils, oregano had the strongest inverse correlation ($r = -0.91$), followed by thyme ($r = -0.88$), clove ($r = -0.84$), and cinnamon ($r = -0.79$), all statistically significant.

Table 5. Correlation Analysis Between Antibacterial Activity, MIC, and Bacterial Load

Variable Comparison	Pearson r	p-value
MIC vs Inhibition Zone	-0.689	<0.001
CFU/g vs Inhibition Zone	-0.812	<0.001
Oregano MIC vs Zone	-0.91	<0.001
Thyme MIC vs Zone	-0.88	<0.001
Clove MIC vs Zone	-0.84	<0.001
Cinnamon MIC vs Zone	-0.79	<0.01

In Table 6, Gram-positive bacteria were slightly more susceptible to the essential oils with a mean inhibition zone value of 18.4 ± 3.7 mm as compared to the mean inhibition zone value of the Gram-negative bacteria (17.2 ± 4.1 mm). Both Gram-positive and Gram-negative bacteria were susceptible to

the essential oils used as antimicrobial agents, with Gram-positive being the most susceptible. Statistical analysis confirmed a significant difference between both groups in inhibition zones ($p = 0.032$) and MIC values ($p = 0.041$).

Table 6. Association Between Gram Reaction and Susceptibility to Essential Oils

Bacterial Group	Mean Inhibition Zone (mm)	MIC (µg/mL)	Range
Gram-positive (<i>S. aureus</i> , <i>Bacillus</i> spp.)	18.4 ± 3.7	0.39–6.25	
Gram-negative (<i>E. coli</i> , <i>Pseudomonas</i> spp., <i>Salmonella</i> spp.)	17.2 ± 4.1	0.39–6.25	
p-value	0.032*	0.041*	

As demonstrated in table 7, ANOVA analysis demonstrated that meat type had a significant effect on the amount of bacteria present ($F = 42.67$, $p < 0.001$), as did the source of meat ($F = 7.82$, $p < 0.002$). There were also significant differences in inhibition zone diameter ($F = 78.40$, $p <$

0.001) due to the type of essential oil used, and there were significant differences in susceptibility to antimicrobial action by bacteria ($F = 36.90$, $p < 0.001$) based on the species of bacteria used. A chi-square analysis demonstrated that there were significant relationships between the type of

meat and the prevalence of the bacteria in the samples ($\chi^2 = 8.91$, $p < 0.031$). There was a significant negative correlation between the level of antimicrobial resistance as measured by the MIC and the size of the inhibition zone ($r = -0.689$, $p < 0.001$), and there was also a significant negative

correlation between the amount of bacteria present as estimated by the amount of CFU (colony-forming units) per gram of meat and the size of the inhibition zone ($r = -0.812$, $p < 0.001$), indicating that as the amount of bacteria increases, the amount of antibacterial action decreases.

Table 7. Inferential Statistical Analyses

Variable Compared	Statistical Test	Statistic	p-value
Meat type vs bacterial load	One-way ANOVA	F = 42.67	<0.001
Source vs bacterial load	One-way ANOVA	F = 7.82	0.002
Essential oil type vs inhibition zone	One-way ANOVA	F = 78.40	<0.001
Bacterial species vs inhibition zone	One-way ANOVA	F = 36.90	<0.001
Meat type vs bacterial prevalence	Chi-square	$\chi^2 = 8.91$	0.031
MIC vs inhibition zone	Pearson correlation	$r = -0.689$	<0.001
CFU/g vs inhibition zone	Pearson correlation	$r = -0.812$	<0.001

Discussion:

Interest in using natural preservatives is on the rise due to the increased consumer demand for safe, high-quality meat products. Meat product safety is a concern for consumers because meat products are very vulnerable to microbial contamination, which leads to spoilage, decreased shelf life, monetary loss to processors, and potential harm to consumers. As a result of these concerns, essential oils have received considerable attention for their broad-spectrum antimicrobial properties based on the presence of natural bioactive constituents. Therefore, the determination of essential oils' antibacterial efficacy against meat spoilage microbes is important in the development of safe and sustainable forms of preservation for the meat industry. The results of this study indicated a considerable amount of variation between the microbiological quality of beef, fresh chicken, and frozen meat products, and the level of bacterial contamination varied greatly based upon the type of meat and the

method of storage used. The level of bacterial contamination found in fresh chickens was higher than in beef or frozen meat products, which are consistent with the biological characteristics of poultry and the complexities associated with the processing of the poultry. Several recent studies have also described that generally poultry meat contains a greater number of bacteria than beef (or other red meats) due in part to differences in the way in which poultry are slaughtered, the processing environment, and how the birds are handled post-processing (13). The bacterial counts found in frozen meat were lower than those in many other types of meat. This is due to the fact that freezing greatly affects the metabolism of bacteria by inhibiting both their division and the growth of their cells. As a result, until the meat is sold to consumers (provided that the cold chain is maintained during storage and distribution), bacterial counts found on frozen meat have consistently been lower than those found on

fresh meat. A number of publications have pointed out that maintaining appropriate temperature controls representative of the cold chain will continue to be one of the most effective ways to minimize microbial growth within meat products and extend their shelf life (14). The high levels of *E. coli* found in the current study (many *E. coli* are found in the intestines of mammals) suggest that fecal contamination occurred at some point during slaughtering, evisceration, dressing of the carcass, or through future handling of the carcass. The frequency at which *E. coli* was found to be recovered in this study corresponds with previously identified frequency of *E. coli* being isolated from raw meat products throughout the world even with improvements made in slaughterhouse hygiene practices (15). In addition to *E. coli*, the results from several recent studies indicate that *S. aureus* is also one of the major contaminants of raw meat products and proper hygiene practices should be used to minimize the contamination of raw meat by *S. aureus* in order to reduce the possibility of staphylococcal food poisoning (14). 20% percent of the total isolates in the present study were identified as *Pseudomonas* spp. These organisms were isolated from each category of meat. *Pseudomonas* species are psychrotrophic organisms, which allows them to multiply under refrigeration conditions. Because of this, these microorganisms are also regarded as one of the main spoilage bacteria found on fresh meat. In recent literature, *Pseudomonas* spp. have been reported as being among the most significant spoilage microorganisms in low-temperature meat products and to have been extensively studied for potential control methods to prevent spoilage (16). In contrast, *Salmonella* spp. were only cultured from chicken and frozen meat samples in the

present study, and there were none cultured from any beef samples. This difference in the distribution of *Salmonella* spp. may reflect the differences in potential sources of contamination throughout the poultry industry, as poultry has been considered to be one of the primary sources of *Salmonella*. Furthermore, the absence of *Salmonella* in beef samples may be indicative of more successful hygiene management practices in beef slaughtering or may simply reflect the small sample size of samples in the current study. Many recently published studies indicate that poultry has a higher prevalence of *Salmonella* spp. compared to beef, providing substantiate evidence that poultry meat is one of the most common means of transmitting salmonellosis worldwide (17 & 18). Isolate of *Bacillus* spp. were found on beef and frozen meats; however, there were none present in the chicken sample. Current research has also confirmed that species of *Bacillus* contamination takes place in the processing environments' ways so far as their ability to survive long periods and are resistant throughout the environment (29). The significant association between the type of meat and the way the bacterial contamination occurs indicates that random means exist in terms of bacteria contaminating meats; however, that contamination is strongly influenced by the type of meat and by the method of processing it. It is well established that properly managing bacteria in all parts of the meat production chain, including slaughtering the animals, maintaining cold-temperature storage, and properly handling products during the retail sale, are needed to reduce levels of bacteria in meats and increase the length of time meats remain in an acceptable condition (19). According to research conducted on meat samples, gram-negative bacteria outnumbered gram-positive with (56.7%) of all bacteria

identified in the samples, demonstrating that the sources of the contamination originally came from enteric microorganisms and environmental sources. This finding also correlates with multiple studies, where it has been identified that gram-negative, enteric microorganisms such as *Escherichia coli*, *Pseudomonas* spp., and *Salmonella* spp. are usually the main spoilage microbial species found in fresh meats due to their rapid growth rates and the various adaptations these microorganisms can exhibit while in storage (20; 21). The high percentage (86.7%) of catalase-positive isolates reflects that the majority of bacteria have evolved to contain mechanisms to protect themselves against oxidative stress, helping them survive through the processing and storage of meat products. The relatively low number (36.7%) of oxidase-positive isolates, relative to the nearly all of the gram-negative bacteria observed in this study were classified to the family Enterobacteriaceae, where the production of oxidase activity has typically been correlated with members of the family Pseudomonadaceae spp. (20). The majority of the isolated bacteria were coagulase-positive (30% of total isolates), indicating that *Staphylococcus aureus* (a prevalent foodborne pathogen often found where there has been a lack of hygiene in meat) was present. Similar support for this finding is evident in recent studies that reiterate the significance of proper sanitation for preventing contamination, with the goal of enhancing meat safety (22; 23). Oregano essential oil exhibited the highest level of antibacterial effectiveness (largest inhibition zone and lowest MIC values), followed by thyme, clove and cinnamon. These results corroborate with (24), who suggest oregano oils rank among the most potent of natural antimicrobials due to their ability to disrupt bacterial membrane integrity, alter membrane permeability, and affect basic

cellular function through high carvacrol and thymol content (24). The increased susceptibility of *Salmonella* spp. and *Staphylococcus aureus* compared to the ability of *Escherichia coli* and *Bacillus* spp. to resist these antibiotics reflects previous findings. The lower susceptibility of *E. coli* has been linked to the outer membrane of Gram-negative bacteria, while the higher resistance shown by *Bacillus* spp. is due to the ability of these organisms to form resistant endospores that allow for reduced efficacy of antimicrobial agents (25). The results from the MIC test corroborated those obtained from the agar diffusion method, indicating that the growth of bacteria can be inhibited by using lower concentrations of oregano, thyme and clove oils as compared to cinnamon oil. Similarly, da (26) indicate that the high phenolic content of these oils provides an effective means of inhibiting spoilage bacteria and can greatly contribute to extending the shelf life of meat products. Our data confirms a strong link between the antibacterial properties of essential oils tested in this study to both MIC and bacterial load. The presence of a significant negative correlation ($r = -0.689$, $p < 0.001$) between MIC and inhibitory zones indicated that lower inhibitory concentrations resulted in increased size of the inhibitory zone, which is indicative of greater antibacterial potency. Additionally, the high degree of inverse correlation ($r = -0.812$, $p < 0.001$) between bacterial load and inhibitory zone indicates that higher numbers of microorganisms can decrease the effectiveness of an antimicrobial agent. The oregano essential oil produced the greatest overall correlations when compared to the other essential oils (i.e., thyme, clove, and cinnamon), reinforcing its superior antibacterial capabilities. These results corroborate similar findings by (27) and (28), who showed that both oregano and

thyme essential oils have consistently exhibited low MIC values and high inhibitory activity against foodborne organisms. Additionally, Nieto reported that essential oils with a high phenolic content

Conclusion:

Meat is a very perishable food type that had high levels of microbial contamination and that fresh samples contained much greater bacterial levels than frozen meat. Five isolates were most commonly identified (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* spp., *Salmonella* spp., and *Bacillus* spp.) and represent the complex spoilage microbiota associated with meat from various sources. Additionally, essential oils (EOs), particularly those from oregano and thyme, demonstrated the greatest antimicrobial (bacterial) activity as evidenced by larger average inhibition zones and lower MIC (minimum inhibitory

have superior antimicrobial activity due to their ability to damage the cells of microorganisms and disrupt key metabolic processes.

concentration) values; EOs derived from cinnamon displayed moderate antimicrobial activity by comparison. Dose-dependent relationships between CFU (colony-forming units)/g, MIC, and inhibition zone support the antimicrobial potential of these EOs. In conclusion, EOs are good candidate natural alternatives to synthetic preservatives for increasing meat safety and prolonging shelf life. Future studies should evaluate the combined use of EOs in synergy with each other, through nano-encapsulation systems, and through application in actual food storage environments.

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