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Research Article

# Antibacterial and Antioxidant Activities of Essential Oils and Their Sensory Effect in Chicken Breast Meat

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## Abstract

This study was carried out to evaluate *in vitro* antibacterial and antioxidant activities of Clove, Sweet Marjoram, and Laurel essential oils (EO), as well as their sensory impact in foodstuffs to select candidates to search for effective natural antibacterial and antioxidant additives in the food industry. Eugenol (81.62%), terpinene-4-ol (29.13%), and 1,8-Cineole (42.3%) were detected by gas chromatography-mass spectrometry analysis as the main components of clove, sweet Marjoram, and Laurel essential oils EOs, respectively. The antioxidant activity was carried by β-carotene–linoleic acid bleaching test and Clove EO showed the best antioxidant activity (AAC=138‰±0,313). The antibacterial activity was detected using the disc diffusion method against four pathogens bacteria (*Citrobacter freundii*, *Enterobacter cloacae*, *Salmonella typhimurium*, and *Staphylococcus aureus)*. Results showed that S.aureus was the most inhibited bacterium with respective inhibition diameters of 21.00±2.886 and 19.67±3.605 for Clove and Marjoram essential oils. Sensory analysis indicated changes in chicken breast flavor, color, and odor by all EO treatments. However, no significant difference in the global acceptance of untreated and EO-treated breasts was observed. In conclusion, Clove EO could be served as a natural alternative improving meat quality and being appreciated by the consumer.

## Keywords

Antibacterial, Antioxidant, Chicken Breast, Clove, Consumer's Acceptance, Essential Oils, Laurel, Sweet Marjoram

## 1. Introduction

The incorporation of essential oils (EOs) in food preservation has gained substantial attention due to their natural origin and multifunctional properties, including potent antibacterial and antioxidant activities. These natural compounds, derived from various plants, offer a promising alternative to synthetic preservatives, addressing consumer concerns over food safety and health. This study investigates the antibacterial and antioxidant activities of selected essential oils and

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their sensory effects on chicken breast meat, a widely consumed protein source vulnerable to microbial spoilage and oxidation.

Chicken breast meat is highly susceptible to deterioration because of its high moisture content and neutral pH, which create favorable conditions for microbial growth and lipid oxidation. Traditional preservation methods, such as refrigeration and synthetic additives, though effective, pose potential health risks and have led to an increased demand for natural preservation alternatives. Essential oils, recognized for their Generally Recognized As Safe (GRAS) status, present a viable solution with additional health benefits [1].

Recent studies have highlighted the efficacy of essential oils like oregano, thyme, and rosemary in combating foodborne pathogens and reducing oxidative spoilage. Key compounds such as thymol, carvacrol, and eugenol exhibit strong antimicrobial activity against a range of bacteria, including Salmonella, Escherichia coli, and Listeria monocytogenes [2]. Additionally, these essential oils possess significant antioxidant properties, which are crucial in mitigating lipid oxidation, thereby preserving the flavor, color, and overall quality of meat products [3].

However, the use of essential oils in food systems is not without challenges, particularly regarding their strong aromas and potential impact on sensory attributes. Consumer acceptance is pivotal, as the sensory qualities imparted by essential oils can influence the overall palatability of the meat. Thus, it is essential to balance antimicrobial efficacy with sensory acceptability to ensure the successful application of essential oils in meat preservation [4].

This study aims to evaluate the antibacterial and antioxidant activities of selected essential oils from Clove (*Eugenia caryophyllus, Fam. Myrtaceae)*, Marjoram (*Origanummajorana* L.), and Laurel (*Laurus nobilis*) in chicken breast meat and assess their impact on sensory properties. By elucidating the dual role of essential oils in enhancing microbial safety and preserving meat quality, this research contributes to the development of natural preservation strategies that align with consumer preferences for safer and more natural food products.

## 2. Material and Methods

*Material*

Clove, Sweet Marjoram, and Laurel EO were provided by a local commercial company (Carthago, Sousse, Tunisia). They were extracted by hydrodistillation process. Raw chicken breast meat fillets were purchased from a local store (Tunis, Tunisia).

*Composition of the essential oils*

The composition of the essential oils was determined using gas chromatography (GC; Agilent 6890N, Agilent Technologies, Paris, France) interfaced with mass spectroscopy (MS; Agilent 5973N, Agilent Technologies). The capillary column used was the HP5-MS 5% phenyl methyl siloxan (length: 30 m; internal diameter: 0.25 mm; film thickness: 0.25 µm) and an automatic passer (Agilent 7683B; Agilent technologies). Helium was the carrier gas at a flow rate of 1 mL/min. The column temperature was initially adjusted at 5 ℃ (during 1 min) then increased progressively at a rate of 2 ℃/min to reach 300 ℃ within 130 min. The samples were diluted in ethanol (1/10) then 1 µL was injected into GC-MS [5]. The components were identified by comparing their relative retention times and mass spectra with the standard data (NIST05, Mass Spectra Library, National Institute of Standards and Technology, Gaithersburg, MD). The GC-MS analyses were conducted at the National Institute of Physicochemical Analyses and Research (Sidi Thabet, Tunisia).

*Antibacterial activities*

*Bacterial strains*

The bacterial support used for the microbiological tests, provided by the Pasteur Institute department of the foodstuff, consists of 4 referenced strains belonging to two classes: Gram + and Gram- presented in the following table 1.

***Table 1.*** *Morphology of the bacterial strains used.*

| Strains | Culture medium | Gram type | Microscopic morphology | Reference | Condition and temperature |
|---|---|---|---|---|---|
| Staphylococcus. Aureus | TSB | + | Coccobacillus in grape clusters | ATCC (25923) | 30 ℃ Without agitation |
| Salmonella. Typhimuim | TSB | -- | Aerobic flagellate stick | ATCC (14028) | 30 ℃ Without agitation |
| Citrobacter. Freundii | TSB | _ | Straight bacilli | ATCC (8090) | 30 ℃ Without agitation |
| Enterobacter Cloacae | TSB | _ | Bacilli | ATCC (25922) | 30 ℃ Without agitation |

The choice of strains was made for their high frequency to contaminate foodstuffs and especially meat and meat products and for their natural resistance to various types of antimicrobial agents.

*Determination of zone of inhibition method*

Antibacterial activity was evaluated *in vitro* using the disc diffusion method against four pathogens, as described by [6].

To produce the aromatogram, 0.1 ml of the bacterial culture is seeded on the surface of a special agar of Trypticase soy agar. The dishes are then left to stand for 30 min at room temperature to fix the bacterial sheet. Disks pre-impregnated with either essential oil or antibiotic are deposited on the surface of the agar. The solution, therefore, diffuses from the disk by creating a concentration gradient. The determination of the diameter of the inhibition zone makes it possible to estimate the inhibitory effect of these extracts.

The breakpoints for susceptible, intermediate, and resistant isolates were set as ≥19 mm (susceptible: S), 18-11 mm (intermediate resistant: IR), and >10 mm or no zone of inhibition (resistant: R) [7].

*Minimum Inhibitory Concentration (MIC)*

MIC was defined as the lowest concentration that inhibited visible microbial growth [8]. This technique consists of inoculating, by a standardized inoculum, a decreasing concentration range of essential oil. After incubation, observation of the range provides access to the Minimum Inhibitory Concentration (MIC), which corresponds to the lowest concentration of essential oil capable of inhibiting bacterial growth.

*Antioxidant activity*

β-carotene–linoleic acid bleaching test was carried out to determine the antioxidant activity of EO, as described by Essaidi et al. [9]. 0.2 mg of β-carotene, 20 mg of linoleic acid, and 200 mg of Tween 40 are dissolved in 0.5 ml of chloroform, the solvent is then evaporated in vacuo. The mixture obtained is diluted in 50 ml of distilled water previously saturated with oxygen. A vigorous agitation is achieved. The emulsion obtained was divided into test tubes (capped and protected from light) at a rate of 4 ml per tube.

Solutions of the various essential oils in ethanol are prepared in a proportion of 2 g / l distributed in the tubes at the rate of 0.2 ml of solution per tube. The BHT is used as a positive control, 0.2 ml of a solution in ethanol is added to a tube which will serve as a control.

A control tube containing 4 ml of the starting solution to which 0.2 ml of ethanol is added. A second emulsion is prepared without β-carotene, it is used for the blank which corresponds to 4 ml of this emulsion with 0.2 ml of ethanol added.

The test tubes are placed in a water bath at 50 °C., the absorbance is measured at a wavelength λ = 470 nm at time t = 0, and then every 15 minutes up to 120 minutes using a spectrophotometer.

$$AAC = [AA\ (120) - AC\ (120) / AC\ (0) - AC\ (120)] * 1000$$

Where AA (120) is the absorbance of the antioxidant at 120 min, Ac (120) is the absorbance of the control at 120 min, and AC (0) is the absorbance of the control at 0 min.

*Sensory analysis*

Fillets were treated with EO at a dose of 0.5% (v/w) and cooked in aluminum in a conventional electric oven at 180 ℃ for 20 minutes. Quantitative descriptive analysis was conducted by 10 trained panelists using a 7 points scale for 5 attributes: taste, odor, color, flavor, and global acceptance.

*Statistical analysis*

Data (mean ± STD) were subjected to ANOVA at α=0.05 using Prism GraphPad software.

## 3. Results and Discussion

*Chemical Composition*

The components of the oils are reported in Table 2. The different constituents of the samples were identified and quantified by GC and GC/MS.

***Table 2.*** *Chemical composition of the essential oils.*

| Compound | KI | *Eugenia caryophyllus* | *Origanummajorana* | *Laurusnobilis* |
|---|---|---|---|---|
| α-Thujene | 932 | - | - | 0.5 |
| α-Pinene | 940 | - | - | 7.8 |
| Camphene | 958 | - | - | 0.3 |
| Sabinene | 980 | - | - | 5.4 |
| β-Pinene | 986 | - | - | 5.9 |
| α-Phellandrene | 1012 | - | - | 0.7 |
| Car-3-ene | 1018 | - | - | 0.1 |
| α-Terpinene | 1024 | - | - | 0.6 |
| p-Cymene | 1034 | - | - | 0.6 |
| 1,8-Cineole | 1046 | - | - | 42.3 |

| **Compound** | **KI** | ***Eugenia caryophyllus*** | ***Origanummajorana*** | ***Laurusnobilis*** |
|---|---|---|---|---|
| γ-Terpinene | 1067 | - | - | 0.6 |
| Linalool | 1103 | - | - | 2.5 |
| Sabinene | 1109 | - | 0.18 | - |
| Δ-3-carene | 1135 | - | 3.01 | - |
| Camphene | 1154 | 0.13 | - | - |
| myrcene | 1162 | - | 1,03 | - |
| α-terpinene | 1171 | - | 3,12 | - |
| limonene | 1183 | - | 0.95 | - |
| trans-2-hexenal | 1190 | - | 1.47 | - |
| Terpinen-4-ol | 1192 | - | - | 2.5 |
| α-Terpineol | 1203 | - | - | 2.1 |
| γ-terpin ène | 1233 | - | 6.18 | - |
| p-cymene | 1268 | - | 0.57 | - |
| terpinol ène | 1274 | - | 1.26 | - |
| Bornylacetate | 1297 | - | - | 0.4 |
| α-Terpinylacetate | 1333 | - | - | 11.2 |
| Eugenol | 1369 | 81.62 | - | - |
| Eugenylacetate | 1372 | 9.61 | - | - |
| α-Caryophyllene | 1374 | 0.75 | - | - |
| Naphtalene | 1401 | 0.40 | - | - |
| α-Copaene | 1407 | - | - | 0.4 |
| α-Cubebene | 1408 | 0.32 | - | - |
| β-Elemene | 1410 | - | - | 1.3 |
| Methyleugenol | 1415 | - | - | 3.5 |
| β-Caryophyllene | 1419 | 6.24 | | |
| β-Caryophyllene | 1446 | - | - | 1.3 |
| allo-Aromadendrene | 1466 | - | - | 0.3 |
| Caryophylleneoxide | 1470 | 0.34 | - | - |
| α-Humulene | 1481 | - | - | 0.2 |
| Germacrene D | 1508 | - | - | 0.8 |
| linalool | 1550 | - | 24.66 | - |
| linalylacetate | 1554 | - | 3.09 | - |
| Bornylacetate | 1560 | - | 1.97 | - |
| δ-Cadinene | 1563 | - | - | 0.8 |
| β-elemene | 1568 | - | 0.23 | - |
| terpinene-4-ol | 1573 | - | 29.13 | - |
| β-caryophyllene | 1595 | - | 0.26 | - |
| α-humulene | 1610 | - | 0.89 | - |
| 2',3',4'Trimethoxyacetophenone | 1616 | 0.30 | - | - |

| Compound | KI | *Eugenia caryophyllus* | *Origanummajorana* | *Laurusnobilis* |
|---|---|---|---|---|
| Myrtenylacetate | 1655 | - | 0.33 | - |
| Geranylacetate | 1750 | - | 7.09 | - |
| γ-cadinene | 1766 | - | 1.59 | - |
| bicyclogermacrene | 1791 | - | 0.19 | |
| nerol | 1811 | - | 0.14 | - |
| geraniol | 1851 | - | 0.67 | - |
| β-selinene | 1875 | - | 0.27 | - |
| eicosane | 2145 | - | 0.24 | - |

Table 2 shows that the Clove essential oil had a high concentration of Eugenol (81.62%) and Eugenyl acetate (9.61%). The primary component of the sesquiterpenes group was Caryophyllene, accounting for 6.24%. These findings are consistent with previous research, taking into account the variability of essential oils [10, 11]. For instance, Chaieb et al. [10] reported eugenol content of 88.58%, eugenyl acetate content of 5.62%, and β-caryophyllene content of 1.39%.

Linalool (24.6%), terpinen-4-ol (29%), γ-terpinene (6.1%) are predominant in the oil of Origanum majorana. Marjoram EO had the same chemical composition as found by [12-14].

The main components of Laurel essential oil are 1,8-cineole (42.3%) and α-terpinyl acetate (11.2%). Laurel oil had a high content of sesquiterpene hydrocarbons (5.1%). The results presented here were in line with those of [15].

*Antibacterial activity*

Table 3 displays the inhibition zone diameters of the essential oils (EO) and the minimum inhibitory concentration (MIC) required to halt the growth of certain bacterial strains.

***Table 3.*** *Effect of Clove, Sweet Marjoram, and Laurel EO on the growth of some bacterial strains.*

| | Clove | Marjoram | Laurel | Gentamycin |
|---|---|---|---|---|
| Diameter of inhibition zone (mm) | | | | |
| *Staphylococcus aureus* ATCC 25923 | 21.00 ±2.67 | 19.67 ±3.78 | 12.33 ±3.11 | 33.33 ±2.22 |
| *Citrobacterfreundii* ATCC 8090 | 11.67 ±2.22 | 14.00 ±0.67 | 10.00 ±0.00 | 30.00 ±4.67 |
| *Enterobactercloacae* | 11.33 ±1.78 | 15.00 ±3.33 | 11.33 ±1.78 | 26.00 ±4.67 |
| *Salmonella typhimurium*ATCC 14028 | 11.67 ±2.22 | 11.00 ±1.33 | 11.00 ±1.00 | 23.33 ±5.56 |
| Minimum inhibitory concentration (mg/ml) | | | | |
| *Staphylococcus aureus* ATCC 25923 | 2.5 | 2.5 | 5 | - |
| *Citrobacterfreundii* ATCC 8090 | 5 | 2.5 | 5 | - |
| *Enterobactercloacae* | 2.5 | 5 | 5 | - |
| Salmonella typhimuriumATCC 14028 | 10 | 5 | 5 | - |

Clove EO demonstrated the most potent antibacterial activity against the Gram-positive bacterium Staphylococcus aureus, with the largest inhibition zones (19.67-21.00 mm) and the lowest MIC values (2.5 mg/mL), followed by Marjoram EO. Furthermore, Marjoram EO was capable of inhibiting the growth of Gram-negative Citrobacter and Enterobacter, with respective MIC values of 5 and 2.5 mg/mL. Two-way ANOVA revealed significant differences for both the EO factor ($p<0.001$) and the bacterial strain factor ($p<0.001$), as well as a significant interaction between the two factors ($p<0.01$).

These findings support previous studies by Smith-Palmer et al. [16-19, 14, 7], which have also reported the antibacterial activity of Clove and Marjoram EO. However, all tested

EO were found to be weakly effective against Salmonella, consistent with the results of Smith-Palmer et al. [16] and the ineffectiveness of Marjoram EO against Salmonella enteritidis. Other studies have shown the antimicrobial activity of Clove EO against Salmonella enteridis [16] and Salmonella paratyphi [18]. Laurel EO showed the lowest overall activity against the tested bacteria, contradicting the findings of Derwich et al. [20] and EI et al. [21], which reported S. aureus as the most sensitive strain to the Laurel EO. This difference may be due to variations in EO extraction processes and environmental conditions.

Clove and Marjoram EO demonstrated the best antibacterial activity against Gram-positive Staphylococcus aureus, a common microorganism involved in food poisoning. The mechanism of action of these oils is thought to be related to the structure of the bacterial wall and membrane permeability of Gram-positive and Gram-negative bacteria. Research by Rayour [22] and Burt [23] suggest that active EO interferes with the lipid bilayer of target cells, leading to loss of cellular constituents and ultimately, bacterial death. The antimicrobial activity of Clove EO may be due to its main component, Eugenol, which has been shown to exhibit antibacterial and antifungal activity [24, 25] by interacting with the bacterial cell membrane. Additionally, studies by Ramos et al [26] suggest that cis-sabinene hydrate is a significant compound responsible for the inhibition of bacterial growth in Origanum majorana EO. The antimicrobial action of EO is thought to occur in three phases: attack of the bacterial wall, acidification of the cell interior, and destruction of genetic material, leading to bacterial death [27].

*Antioxidant Activity*

The order of antioxidant activity observed in this study was as follows: BHT > Clove EO > Marjoram EO > Laurel EO, with ACC values of 141‰ ±0.32, 138‰±0.31, 91‰±0.29, and 31‰±0.33, respectively (Figures 1, 2).

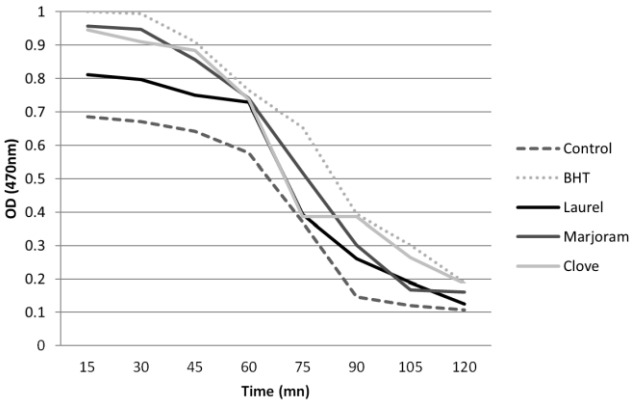


***Figure 1.*** *Evolution of the optical density of Clove, Marjoram, and Laurel EO and synthetic antioxidant (BHT) in the β-carotene bleaching test. (n=3).*

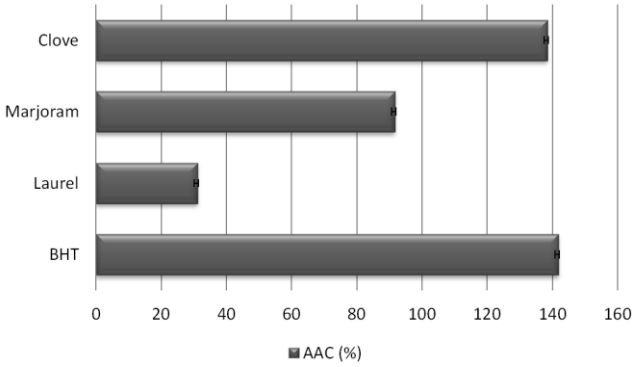


***Figure 2.*** *The antioxidant activity coefficient of Clove, Marjoram, and Laurel EO and synthetic antioxidants in the β-carotene bleaching test. (n=3).*

Clove EO exhibited effective antioxidant activity, which was greater than the other samples, making it a powerful antioxidant. This activity is attributed to the presence of eugenol in clove EO [28]. Clove EO is generally regarded as safe by the FDA as a food additive [29].

Marjoram EO's antioxidant activity is likely due to the presence of sabinene hydrate, which has been shown to prevent peroxide formation [30]. The main components of Laurel EO, 1,8-cineole, 1-(S)-α-pinene, and R-(+)-limonene, have also been found to exhibit antioxidant activity [31]. The tested essential oils may be useful for preserving unsaturated fats and oils in the food industry, as they can prevent lipid peroxidation and rancidity.

*Sensory analysis*

The results of the sensory analysis revealed that the use of EO treatment had a noticeable impact on the flavor, color, and odor characteristics of the chicken meat, as illustrated in Figure 3. However, it is noteworthy that the panelists rated the untreated and Clove EO-treated fillets as having better acceptance than the other samples. This could be due to the strong and distinct flavor and aroma of some of the essential oils used in the study, which may not be preferred by all consumers. The findings suggest that while EO treatment can have a significant impact on the sensory properties of chicken meat, the choice of EO and its concentration must be carefully considered to ensure consumer acceptability.

## 4. Conclusion

According to the results of this study, Clove EO demonstrated the most potent antibacterial activity against Staphylococcus aureus, as well as the highest antioxidant activity, and a better overall acceptance from consumers when used on chicken breast meat. This finding suggests that Clove EO has the potential to be utilized in the food industry to prolong the shelf-life of food products, as well as provide consumers with natural and perceived healthier food additives, compared to synthetic alternatives.

However, further studies are needed to investigate the ef-

fectiveness of Clove EO against a broader range of foodborne and spoilage microorganisms under specific storage, environmental, and food processing conditions. These studies will enable researchers to identify the optimal conditions and concentrations required to maximize the effectiveness of Clove EO in food preservation, as well as any potential side effects or limitations to its use.

## Abbreviations

| | |
|---|---|
| EO | Essential Oil |
| MIC | Minimum Inhibitory Concentration |
| BHI | Brain Heart Infusion |
| AAC | Antioxidant Activity |
| ATCC | American Type Culture Collection |
| TSB | Tryptic Soy Broth |
| ANOVA | Analysis of Variance |

## Conflicts of Interest

The authors declare no conflicts of interest.

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