Libyan Journal of Health, Science, and Development

Volume 2, Issue 3, 2026, Pages: 23-28

Journal homepage: https://ljhsd.org.ly/index.php/ljhsd/index

Directory of Online Libyan Journals

(ARTICLE)

Comparative Antibacterial and Preservative Effects of Aqueous Garlic and Clove Extracts, Sodium Nitrite and Sodium Benzoate in Fresh Minced Meat

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مقارنة الفعالية المضادة للبكتيريا والحافظة لمستخلصي الثوم والقرنفل المائيين ونترات الصوديوم وبنزوات الصوديوم في لحم البقر المفروم الطازج

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Publication history: Received *on 15 October 2026;* ***Accepted*** *on 04 October 2026;* ***Published*** *on 05 November 2026*

Abstract

This study aimed to evaluate the antibacterial and preservative efficacy of aqueous extracts of fresh garlic (*Allium sativum* L.) and clove (*Syzygium aromaticum*), used individually and in combination, and to compare their efficacy with the synthetic preservatives sodium nitrite and sodium benzoate in fresh minced beef. Bacteria associated with meat spoilage were isolated and identified, including *staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Vibrio mimicus.* The antibacterial activity of the plant extracts was evaluated using the agar well diffusion assay at concentrations ranging from 10% to 100%. preservative efficacy was further assessed in experimentally inoculated minced meat stored at 4 and 25 C^0 for 24 and 48h.The results showed that garlic extract exhibited notable antibacterial activity, with inhibition zones reaching up to 40mm, while Clove demonstrated considerable activity, particularly against *V.mimicus*. The garlic- clove combination exhibited the highest overall antibacterial activity among the plant extracts, with enhanced efficacy under refrigerated conditions. Complete inhibition of *E. coli* and *V. mimicus* and marked reduction in *S. aureus* counts were observed following treatment with the garlic- clove combination. Among the synthetic preservatives, sodium nitrite demonstrated the highest preservative efficacy, completely inhibiting the tested bacteria under refrigerated conditions. Overall, the findings indicate that garlic and clove, particularly when used in combination, have promising potential as natural preservatives for minced meat and may contribute to reducing reliance on synthetic chemical preservatives while maintaining product quality.

Keywords: Garlic 1, Clove 2, Natural preservative 3, Antibacterial activity 4, Minced meat 5, Food spoilage bacteria 6, Sodium nitrite7, Sodium benzoate8.

الملخص

هدفت هذه الدراسة إلى تقييم الفعالية المضادة للبكتيريا والفعالية الحافظة للمستخلصات المائية للثوم الطازج (*Allium sativum* L.) والقرنفل (*Syzygium aromaticum*)، منفردة وبصورة مشتركة، ومقارنتها بفعالية المواد الحافظة الكيميائية، نترات الصوديوم وبنزوات الصوديوم، في لحم البقر المفروم الطازج. تم عزل عدد من البكتيريا المرتبطة بفساد اللحوم، وشملت *Pseudomonas. aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *vibrio mimics* تم تقييم النشاط المضاد للبكتيريا للمستخلصات النباتية باستخدام طريقة الانتشار في الابار الاغارية بتراكيز تراوحت بين 10 % و100 %. كما تم تقييم كفاءة المواد الحافظة في الحد من نمو البكتيريا في اللحم الملقح تجريبيا أثناء التخزين عند درجتي 4م^0, 25م^0 لمدة 24 و48 ساعة.أظهرت النتائج أن مستخلص الثوم امتلك

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نشاطا مضادا للبكتيريا، حيث بلغت مناطق التثبيط ما يصل إلى 40 مم، في حين أظهر القرنفل فعالية ملحوظة، خاصة ضد *V. mimicus*. كما أظهر خليط الثوم والقرنفل أعلى فعالية مضادة للبكتيريا بين المستخلصات النباتية المختبرة، مع تعزيز واضح للنشاط تحت ظروف التبريد، حيث أدى إلى تثبيط كامل لنمو *E. coli* و *V. mimics*وتقليل أعداد *S. aureus* في المقابل، أظهرنترات الصوديوم أعلى كفاءة حفظ بين المواد المختبرة، مع تثبيط كامل للبكتيريا المختبرة عند 4م^0. وتشير النتائج إلى إمكانية استخدام الثوم والقرنفل، ولا سيما مزيجهما، كبدائل طبيعية واعدة للمواد الحافظة الكيميائية في حفظ اللحوم، مع الحفاظ على جودة المنتج وتقليل الاعتماد على المواد الحافظة الاصطناعية.

الكلمات المفتاحية: الثوم1، القرنفل 2، المواد الحافظة الطبيعية 3، النشاط المضاد للبكتيريا 4، اللحم المفروم 5، بكتيريا فساد الأغذية، نترات الصوديوم 6 ، بنزوات الصوديوم 7.

Introduction

Microbial spoilage is a major challenge to the global food industry, reducing products, shortening shelf life, and increasing economic losses and public health risks. Meat and meat products are particularly vulnerable to microbial contamination because of their high-water activity, nutrient-rich composition, and near-neutral pH, which provide favorable conditions for the growth of spoilage and pathogenic bacteria, including *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa,* and *Vibrio mimicus* (Bai *et al*., 2023).The growing demand for clean-label foods has increased interest in natural antimicrobial agents as alternatives to synthetic preservatives. Among these, plant-derived essential oils and extracts have attracted considerable attention owing to their antimicrobial efficacy, safety, and consumer acceptance. clove (*Syzygium aromaticum*) and garlic (*Allium sativum*) are rich in bioactive compounds, particularly eugenol and allicin, respectively, which exhibit broad-spectrum antimicrobial activity through disruption of essential microbial structures and metabolic pathways (Wada *et al*., 2021; Zaqzouq *et al*., 2022). Despite extensive research on plant-based antimicrobials, further evaluation of their effectiveness against spoilage and pathogenic bacteria associated with meat products remains necessary. Therefore, this study aimed to investigate the antibacterial activity of clove and garlic against selected meat- associated bacteria and to assess their potential as natural preservatives for improving microbial safety and extending the shelf life of meat products.

Material and methods

Collection of Plant Materials and Meat Samples

Fresh garlic bulbs (*Allium sativum*) were obtained from a local market in Benghazi, Libya. The cloves were peeled, rinsed with sterile distilled water, chopped into small pieces, homogenized using a sterile blender, and stored at 4°C until extraction. Dried flower buds of clove (*Syzygium aromaticum*) was also purchased from local markets in Benghazi. The Plant materials were cleaned to remove foreign impurities, dried, when necessary, ground into fine powders, and stored separately in sterile airtight containers at room temperature until extraction.

Fresh minced beef samples were collected aseptically from four retail butcher shops in Benghazi, Libya. Samples were immediately transferred to sterile polyethylene bags, transported to the Microbiology Laboratory in insulated ice containers, and processed within 2 h of collection. All experiments were conducted between August 2023 and August 2024.

Isolation and Purification of Bacterial Isolates

Bacterial isolation was performed using standard microbiological procedures. Briefly, 1 g of each minced meat sample was homogenized in 9 mL of sterile distilled water. Ten-fold serial dilutions were prepared, and 0.1 mL aliquots from the 10^{-2}, 10^{-3}, and 10^{-4} dilutions were spread onto Blood Agar, MacConkey Agar, and Mannitol Salt Agar (Oxoid Ltd., Basingstoke, UK). Plates were incubated aerobically at 37°C for 24–48 h. Representative colonies with distinct morphological characteristics were repeatedly subcultured to obtain pure isolates, which were maintained on nutrient agar slants at 4°C until further analysis.

Preparation of Plant Extracts

Aqueous extracts were prepared according to Vivian *et al*. (2020). Briefly, 100 g of each powdered plant material was mixed with 100 mL of sterile distilled water (1:1, w/v) and incubated at 37°C for 24 h with intermittent stirring. The mixtures were filtered through Whatman No. 1 filter paper, and the resulting filtrates were used as crude extracts. Working solutions were prepared at concentrations of 10% 25%, 50%, 75%, and 100% (v/v). Fresh garlic extract was prepared immediately before use.

Identification of Bacterial Isolates

Bacterial isolates were initially identified based on colony morphology, Gram staining characteristics, and conventional biochemical tests following the methods described by Cheesbrough (2006). Species level identification was subsequently confirmed using the VITEK® 2 Compact automated identification system (bioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions.

Meat Preservation Assay

The preservative efficacy of selected plant extracts and synthetic preservatives was assessed according to Elgendy *et al*. (2019). Fresh minced beef (4.8 kg) was divided into four equal groups (1.2 kg each), and each group was further subdivided into six portions (200 g each). Meat samples were experimentally inoculated with approximately 1×10^8 CFU/g of *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio mimicus*, or *Staphylococcus aureus*. Inoculated samples were treated with the most effective plant extracts (garlic, clove, and the garlic–clove combination) or with sodium benzoate and sodium nitrite. Untreated inoculated samples served as controls. Samples were stored at room temperature (25 ± 2°C) and under refrigeration (4 ± 1°C) for 24 and 48 h. Bacterial counts were determined at each storage interval using serial dilution and standard plate count methods following FDA (2002) and Fik *et al*. (2007). Results were expressed as colony-forming units per gram (CFU/g).

Statistical Analysis

Statistical analyses were performed using SPSS version 28. The Chi-square test (χ^2) was used to evaluate the association between preservative type (natural or synthetic) and bacterial species isolated from minced meat. statistical significance was established at $P< 0.05$.

Results and discussion

Table 1 Effect of Garlic on bacterial growth inoculated to meat at $4C^0$ &$25C^0$ for 48hr

Bacteria	colony forming unit /gram (CFU/g)			
	Garlic		Control	
	$4C^0$	$25C^0$	$4C^0$	$25C^0$
Staphylococcus aureus	2×10^4	UN C.	1.95×10^6	UN C.
Pseudomonas aeruginosa	Un C.	Un C.	1.1×10^5	UN C.
Escherichia coli	1.7×10^5	Un C.	4×10^4	UN C.
Vibrio mimicus	6.0×10^5	Un C.	7×10^4	UN C.

Un C.=Un countable

Table 11 Effect of garlic and clove mixture on bacterial growth inoculated to meat at $4C^0$ and$25C^0$ for 48hr

Bacteria	colony forming unit /gram (CFU/g)			
	Garlic and clove mixture		Control	
	$4C^0$	$25C^0$	$4C^0$	$25C^0$
Staphylococcus aureus	1×10^4	UN C.	1.95×10^6	UN C.
Pseudomonas aeruginosa	$10.\times10^5$	Un C.	1.1×10^5	UN C.
Escherichia coli	ND.	6×10^4	4×10^4	UN C.
Vibrio mimicus	ND.	2×10^4	7×10^4	UN C.

Un C.=Un countable
ND. = Not Detected



Figure 1: Representative image of meat before the addition of sodium nitrite



Figure II: Meat discoloration observed in samples mixed with sodium nitrite

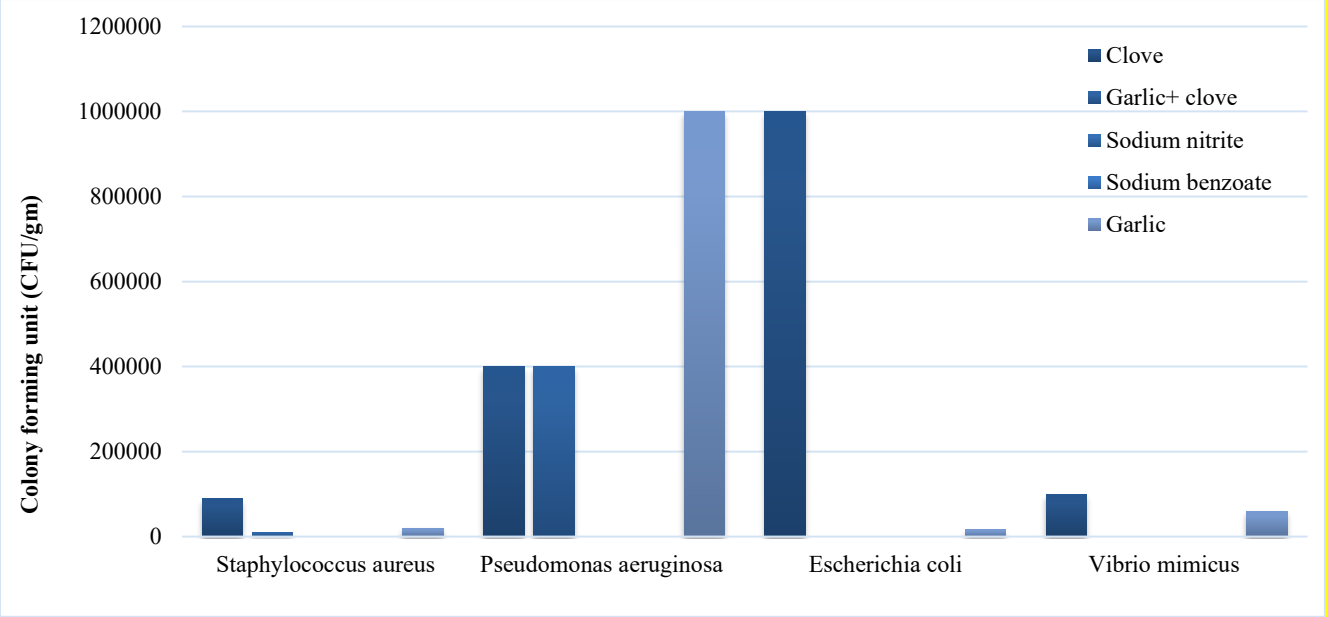


Figure III: Comparison between plant extracts and chemical preservatives on bacterial growth in meat at 25C°

Application of the tested agents to meat samples demonstrated that refrigeration ($4C^0$) markedly enhanced antimicrobial efficacy. Garlic extract effectively reduced bacterial counts, particularly *S. aureus*, whereas its activity decreased significantly at $25C^0$ because of the thermal instability of allicin, in agreement with previous reports (schweigger *et al*., 2007; Atsamnia *et al*., 2017). Clove extract also showed greater effectiveness at refrigeration temperature but lost most of its activity at room temperature. antibacterial activity at $4C^0$ than at $25C^0$, with *S. aureus* being the most susceptible microorganisms. The enhanced efficacy under refrigeration is likely associated with reduced bacterial metabolism, which prolongs exposure to bioactive compounds. The garlic-clove combination demonstrated enhanced antibacterial activity under refrigerated conditions, completely inhibiting *E. coli* and *V. mimicus* while markedly reducing *S. aureu* counts, indicating a synergistic interaction between the bioactive constitutes of both extracts. In contrast, *p*. *aeruginosa* remained the most resistant bacterium, likely due to its low outer membrane permeability and efficient efflux systems. Among the chemical preservatives, sodium nitrite exhibited the strongest antibacterial activity, achieving complete inhibition of all tested bacteria at 4 C^0 and maintained high efficacy against *S. aureus* and *V. mimicus* at $25C^0$. Sodium benzoate also showed effective inhibition of *S. aureus, E. coli* and *V. mimicus,* whereas *p. aeruginosa* displayed comparatively high resistance. These findings are consistent with previous studies indicating that refrigeration enhances preservative efficacy by reducing bacterial metabolic activity (poole *et al*., 2011; Ferysiuk *et al*., 2020; Tompkin 2005).

Conclusion

These results indicate that spice extracts offer promising natural alternatives to synthetic preservatives in meat preservation, exhibiting antibacterial activity with minimal effects on product quality. Although chemical preservatives showed greater efficacy in controlling bacterial growth, plant -based extracts represent potentially safer and more sustainable options for extending meat shelf life.

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