Comparative analysis of antibacterial activity and chemical composition of essential oils from *Salix aegyptiaca* male inflorescence and leaves

Running title: Antibacterial activity and chemical activity of *Salix aegyptiaca* essential oil

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Abstract

Background: The rise of antibiotic-resistant bacterial strains and increasing consumer demand for natural food preservatives have driven research into plant-based antimicrobial agents. *Salix aegyptiaca* (*S. aegyptiaca*), commonly known as *Musk Willow*, has shown potential as a source of bioactive compounds, but its antibacterial properties remain underexplored. This study aims to investigate the chemical composition and antibacterial efficacy of essential oils extracted from the leaves and male inflorescence of *S. aegyptiaca* against key foodborne pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enteritidis*.

Methods: Essential oils were extracted from *S. aegyptiaca* leaves and male inflorescence using hydrodistillation and analyzed through Gas Chromatography-Mass Spectrometry (GC-MS) to identify bioactive compounds. Antibacterial activity was evaluated using Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and diffusion methods (Agar Disk and Agar Well Diffusion).

Results: GC-MS analysis revealed high concentrations of 1,4-Dimethoxybenzene, Citronellol, and Eugenol in leaf oil and Carvone in male inflorescence oil. The leaf oil exhibited stronger antimicrobial effects, with MIC values as low as 1250 µg/mL against *Staphylococcus aureus*. Both oils showed limited efficacy against Gram-negative. *Staphylococcus aureus* was the most susceptible strain, while *Escherichia coli* displayed the highest resistance.

Conclusion: The essential oils of *S. aegyptiaca*, particularly from the leaves, demonstrate notable antibacterial activity against common foodborne pathogens. These findings suggest their potential as natural food preservatives, offering an alternative to synthetic additives. Further research into their application in food systems and toxicological profiles is warranted to fully harness their benefits.

Keywords: *Salix aegyptiaca*, Essential oil, Antibacterial activity, Foodborne pathogens, Natural preservative, Male inflorescence

Introduction

Foodborne illnesses continue to be a major global health challenge, with bacterial pathogens causing millions of infections and substantial economic losses each year. These illnesses are primarily caused by bacteria such as *Salmonella enteritidis (S. enteritidis)*, *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. monocytogenes*), and *Staphylococcus aureus* (*S. aureus*), which are frequently transmitted through contaminated food products. Addressing these bacterial pathogens has traditionally involved synthetic preservatives and antibiotics (1). However, the emergence of antibiotic-resistant bacterial strains has limited the effectiveness of these approaches, prompting a search for natural antimicrobial agents that can serve as safer and more sustainable alternatives. Natural plant extracts, particularly essential oils, have attracted increasing scientific interest due to their potent antimicrobial, antioxidant, and preservative properties. Essential oils are complex mixtures of volatile compounds produced by plants as secondary metabolites (2). These oils serve as a defense mechanism against pathogens, pests, and environmental stressors, thus offering an inherent antimicrobial capacity that can be exploited in food preservation. Numerous studies have demonstrated that essential oils from plants such as thyme, oregano, and clove exhibit strong antibacterial properties. However, the potential of many other essential oils, including those derived from traditionally used medicinal plants, remains underexplored in the context of foodborne pathogens.

One such plant is *Salix aegyptiaca* (*S. aegyptiaca*), commonly known as Musk Willow. Traditionally, *S. aegyptiaca* has been used in herbal medicine across various cultures, particularly in Middle Eastern and Central Asian countries. It is known for its anti-inflammatory, analgesic, and antioxidant properties, making it valuable in treating headaches, digestive disorders, and respiratory ailments (3, 4). The male inflorescence of *S. aegyptiaca*, is particularly valued and traditionally harvested for its aromatic properties and medicinal uses, often extracted for its essential oil (5).The leaves of *S. aegyptiaca* have also been traditionally used for their therapeutic effects, including wound healing and as an antipyretic (3). Previous research has highlighted the presence of various bioactive compounds in different parts of *S. aegyptiaca*, including phenolic compounds, flavonoids, and tannins, which are known for their diverse pharmacological activities, including antimicrobial efficacy (5, 6). For instance, studies on other *Salix* species have reported significant antimicrobial effects, supporting the potential of this genus as a source of natural antimicrobials (7). This study seeks to bridge this gap by evaluating the antibacterial activity of *S. aegyptiaca* essential oil against several major foodborne bacteria. Specifically, the study focuses on pathogens commonly associated with food spoilage and foodborne infections, including *S. aureus*, *E. coli*, *L. monocytogenes*, *S. enteritidis*, *Pseudomonas aeruginosa*, and others. By exploring the effectiveness of *Salix aegyptiaca* essential oil through multiple antimicrobial assays, such as the MIC, MBC, Agar Disk Diffusion, and Agar Well Diffusion, this research aims to provide a scientific basis for its application as a natural preservative in the food industry (8-11). Given the increasing consumer demand for natural and safe food products, the exploration of *S. aegyptiaca* essential oil as a preservative is both timely and relevant. These findings from this study could potentially support the development of this essential oil as an effective and sustainable alternative to synthetic food preservatives, enhancing food safety while reducing reliance on conventional antibiotics.

Methods

Plant material collection and identification

Fresh *S. aegyptiaca* leaves and male inflorescences were collected from the Zagros Mountains

region of Iran during the early spring flowering season to ensure peak phytochemical content. Botanical identification of the plant specimens was conducted by agricultural specialists at Gorgan University of Agricultural Sciences and Natural Resources. After collection, the plant materials were carefully cleaned and air-dried at room temperature in a shaded, well-ventilated area to prevent degradation of volatile compounds.

Essential oil extraction

The essential oils from both the male inflorescences and leaves were extracted using hydro distillation with a Clevenger-type apparatus, following the method described by the European Pharmacopoeia. For each extraction, 100 grams of the dried plant material (male inflorescences and leaves separately) were placed in a 2-liter round-bottom flask with 1.5 liters of distilled water. The mixture was heated, and the steam carrying the volatile oils was condensed and collected. The extraction process was carried out for 4 hours to ensure maximum recovery of the essential oils. Considering that the male inflorescence of this plant contains aromatic compounds primarily in the fresh flowering stage, extraction was performed promptly after collection from fresh plant material to ensure the capture of volatile constituents. The oils were subsequently separated from the aqueous layer, dried over anhydrous sodium sulfate, filtered, and stored in dark glass vials at 4°C to prevent oxidative degradation. The yield of essential oils was calculated as a percentage of the dry weight of the plant material (12).

Chemical analysis of essential oils

The chemical composition of the essential oils was analyzed using gas chromatography coupled with mass spectrometry (GC-MS). The GC-MS system used was equipped with a fused silica capillary column (30 m × 0.25 mm, film thickness of 0.25 μm). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The temperature of the GC oven was programmed to increase from 60°C to 240°C at a rate of 3°C/min, and the injector temperature was set at 250°C (13).

Bacterial strains and preparation

The antibacterial activity of the essential oils was tested against ten bacterial strains known to cause foodborne illnesses or food spoilage. These strains included *S. aureus* (PTCC 1917), *E. coli* (PTCC 1338), *L. monocytogenes* (PTCC 1783), *Pseudomonas aeruginosa* (PTCC 1310), *S. enteritidis* (PTCC 1787), *Shigella dysenteriae* (PTCC 1188), *Klebsiella pneumoniae* (PTCC 1053), *Alcaligenes faecalis* (PTCC 1624), *Serratia marcescens* (PTCC 1621), and *Streptococcus pyogenes* (PTCC 1762). The strains were obtained from the Persian Type Culture Collection (PTCC). Bacterial suspensions were prepared by growing each strain in Brain Heart Infusion (BHI) broth at 37°C for 18 hours to reach the exponential growth phase. The bacterial concentration was adjusted to approximately 10^6 CFU/mL by optical density measurement at 600 nm using a spectrophotometer. These standardized inoculums were used for all subsequent assays.

Antibacterial assays

MIC and MBC

The MIC and MBC of the essential oils were determined using the broth microdilution method. A series of two-fold dilutions of the essential oils were prepared in 96-well microtiter plates containing 100 μL of BHI broth. Bacterial suspensions (10 μL, 10^6 CFU/mL) were added to each well, and the plates were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of essential oil that completely inhibited visible bacterial growth. To determine the MBC, aliquots from wells showing no visible growth were plated on nutrient agar and incubated for 24 hours. The MBC was defined as the lowest concentration of essential oil that killed 99.9% of the bacteria. Each experiment was performed in triplicate, and the results were averaged (14).

Agar disk diffusion assay

The antibacterial activity of the essential oils was assessed using the agar disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI). Bacterial cultures grown overnight were diluted in sterile saline to reach a turbidity equivalent to 0.5 McFarland standard (approximately 10^6 CFU/mL). A sterile cotton swab was used to evenly spread the bacterial suspension onto Mueller-Hinton agar plates. Sterile paper disks (6 mm in diameter) were impregnated with 10 µL of each essential oil (diluted in dimethyl sulfoxide) and placed on the inoculated agar surface. Plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zones was measured using a digital caliper. Gentamicin (10 µg/disc) and chloramphenicol (30 µg/disc) were used as positive controls, while disks containing only DMSO served as the negative control. Each experiment was performed in triplicate, and results were expressed as the mean ± standard deviation (15).

Agar well diffusion assay

The agar well diffusion method was also employed to determine the antibacterial activity of the essential oils. Wells (6 mm in diameter) were punched into Mueller-Hinton agar plates previously inoculated with bacterial suspensions. Different concentrations of essential oils (diluted in DMSO) were added to the wells (50 µL per well). Plates were incubated at 37°C for 24 hours, and the inhibition zones around the wells were measured. Positive and negative controls were included as described for the disk diffusion assay. This method allowed for the evaluation of the antibacterial activity of various oil concentrations (16).

Statistical analysis

Data from the antibacterial assays were analyzed using SPSS software to assess the significance of the essential oils' effects on different bacterial strains. Normality of the data was first confirmed with the Kolmogorov-Smirnov test, and variance homogeneity was checked with Levene's test. One-way ANOVA followed by Tukey's post hoc test was used to determine statistically significant differences between groups, with a significance threshold set at $p < 0.05$. Results were expressed as mean ± standard deviation (SD) based on three independent experiments.

Results

The study results highlight the chemical composition of *S. aegyptiaca* essential oils from leaves and male inflorescence and their antibacterial activity against selected foodborne pathogens. The findings are presented in terms of chemical profile, MIC and MBC values, and the inhibition zones observed in the agar disk and well diffusion assays.

Chemical composition of *Salix aegyptiaca* essential oils

The GC-MS analysis of the essential oils revealed a complex mixture of bioactive compounds in both the leaf and male inflorescence oils. As described in Table 1 the predominant components in the leaf oil were 1,4-Dimethoxybenzene (34.78%), Citronellol (13.53%), and Eugenol (5.29%). In contrast, the male inflorescence oil was primarily composed of 1,4-Dimethoxybenzene (28.46%), Citronellol (10.75%), and Carvone (5.12%). These compounds are known for their antimicrobial properties, which may explain the effectiveness of the oils against bacterial strains tested in this study. The differences in composition between the leaf and male inflorescence oils suggest that the bioactivity may vary depending on the source of the essential oil within the plant (17, 18).

MIC and MBC results

The MIC and MBC values for each bacterial strain are summarized in Tables 2, indicating the oil concentrations required to inhibit and kill each bacterial strain.

Table 1. Chemical composition of *Salix aegyptiaca* essential oils (GC-MS Analysis)

Compound	Leaf oil (%)	Male inflorescence oil (%)
1,4-Dimethoxybenzene	34.78	28.46
Citronellol	13.53	10.75
Eugenol	5.29	-
Carvone	-	5.12
Others	46.40	55.67
Total identified (%)	100	100

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Salix aegyptiaca* essential oils against bacterial strains

Bacterial strain	Leaf oil MIC (µg/mL)	Leaf oil MBC (µg/mL)	Male inflorescence oil MIC (µg/mL)	Male inflorescence oil MBC (µg/mL)
Staphylococcus aureus	1250	2500	2500	2500
Escherichia coli	5000	5000	5000	10000
Listeria monocytogenes	2500	5000	5000	5000
Pseudomonas aeruginosa	2500	5000	5000	5000
Salmonella enteritidis	5000	5000	5000	10000
Shigella dysenteriae	5000	5000	5000	10000
Klebsiella pneumoniae	2500	5000	5000	5000
Alcaligenes faecalis	2500	5000	5000	5000
Serratia marcescens	2500	2500	2500	2500
Streptococcus pyogenes	2500	5000	5000	5000

Leaf essential oil:

The MIC values ranged from 1250 µg/mL for *Staphylococcus aureus* to 5000 µg/mL for *S. enteritidis* and *Shigella dysenteriae*. MBC values followed a similar trend, with the lowest MBC recorded for *S. aureus* (2500 µg/mL), whereas *S. enteritidis* and *Shigella dysenteriae* required higher concentrations (5000 µg/mL) to achieve bactericidal effects. These results indicate that *S. aureus* is the most susceptible strain to the leaf oil, while *S. enteritidis* and *Shigella dysenteriae* are more resistant.

Male inflorescence essential oil

The MIC and MBC values for the male inflorescence oil were generally higher compared to the leaf oil, indicating slightly lower antimicrobial efficacy. The lowest MIC was observed for *Serratia marcescens* (2500 µg/mL) and *S. aureus* (2500 µg/mL), while the highest MIC values (5000

µg/mL) were recorded for *E. coli*, *Salmonella enteritidis*, and *Shigella dysenteriae*. The MBC values for male inflorescence oil were lowest for *S. marcescens* and *S. aureus* (2500 µg/mL) but increased to 10,000 µg/mL for *S. enteritidis*.

Agar disk diffusion assay

The agar disk diffusion assay results (Tables 3) showed inhibition zones varying by bacterial strain and type of essential oil used. In general, the leaf oil demonstrated larger inhibition zones than the male inflorescence oil.

Leaf oil

The largest inhibition zone was observed against *S. aureus* (9.38 ± 0.15 mm), indicating strong antibacterial activity. In contrast, *E. coli* exhibited the smallest inhibition zone (7.59 ± 0.20 mm), suggesting lower susceptibility. Other strains, such as *Pseudomonas aeruginosa* and *Serratia marcescens*, showed moderate sensitivity with inhibition zones of 9.28 ± 0.15 mm and 9.12 ± 0.15 mm, respectively.

Male inflorescence oil

The inhibition zones for the male inflorescence oil were generally smaller than those for the leaf oil. The largest zone was again observed against *S. aureus* (8.56 ± 0.20 mm), while the smallest was recorded for *Streptococcus pyogenes* (7.82 ± 0.12 mm). These results reinforce the finding that *S. aureus* is particularly susceptible to both oils, with slightly higher efficacy observed in the leaf oil.

Table 3. Inhibition zones of *Salix aegyptiaca* essential oils (Agar disk diffusion)

Bacterial strain	Leaf oil inhibition zone (mm)	Male inflorescence oil inhibition zone (mm)
Staphylococcus aureus	9.38 ± 0.15^{Aa}	8.56 ± 0.20^{Ab}
Escherichia coli	7.59 ± 0.20^{Ba}	7.82 ± 0.12^{Ba}
Listeria monocytogenes	8.92 ± 0.18^{Ca}	8.10 ± 0.13^{Cb}
Pseudomonas aeruginosa	9.28 ± 0.15^{Aa}	8.46 ± 0.15^{Ab}
Salmonella enteritidis	7.80 ± 0.19^{Da}	7.24 ± 0.12^{Db}
Shigella dysenteriae	8.23 ± 0.17^{Ea}	7.75 ± 0.15^{Bb}
Klebsiella pneumoniae	8.34 ± 0.18^{Ea}	8.05 ± 0.14^{Ca}
Alcaligenes faecalis	8.51 ± 0.14^{Fa}	8.12 ± 0.16^{Cb}
Serratia marcescens	9.12 ± 0.15^{Aa}	8.50 ± 0.14^{Ab}
Streptococcus pyogenes	8.68 ± 0.16^{Ca}	7.82 ± 0.12^{Bb}

Different Capital letters in each column indicate a statistically significant difference ($P < 0.05$).
Different small letters in each row indicate a statistically significant difference ($P < 0.05$).

Agar well diffusion assay

The results of the agar well diffusion assay, illustrated in Figure 1, confirm the antibacterial activity observed in the disk diffusion test and further demonstrate the variance in susceptibility among bacterial strains.

Leaf oil

In the well diffusion assay, *S. aureus* again displayed the largest inhibition zone (9.38 ± 0.15 mm), confirming its high sensitivity. Conversely, *E. coli* showed the smallest inhibition zone (7.59 ± 0.20 mm), highlighting its relative resistance. Other strains such as *Pseudomonas aeruginosa* and *Serratia marcescens* exhibited moderate inhibition zones (9.28 ± 0.15 mm and 9.12 ± 0.15 mm,

respectively), suggesting variable antibacterial effects based on bacterial species.

Male Inflorescence Oil

Similar trends were observed in the agar well diffusion assay for the male inflorescence oil, with the most significant inhibition against *S. aureus* (8.56 ± 0.20 mm) and the least against *Shigella dysenteriae* (7.24 ± 0.12 mm). This consistency in results across both diffusion methods emphasizes *S. aureus* as the most susceptible bacterium, with the male inflorescence oil showing slightly reduced efficacy compared to the leaf oil. Statistical analysis confirmed significant differences ($p < 0.05$) between the antibacterial activities of the leaf and male inflorescence oils against specific bacterial strains. Variability in inhibition zones, MIC, and MBC values were statistically significant across different strains, with *S. aureus* consistently demonstrating higher sensitivity, while *E. coli* and *S. enteritidis* exhibited lower sensitivity to both essential oils. These findings underline the potential use of *S. aegyptiaca* essential oils, particularly from leaves, as effective antimicrobial agents.

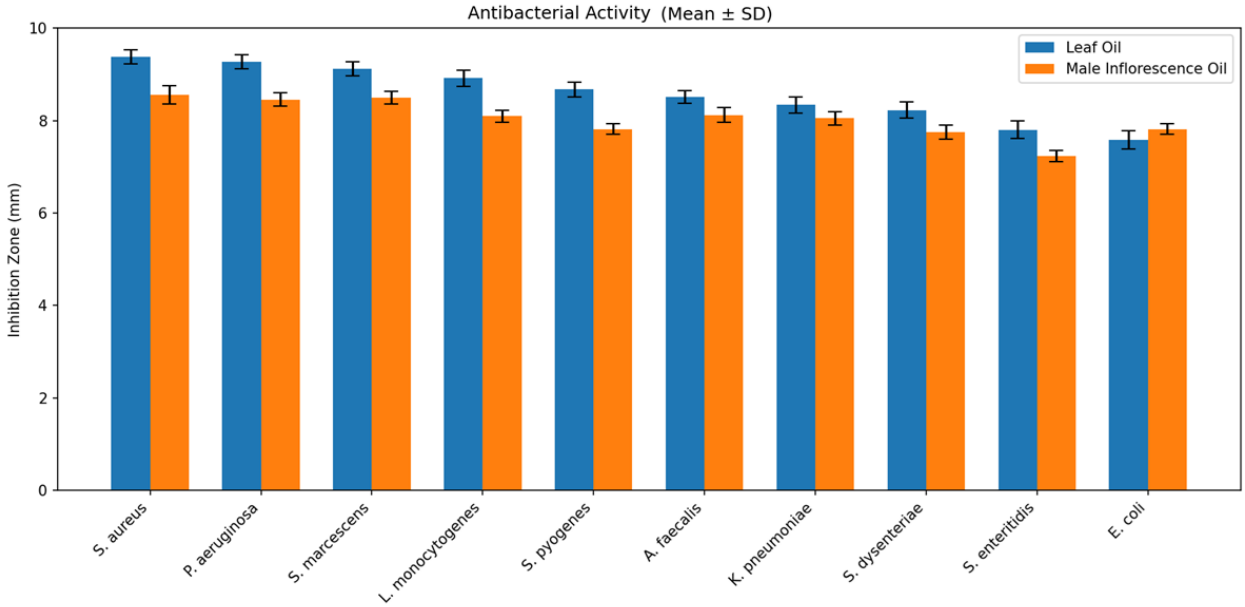

Figure 1. Comparative antimicrobial activity of *Salix aegyptiaca* leaf and male inflorescence essential oils against various bacterial strains, as measured by inhibition zone diameters (mm) using the agar well diffusion method.

Discussion

The present study provides a comparative analysis of the chemical composition and antibacterial properties of essential oils derived from the leaves and male inflorescence of *S. aegyptiaca* against several bacterial strains responsible for foodborne diseases and food spoilage. The findings indicate that these essential oils, particularly those from the leaves, possess significant antibacterial activity against both Gram-positive and Gram-negative bacteria, although the efficacy varies depending on the bacterial species (19). While the investigation of antibacterial effects of plant extracts is a field with numerous contributions, this study offers specific insights into the comparative potential of different parts of *S. aegyptiaca*, a plant with traditional medicinal importance, against a panel of relevant foodborne pathogens. The observed differences in chemical profiles and corresponding bioactivities between leaf and male inflorescence oils contribute to a better understanding of how to optimally utilize this plant resource.

The GC-MS analysis revealed that 1,4-Dimethoxybenzene, Citronellol, and Eugenol were the

dominant compounds in the leaf oil, while the male inflorescence oil was rich in 1,4-Dimethoxybenzene, Citronellol, and Carvone. These compounds are well-known for their antimicrobial properties, which can be attributed to their chemical structures and interaction with bacterial cell membranes. For instance, Eugenol and Citronellol are phenolic and monoterpenoid compounds, respectively, with known ability to disrupt bacterial cell walls, increase membrane permeability, and interfere with intracellular functions, ultimately leading to cell death (20, 21). Previous studies have reported that 1,4-Dimethoxybenzene exhibits antimicrobial effects, though its activity varies depending on the bacterial strain and concentration. The higher percentage of this compound in the leaf oil, along with the presence of Eugenol (absent in the male inflorescence oil), may contribute to the superior antibacterial activity observed compared to the male inflorescence oil. This aligns with research showing that the efficacy of essential oils is often directly related to the concentration and synergy of their active constituents (22). The distinct chemical profile of the male inflorescence oil, characterized by Carvone, also contributes to its antimicrobial action, albeit to a lesser extent than the leaf oil in this study.

The results demonstrated that *S. aureus*, a Gram-positive bacterium, was the most susceptible to both leaf and male inflorescence oils. In contrast, *E. coli* and *S. enteritidis*, both Gram-negative bacteria, were less affected, as evidenced by their higher MIC and MBC values. This differential sensitivity is a commonly observed phenomenon and may be attributed to structural differences between Gram-positive and Gram-negative bacteria (23). Gram-negative bacteria possess an outer lipopolysaccharide layer that acts as a protective barrier, limiting the penetration of hydrophobic molecules, including essential oil compounds (24). Conversely, Gram-positive bacteria lack this outer membrane, allowing for easier access of essential oils to their cell walls and plasma membranes. These findings are consistent with other studies reporting that essential oils tend to be more effective against Gram-positive bacteria (25).

The essential oils of *S. aegyptiaca* likely exert their antibacterial effects through multiple mechanisms. Phenolic compounds such as Eugenol are known to cause cell membrane disruption, protein denaturation, and enzyme inhibition (26). Additionally, terpenoids like Citronellol and Carvone have been reported to cause structural damage to bacterial cell walls and membranes, leading to increased permeability and leakage of cellular contents (27). This multi-target approach makes essential oils particularly promising as antimicrobial agents because bacteria are less likely to develop resistance when subjected to multiple simultaneous stresses (28). Given the varied composition of the essential oils from leaves and male inflorescences, it is plausible that the oils' antimicrobial activity results from a synergistic effect among the different constituents. This synergy could enhance the overall efficacy of the oil beyond the additive effect of individual components (29).

The results of the disk and well diffusion assays indicate that the inhibition zones produced by *S. aegyptiaca* oils, especially from the leaves against *S. aureus*, were substantial, though generally smaller than those observed for gentamicin, the antibiotic used as a positive control. However, essential oils offer certain advantages over synthetic antibiotics, including their natural origin, lower likelihood of contributing to antibiotic resistance, and broader acceptance by consumers in the context of natural and organic foods (30, 31). While antibiotics target specific cellular pathways, often resulting in resistance over time, essential oils have a broader mechanism of action and can act on multiple targets within the bacterial cell. This may reduce the risk of resistance development, making essential oils a valuable alternative for combating antibiotic-resistant bacterial strains (32, 33).

The justification for this work lies in its potential contribution to the food industry by exploring

natural alternatives for food preservation, which is a growing area of interest due to consumer preferences and concerns about synthetic additives. While this study has demonstrated promising antibacterial properties of *S. aegyptiaca* essential oils, several limitations should be noted. First, the study was conducted in vitro, which may not fully represent the oil's performance in complex food matrices where interactions with other food components can affect efficacy. Future research should investigate the oils' antimicrobial effects in real food systems to better assess their practical applicability as preservatives. Moreover, although the chemical composition of the oils was analyzed, the potential synergistic effects of individual compounds were not specifically examined. Fractionation studies and combination assays with individual compounds provide more detailed insights into the contributions of specific bioactive compounds. Finally, toxicity studies are essential to ensure that the use of these essential oils at effective antimicrobial concentrations does not pose risks to human health. While the finding that different plant parts exhibit varied oil compositions and activities might not be entirely novel in a broad sense, the specific data for *S. aegyptiaca* male inflorescence versus leaves against a range of foodborne pathogens adds valuable information to the existing body of knowledge on plant-derived antimicrobials.

Conclusion

This study demonstrates that essential oils derived from the leaves and male inflorescence of *S.aegyptiaca* possess notable antibacterial activity against a range of foodborne pathogens. The leaf essential oil, rich in 1,4-Dimethoxybenzene, Citronellol, and Eugenol, exhibited superior efficacy compared to the male inflorescence oil. *S. aureus* was the most susceptible bacterium, while Gram-negative bacteria like *E. coli* showed greater resistance. These findings highlight the potential of *S. aegyptiaca* essential oils, particularly from the leaves, as natural antimicrobial agents for applications such as food preservation. Practical applications could involve incorporating these oils into food packaging materials or as direct food additives to extend shelf-life and enhance safety. Future research should focus on in-situ studies within food matrices, exploring synergistic effects, and conducting comprehensive toxicological assessments to pave the way for their safe and effective utilization in the food industry as an alternative to synthetic preservatives.

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Declarations

Not applicable.

Ethics statement

This study did not involve human or animal clinical trials. Therefore, an ethics statement regarding patient consent or clinical trial registration is not applicable.

Conflict of interest

All authors declare no conflict of interest.

Author contributions

M.R: Writing - original draft, Methodology, Investigation, Formal analysis, Conceptualization. F.H: Writing - original draft, Methodology, Investigation, Conceptualization, M.G: Writing - original draft, Methodology, Investigation, Conceptualization. M.A.M: Writing - original draft, Investigation. N.M: Writing - review and editing, Writing - original draft.

Data availability statement

Not applicable.

Highlights

- *Salix aegyptiaca* (Musk Willow) essential oils from leaves and male inflorescence were investigated for antibacterial properties.
- GC-MS analysis identified 1,4-Dimethoxybenzene, Citronellol, and Eugenol as major bioactive components in leaf oil, and 1,4-Dimethoxybenzene, Citronellol, and Carvone in male inflorescence oil.
- The leaf oil displayed stronger antibacterial effects compared to the male inflorescence oil, particularly against *Staphylococcus aureus*.
- MIC values as low as 1250 µg/mL were observed for *S. aureus*, demonstrating the efficacy of the leaf oil.
- Both leaf and male inflorescence oils showed limited efficacy against Gram-negative bacteria, such as *E. coli*, due to the structural protection provided by the outer lipopolysaccharide layer.
- Antibacterial activity of *S. aegyptiaca* essential oils involves mechanisms like membrane disruption and enzyme inhibition.
- Potential application as a natural preservative in the food industry was demonstrated, with implications for replacing synthetic additives and reducing reliance on conventional antibiotics.

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