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Analysis of the Antibacterial Activity of Essential Oils Against Enteropathogens

Adriana Froes do Nascimento Souto[1], Leonardo Ferreira Oliveira[1], Agueda Maria de França Tavares[1], Franciane Gabrielle dos Santos[1], Alessandro Soares Fonseca de Matos[2], Ageu Emerson Braz do Carmo[1], Suze Adriane Fonseca[1], Joao Bosco de Souza Júnior[3], Lorena Bianca Chaves Barbosa[1], Sabrina Ferreira Simões do Nascimento[1], Maria Júlia Ribeiro Magalhães[1], Meriane Gonçalves Resende[4], Ana Elisa Lelis de Oliveira[5], Nayara Gonçalves Pereira[6], Anna Christina de Almeida[1]

[1]*(Institute of Agricultural Sciences, Federal University Of Minas Gerais, Brazil)*
[2]*(Zootechnician, Federal University Of Minas Gerais, Brazil)*
[3]*(Chemical Engineering, Faculty of Science and Technology of Montes Claros, Brazil)*
[4]*(Bachelor of Pharmacy, Ibituruna Health College, Brazil)*
[5]*(Degree in Pharmacy, FIPMoc University Center,Brazil)*
[6]*(PhD in Biotechnology, State University of Montes Claros, Brazil)*

Abstract:
Background*: The increasing prevalence of antibiotic-resistant bacteria and consumer concerns over chemical preservatives have driven interest in plant-derived essential oils as natural antimicrobials. S. aureus and E. coli are common causes of foodborne illnesses, highlighting the need for effective natural control measures.*
Materials and Methods*: Aromatogram assays (disk diffusion technique) were conducted using 10 µl of each essential oil applied to filter paper disks on Mueller-Hinton agar inoculated with standardized bacterial suspensions. Plates were incubated at 35 ± 2 °C for 24 hours, and inhibition zones were measured in millimeters. All experiments were performed in triplicate.*
Results*: Chinese cinnamon oil (Cinnamomum cassia) showed the strongest antibacterial effect, producing 23 mm inhibition zones against both bacteria. Clove oil (Eugenia caryophyllus) was effective against S. aureus (22 mm), but inactive against E. coli. Most other oils, including peppermint and tea tree, exhibited little or no antibacterial activity. Oils were generally more effective against the Gram-positive S. aureus than against E. coli.*
Conclusion: *Essential oils, especially Chinese cinnamon and clove, demonstrate promising antibacterial properties and could serve as natural alternatives for food preservation. However, their effectiveness varies by oil and pathogen type, indicating the need for targeted application and further investigation into mechanisms of action.*
Key Word*: Antibacterial, Aromatogram, Essential oils, E. coli, S. aureus.*

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I. Introduction

Food safety and quality are top priorities in the food production industry, especially since microbial contamination remains one of the leading causes of foodborne illness around the world[1]. Among the many pathogens that pose a threat, Escherichia coli and Staphylococcus aureus are particularly concerning due to how frequently they occur and their ability to cause serious intestinal infections. These bacteria not only endanger public health but also create significant challenges for food manufacturers working to ensure product safety and extend shelf life[2,3,4].

Outbreaks of food poisoning and toxin-related infections remain a serious public health issue, often linked to the consumption of food contaminated with harmful bacteria or their toxins[5,6]. These incidents can cause sudden and widespread cases of gastroenteritis, leading to symptoms like stomach cramps, diarrhea, nausea, and vomiting. While most cases are mild and resolve on their own with rest and hydration, some can be more severe - especially in young children, older adults, and people with weakened immune systems -

sometimes requiring hospitalization. In certain situations, particularly when the infection is invasive or persistent, antibiotics may be prescribed to help manage the illness[7]. However, the rise in antibiotic-resistant bacteria has made treatment more challenging, underscoring the importance of using antibiotics responsibly and exploring safer, more natural alternatives to help prevent and control foodborne diseases[8].

Traditionally, antimicrobial agents have been used to control the spread of harmful microbes. However, growing concerns about antibiotic resistance and chemical residues in food have led to an increased interest in safer, more natural alternatives. One such alternative is essential oils - aromatic compounds extracted from plants - which have shown promising antimicrobial properties. Thanks to their natural origin, environmental friendliness, and broad-spectrum activity, essential oils are being explored as potential tools for food preservation[9,10].

This study investigates the in vitro antibacterial activity of selected essential oils against two major foodborne pathogens: Staphylococcus aureus ATCC 13565 and Escherichia coli O86:H35 (EPEC). By evaluating their effectiveness, we aim to explore how essential oils could serve as natural antimicrobial agents, helping to enhance food safety and reduce the risk of foodborne diseases.

II. Material And Methods

Selection and Characterization of Bacterial Strains

Two clinically and epidemiologically significant enteropathogenic bacterial strains were selected for this study: *S. aureus* (ATCC 13565) and *E. coli* O86:H35, classified as Enteropathogenic *Escherichia coli* (EPEC). These strains were chosen based on their documented involvement in foodborne illness outbreaks and their distinct pathogenic mechanisms and antimicrobial resistance profiles. Stock cultures were preserved at -20 °C in Brain Heart Infusion (BHI) broth supplemented with 20% glycerol. Prior to testing, the strains were reactivated in liquid BHI and incubated at 35 ± 2 °C for 18 to 24 hours to ensure optimal growth.

Preparation of Bacterial Inoculum

Following reactivation, bacterial cultures were diluted in sterile saline solution (0.85% NaCl) to achieve a turbidity equivalent to the 0.5 McFarland standard, corresponding to an approximate bacterial concentration of 1.5 × 10 CFU/mL. Turbidity standardization was performed visually using a commercial reference composed of 1.175% barium chloride (BaCl) and 1% sulfuric acid (H SO), ensuring consistent bacterial loads across all test plates.

Culture Medium

Mueller-Hinton agar was used as the culture medium throughout the experiments. This medium is widely recognized as the gold standard for antimicrobial susceptibility testing, as recommended by the Clinical and Laboratory Standards Institute (CLSI). Its low thymidine and thymine content, pH stability, and consistent performance across bacterial species make it especially suitable for diffusion-based assays.

Disk Diffusion Assay for Antimicrobial Activity (Aromatogram)

The antimicrobial potential of selected essential oils was assessed using a disk diffusion method adapted for volatile compounds - commonly referred to as an Aromatogram. This methodology follows the protocol described by Guimarães *et al.*[11]. Sterile paper filter disks (6 mm in diameter) were impregnated with 10 µL of undiluted essential oil and placed onto the surface of Mueller-Hinton agar plates previously inoculated with the standardized bacterial suspension. Inoculation was performed using a sterile swab to evenly spread the bacterial cells across the entire plate, ensuring uniform exposure.

Incubation Conditions

Once prepared, the plates were incubated in an inverted position at 35 ± 2 °C for 24 hours in a conventional incubator. This incubation period allowed sufficient time for bacterial growth and for the volatile compounds in the essential oils to diffuse through the agar. The interaction between the oils and the bacteria could then be observed as zones of inhibited growth surrounding the disks.

Measurement and Interpretation of Results

After the incubation period, the diameter of the inhibition zones around each disk was measured using a ruler with millimeter precision. Results were recorded in millimeters (mm) and tabulated for analysis. All tests were performed in triplicate to ensure reliability, and the average of the three measurements was reported for each sample. The presence of a clear inhibition zone was interpreted as an indication of bacterial susceptibility to the essential oil tested. Conversely, the absence of a halo was considered a sign of bacterial resistance or insufficient antimicrobial activity under the conditions tested.

III. Result

The data presented in Table no 1 offer valuable insight into the antibacterial effectiveness of the essential oils tested against the selected pathogens. Inhibition zone diameters varied widely among the oils, with Chinese cinnamon (*Cinnamomum cassia*) standing out as the most effective. It produced a 23 mm inhibition zone against both *S. aureus* and *E. coli*, suggesting strong broad-spectrum antibacterial activity. This performance positions it as a promising candidate for applications in food safety and natural preservation strategies.

In contrast, several oils - including peppermint (*Mentha piperita*), cajeput (*Melaleuca leucadendron*), and tea tree (*Melaleuca alternifolia*) - did not produce significant zones of inhibition. These findings suggest limited or no antibacterial activity under the experimental conditions, highlighting the importance of selecting appropriate oils based on targeted efficacy.

The results also pointed to differences in oil activity depending on the bacterial strain. For example, clove leaf oil (*Eugenia caryophyllus*) was effective only against *S. aureus*, producing a 22 mm inhibition zone, but had no measurable effect on *E. coli*. Interestingly, none of the oils tested showed selective activity against *E. coli* alone; all oils that inhibited *E. coli* also inhibited *S. aureus*, indicating a broader or preferential effect toward Gram-positive bacteria.

Table no 1. Antibacterial Activity of Essential Oils against *Staphylococcus aureus* and *Escherichia coli*.

Essential Oil	Scientific Name	Origin EO	*S.aureus* 13565	*E. coli O86:H35*
Peppermint	*Mentha piperita*	India	0 mm	0 mm
Rosemary	*Rosmarinus officinalis*	India	0 mm	0 mm
Palmarosa	*Cymbopogon martini*	India	9 mm	10 mm
Star Anise	*Illicium verum*	China	0 mm	0 mm
Cajeput	*Melaleuca leucadendron*	India	0 mm	0 mm
Clove Leaves	*Eugenia caryophyllus*	Singapore	22 mm	11 mm
White Copaiba	*Copaífera officinalis*	Brazil	0 mm	8 mm
Litsea Cubeba	*Litsea cubeba*	China	10 mm	10 mm
Copaiba	*Copaifera reticulata*	Spain	0 mm	0 mm
Tea Tree	*Melaleuca alternifolia*	Australia	0 mm	0 mm
Petitgram	*Citrus aurantium*	Paraguay	0 mm	0 mm
Chinese Cinnamon	*Cinnamomum cassia*	China	23 mm	23 mm
Eucalyptus Staigeriana	*Eucalyptus staigeriana*	Brazil	6 mm	6 mm
Artemisia	*Artemisia vulgaris*	USA	0 mm	5 mm
Eucalyptus Citriodora	*Eucalyptus citriodora*	Brazil	0 mm	5 mm
Cardamom	*Elettaria cardamomum*	Guatemala	6mm	0 mm
Basil	*Ocimum basilicum*	India	0 mm	0 mm
Coriander	*Coriandrum sativum*	Liechtenstein	0 mm	0 mm
Sandalwood Amyris	*Amyris balsamifera*	Haiti	0 mm	7 mm
Cypress	*Cupressus sempervirens*	Spain	0 mm	0 mm
Sicilian Lemon	*Citrus limon*	Italy	0 mm	0 mm
Sage	*Salvia sclarea*	Spain	0 mm	7 mm
Lemongrass	*Cymbopogon citratus*	Morocco	14 mm	10 mm
Citronella	*Cymbopogon winterianus*	Singapore	6 mm	6 mm
Fennel	*Foeniculum vulgare*	Moldova	0 mm	0 mm
Ginger	*Zingiber officinale*	China	0 mm	0 mm
Patchouli	*Pogostemon cablin*	Indonesia	0 mm	7 mm
Geranium	*Pelargonium graveolens*	Egypt	7 mm	7 mm
Bergamot	*Citrus bergamia*	Italy	0 mm	0 mm
Lavender	*Lavandula hybrida*	Spain	0 mm	0 mm
Sweet Orange	*Citrus Sinensis*	Brazil	0 mm	0 mm
Lavender	*Lavandula angustifólia*	Spain	0 mm	0 mm
Lemongrass	*Cymbopogon flexuosus*	India	17mm	0 mm
Virginia Cedar	*Juniperus virginiana*	USA	0 mm	0 mm
Atlas Cedar	*Cedrus atlântica*	Morocco	0 mm	0 mm

Overall, these findings suggest that some essential oils, particularly Chinese cinnamon and clove, hold notable potential for controlling *S. aureus*, while activity against *E. coli* appears more limited. The ability to target either Gram-positive or Gram-negative bacteria could inform future research and product development, especially in the context of natural antimicrobials for food preservation. The observed variability in oil performance also underscores the need for further studies to better understand their antimicrobial properties and mechanisms of action.

IV. Discussion

The search for natural alternatives to synthetic antimicrobials has driven increasing interest in essential oils, whose efficacy against various pathogenic microorganisms has been widely documented in the scientific literature. The data presented show significant variations in the antibacterial activity of different essential oils against both *E. coli*, a Gram-negative bacteria, and *S. aureus*, a Gram-positive bacteria.

Among the oils analyzed, *C. cassia* stood out for its strong inhibitory action, with inhibition zones measuring 23 mm against both tested strains. This performance is consistent with previous studies reporting low minimum inhibitory concentrations (MICs) for this oil against *E. coli*, ranging from 0.025% to 0.28 mg/mL[12]. Its potent antibacterial activity is largely attributed to its high content of phenolic compounds, particularly cinnamaldehyde, which is known to disrupt bacterial membrane permeability and induce irreversible structural damage.

Another essential oil that demonstrated notable performance was *E. caryophyllus*, which produced inhibition zones ranging from 11 mm to 40 mm, showing greater efficacy against *S. aureus*[13,14]. This variation suggests a more targeted antimicrobial activity against Gram-positive bacteria. Such behavior is likely related to the presence of eugenol, a bioactive phenol with bactericidal properties that disrupts cell wall integrity and inhibits key metabolic enzymes.

The findings align with previous research highlighting the effectiveness of various essential oils against both Gram-positive and Gram-negative microorganisms[15]. The significant inhibition of *E. coli* and *S. aureus* growth reinforces the hypothesis that these natural compounds may serve as promising allies in infection control, particularly in light of the alarming rise in bacterial resistance to conventional antibiotics.

In addition, recent studies have emphasized the importance of nanotechnology in the formulation of essential oils, especially in the form of nanoemulsions[16,17]. These formulations not only improve the solubility of active compounds but also allow for controlled and sustained release, thereby extending antimicrobial effects and enhancing therapeutic efficacy.

The evidence discussed suggests that essential oils such as those from *C. cassia* and *E. caryophyllus* hold strong potential for the development of new antimicrobial agents, whether as phytotherapeutic products, therapeutic cosmetics, or adjuvants in pharmaceutical formulations. Beyond their bactericidal activity, their ability to inhibit biofilm formation - structures often linked to chronic and recurrent infections - is especially relevant and warrants further investigation[15].

Another promising aspect is the synergistic interaction between essential oils and antibiotics. Studies have shown that such combinations can enhance antimicrobial efficacy, even against multidrug-resistant strains[18]. This strategy could help reduce the required doses of antibiotics, minimize adverse effects, and delay the emergence of new bacterial resistance mechanisms.

V. Conclusion

This study shows that certain essential oils, especially Chinese cinnamon, have strong antibacterial effects against foodborne bacteria like *S. aureus* and *E. coli*, making them promising natural alternatives for food preservation. However, effectiveness varies by oil and bacteria type, so careful selection is needed. Further research is necessary to understand how these oils work and how well they perform in real food systems.

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