

**EVALUATION OF THE TOXICITY AND
ANTIBACTERIAL EFFECTS OF PLANT
ESSENTIAL OILS AGAINST BACTERIAL-
CONTAMINATED TOMATOES AND LETTUCE**

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UNIVERSITI SAINS MALAYSIA

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by

KIAN SEDRICK SOOSAY

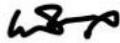
**Dissertation submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Health Science (Honours)
(Biomedicine)**

January 2025

CERTIFICATE

This is to certify that the dissertation entitled “Evaluation of the Toxicity and Antibacterial Effects of Plant Essential Oils against Bacterial-contaminated Tomatoes and Lettuce” is the bona fide record of research work done by Kian Sedrick Soosay during the period from August 2024 to January 2025 under my supervision. I have read this dissertation and, in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Health Science (Honours) (Biomedicine).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.



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**PENILAIAN TERHADAP KETOKSIKAN DAN KESAN ANTIBAKTERIA
MINYAK PATI TUMBUHAN TERHADAP TOMATO DAN SELADA YANG
TERCEMAR BAKTERIA**

ABSTRAK

Minyak pati tumbuhan (EO) boleh digunakan sebagai pengawet semulajadi untuk menggantikan bahan pengawet sintetik atau tiruan. Beberapa kajian telah mengkaji aktiviti antimikrob EO terwap dalam merawat makanan yang tercemar bakteria. Kajian ini bertujuan untuk menilai ketoksikan dan kesan antibakteria tiga EO tumbuhan termasuk serai EO (LEO), cengkih EO (CLEO) dan kayu manis EO (CNEO) terhadap tomato dan selada yang tercemar bakteria. Wap dripada EO (LEO, CLEO dan CNEO) telah disaring untuk ketoksikannya terhadap *Artemia salina* dengan menggunakan ujian kematian udang air garam (BSLT). Saringan aktiviti antibakteria ditentukan dengan mengukur perencatan pertumbuhan *Bacillus cereus* pada agar nutrien (NA) selepas terdedah kepada wap EO pada kepekatan bersiri antara 0.38% v/v hingga 100% v/v. Selepas itu, EO yang paling tidak toksik dan paling berkesan telah dipilih untuk rawatan tomato dan selada yang disuntik *B. cereus* pada suhu sejuk (4°C) dan bilik. Bacaan ketumpatan optik (OD) diambil untuk setiap sampel pada hari ke-1, ke-3, dan ke-7 rawatan untuk mengukur pertumbuhan bakteria. LEO, CLEO, dan CNEO tidak menunjukkan ketoksikan berdasarkan klasifikasi Clarkson dan Meyer dengan nilai LC₅₀ melebihi 10000 ppm. Wap LEO menunjukkan nilai LC₅₀ tertinggi iaitu 122700 ppm dan kesan perencatan lebih daripada 50% pada kepekatan 6.25% v/v. Tomato dan salad yang tercemar *B. cereus* dirawat dengan 6.25% v/v wap LEO merekodkan bacaan OD pertumbuhan bakteria yang lebih rendah berbanding sampel yang tidak dirawat, terutama pada suhu bilik dalam tempoh 7 hari selepas pengeraman.

Kajian ini mencadangkan potensi LEO sebagai pengawet makanan semula jadi yang lebih selamat dan berkesan, tetapi penyasatan lanjut diperlukan untuk mengkaji jika ia boleh digunakan sebagai pengawet alternatif untuk makanan pada suhu bilik.

EVALUATION OF THE TOXICITY AND ANTIBACTERIAL EFFECTS OF PLANT ESSENTIAL OILS AGAINST BACTERIAL-CONTAMINATED TOMATOES AND LETTUCE

ABSTRACT

Plant essential oils (EOs) can be used as natural preservatives to replace synthetic or artificial food preservatives. Some studies have examined the antimicrobial activity of vaporized EOs in treating bacterial-contaminated food. This study aimed to evaluate the toxicity and antibacterial effects of three plant EOs which include lemongrass EO (LEO), clove EO (CLEO) and cinnamon EO (CNEO) against bacterial-contaminated tomatoes and lettuce. The plant EO (LEO, CLEO and CNEO) were screened for their toxicities against *Artemia salina* by using the brine shrimp lethality test (BSLT). The antibacterial activity screening was determined by measuring *Bacillus cereus* growth inhibition on nutrient agar (NA) after being exposed to EO vapors at serial concentrations ranging from 0.38% v/v to 100% v/v. Subsequently, the least toxic and most potent EO was selected for the treatment of *B. cereus*-inoculated tomato and lettuce at cold (4°C) and room temperatures. Optical density (OD) readings were taken for each sample on the 1st, 3rd, and 7th day of treatment to measure the bacterial growth. LEO, CLEO, and CNEO showed no toxicity based on Clarkson and Meyer classification with LC₅₀ values above 10000 ppm. LEO vapors showed lesser toxicity with the highest LC₅₀ value of 122700 ppm and an inhibitory effect of more than 50% at 6.25% v/v concentration. *B. cereus*-contaminated tomato and lettuce treated with 6.25% v/v LEO vapor recorded lower OD readings of bacterial growth than those of untreated samples, particularly at room temperature within 7 days of incubation. This study suggests the potential of LEO as a safer and more effective natural food

preservative, but further investigations are needed to examine if it can be used as an alternative preservative for foods at room temperature.

CHAPTER 1

INTRODUCTION

1.1 Study Background

The increasing prevalence of foodborne illnesses caused by pathogenic bacteria, particularly *Bacillus cereus*, has raised significant concerns among public health. A Gram-positive bacterium called *B. cereus* is frequently found in various fruits and vegetables and is linked to gastrointestinal disorders and food spoilage. It is commonly found to be prone to contaminating tomatoes and lettuces. However, conventional approaches to limiting bacterial growth in food products are losing their effectiveness, prompting the need for alternative antimicrobial strategies (Duan et al., 2023; Jessberger et al., 2020; Yu et al., 2020).

Nevertheless, plant essential oils (EOs) have garnered deep interest in their potential antibacterial properties. Plants naturally produce these oils as secondary metabolites, which are intricate blends of volatile substances. On top of that, numerous studies have demonstrated that EOs have a well-established antimicrobial efficacy, which is effectively inhibiting a variety of pathogens, including *B. cereus* (Lopez-Romero et al., 2015; Swamy et al., 2016). By rupturing bacterial cell membranes and preventing metabolic processes of bacteria which are caused by the bioactive substances found in EOs such as phenolics, terpenes, and aldehydes, which contribute to their antibacterial mechanism (Swamy et al., 2016; Nascimento et al., 2000).

While EOs are frequently regarded as safer substitutes for artificial preservatives, it is very important to assess their toxicity profiles, particularly when applying them to food. EOs can exhibit cytotoxic effects at high concentrations or

prolonged exposure (Nascimento et al., 2000; Swamy et al., 2016). Therefore, their use in food safety depends on an understanding of the trade-off between possible toxicity and effective antibacterial action.

This study is significant as it addresses both the growing concern over foodborne pathogens like *B. cereus* and the need for safer alternatives to carcinogenic chemical preservatives. This study could result in novel ways to improve food safety while lowering the health risks connected with artificial additives by assessing the toxicity and antibacterial properties of plant EOs.

1.2 Problem Statement

The preservation of fruits and vegetables poses significant challenges in maintaining their freshness over extended periods without the usage of artificial preservatives. Fresh produce is extremely perishable and has a short shelf life due to variables like humidity, temperature changes, and exposure to ethylene gas, which accelerates ripening. Food insecurity and financial losses in the agriculture industry are exacerbated by these conditions, which frequently result in spoilage and waste. Thus, effective preservation techniques that would not jeopardize food safety or quality are desperately needed as the demand for fresh produce around the world continues to rise (Hussain & Gooneratne, 2017).

Despite artificial preservatives are frequently used to increase shelf life, their detrimental effects on human health and potential negative reactions have drawn criticism. Studies have linked certain synthetic additives to various health issues, therefore prompting consumers to seek cleaner and safer alternatives. Recent outbreaks of foodborne illness linked to tainted fresh produce, have led to an increased

awareness of these risks that have increased scrutiny of food safety procedures. When fruits and vegetables are handled incorrectly, pathogens like *B. cereus* can flourish and pose a major health risk to consumers. In response to these challenges, there is a pressing need for innovative solutions which prioritize food safety without relying on harmful chemicals. Plant EOs have emerged as a promising alternative because of their inherent antibacterial qualities and capacity to prolong the shelf life of fresh produce. However, the effectiveness and safety of these oils must be rigorously evaluated to ensure they can be utilized effectively in commercial applications (Ungureanu et al., 2023).

1.3 Study Rationale

Research has demonstrated that EOs possess significant antibacterial activity against a range of foodborne pathogens, including *B. cereus*, which is commonly associated with spoilage in fruits and vegetables (Mohd Israfi et al., 2022; Pandey et al., 2017). It has been demonstrated that the bioactive ingredients in EOs, such as terpenes and phenolics, break down microbial cell membranes and suppress metabolic processes, thus preventing food spoilage and increasing shelf life (Gabriela Aguiar Campolina et al., 2023). For instance, research has indicated that essential oils from plants such as cinnamon, oregano, and thyme have strong antibacterial properties, which makes them a good choice for food preservation applications (De-Montijo-Prieto et al., 2021). The multifunctional properties of these natural compounds not only support food quality preservation but also satisfy consumer demands for healthier, chemical-free options.

Despite the promising potential of plant EOs, further research is needed before they can be used in food preservation. To maximize their effectiveness, factors like the concentration of EOs, application technique and compatibility with different food matrices must be investigated. Additionally, consumer acceptance and regulatory considerations play crucial roles in determining the feasibility of integrating EOs into commercial food preservation practices. A systematic evaluation is necessary to determine the most effective formulations for particular fruits and vegetables because research has shown that the effectiveness of various plant EOs can vary greatly depending on their chemical compositions and extraction techniques (Gabriela Aguiar Campolina et al., 2023). To further protect consumer safety, it is essential to comprehend the possible toxicity of specific EOs at different concentrations. Despite EOs being thought to be safe, comprehensive studies are required to determine how they affect human health when added to food products as preservatives. The purpose of this study was to assess the toxicity and antibacterial properties of lemongrass EO (LEO), cinnamon EO (CNEO) and clove EO (CLEO) against *B. cereus* in tomatoes and lettuce. The results of the study would provide insights into sustainable food preservation techniques that not only improve food safety but also satisfy the growing consumer demand for natural substitutes for synthetic additives by examining the effectiveness and real-world applications of these natural preservatives (Yaman Fadhil, 2023).

1.4 Research Questions

1. What are the toxicity effects of the tested plant EO vapours on brine shrimps?
2. What are the inhibitory percentages of the tested plant EO vapours against *B. cereus* growth on the nutrient agar?

3. Is there any significant difference in growth optical density (OD) readings between EO-treated *B. cereus*-contaminated tomatoes incubated at cold temperature and those at room temperature on three different days?
4. Is there any significant difference in growth OD readings between EO-treated *B. cereus*-contaminated lettuce incubated at cold temperature and those at room temperature on three different days?

1.5 Hypothesis

H₀: There is no significant difference in growth OD readings between EO-treated *B. cereus*-contaminated tomatoes incubated at cold temperature and those at room temperature on three different days.

H₁: There is a significant difference in growth OD readings between EO-treated *B. cereus*-contaminated tomatoes incubated at cold temperature and those at room temperature on three different days.

H₀: There is no significant difference in growth OD readings between EO-treated *B. cereus*-contaminated lettuce incubated at cold temperature and those at room temperature on three different days.

H₁: There is a significant difference in growth OD readings between EO-treated *B. cereus*-contaminated lettuce incubated at cold temperature and those at room temperature on three different days.

1.6 Study Objectives

1.6.1 General Objective

To evaluate the toxicity and antibacterial effects of plant EO vapours (CLEO, LEO and CNEO) against *B. cereus*-contaminated tomatoes and lettuce.

1.6.2 Specific Objectives

1. To determine the 50% lethal concentration (LC₅₀) of the selected plant EO vapours using the brine shrimp lethality test (BSLT).
2. To determine the percentage inhibition of plant EO vapours at different concentrations against *B. cereus* growth on nutrient agar.
3. To compare the difference in growth OD readings between EO-treated *B. cereus*-contaminated tomatoes incubated at cold temperature and those at room temperature on three different days.
4. To compare the difference in growth OD readings between EO-treated *B. cereus*-contaminated lettuce incubated at cold temperature and those at room temperature on three different days.

1.7 Study Flowchart

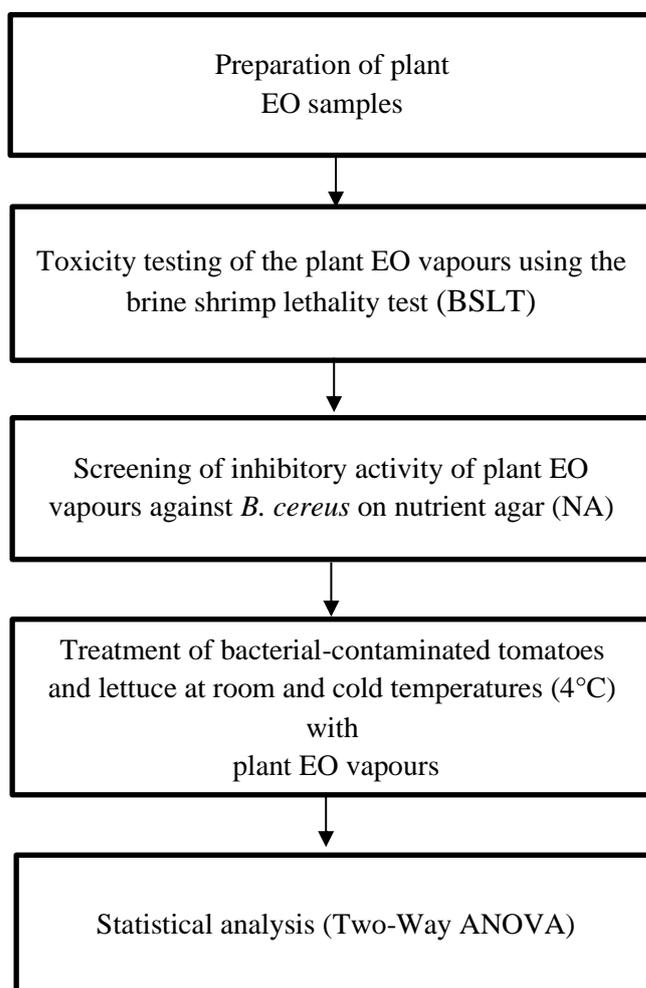


Figure 1.1: Study flowchart

CHAPTER 2

LITERATURE REVIEW

2.1 Natural and Artificial Preservatives

Preservatives are essential for prolonging the shelf life of food products, preventing spoilage, and maintaining safety by inhibiting the growth of microorganisms. The application of preservatives can be divided into two primary categories which are natural preservatives which come from organic sources and artificial preservatives which were created from chemical methods. This literature review focuses on the traits, advantages, and disadvantages as well as the uses of both natural and artificial preservatives, emphasizing their influence on food safety and consumer health. Both natural and synthetic preservatives are regulated by health agencies like the Food and Drug Administration (FDA) in the U.S. These regulations guarantee that all food additives comply with safety standards before their commercial use. Nonetheless, the regulatory landscape can differ considerably between natural and artificial substances due to differing perceptions about their safety profiles (Taylor, 2022).

The practice of using preservatives dates back to ancient times when early methods included salting, smoking, and fermenting foods. Salt was among the earliest preservatives known, which was used to prevent microbial growth by removing moisture from food items like fish and meat (Henney et al., 2010). Additionally, other natural substances, such as honey and vinegar, were used for their preservative properties due to their ability to lower pH and create an environment unfavorable for spoilage organisms (Baptista et al., 2020). The understanding of microbial spoilage advanced significantly in the 19th century, resulting in innovations in preservation technology (Dr. Luxita Sharma & Sharma, 2024). The invention of canning during the

Napoleonic Wars marked a significant advancement in food preservation, as it combined airtight sealing with heat treatment to avert spoilage (Featherstone, 2012). This era also witnessed the rise of chemical preservatives in response to the increasing scale of industrial food production.

Natural preservatives are substances that are derived from various organic sources including plants and animals. Common examples include salt, sugar, vinegar, citric acid, essential oils (EOs), and extracts from plants such as rosemary and cinnamon. These preservatives typically have natural antimicrobial properties that help to restrict the growth of bacteria, molds, and yeasts (Teshome et al., 2022). In contrast, artificial preservatives are synthetic compounds formulated to prevent spoilage and prolong shelf life. Some examples are sodium benzoate, potassium sorbate, and butylated hydroxytoluene (BHT) (Kumar et al., 2022). These synthetic substances are often more effective in smaller amounts compared to natural ones and can offer broad-spectrum protection against different microorganisms (Teshome et al., 2022). Significantly, many natural preservatives function by modifying the pH of food items or establishing an environment that is unfavorable for the growth of microorganisms (Kumar & Rajput, 2023). For example, salt draws moisture out from foods via osmosis, which prevents bacterial growth. Essential oils possess phenolic compounds that disrupt cell membranes and metabolic activities in bacteria (Nazzaro et al., 2013). Synthetic preservatives generally work by blocking enzymes essential for microbial metabolism or by disrupting DNA replication in bacteria. For instance, sodium benzoate is effective at lower pH levels and hinders the development of yeast and mold by interfering with their cellular processes (Walczak-Nowicka & Herbet, 2022).