CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

In vitro growth-inhibitory effect of essential oils from spices against *Bacillus cereus* in vapor phase

BACHELOR'S THESIS

Prague 2024

Author: Jan Gábor

Supervisor: prof. Ing. Ladislav Kokoška, Ph.D.

Declaration

I, Jan Gábor, hereby declare that I have done this thesis entitled “*In vitro* growth-inhibitory effect of essential oils from spices against *Bacillus cereus* in vapor phase” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 17th April 2024

....

Jan Gábor

Acknowledgements

I wish to extend my sincere appreciation to prof. Ing. Ladislav Kokoška, Ph.D., for his patience, invaluable guidance, and insightful comments throughout the process of writing my bachelor's thesis. I am grateful for the opportunity to work in the Laboratory of Ethnobotany and Ethnopharmacology, which enabled me to contribute to valuable research. Additionally, I am indebted to Ayesha Tajammul for her mentorship during my laboratory experiments and for sharing her expertise in this area. Lastly, I would like to express my gratitude to all members of the LEE laboratory for their assistance and constructive feedback.

Abstract

Foodborne diseases pose significant public health risks worldwide, emphasizing the importance of safeguarding food against bacterial spoilage and contamination in the food sector. Spice essential oils (SEOs), known for their antimicrobial activity in the vapor phase and utilized as flavouring agents in food, have been proposed as active agents in antimicrobial atmosphere packaging. This study aimed to determine the minimum inhibitory concentrations (MICs) of hydro-distilled SEOs from various spices (*Amomum subulatum*, *Carum carvi*, *Cinnamomum cassia*, *Curcuma longa*, *Laurus nobilis*, *Myristica fragrans*, *Pimenta dioica*, *Piper nigrum*, *Rosa x damascena* ‘Amadeus’, *Sesamum indicum*, *Syzygium aromaticum*, and *Thymus vulgaris*) commonly used in kitchen environments against foodborne bacterial pathogens (*Bacillus cereus* ATCC 11778; CCM 2010) in both liquid and vapor phases using an *in vitro* broth-microdilution volatilization method. Among the SEOs tested, *C. cassia* exhibited the most potent antimicrobial effect in both liquid and vapor phases against *B. cereus* ATCC 11778 (MIC = 128 µg/mL) and in the liquid (MIC = 128 µg/mL) and vapor (MIC = 256 µg/mL) phase against *B. cereus* CCM 2010. These results indicate that SEO from *C. cassia* holds promise as a source of volatile antibacterial agents for further exploration in the development of natural and non-toxic food preservatives.

Key words: antimicrobial activity, foodborne disease, spice essential oil, vapor phase

Contents

1. Introduction... 1

2. Literature Review 2

2.1. *Bacillus cereus*... 2

2.1.1. Microbiology 2

2.1.2. Epidemiology and pathogenesis 4

2.1.3. Prevention and treatment 6

2.2. Spice essential oils 11

2.2.1. Botany 12

2.2.2. Chemistry 13

2.2.3. Antimicrobial activity 16

2.3. Antimicrobial activity in vapor phase 17

2.3.1. The use in food preservation 18

2.3.2. Methods of antimicrobial susceptibility testing in vapor phase 20

2.3.3. Plant-derived volatile antimicrobials 24

2.4. Spices and their uses in foods 25

3. Aims of the Thesis 37

4. Material and Methods 37

4.1. Plant material 37

4.2. Distillation of spice essential oils 39

4.3. Moisture determination 39

4.4. Media and microorganisms 39

4.5. Chemicals 40

4.6. Antimicrobial assay 40

5. Results and Discussion 43

6. Conclusion 47

7. References 48

List of tables

Table 1. Botanical data and physical characteristics of spice essential oils 38
Table 2. *In vitro* growth-inhibitory effect of essential oils from spices against *Bacillus cereus* in liquid and vapor phase 46

List of figures

Figure 1. The 96-well microtiter plate: experimental setup with flat-bottom wells....... 41
Figure 2. The 96-well microtiter plate lid: experimental setup with flat-bottom flanges 42

List of the abbreviations used in the thesis

AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
CCM	German Collection of Microorganisms
CLSI	Clinical and Laboratory Standards Institute
DMSO	Dimethyl Sulfoxide
EO	Essential Oil
MH	Mueller–Hinton
MIC	Minimum Inhibitory Concentration
MTT	Thiazolyl Blue Tetrazolium Bromide Dye
PDVA	Plant-derived Volatile Antimicrobials
SEO	Spice Essential Oil

1. Introduction

The rise in food-related illnesses due to foodborne pathogens presents a significant public health concern worldwide. Among the various harmful organisms, *Bacillus cereus*, a common gram-positive bacterium, has long been acknowledged as a significant cause of gastrointestinal illnesses upon ingestion of contaminated food accounting for a substantial number of outbreaks (Ehling-Schulz *et al*. 2019). Its wide distribution across diverse environments and its ability to form heat-resistant spores render *B. cereus* a formidable challenge in ensuring food safety and hygiene. Understanding the characteristics and behaviour of such pathogen is essential for implementing effective control measures to minimize the risk of foodborne illnesses.

In recent years, there has been a growing interest in exploring natural compounds, particularly essential oils (EOs), for their antimicrobial properties against foodborne pathogens. While numerous studies have investigated the antimicrobial activity of EOs in the liquid phase, research on their efficacy in the vapor phase remains limited (Bassolé & Juliani 2012; Reyes-Jurado *et al*. 2020; Vihanova *et al*. 2021). The vapor phase represents a unique mode of antimicrobial action where volatile compounds from EOs are dispersed as airborne molecules, potentially exerting inhibitory effects on microorganisms in the surrounding environment. For example, EOs obtained from spices (SEOs) have emerged as promising antimicrobial agents, owing to their intricate chemical compositions, prompting investigations into their efficacy against *B. cereus*. The use of EOs as antimicrobial agents presents a natural and non-toxic approach to food preservation (Bakkali *et al*. 2008). In a time where consumers are increasingly seeking natural and sustainable alternatives, understanding the inhibitory effects of EOs from spices on this pathogen can potentially lead to the development of safer, non-toxic, and overall, more effective antimicrobial strategies in food production and storage (Li *et al*. 2022). This study aims to assess the effectiveness of EO in inhibiting the growth of *B. cereus*, shedding light on potential strategies for mitigating the risks associated with this pathogen in food safety. By investigating the mechanisms and efficacy of these EOs under vapor phase conditions, this study aims to contribute to the fields of food microbiology and antibacterial research.

2. Literature Review

2.1. *Bacillus cereus*

The genus *Bacillus* includes bacterial species such as *B. anthracis*, *B. subtilis*, *B. thuringiensis* and *B. cereus*, which are causing serious human diseases. *B. cereus* is a motile, rod-shaped, gram-positive bacterium with ubiquitous presence throughout the world (Liu *et al*. 2015), which was identified in 1887 by the Frankland & Frankland company (Rangan 2008). *B. cereus* exhibits its primary distinctive feature through the formation of endospores, which grants it the capacity to endure challenging conditions and maintain resilience across diverse environments making it a problematic factor during food processing and storage. It can be found in soil and therefore in plants and their products. This bacterium can be both harmful and beneficial, thus showing a dual nature. While some strains are harmless and are even used in probiotics and numerous food fermentation processes, others are known to cause foodborne illnesses due to the production of toxins (Kotiranta *et al*. 2000). Research efforts are ongoing to unravel the genetic determinants of virulence and design effective targeted therapeutic interventions (Coburn *et al*. 2020). Although this bacterium is well documented for its role in the environment, its renown notoriety also stems from its pathogenic potential. It can contribute to foodborne illness and opportunistic infections in humans. In addition to its clinical implications, *B. cereus* has attracted attention for its positive contributions to industrial processes such as food processing, thus marking it as a versatile microorganism with benefits as well as potential risks (Reyes-Jurado *et al*. 2020).

2.1.1. Microbiology

B. cereus is characterized by a rod-shaped morphology, which is a common feature of the genus *Bacillus* (Liu *et al*. 2015). The cells of this bacterium typically appear as elongated rods that are straight or slightly curved and lack the complex shapes or structures seen in some other bacterial species. These cells are arranged independently or in chains, reflecting the typical morphology of bacteria of the genus *Bacillus*. The width of the individual cells is most commonly ranging from 0.5 to 2.0 μm and the length is raging from 3.0 to 5.0 μm making it a medium-sized bacterium. As a gram-positive bacterium, it has a unique cellular structure of its cell wall. This unparalleled property is due to the high concentration of polysaccharides, especially the polysaccharide peptidoglycan. The appearance of *B. cereus* cells can be observed using a variety of microscopic techniques, including light microscopy and electron microscopy (Clarke & Eberhardt 2002). The *B. cereus* has a noteworthy ability of forming endospores. These endospores can be observed under a microscope and appear as oval or cylindrical structures within the bacterial cell. This innate ability for formation of endospores significantly affects the survival, adaptability, and pathogenic potential of the bacterium (Ryu & Beuchat 2005).

The *B. cereus* is an aerobically respiring organism and uses oxygen for metabolic processes. Its metabolic versatility allows it to metabolize a wide variety of organic compounds, demonstrating its adaptability to a variety of food sources (Vidic *et al*. 2020). This metabolic flexibility contributes to the bacterium's ability to thrive in highly diverse ecological niches and underscores its ecological importance in decomposition processes and nutrient cycling. Another crucial ability which the *B. cereus* possesses is a capability of forming biofilm. For *B. cereus*, a biofilm formation is another crucial aspect of the bacteria's lifestyle. This ability allows the bacterium to adhere to various surfaces and form structured communities of cells which are enclosed in a matrix of extracellular polymeric substances which establish the functional and structural integrity of the biofilm as such (Karunakaran & Biggs 2011). Biofilm formation by *B. cereus* increases the bacterium's resistance to environmental stresses and may contribute to its persistence in different environments (Majed *et al*. 2016). As researchers delve deeper into the molecular and cellular complexities of this bacterium, new avenues continue to open to harness its capabilities and minimalize its risks. To understand the microbiology of *B. cereus* it is crucial to reveal its pathogenic potential and its role in the ecosystem.

B. cereus can benefit very positively in a wide range of environmental areas. Various environmental factors influence the abundance of *B. cereus*. Temperature, pH, and nutrient availability play a key role in its growth and sporulation. The bacterium's ability to form endospores allows it to tolerate adverse conditions, which contributes to its persistence in various habitats (Karunakaran & Biggs 2011). This bacterium has a close connection with soil and water sources such as lakes and rivers which serves as a primary containment area where the bacteria can actively participate in decomposition processes and nutrient cycling. Factors such as temperature, humidity, and agricultural practices can affect the number of bacteria in the soil and water. In addition, *B. cereus* also engages in a bacterial communication system known as quorum sensing, a mechanism by which bacterial cells communicate and coordinate their behaviour based on their population density. Quorum sensing in *B. cereus* involves the production and detection of signalling molecules called autoinducers, which allows the bacterium to modulate gene expression and swiftly adapt its behaviour in response to the surrounding microbial community (Kalia 2013).

2.1.2. Epidemiology and pathogenesis

Epidemiology

The negative impact of *B. cereus* is primarily linked to foodborne illnesses, attributed to its common occurrence in food processing settings owing to the adhesive properties of its endospores (Bottone *et al*. 2010). As of 2024, a comprehensive global database that precisely documents the number of individuals affected by *B. cereus* infections remains unavailable. Nevertheless, estimates suggest that on a global scale, an annual mortality rate of approximately 10 to 1000 individuals can be attributed to *B. cereus* infections (Todd 1996). Between 1993 and 1998, a survey conducted in Europe revealed 278 outbreaks attributed to *B. cereus*, constituting approximately 1.3% of the total reported foodborne outbreaks (WHO 2000). In North America, Europe, and Japan, *B. cereus* has been linked to a range of reported foodborne outbreak cases accounting for approximately 1% to 22% of incidents in the period 1988-2008 (Pexara & Govaris 2018). Notably, within the broader timeframe of 2010 to 2016, a significant 86% of outbreaks in the EU were associated with enterotoxins produced by *Clostridium*, *Staphylococcus*, and *B. cereus*. Data spanning from 2011 to 2015 across various member states of the European Union indicated 220 to 291 reported food poisoning outbreaks linked to *B. cereus*, comprising 3.9% to 5.5% of total food poisoning incidents (Yang *et al*. 2023). According to the annual report of the European Food Safety Authority, *B. cereus* accounted for approximately 16% to 20% of food poisoning outbreaks in the member states caused by bacterial toxins in 2017 (Authority 2018). The majority of these instances are connected to the consumption of cooked foods that underwent slow cooling and which were stored inadequately under refrigeration. *B. cereus* is increasingly recognized as a cause of serious gastrointestinal and potentially fatal non-gastrointestinal infections (Bottone 2010). It is essential to acknowledge that these figures may vary due to regional reporting practices, healthcare infrastructure, and the evolving nature of epidemiological data.

The sources of *B. cereus* contamination have a big field of variation, from agricultural practices to food processing and distribution. Improper storage and abuse of temperature further increases the risk of food outbreaks. Certain conditions increase the likelihood of contamination with *B. cereus*. Poor hygiene practices in the food handling environment, inadequate refrigeration, and extended storage time can create an ideal environment for the growth of this bacterium and its production of toxins (Vidic *et al*. 2020; Veysseyre *et al*. 2015). Food handling practices in institutional settings such as hospitals and cafeterias can also increase the risk of foodborne illness outbreaks. *B. cereus* has earned notoriety as a foodborne pathogen with the potential to contaminate a variety of food products. Foods such as rice, pasta, dairy products, vegetables, and spices are particularly susceptible. Contamination can occur during cultivation, harvesting, processing or storage, and spores can resist cooking processes, germinate under inappropriate storage conditions, and produce toxins that cause food poisoning. Contact with soil during cultivation, irrigation with contaminated water, and poor food handling hygiene contribute to the introduction of *B. cereus* into the food chain (McDowell *et al*. 2023; Vidic *et al*. 2020).

Pathogenesis

B. cereus exhibits a wide spectrum of pathogenicity, from mild gastrointestinal symptoms to severe systemic infections. Unravelling the mechanisms of *B. cereus* pathogenesis involves investigating toxin production, adhesion capabilities, and the interplay between the bacterium and the host's immune system. Understanding the epidemiology and pathogenesis of *B. cereus* is paramount to implementing effective preventive measures, managing infections, and investigating therapeutic interventions. Ongoing research continues to unravel the complexity of its interactions with the environment and host (Veysseyre *et al*. 2015), shedding light on both its beneficial roles and potential threats to human health.

Toxins are essential for the pathogenicity of *B. cereus* and their bacterial production, and thus bacterial multiplication, takes place in both small and large intestine. The *B. cereus* can produce two distinct types of toxins which is the heat-stable emetic toxin and the heat-labile diarrheal toxin. The emetic toxin causes vomiting, while diarrheal toxin leads to diarrhoea (Ehling-Schulz *et al*. 2019). These toxins are produced in the digested food matrix or the intestines, contributing to the characteristic symptoms of *B. cereus* food poisoning. In addition, *B. cereus* has adhesion molecules that facilitate its attachment to host cells (Majed *et al*. 2016). Although some strains are primarily associated with gastrointestinal infections, they have demonstrated the ability to invade intestinal epithelial cells, potentially contributing to the severity of infections. *B. cereus* has evolved strategies to evade the host immune system. The formation of biofilms allows bacteria to resist immune responses and persist in different environments. In addition, the production of proteases and other virulence factors contributes to immune evasion. The susceptibility to *B. cereus* infections varies among individuals. One specific group of people which are immunocompromised individuals, such as patients undergoing chemotherapy or organ transplant recipients, are at higher risk of serious infections. The outcome of *B. cereus* infections is influenced by host factors, such as the strength of the immune system and the presence of underlying medical conditions (Veysseyre *et al*. 2015).

2.1.3. Prevention and treatment

Mitigation of *B. cereus* infections requires the use of a mix of strategies that focus on reducing environmental contamination, adopting good food handling practices, and promoting awareness of associated risk factors. A thorough prevention strategy integrates environmental and individual measures to mitigate the risk of foodborne illness and other infections. Key prevention approaches for *B. cereus* include maintaining hygiene and sanitation, implementing proper food storage and handling practices, temperature control, quality assurance in the food production environment, monitoring environmental sources, and promoting general knowledge and awareness of the bacterial transmission (Vidic *et al*. 2020).

Prevention

Adherence to strict hygiene practices is essential to avoid contamination with *B. cereus*. This includes regular hand washing, especially in food handling environments, and thorough cleaning of surfaces and equipment. Ensuring adequate hygiene in domestic and commercial environments is a vital aspect of minimizing the risk of bacterial contamination. Correct food handling practices play a key role in the prevention of foodborne illness associated with *B. cereus*. This includes cooking food thoroughly, cooling perishable food quickly, and preventing excessive heat during food preparation and storage. *B. cereus* spores are heat resistant, so temperature control is a critical aspect of prevention. Proper cooking temperatures and cooling procedures inhibit the growth and production of *B. cereus* toxins (Veysseyre *et al*. 2015). The implementation of strict quality control measures in the food industry is essential to prevent contamination with *B. cereus*. Enforcing prevention includes regular bacterial testing and strict adherence to hygiene protocols, which significantly contribute to the safety of food products. In addition, monitoring environmental reservoirs such as soil and water helps to understand the prevalence and distribution of *B. cereus* (Kramer & Gilbert 1989). This surveillance offers valuable insights for anticipating potential sources of contamination and guiding the implementation of targeted preventive measures. Awareness campaigns and educational initiatives are an integral part of prevention efforts. Educating individuals, especially those in high-risk groups, about the dangers associated with *B. cereus* and the importance of good food handling practices enables them to make informed decisions and adopt preventive behaviours in a variety of activities, including food preparation (Rivera *et al*. 2018).

Treatment

The solution to the contamination of *B. cereus* bacteria in food requires the implementation of various measures designed to prevent and eradicate this bacterium and its toxins. The germination and multiplication of *B. cereus* spores can occur when food is stored within the temperature range of 10°C to 50°C (Vidic *et al*. 2020; Juneja *et al*. 2019). It is crucial to prevent temperature abuse, including leaving cooked food at room temperature for prolonged durations, to avert bacterial growth and toxin production. Ensuring adequate cooking and temperature management is essential in averting foodborne illnesses associated with *B. cereus*. Thoroughly cooking food, particularly items like rice and pasta, is necessary in deactivating spores and eliminating any present bacteria. Following the cooking process, it is crucial to promptly cool food to deter the multiplication of *B. cereus*. Keeping the food refrigerated at temperatures below 5°C effectively hinders the growth of the bacterium (Granum & Lindbäck 2012). It is recommended to refrigerate cooked food within two hours of preparation, or within one hour if the ambient temperature is elevated (Juneja *et al*. 2019).

Ensuring the proper storage of leftovers is essential in averting illnesses associated with *B. cereus*. It is advisable to divide sizable amounts of food into smaller portions for refrigeration, storing them in airtight containers. Thoroughly reheating leftovers before consumption is also recommended as an additional precaution. Additionally, maintaining appropriate temperatures during both storage and serving, whether hot or cold, is vital to impede the growth and toxin production of *B. cereus*. Emphasizing the significance of prevention is crucial in the context of *B. cereus* in food, given the limited treatment options for contaminated food. Once toxins have been produced, their complete elimination through cooking or reheating may not be guaranteed (Schneider *et al*. 2017). Consequently, placing a strong emphasis on preventive measures during food preparation, storage, and handling is paramount to ensuring food safety and mitigating the risk of *B. cereus* related foodborne illnesses. Vital to the prevention of foodborne illnesses is the education of food handlers, consumers, and individuals in food service establishments about the risks linked to *B. cereus* contamination. Fostering awareness regarding appropriate food storage, handling, and cooking practices plays a crucial role in this preventive effort (Juneja *et al*. 2019).

For centuries, traditional food preservation techniques have remained integral to numerous cultures globally, enduring through time. These techniques hinge upon natural processes to preserve food products (Seervi *et al*. 2014). For example, dehydration, is a drying process that involves exposing food to hot air or sunlight to remove moisture, hindering enzymatic processes and microbial growth. Another method of drying involves extracting moisture from food using salt, creating an environment unfavourable for bacteria and fungi. Due to its affordability and diverse properties, salt is extensively utilized in the food industry, primarily for its ability to lower water activity levels, leading to preservative and antimicrobial effects. (Albarracín *et al*. 2011). Fermentation, on the other hand, enables beneficial microorganisms to transform sugars and starches into acids or alcohol, thereby preserving food while also potentially enriching its flavour profile. Additionally, sealing foods in airtight containers and heating them to eliminate pathogens and enzymes, thus extending shelf life, can be an effective way of food preservation. However, ensuring that the canning process transpires in a completely sterile environment is crucial to maintaining its efficacy. While traditional food preservation techniques have been employed for centuries, modern alternatives offer greater efficiency, requiring less time while yielding enhanced effectiveness (Farkas 2006).

Technological advancements have given rise to modern preservation techniques such as refrigeration, freezing, vacuum packaging, and irradiation. Currently, refrigeration, a prevalent method in households, involves storing food below 5°C to slow microbial growth and enzymatic reactions, thus maintaining freshness and quality for a specified duration (Granum & Lindbäck 2012). Another approach to food preservation is freezing, resembling the process of refrigeration but differing primarily in storage temperature. This method involves keeping food at sub-zero temperatures to halt microbial activity and effectively preserve its texture, flavour, and nutritional content (Zeuthen & Bøgh-Sørensen 2003). Another method, vacuum packaging, involves removing air from the package to create a vacuum that serves to inhibit oxidation and microbial contamination. For example, the volatile nature of EOs makes them well-suited for use in antibacterial packaging systems. Antibacterial films are commonly produced by incorporating compounds such as bacteriocins, enzymes, antioxidants and EOs into liquid solutions or by using tape casting technology (Chawla *et al*. 2021; Zeuthen & Bøgh-Sørensen 2003). This method enables controlled release of EOs over time and helps avoid any direct contact between EOs and food (Ju *et al*. 2019). In contrast, irradiation involves exposing food to ionizing radiation, such as X-rays generated by machinery sources and gamma rays emitted from radioisotopes like 60Co and 137Cs, or high-energy electrons. This process aims to eliminate bacteria, parasites, and insects, thereby extending shelf life and enhancing food safety (Farkas 2006). These methods are in the modern world essential for averting food spoilage, minimizing microbial contamination, and securing food supplies worldwide. While traditional and modern food preservation methods remain effective, contemporary demands require the development of even more efficient and superior techniques to supplant the commonly used methods (Seervi *et al.* 2014).

EOs have over time garnered attention among food preservation methods because of their innate antimicrobial properties (Vihanova *et al*. 2021). EOs can serve as natural preservatives with antimicrobial effects similar to conventional options, while possessing additional benefits such as safety, non-toxicity, and environmental friendliness. Utilizing

EOs in various food preservation methods, including packaging, coating, and immersion treatments, demonstrates effectiveness in prolonging the shelf life of perishable foods such as fruits, vegetables, meats, and dairy products (Li *et al*. 2022). By inhibiting microbial growth and slowing spoilage, EOs play a crucial role in preserving food quality. Furthermore, the antimicrobial properties of EOs operate through various pathways, including membrane disruption or interference with cell metabolism, (Bassolé & Juliani 2012). This multifaceted approach enables EOs to penetrate microbial biofilms, thereby enhancing their effectiveness against resilient pathogens such as *B. subtilis*, *B. anthracis* and *B. cereus* (Pandey *et al*. 2017; Reyes-Jurado *et al*. 2020). By integrating EOs into food preservation techniques, we can elevate food safety, diminish food waste, and fulfil the requirements of contemporary consumers for natural and sustainable food preservation alternatives.

Treatment for *B. cereus* infections varies depending on the symptoms and severity of the disease. While many cases of gastroenteritis associated with *B. cereus* resolve naturally without targeted treatment, more serious infections require medical intervention (Jessberger *et al*. 2020). Specialized care, known as supportive care, is tailored to relieve symptoms. In mild cases of gastroenteritis, supportive care favours maintaining hydration, often including oral rehydration solutions to prevent dehydration from vomiting and diarrhoea (McDowell *et al*. 2023). Serious systemic infections, such as bacteraemia, endocarditis, or central nervous system infection, usually require antibiotic treatment. Identifying a specific strain and understanding its antibiotic susceptibility pattern is essential to guide targeted treatment. In cases of more serious infections, such as endocarditis involving the heart valves, surgery involving procedures such as valve replacement may be required to address complications associated with the infection (Ngow & Khairina 2013).

Treatment of complications related to severe infections, such as sepsis or organ failure, is critical (Dabscheck *et al*. 2015). Systemic infections occur when *B. cereus* gains access to the bloodstream or other internal organs, leading to a wider and more severe impact on the body. Depending on the clinical presentation, this may include intensive care measures such as mechanical ventilation, vasopressor support, and renal replacement therapy. Although there is no specific antitoxin for *B. cereus* toxins, supportive measures and antitoxin therapy may be considered in severe cases (McDowell *et al*. 2023).

However, the effectiveness of antitoxin therapy is still a subject of research. It is important to note that prevention remains a key focus in the treatment of *B. cereus* infections. Early and appropriate medical care, especially for severe systemic infections, is essential to optimize patient outcomes (O'Day *et al*. 1981).

While a variety of methods and practices are presently employed to address pathogens like *B. cereus*, there is still a growing demand for a preventive approach that is natural and non-toxic.

2.2. Spice essential oils

EOs are natural plant products obtained from aromatic plants commonly located in areas spanning from temperate to tropical climates. They are defined as volatile, natural, complex mixtures of compounds characterized by a strong odour and formed by aromatic plants as secondary metabolites (Bakkali *et al*. 2008). SEOs, on the other hand, specifically denote EOs extracted from spices employed to flavour food and are mainly acquired through process of steam or hydro-distillation from various plant parts (Bassolé & Juliani 2012; Tisserand & Young 2013; Tongnuanchan & Benjakul 2014). The aromatic nature of SEOs is characterized by their distinctive fragrances, attributed to specific mixtures of compounds, which play a critical role in determining the unique aroma and therapeutic attributes. The compound mixtures within SEOs may exhibit antimicrobial properties, aiding in the inhibition of pathogenic bacteria, viruses, and fungi, suggesting their potential application in sanitation, strengthening of immune functions, and combating diseases (Oyen & Dung 1999; Selmi *et al*. 2022).

Throughout the history, EOs played a crucial role in traditional medicine, dating back to ancient civilizations, utilized for preventing as well as treating illnesses (Shaaban *et al*. 2012). In recent years, EOs have found extensive use across various sectors including aromatherapy, cosmetics, and perfume industries with the addition of pharmaceutical and agronomic industries which use them for their medicinal applications in human healthcare, which are attributed to their antibacterial, antiviral, antioxidant, anticarcinogenic, and pain-relieving properties (Baser & Buchbauer 2010; Selmi *et al*. 2022). While extensively utilized in medical practices across Asia, in Western medicine, they are primarily employed to enhance the palatability of medications and in household applications like liniments to alleviate muscle pain or disinfectants for cold relief (Bakkali *et al*. 2008; Kokoska 2003).

2.2.1. Botany

Until now, approximately 60 families of EO-producing plants have been identified, with an estimated number of over 3,000 EOs, of which approximately 300 are of commercial importance (Bassolé & Juliani 2012; Shaaban *et al.* 2012). The families of EO-producing plants that hold the most economic and financial significance include Apiaceae, Asteraceae, Geraniaceae, Lamiaceae, Lauraceae, Myristicaceae, Myrtaceae, Oleaceae, Pinaceae, Piperaceae, Poaceae, Rosaceae, Rutaceae, Santalaceae, and Zingiberaceae (Houdkova & Kokoska 2020; Oyen & Dung 1999; Rangan & Barceloux 2008). The composition of EOs can vary widely depending on several factors, including plant species, geographical location, climate, soil conditions, and extraction methods.

EOs are typically stored in specialized structures within plant tissues, such as oil cells (e.g., Lauraceae, Zingiberaceae), glandular trichomes (e.g., Labiateae), secretory canals (e.g., Compositae, Umbelliferae), and secretory cavities (e.g., Myrtaceae, Rutaceae) (Kokoska 2003; Oyen & Dung 1999) and can be found within various plant components such as flower buds (e.g., *Syzygium aromaticum*, *Rosa chinensis*), petals and flowers (e.g., *Chamaemelum nobile*, *Rosa damascena*), fruits (e.g., *Pimenta dioica*, *Piper nigrum*), seeds (e.g., *Myristica fragrans*, *Pimpinella anisum*), leaves (e.g., *Laurus nobilis*, *Mentha citrata*), bark (e.g., *Cinnamomum cassia*, *Cinnamomum zeylanicum*), wood (e.g., *Aniba rosaeodora*, *Cedrus atlantica*), and roots or rhizomes (e.g., *Acorus calamus*, *Zingiber officinale*) (Bassolé & Juliani 2012; Tisserand & Young 2013; Tongnuanchan & Benjakul 2014).

The exact functions of EOs are not entirely understood. EOs play roles in plant metabolism with some studies suggesting that many of these compounds are simply only byproducts of the plant biosynthesis. However, it is theorized that floral fragrances aid in natural selection by attracting specific insects, EOs found in leaves, wood, and roots may act as defence mechanisms against plant parasites or animal predators, and when a tree trunk is wounded, oleoresinous exudations can prevent cell sap loss and create a protective barrier against parasites and pathogens (Houdkova & Kokoska 2020; Oyen & Dung 1999).

2.2.2. Chemistry

EOs are soluble in alcohol, ether, and non-drying oils, but insoluble in water (Başer & Buchbauer 2010). They typically possess lower density than water and exhibit lipophilic properties allowing for their separation through decantation (Dhifi *et al*. 2016). The vapor pressure of EOs at room temperature and atmospheric pressure suggests that they exist partially in the vapor state. They also commonly possess a varied fragrance from subtle scent to intense fragrance and vary in colour from transparent and translucent to shades of yellow, ranging from bright to deep hues (Bakkali *et al.* 2008).

In the chemical compositions of EOs, a wide array of compounds from diverse sources is commonly present, typically containing around 20 to 60 components. Among the primary components found in EOs are benzenoids, terpenes, terpenoids and miscellaneous compounds, along with aliphatic and aromatic compounds (Houdkova & Kokoska 2020; Shaaban *et al.* 2012; Bassolé & Juliani 2012). It's typical for two or three major components to have higher concentrations (20%-70%) compared to the rest, while the remaining components are present in trace amounts (<0.1%) (Bakkali *et al*. 2008; Shaaban *et al.* 2012). Generally, these primary components largely influence the biological properties of EOs. Examples of components found at relatively high concentrations include limonene (43.5%) and carvone (32.6%) in *Carum carvi* EO, cinnamaldehyde (69.15%) and cinnamyl (E)-acetate (7.12%) in *C. cassia* EO or eugenol (48.67%) and β-pinene (18.52%) in *P. dioica* EO (ALrashidi *et al*. 2022; Jiang *et al*. 2011; Jose *et al*. 2019; Ju *et al.* 2018; Oyen & Dung 1999; Shaaban *et al*. 2012).

Terpenes, including monoterpenes, sesquiterpenes, and diterpenes, are the predominant hydrocarbons in EOs, comprised of multiple C_5 base units referred to as isoprene (Ni *et al*. 2021). Isoprene is one of the basic compounds in animal and plant biochemistry with carbon numbers ranging from C_{10} to C_{40}, contributing to its complexity as a compound (Oyen & Dung 1999). Monoterpenes (C_{10}) and sesquiterpenes (C_{15}) are the predominant constituents of EOs, while diterpenes are encountered infrequently (Finar 1994). Encompassing acyclic, monocyclic, and bicyclic forms, terpenes display a diverse array of compounds, embodying the predominant chemical functionalities found in organic materials. Monoterpenes can manifest as acyclic (geraniol, linalool), monocyclic (thymol, eugenol), and bicyclic (α-pinene, myrtenal). Sesquiterpenes, on the other hand, are compounds constructed from three isoprene units and, like monoterpenes, can occasionally adopt cyclic structures. Because of their abundance, sesquiterpenes are a category of terpenes characterized by a molecular structure that defies generalization (Kokoska 2003). However, some examples of tricyclic monoterpenes, like tricyclene and cyclofenchene, are also present (Bakkali *et al*. 2008; Zielińska-Błajet & Feder-Kubis 2020).

Aromatic compounds, on the other hand, derived from phenylpropane (C_6–C_3), are less prevalent when compared to terpenes, encompassing substances like anethole, estragole, eugenol, and vanillin, found notably in EOs of *P. anisum*, *Foeniculum vulgare*, *Petroselinum crispum*, *Ocimum basilicum*, *S. aromaticum*, and *Vanilla planifolia* among others (Ni *et al*. 2021). The main differences lie in varying numbers and positions of hydroxyl and methoxy groups (Sakkas & Papadopoulou 2017). Their assorted functionalities may be carried over during hydro-distillation such as acyclic esters like butyl acetate, prevalent in apples, isoamyl acetate, common in the fruits of *Musa* spp., heptane and paraffin in EO of *Matricaria* spp., and 1-octen-3-ol in EO of *Lavandula* spp. (Selmi *et al*. 2022).

Miscellaneous compounds refer to a diverse group of chemical substances that do not fit into specific categories like hydrocarbons, terpenes, or oxygenated compounds (Oyen & Dung 1999). In the context of EOs and natural products, miscellaneous compounds can include a wide range of molecules such as sulphur-containing compounds, nitrogen-containing compounds, heterocyclic compounds, and complex polyphenols. These compounds may contribute to the aroma, flavour, and therapeutic properties of EOs (Warburton 1984).

Aliphatic compounds are organic compounds characterized by straight or branched carbon chains. These chains can vary in length and may contain single (alkanes), double (alkenes), or triple (alkynes) bonds between carbon atoms (Başer & Buchbauer 2010). Aliphatic compounds are abundant in nature and have various applications in industries such as petrochemicals, pharmaceuticals, and agriculture. They are essential components of many everyday substances, including plastics, solvents, fuels and play significant roles in biological processes, serving as building material for lipids, amino acids, and carbohydrates. The odour of most aliphatic alcohols is weak and their role as components in fragrance compositions is limited (Oyen & Dung 1999).

Benzenoids, or benzene derivatives, are a class of organic compounds that contain one or more benzene rings with alternating single and double bonds in their chemical structure (Gutman & Cyvin 2012). This arrangement gives benzene and its derivatives unique stability and aromatic properties. Benzenoids are widely distributed in nature and are found in various plant species being important building materials in organic chemistry and used in the synthesis of numerous pharmaceuticals, fragrances, dyes, and other industrial chemicals. Common examples of benzenoids include benzene, toluene, xylene, phenol, aniline, and various substituted derivatives. These compounds exhibit diverse chemical and physical properties, making them valuable in a wide range of applications across different industries (Oyen & Dung 1999).

Extraction methods

The extraction methods play an essential part in defining the quality of EOs. Improper methods and processes can alter or degrade the chemical composition, compromising their natural and biological properties (Bakkali *et al*. 2008). In severe instances, this may lead to physical changes, flavour alteration, and discoloration underscoring the importance of employing suitable extraction methods to preserve the integrity and main aspects of EOs. The extraction of EOs typically relies on the characteristics of plant species and the hydrophilic or hydrophobic nature of the desired constituents. The quality of the product is influenced by several criteria, specifically internal factors such as type of the plant organ, age, phase of the vegetative cycle and external factors such as climate and soil composition (Juteau *et al*. 2003). The primary extraction methods employed include steam and hydro-distillation, expression, cold pressing, solvent extraction, and supercritical fluid extraction (Kokoska 2003, Reyes-Jurado *et al*. 2015). Steam distillation is probably the most common method used for extracting EOs from aromatic plants, where steam is passed through the plant material to vaporize the volatile compounds, which are then condensed and collected as EO. However, each extraction method yields varied EOs from identical plant material due to variations in the extraction efficiency of different components or alterations that certain components may undergo during extraction. While these differences are typically minor, they significantly influence the quality of the extracted EO (Oyen & Dung 1999).

2.2.3. Antimicrobial activity

In recent years, there has been a notable increase in studies focusing on biologically active compounds, driven by the the increasing resistance of microorganisms to antimicrobial agents (Ogwuche *et al*. 2014; Kang *et al*. 2018). The antimicrobial efficacy of EOs is typically assessed through minimum inhibitory concentration (MIC) values, which is defined as the lowest concentration of an EO that inhibits visible microbial growth under specific and well-defined conditions. The effectiveness of EOs when applied against microbes is significantly influenced by the quality and quantity of the EO and its constituent components (Reyes-Jurado *et al*. 2020). Furthermore, the prooxidant activity can enhance their cytotoxic capability, rendering them effective antiseptic and antimicrobial agents suitable for various personal applications, including air purification and personal hygiene, with the addition of insecticidal applications in crop and food stock preservation (Bakkali *et al*. 2008; Ni *et al*. 2021).

Examining the impact of EOs on combating food spoilage and pathogenic bacteria, including strains classified as gram-negative and gram-positive, has been a significant focus in recent years (El Atki *et al*. 2019; Oulkheir *et al*. 2017). Gram-positive bacteria typically exhibit cell walls composed of approximately 90% to 95% of peptidoglycans. Gram-negative bacteria on the other hand, possess more complex structures characterized by a thinner peptidoglycan layer and an additional outer membrane consisting of a double layer of phospholipids, which concludes that gram-positive bacteria are more susceptible to EOs than gram-negative bacteria, attributed to differences in their cell wall structures (Nazzaro *et al*. 2013). In certain research projects, EOs derived from spices such as *S. aromaticum*, *Coriandrum sativum*, *F. vulgare*, *M. fragrans*, *T. vulgaris*, and *Salvia rosmarinus* have exhibited potent antibacterial properties against pathogens including *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (El Atki *et al*. 2019; Oulkheir *et al*. 2017), with *S. aromaticum* EO proving to be the most efficient. Numerous studies have demonstrated the antibacterial properties of EOs against various bacterial strains, including *Salmonella typhimurium*, *Escherichia coli* or *B. cereus* (Ju *et al.* 2018; Shaaban *et al*. 2012). As scientific research continues to unravel their mysteries, EOs are poised to play an increasingly important role in promoting holistic health and vitality (Reyes-Jurado *et al*. 2020).

2.3. Antimicrobial activity in vapor phase

Antimicrobial agents are a vital element in combating microbial infections and preserving food quality. In the past, microbial control often depended on direct contact with antimicrobial agents. However, recent studies emphasize the efficacy of EOs in vapor phase, which can combat microorganisms without direct contact, highlighting the significance of the volatility of their chemical components for their effectiveness (Hammerbacher *et al*. 2019; Ju *et al*. 2019). In contrast to typical antimicrobial substances such as conventional antibiotics, EOs demonstrate quite unique physical and chemical characteristics. These properties of EOs, mainly limited water solubility and high volatility, significantly enhance their efficacy when deployed in the vapor phase (Reyes-Jurado *et al*. 2020; Selmi *et al*. 2022). Understanding their effectiveness can be explained by examining the bioactive compounds present in EOs as well as their volatility. Upon volatilization, these compounds disperse and exert antimicrobial effects without the need for direct contact. EO vapors disrupt microbial cell membranes, hamper essential enzyme activity, and induce oxidative stress, leading to microbial death (Kokoska 2003; Li *et al*. 2022).

Multiple factors influence the antimicrobial effectiveness of EOs in the vapor phase, including vapor pressure, chemical composition, humidity, temperature, and the concentration gradient between oil vapors and microbial organisms (Selmi *et al*. 2022). Microorganisms vary in their susceptibility, with some strains displaying heightened sensitivity to specific EO components such as terpenes or oxygenated compounds (Reyes-Jurado *et al*. 2020). The antimicrobial potential of EOs in the vapor phase extends across various applications. In healthcare settings, vapor-phase interventions offer promise for managing respiratory infections and controlling airborne pathogens (Mahmoudi *et al*. 2020). Similarly, within the food industry, vapor-phase treatments provide a non-contact approach to preserving food quality and potentially extending shelf life of food products. EOs, derived from aromatic plants, have long been recognized for their antimicrobial properties. While historically applied through direct contact methods, recent investigations have illuminated their potential in the vapor phase, offering a promising avenue for antimicrobial intervention (Reyes-Jurado *et al*. 2020).

2.3.1. The use in food preservation

Many food products require protection against microbial spoilage over their storage period. Given consumers' escalating preference for safe, chemical-free items, there has been extensive need to explore the viability of mild preservation techniques (Goñi *et al*. 2009). The main goal is to enhance the microbial quality and safety of products while preserving their nutritional and sensory characteristics. Thermal processing is a widely employed method in the food industry to eradicate microorganisms and extend the shelf life of food products. However, traditional thermal processing methods can lead to a reduction of certain bioactive compounds such as vitamin C and carotenoids (Rawson *et al*. 2011). Emerging nonthermal technologies like high hydrostatic pressure, ozone processing, and ultraviolet treatment offer promising avenues for preserving the nutritional and sensory qualities of food products. Dense phase carbon dioxide technology is typically utilized for liquid foods. This technology presents a significant drawback due to the potential tissue damage inflicted by the high pressure utilized during processing, rendering them unsuitable for sale (Aneja *et al*. 2016).

Packaging, on the other hand, serves as a shield against environmental factors and microbial contamination, ensuring the preservation of food quality and safety. Employing bioactive packaging such as antimicrobial atmosphere packaging helps to prevent food spoilage and contamination, which can have a substantial effect on public health, while also extending the shelf life of food products, especially those prone to microbial decay (Papadochristopoulos *et al*. 2021; Véronique 2008). Unlike conventional packaging, which primarily acts as a barrier to the exchange of air gases, moisture, and aromatic compounds between the food and its surroundings, bioactive packaging differs in incorporating antimicrobial activity in extending shelf life and thus ensuring food safety (Salmieri *et al*. 2014). Enhancing the safety and quality of packaged food through the integration of natural antimicrobial and antioxidant compounds is an active area of research. However, their direct incorporation into food packaging materials is hindered by their hydrophobic nature and low stability against environmental conditions during food processing, distribution, and storage. Moreover, the uncontrolled release of volatile active constituents from EOs can compromise their beneficial properties.

To address these challenges, various carriers and encapsulation techniques have been developed. The most commonly used encapsulation methods for EOs involve enclosing the volatile compounds within a protective matrix to enhance stability, control release, and facilitate various applications (Mukurumbira *et al*. 2022). These methods include techniques such as microencapsulation, which involves enclosing EOs within micro-sized particles, offering protection against environmental factors and controlled release, nanoencapsulation, which operates on a smaller scale, using nanoparticles to encapsulate EOs for improved bioavailability and targeted delivery, emulsification, which involves dispersing EOs in water or oil to create stable emulsions, suitable for various applications like food and cosmetics, and inclusion complexation, which utilizes carrier molecules to form complexes with EOs, enhancing their stability and solubility. These encapsulation methods play a vital role in extending the shelf life of EOs, enhancing their efficacy, and broadening their applicability in diverse industries (El Asbahani *et al*. 2015; Yousefi *et al*. 2023).

EOs and their active constituents play a crucial role in averting poisoning and illnesses, as well as mitigating bacterial dissemination within the food industry (Mahmoudi *et al*. 2020). These volatile mixtures of compounds as substitutes for chemical preservatives are gaining popularity because of their better tolerance in the human body, inherent superiorities for the food industry, and their ability to be applied in the vapor phase (Liu *et al*. 2017). The industrial use of EOs is often limited by their vulnerability to environmental factors like light, oxygen, and temperature, coupled with their low solubility in water, high volatility, and propensity to evaporate rapidly (Ju *et al*. 2019). However, utilizing the antimicrobial potential of vapor-phase activity presents a promising avenue for improving food preservation (Bakkali *et al*. 2008). By capitalizing on the antimicrobial capabilities of volatile compounds, food producers have the opportunity to devise innovative preservation methods that guarantee the safety, quality, and prolonged shelf life of food items, all while satisfying consumer's preferences for natural, non-toxic and maximally non-processed foods. However, the lack of laboratory methods suitable for evaluation of antimicrobial action in the vapor phase is an obstacle for the process of development of innovations for food preservation based on plant antimicrobial volatiles.

2.3.2. Methods of antimicrobial susceptibility testing in vapor phase

Antimicrobial susceptibility testing (AST) is a fundamental practice within clinical microbiology laboratories, essential for evaluating the effectiveness of antimicrobial agents against pathogenic microorganisms (Gajic *et al*. 2022). AST holds significant importance in microbiological analyses, providing vital data to guide treatment decisions and monitor antimicrobial resistance. Consequently, it aids in the judicious selection and application of suitable antibiotic therapy to address bacterial infections effectively (Pulido *et al*. 2013). Surveillance of antimicrobial resistance is based on routine clinical antimicrobial susceptibility data from microbiological laboratories collected by numerous global surveillance systems such as GLASS provided by the WHO and AR Lab Network of the Centers for Disease Control and Prevention (Iskandar *et al*. 2021). While national and international surveillance of resistance is beneficial for public health, the importance of understanding local resistance rates holds even greater significance. As a result, there is a consensus emerging regarding the necessity to create annual antibiograms customized for each hospital. This approach is especially critical for hospital departments with heightened resistance rates, such as intensive care units, and should be applied as soon as possible (Gajic *et al*. 2022).

Historically, multiple European national antimicrobial breakpoint Committees developed distinct AST protocols according to individual Committee's requirement (Gajic *et al*. 2022). Nowadays, this standardization is facilitated by internationally recognized guidelines for antimicrobial susceptibility testing, such as guidelines provided by the Clinical Laboratory Standards Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Numerous commercial vendors offer pre-prepared reagents tailored for some of these techniques in convenient ready-to-use formats aimed at laboratories handling significant workloads (Khan *et al*. 2019; Leclercq *et al*. 2013; Pulido *et al*. 2013). A multitude of techniques are employed in AST, with several commercially available methods dominating the field of testing. These include the antimicrobial gradient method such as the E-test (bioMérieux AB BIODISK, Marcy-l'Étoile, France), automated instrument systems such as MicroScan WalkAway system (Beckman Coulter Inc., California, USA) and BD Phoenix Automated Microbiology System (Becton Dickinson Diagnostic Systems, Pont de Claix, France), broth and agar dilution tests, and the disc diffusion test. (Leclercq *et al*. 2013; Reller *et al*. 2009).

However, when it comes to the vapor phase, alternative methods for AST must be employed, as the commonly used commercial methods lack the capability to evaluate samples in this phase.

Solid matrix volatilization methods

Disc volatilization assay is an improved method for the use in the vapor phase based on the standardised disc diffusion method and is also the most frequently used method to assess the antimicrobial effects of volatiles for testing in the vapor phase. For this method, sterile filter paper discs are saturated with the volatile compound at a desired concentration and placed onto the lid of petri-dishes containing solidified medium inoculated with the test-microorganism. The dishes are immediately inverted and sealed with parafilm or sterile tape to prevent the leakage of the vapor phase active compound. Incubation follows under appropriate conditions for the target microorganisms. Generally, the antimicrobial agents diffuse from the discs into the atmosphere within the petri dishes and subsequently into the agar, inhibiting the growth of the microorganisms. The diameter of the resulting inhibition zones is indicative of the antimicrobial activity, with interpretations based on predefined criteria: weak activity if the zone is ≤ 12 mm, moderate activity between 12 mm and 20 mm, and strong activity ≥ 20 mm (Houdkova & Kokoska 2020).

Another variation of the standardised disc diffusion method is the dressing model volatilization test. This method is a more specified alternation, which modifies the matrix from which the tested compound evaporates. This method involves placing a bacterial suspension on an agar plate covered with layers mimicking common materials used in treating skin infections, including those containing volatile antimicrobials. Following an incubation period, inhibition zones on the agar surface are measured. While this assay method effectively replicates the conditions of volatile antimicrobial application in medical settings, its limitation may lie in the potential interference of the tested agents with the dressing models (Edwards-Jones *et al*. 2004; Houdkova & Kokoska 2020).

Lastly, airtight apparatus disc volatilization method is a variation of the disc volatilization assay method improved by the utilization of an airtight box for the petri-dishes. The inner part of the box is shielded with aluminium foil to prevent absorption of volatile antimicrobials by plastic and safeguard the container walls from direct contamination. Paper discs soaked in volatile antimicrobial solutions are then positioned at the top of the airtight box, away from the petri dish containing the inoculated medium. The method typically employs 9 cm paper discs for a 1.3 L volume airtight box, although an alternative approach involves placing the pure volatile antimicrobial in a glass vessel inside the box. Following incubation under specified conditions, the antimicrobial potential can be evaluated. While this method offers the advantage of accommodating various inoculated materials and larger objects for surface decontamination assessment, conducting experiments with multiple boxes to assess the antimicrobial potential of essential oils across different concentrations necessitates ample space (Houdkova & Kokoska 2020; Inouye *et al*. 2006).

Liquid matrix volatilization methods

Broth microdilution volatilization method is a newly designed method by Houdkova *et al*. (2017) based on the broth microdilution and disc volatilization methods. The experimental setup utilizes standard 96-well microtiter plates with tight-fitting lids featuring flanges to minimize evaporation. Initially, agar is dispensed into each flange on the lid and inoculated with bacterial suspension once solidified. In the subsequent step, seven twofold serially diluted concentrations of volatile compounds are prepared on a microtiter plate and subsequently inoculated with bacterial suspensions. To secure the plate and lid together, stainless steel clamps are employed, along with handmade wooden pads for added stability. The microtiter plates are then incubated under specified conditions. MICs are determined through visual assessment of bacterial growth, with metabolically active bacterial colonies stained using MTT.

Microplate patch volatilization assay, originally named the vapour-phase-mediated patch assay (Feyaerts *et al*. 2017), is a method for the detection of the volatile agents' antimicrobial activity in the vapor phase. This method utilizes the U-shaped, 96-wells microtiter plates, where a patch is defined as the set of wells in an area (square) surrounding one or more test wells. Initially, microbial inoculum is dispensed into all wells, followed by the addition of the desired volume of the compound under investigation or its solution at the centre of a squared patch containing either 9 or 36 wells. Wells positioned outside of the patch function as internal negative controls. This well arrangement permits testing of only one or two samples per plate. Optionally, half of the patch and corresponding control wells can be sealed with a vapor barrier. Subsequently, the plate is covered with a lid and incubated under specified conditions. Evaluation of results involves optical density scanning of each well using a reader (Houdkova & Kokoska 2020).

In addition, agar plug-based vapour phase assay, developed by Amat *et al*. (2017), is a method providing qualitative as well as quantitative measurements on the activities of the volatile agents in the vapor phase. This method involves the use of two separate agar plates. Initially, the pathogen is inoculated onto the first plate and allowed to incubate for 1 hour. Subsequently, agar plugs with a diameter of 13 mm are extracted from this plate. The second plate is divided into four sections, each with portions of agar removed (10 mm in diameter), allowing for the insertion of sterile caps from 1.5 mL disposable or conical freestanding microtubes containing the volatile compounds. The agar plugs prepared from the first plate are then positioned atop these caps. Following a 24-hour incubation period, visual examination is conducted to assess bacterial growth. If a quantitative assessment of antibacterial activity is desired, agar plugs containing bacterial cells are immersed in broth for 10 minutes and subsequently plated on agar to assess cell viability and enumerate bacterial colonies in comparison with the growth control plate (Houdkova & Kokoska 2020).

Lastly, the airtight apparatus liquid volatilization method, described by Sekiyama *et al*. (1996) and originally named the vapour-agar contact method, entails placing an inoculated agar plate and a petri dish containing the volatile compound within a sealed container. After incubation under specified conditions, the inhibitory effect is assessed by measuring the diameter of colonies formed by the pathogenic strains. Moreover, Krumal *et al*. (2015) enhanced this approach by introducing an additional petri dish filled with a solution of distilled water and sodium chloride to maintain consistent relative humidity during the experiment (Houdkova & Kokoska 2020)

2.3.3. Plant-derived volatile antimicrobials

Volatile antimicrobials derived from plants (PDVAs) are abundant chemicals that are emitted by plants as an important factor, allowing for communication and interaction among plants, microorganisms, and the environment (Houdkova & Kokoska 2020). PDVA constitute a varied category of natural compounds showing potential for diverse applications across several industries such as healthcare and food preservation. PDVAs are attracting attention as sustainable alternatives to synthetic antimicrobial agents, given their natural origin and broad-spectrum activity against microbial pathogens. Various PDVAs including aldehydes, phenols, and terpenes demonstrate antimicrobial activity in the vapor phase by disrupting microbial cell membranes, inhibiting enzyme function, and interfering with microbial metabolic processes (Bakkali *et al*. 2008; Popović-Djordjević *et al*. 2019).

EOs stand out as key representatives among volatile agents with a wide range of biological effects, offering potential applications across human, animal, and plant health, as well as food quality and preservation (Houdkova & Kokoska 2020). Typically, these substances are housed within specialized plant secretory structures like glandular trichomes, secretory cavities, secretory idioblasts or resin ducts serving as intrinsic defences against threats and injuries (van Schie *et al*. 2006). Moreover, EOs consist of complex mixtures capable of producing synergistic antimicrobial effects, which could help address microbial resistance issues (Baser & Buchbauer 2010). However, it's important to consider potential respiratory, allergic, and immune-related effects associated with EO inhalation, particularly in infants and children. Therefore, conducting thorough safety evaluations of PDVAs is imperative before their practical implementation. Despite their historical medicinal use, regulatory bodies such as the Ph. Eur. (European Pharmacopoeia, Council of Europe, Strasbourg, France) and the USP-NF (United States Pharmacopeia-National Formulary, Frederick, USA) only acknowledge a limited selection of EO-bearing plants and their preparations as medicinal (Maffei *et al*. 2011). Moreover, the primary benefits of EOs include not requiring systemic application to the body or direct application to agricultural or food products, as well as their natural tendency to be consistently dispersed in the air of the intended environment (Houdkova & Kokoska 2020). However, to make further progress, it's essential to carefully select the appropriate EO-producing plants depending on the desired application and outcome.

2.4. Spices and their uses in foods

Spice plants, also known as culinary herbs and spices, are botanical species valued for their aromatic, flavourful, and sometimes medicinal properties. These plants have been an integral part of human civilization for thousands of years, used in cooking, medicine, religious rituals, and perfumery (Czarra 2009; De Guzman & Siemonsma 1999). Spice plants encompass a wide range of botanical families and species, each offering unique flavours and fragrances. Common spice plants include culinary herbs (e.g., *O. basilicum*, *Thymus vulgaris* and *Salvia rosmarinus)* as well as spice condiments (e.g., *Cinnamomum verum*, *S. aromaticum* and *Zingiber officinale)*. These plants are cultivated in various regions around the world, each contributing to the rich tapestry of global cuisines. In addition to their culinary uses, many spice plants have long been utilized for their medicinal properties thanks to their aromatic compounds, concentrated within EOs, responsible for the distinctive flavours and scents (Ertürk 2006).

Moreover, these plants have played significant roles in shaping human history and culture. They have been traded along ancient spice routes, leading to the exploration and colonization of new territories, and used in religious ceremonies and cultural traditions, symbolizing prosperity, healing, and spiritual purification. Today, spice plants continue to be essential ingredients in kitchens around the world, adding depth, complexity, and vibrancy to dishes. They are also valued for their health benefits and are increasingly being studied for their potential therapeutic applications. Scientists are exploring various aspects of spice plants to understand their potential benefits and optimize their utilization (Ceylan & Fung 2004; De Guzman & Siemonsma 1999). In recent years, several studies have highlighted the potential of EOs derived from various spice plants as antimicrobial agents for food preservation in the vapor phase. For instance, Shahbazi *et al*. (2018) demonstrated that the EO extracted from *Ziziphora clinopodioides* exhibited significant growth-inhibiting effects against bacterial pathogens Listeria monocytogenes and S. aureus when applied to refrigerated raw chicken meatballs as a food preservative. Similarly, Valkova *et al*. (2021) found that *Mentha* x *piperita* EO displayed strong growth-inhibiting activity against fungal pathogens *Penicillium* (*P.*) *expansum* and *P. crustosum* when applied to bread. These findings underscore the potential of spice plants as non-toxic and natural food preservatives, with the ability to be utilized in the vapor phase, offering promising prospects for food processing as well as food storage.

In culinary practice, spices are often incorporated into the foods not as individual components but rather as part of complex spice blends. In general, spice blends are combinations of various herbs, spices, and sometimes other flavouring agents, carefully curated to enhance the taste, aroma, and overall culinary experience of dishes. These blends often carry cultural significance and are integral to many cuisines around the world. They can vary in composition, reflecting the unique culinary traditions and preferences of different regions and cultures (Norman 2021). Some of the notable spice blends widely utilized in culinary contexts include Advieh, Baharat, Garam masala, Ras El Hanout, and Za'atar mixtures.

Advieh is a traditional Persian spice blend renowned for its rich and aromatic flavour profile. This blend is widely used in Persian cuisine to season a variety of dishes, including rice, stews, meats, and vegetable dishes (Abidin *et al*. 2020). The exact composition of Advieh can vary depending on regional preferences and individual recipes, but it typically includes a combination of ground spices and herbs such as *C. cassia*, *A. subulatum*, *S. aromaticum*, *Cuminum cyminum*, *M. fragrans*, *Z. officinale*, and various rose petals or rosebuds (Norman 2021). These spices are typically ground and blended in varying proportions to create the perfect balance of flavours. Advieh is often used as a seasoning for rice dishes, or to be used to flavour stews, grilled meats, kebabs, and vegetable dishes. Overall, Advieh is a versatile spice blend that adds depth, warmth, and complexity to Persian cuisine, making it a cherished ingredient in kitchens across the region and beyond (Abidin *et al*. 2020).

Similarly, Baharat is a versatile spice blend that is widely used in Middle Eastern and Mediterranean cuisines, particularly in countries like Turkey, Lebanon, Syria, and Israel (Macit *et al*. 2017). This aromatic blend typically features a combination of ground spices and herbs, creating a flavour profile that is bold, complex, and warmly spiced. While the exact composition of Baharat can vary from one recipe to another and from one region to another, common ingredients found in this blend include *C. cassia*, *C. cyminum*, *C. sativum*, *Capsicum annuum*, *M. fragrans*, *P. dioica*, *P. nigrum*, and *S. aromaticum* (Norman 2021). Baharat is incredibly versatile and can be used to season a wide variety of dishes, including meats, poultry, fish, vegetables, soups, stews, and rice dishes. Baharat is often used as a dry rub for grilling or roasting meats, as a seasoning for rice pilaffs or couscous, or as a flavouring agent in marinades, sauces, and dips (Macit *et al*. 2017).

Garam masala, on the other hand, is a spice blend that is widely used in Indian cuisine. This aromatic blend typically consists of a combination of ground spices. While the exact composition of garam masala can vary from one recipe to another and from one region to another, common ingredients found in this blend include *A. subulatum*, *C. cassia*, *C. cyminum*, *C. sativum*, *L. nobilis*, *M. fragrans*, *P. nigrum*, and *S. aromaticum* (Norman 2021). Garam masala is incredibly versatile and can be used to season a wide variety of dishes, including curries, stews, rice dishes, soups, and grilled meats. It adds depth, warmth, and complexity to dishes, imparting its rich aroma and flavour to every bite. Garam masala is often added towards the end of cooking or sprinkled on top of dishes as a finishing touch to preserve its delicate flavours (Vasavada *et al*. 2006).

In contrast, Ras El Hanout is a highly aromatic and complex spice blend that originates from North Africa, particularly Morocco. It is often considered the crown jewel of Moroccan spice blends and is prized for its rich and vibrant flavours (Djenane *et al*. 2023). The exact composition of Ras El Hanout can vary widely depending on the region, the spice merchant, and individual preferences, but it typically includes a combination of more than a dozen different spices and herbs such as *A. subulatum*, *C. cassia*, *C. cyminum*, *C. longa*, *C. sativum*, *C. annuum*, *M. fragrans*, *P. dioica*, *P. nigrum*, *S. aromaticum*, *Z. officinale* and rose petals or rosebuds (Norman 2021). These are just a few examples of the many spices and herbs that can be included in Ras El Hanout. This spice blend is incredibly versatile and can be used to season a wide variety of dishes, including tagines, couscous, grilled meats, roasted vegetables, and soups. It adds depth, complexity, and an exotic aroma to dishes, elevating them to new heights of flavour (Djenane *et al*. 2023).

Furthermore, Za'atar is a popular Middle Eastern spice blend that is beloved for its aromatic flavour and versatility in culinary applications. Za'atar spice blend typically consists of a combination of dried herbs, seeds, and spices, and its exact composition can vary depending on regional variations and personal preferences. While the exact recipe for za'atar may vary, the blend often includes the *Rhus coriaria*, *S. indicum*, and *T. vulgaris* (Norman 2021). Sometimes, salt can be added to the blend. Za'atar is incredibly versatile and can be used in a variety of ways in cooking. It is commonly sprinkled over flatbreads, such as pita or mana'eesh, before baking, giving them a flavourful and aromatic crust. Za'atar can also be mixed with olive oil to create a dipping sauce for bread or used as a seasoning for roasted vegetables, meats, or fish. In addition, it can be sprinkled over salads, hummus, or yogurt for added flavour and texture. Beyond its culinary uses, za'atar is also believed to have health benefits and is sometimes used in traditional medicine for its purported medicinal properties. It is rich in antioxidants and is believed to have anti-inflammatory and antimicrobial properties (Khalil *et al*. 2022).

In this study, 12 EO-producing spice plants from various families (Apiaceae, Lamiaceae, Lauraceae, Myristicaceae, Myrtaceae, Pedaliaceae, Piperaceae, Rosaceae and Zingiberaceae) were chosen based on their use in culinary applications with special emphasis on species used in in spice blends.

Amomum subulatum **Roxb.**

A. subulatum, commonly known as black cardamom, is a perennial herbaceous plant native to the eastern Himalayas and neighbouring regions of Southeast Asia belonging to the family Zingiberaceae and found in the Advieh, Bahrat, Garam masala and Ras El Hanout spice blends. It is renowned for its distinctively smoky flavour and aroma, which sets it apart from the more commonly used *Elettaria cardamomum*. The plant features tall, reed-like stems, long lanceolate leaves, and small pale-yellow flowers with purple veins. It typically grows in humid forest understories and is cultivated for its aromatic seeds, which are used extensively as a spice in various culinary dishes, particularly in South Asian cuisine (Satyal *et al*. 2012).

The EO, commonly obtained through steam-distillation or solvent extraction from seed pods, possesses a rich and complex fragrance characterized by earthy, woody, and slightly spicy notes. It is highly valued for its aromatic qualities utilized as a natural fragrance in perfumes, cosmetics, and personal care products. The EO is also known for its potential therapeutic properties to treat various health related problems, including digestive and respiratory tract issues (Jafri *et al*. 2001). In addition, some studies have demonstrated the growth-inhibiting efficacy of *A. subulatum* EO against various bacterial and fungal pathogens (Gilani *et al*. 2006; Joshi *et al*. 2013). The broad-spectrum antimicrobial properties of *A. subulatum* EO render it a valuable natural remedy for combating microbial infections, prompting further investigation into its therapeutic applications in healthcare, food preservation, and other industries (Sabulal & Baby 2021).

***Carum carvi* L.**

C. carvi, commonly known as caraway, is a biennial herbaceous plant native to Europe, Western Asia, and North Africa belonging to the family Apiaceae and commonly found in the Ras El Hanout spice blend. The plant typically grows in sunny locations with well-drained soil and features feathery leaves, small white or pink flowers and crescent-shaped seeds which are widely used as a spice (Agrahari & Singh 2014).

The EO extracted from the fruit of *C. carvi* is highly valued for its distinctive aroma, which is warm, spicy, and slightly sweet. It is also renowned for its digestive benefits and is commonly used to alleviate symptoms of indigestion, bloating, and flatulence (De Guzman & Siemonsma 1999). In addition to its digestive properties, the EO exhibits antimicrobial activity against a range of bacteria and fungi (Sachan *et al*. 2016). Its antimicrobial effects are attributed to compounds like carvone and limonene, which have been shown to inhibit the growth of various pathogenic microorganisms (Gupta *et al*. 2011). Furthermore, the EO can be utilized in natural skincare and haircare products for its antiseptic and cleansing properties. Overall, *C. carvi* EO offers a range of health benefits and is prized for its versatility in culinary, medicinal, and aromatic applications (Sachan *et al*. 2016).

***Cinnamomum cassia* (L.) J. Presl**

C. cassia, also known as the Chinese cinnamon, is an evergreen tree native to southern China belonging to the family Lauraceae and found in Advieh, Garam masala, and Ras El Hanout spice blends. This tree exhibits glossy green leaves, clusters of small, yellow flowers and bark that are commonly employed in Asian cuisine as a spice (Trinh *et al*. 2015; Zhang *et al*. 2019). Native to southern China, *C. cassia* is widely cultivated in other parts of Asia, including Vietnam, Indonesia, and India (Firmino *et al*. 2018). *C. cassia* has been used for centuries in traditional Chinese medicine to treat various ailments, including digestive issues, colds, and menstrual cramps (Huang *et al*. 2014, Jose *et al*. 2019).

The EO from *C. cassia*, mainly extracted from the bark, is valued for its aromatic and therapeutic properties. It contains compounds such as cinnamaldehyde, eugenol and coumarin, which are responsible for its characteristic aroma and is believed to have antimicrobial and antifungal properties (Alam *et al*. 2023). Overall, *C. cassia* bark EO has shown promising potential in antimicrobial efficacy against a wide range of pathogens, making it a potential candidate for use in various applications such as food preservation, pharmaceuticals, and environmental sanitation (Vihanova *et al*. 2021; Zhang *et al*. 2019).

***Curcuma longa* L.**

C. longa, commonly known as turmeric, is a perennial flowering plant native to South Asia belonging to the ginger family, Zingiberaceae found in Advieh, Baharat and Ras El Hanout spice blends. Turmeric grows large, bright green leaves and funnel-shaped yellow flowers. The rhizomes of the plant are harvested, boiled, dried, and grinded to produce the vibrant yellow-orange powder known as turmeric used in medicinal and culinary applications such as to flavour curries, soups, and stews (Verma *et al*. 2018).

Derived from the rhizomes of the plant, the EO is esteemed for its aromatic and therapeutic attributes, including anti-inflammatory, antioxidant, and antimicrobial effects. Its unique aroma and potential antimicrobial efficacy are attributed to constituents such as curcumin and turmerone (Hwang *et al*. 2016). Some studies have demonstrated that the EO exhibits broad-spectrum antimicrobial activity against bacteria, as well as fungi and viruses (Antunes *et al*. 2012; Naz *et al*. 2010). Overall, *C. longa* EO has shown promising antimicrobial efficacy against a wide range of pathogens, making it a potential candidate for use in various applications such as food preservation, pharmaceuticals, and environmental sanitation.

***Laurus nobilis* L.**

L. nobilis, commonly known as bay laurel, is an aromatic evergreen tree native to the Mediterranean region belonging to the family Lauraceae typically found in the Garam masala spice blend. The tree has dark green, glossy leaves that are lance-shaped with smooth edges. The leaves are commonly used dried as a spice, adding a distinctive flavour to soups, stews, and sauces imparting a subtle, slightly bitter taste (Caputo *et al*. 2017; Paparella *et al*. 2022). In addition to its culinary uses, *L. nobilis* has a long history of symbolic and medicinal significance. In ancient Greece and Rome, bay leaves were used to crown victorious athletes, scholars, and poets, symbolizing honour, and achievement. They were also believed to have protective properties and were often hung in homes to ward off evil spirits (Anzano *et al*. 2022).

Extracted primarily from its leaves, the EO is rich in compounds like eugenol, cineole, and linalool, contributing to its aromatic and therapeutic properties (Sırıken *et al*. 2018). The EO offers antioxidant and anti-inflammatory effects, further enhancing its therapeutic potential. Known for its calming and uplifting effects in aromatherapy, this EO is also prized in natural skincare and hair care for its soothing and cleansing benefits (Caputo *et al*. 2017). Multiple studies indicate its potent antimicrobial activity against bacteria, fungi, and viruses, making it a valuable ally in combatting infections and promoting overall well-being (Nabila *et al*. 2022; Sırıken *et al*. 2018).

***Myristica fragrans* Houtt.**

M. fragrans, commonly known as nutmeg, is an evergreen tree native to Indonesia belonging to the family Myristicaceae found in various spice blends such as Baharat, Garam masala and Ras El Hanout. The tree produces small, yellow-white flowers that give way to round, fleshy fruits known as drupes containing the nutmeg seed. The nutmeg seed is encased in a hard, brown shell surrounded by a red, lacy covering called mace (Jaiswal *et al*. 2009). Both nutmeg and mace are widely used as spices to flavour a variety of dishes, including baked goods, custards, sauces, soups, and beverages such as eggnog and mulled wine (Ogunwande *et al*. 2003). Aside from its culinary uses, nutmeg has a long history of medicinal and therapeutic applications. For example, in traditional herbal medicine, it has been used to alleviate digestive issues, promote sleep, relieve pain, and stimulate circulation (De Guzman & Siemonsma 1999).

The EO, extracted from the seed through steam distillation, is used in aromatherapy for its calming, sedative, and analgesic properties (Jaiswal *et al*. 2009). Certain studies have indicated that nutmeg EO exhibits inhibitory effects against a wide range of bacterial and fungal pathogens (Nikolic *et al*. 2021). The antimicrobial activity of the EO is attributed to its chemical composition, which includes compounds such as myristicin, elemicin, and safrole. These bioactive compounds are believed to disrupt the cell membranes of microorganisms, inhibit enzyme activity, and interfere with microbial growth processes, ultimately leading to the inhibition of bacterial and fungal growth (Ogunwande *et al*. 2003). However, it's worth noting that nutmeg EO contains compounds such as myristicin, which in large quantities can be toxic and even psychoactive. Consuming excessive amounts of nutmeg may lead to adverse effects such as hallucinations, nausea, dizziness, and rapid heartbeat. Therefore, it should be used in moderation and with caution (Omoruyi & Emefo 2012; Rahman *et al*. 2015).

***Pimenta dioica* (L.) Merr.**

P. dioica, commonly known as allspice, is an evergreen tree native to the Caribbean islands and Central America belonging to the Myrtaceae family and commonly found in Baharat and Ras El Hanout spice blends. The tree produces small, white flowers that develop into round, green berries, which turn dark brown when ripe. The berries contain seeds that emit a fragrance reminiscent of a blend of spices, including cloves, cinnamon, and nutmeg, hence the name allspice (Rao *et al*. 2012). In addition, *P. dioica* has a long history of medicinal and therapeutic applications. For example, in traditional herbal medicine, it has been used to alleviate digestive issues, soothe muscle aches and pains, and promote overall well-being (ALrashidi *et al*. 2022).

The EO, extracted from the berries through steam distillation, is highly valued in aromatherapy and is also used in various non-culinary products, including perfumes, soaps, and cosmetics, for its aromatic fragrance and potential antimicrobial effect (Zhang & Lokeshwar 2012). Some studies have indicated that the EO possesses antibacterial and antifungal properties (Zabka *et al*. 2009). The antimicrobial activity of allspice EO is attributed to its chemical composition, which includes compounds such as eugenol, methyl eugenol, and myrcene (De Guzman & Siemonsma 1999). These bioactive compounds are believed to disrupt the cell membranes of microorganisms, inhibit enzyme activity, and interfere with microbial growth processes, ultimately leading to the inhibition of bacterial and fungal growth. Overall, the antimicrobial properties of *P. dioica* EO suggest its potential use in healthcare, personal hygiene products, and food preservation as a natural alternative to synthetic antimicrobial agents (Jarquín-Enríquez *et al*. 2021).

***Piper nigrum* L.**

P. nigrum, commonly known as black pepper, is a flowering vine native to South India belonging to the Piperaceae family and frequently found in Baharat, Garam masala and Ras El Hanout spice blends. The vines of *P. nigrum* feature heart-shaped leaves and small, spherical fruits widely cultivated as a spice known as peppercorns. These peppercorns are initially green when immature, turning black when dried (Meghwal & Goswami 2013). For centuries, *P. nigrum* has been prized for its culinary properties. It has a sharp, spicy taste with earthy undertones, making it a popular seasoning in a wide range of dishes, including meats, soups, stews, sauces, and marinades. In addition to its culinary uses, *P. nigrum* has a long history of medicinal applications. For example, in traditional medicine such as Ayurveda, it is believed to aid digestion, improve appetite, and alleviate gastrointestinal discomfort. Beyond its therapeutic uses, *P. nigrum* has also found applications in various production processes such in the production of perfumes, cosmetics, and personal care products (Feitosa *et al*. 2024).

The EO, extracted from the dried berries commonly through steam distillation, is used in aromatherapy for its invigorating and stimulating aroma. Findings from various studies indicate that black pepper EO exhibits significant antibacterial and antifungal effects (Karsha & Lakshmi 2010; Zhang *et al*. 2021). The antimicrobial efficacy of *P. nigrum* EO is mainly attributed to its chemical composition, which comprises bioactive compounds such as piperine, limonene, β-caryophyllene, and α-pinene. The antimicrobial effects of the EO suggest its possible applications in healthcare, personal hygiene products, and food preservation, providing a natural substitute for synthetic antimicrobial agents (Murbach Teles Andrade *et al*. 2014).

***Rosa x damascena* Herrm. 'Amadeus'**

R. x damascena 'Amadeus', a cultivar of the *R. x damascena* commonly known as the Damask rose, is a hybrid deciduous rose shrub that is prized for its fragrant flowers and EO. The rose petals can be found in the spice blends Advieh and Ras El Hanout. It is believed to be a hybrid between *Rosa gallica* and *Rosa moschata*, originating in the Middle East (Elhawary *et al*. 2021). It produces distinctive pink flowers with multiple layers of petals which are mostly harvested for their EO.

The EO is extracted through steam distillation and contains a complex mixture of volatile compounds, including citronellol, geraniol, nerol, and various other terpenes and phenolic compounds (Loghmani-Khouzani 2007). This EO is highly prized in the perfume industry for its rich, floral aroma and is often used as a base note in perfumes and colognes. In addition to its aromatic properties, EO of *R. x damascena* is also believed to have various therapeutic benefits. The EO is commonly used in aromatherapy for its calming and mood-enhancing effects, and it is often incorporated into skincare products for its hydrating and rejuvenating properties. Some studies suggest that the EO may also have antioxidant, anti-inflammatory, and antimicrobial properties, although further research is needed to confirm these potential health benefits (Safaei-Ghomi *et al*. 2009).

***Sesamum indicum* L.**

S. indicum, commonly known as sesame, is an annual flowering plant native to sub-Saharan Africa and the Indian subcontinent belonging to the family Pedaliaceae and typically found in the Za'atar spice blend. This plant features lanceolate leaves and produces tubular pink, purple or blue flowers which develop into small, oblong-shaped seeds within capsules. The seeds are renowned for their rich nutty flavour and high oil content, making them a popular cooking ingredient. They are commonly used as a topping for bread, pastries, and confections, as well as in salads, stir-fries, and sauces (Meghwal & Goswami 2013; Zech-Matterne *et al*. 2015). In addition to their culinary uses, sesame seeds are also pressed to extract sesame oil, which is used in cooking, as a flavouring agent, and in various cosmetic and medicinal products.

Nutritionally, *S. indicum* seeds contain significant amounts of calcium, magnesium, phosphorus, iron, and zinc, as well as antioxidants such as sesamin and sesamolin. In traditional medicine systems such as Ayurveda and traditional Chinese medicine, *S. indicum* seeds have been used for their purported medicinal properties. They are believed to support cardiovascular health, promote digestive function, and nourish the skin and hair (Zech-Matterne *et al*. 2015). Beyond its culinary and medicinal uses, *S. indicum* has various industrial applications such as in the production of soaps, cosmetics, and pharmaceuticals, as well as in the manufacturing of paints, lubricants, and biofuels (Mili *et al*. 2021). While current studies have not yet evaluated the antimicrobial effects of *S. indicum* EO, future research may be able to uncover different findings.

***Syzygium aromaticum* (L.) Perr. & L.M.Perry**

S. aromaticum, or clove, is an evergreen tree native to Indonesia belonging to the family Myrtaceae found in many spice blends such as Advieh, Baharat, Garam masala and Ras El Hanout. The tree bears grayish-brown bark, glossy green leaves, and clusters of pink flowers, which give way to aromatic flower buds that are later harvested and dried to produce the spice known as clove (Mittal *et al*. 2014). Clove buds are characterized by their strong aromatic flavour and contain volatile compounds such as eugenol, which is responsible for their distinctive aroma and flavour. Clove is a popular ingredient in spice blends, such as garam masala and pumpkin pie spice (Barakat 2014). In addition to their culinary uses, cloves have a long history of medicinal use. For example, in traditional medicine systems such as Ayurveda, traditional Chinese medicine, and herbalism it is commonly used to alleviate toothaches, sore throats, respiratory infections, digestive issues, and nausea. Beyond the culinary and medicinal uses, *S. aromaticum* is also widely used in the production of perfumes, cosmetics, and dental products, as well as in the manufacturing for cigarettes (Haro-González *et al*. 2021).

The EO, obtained using steam distillation of clove buds, is used in aromatherapy blends, massage oils, and personal care products. It exhibits potent antimicrobial activity thanks to its main constituent, eugenol, which is responsible for its strong antimicrobial properties against a wide range of bacteria, fungi, and viruses (De Guzman & Siemonsma 1999; Pinto *et al*. 2009). Due to its significant antimicrobial efficacy, *S. aromaticum* EO has potential applications in healthcare, personal hygiene products, and food preservation as a natural alternative to synthetic antimicrobial preservatives (Ayoola *et al*. 2008; Behbahani *et al*. 2019).

***Thymus vulgaris* L.**

Lastly, *T. vulgaris*, commonly known as thyme, is a perennial herbaceous plant native to the Mediterranean region belonging to the family Lamiaceae commonly found in the Za'atar spice blend. The plant typically grows low to the ground, forming dense mats of small, aromatic leaves elliptical to lanceolate in shape, typically green grey in colour, which have a distinct aromatic fragrance when crushed. Thyme is widely used as a culinary herb to flavour a variety of dishes, including soups, meats, and vegetables (Satyal *et al*. 2016). In culinary applications, thyme is prized for its strong flavour and aroma. It is a staple herb in Mediterranean cuisine and is commonly used to season soups, stews, sauces, marinades, meats, vegetables, and breads. Throughout centuries, *T. vulgaris* was valued for its antiseptic, antimicrobial, and expectorant properties, making it a popular remedy for respiratory ailments such as coughs, colds, bronchitis, and sore throats with thyme tea being a common home remedy for respiratory congestion and infections (Oulkheir *et al*. 2017).

Furthermore, *T. vulgaris* EO, extracted from the leaves, is used in aromatherapy and natural medicine for its therapeutic benefits. It is believed to have antimicrobial, antifungal, and anti-inflammatory properties, and is used to treat skin conditions such as acne (Satyal *et al*. 2016). The EO’s antimicrobial effects are primarily attributed to its main bioactive constituents, such as thymol and carvacrol (Fani & Kohanteb 2017). Its antimicrobial activity is believed to disrupt microbial cell membranes, interfere with enzyme function, and inhibit microbial growth processes. *T. vulgaris* EO has strong antimicrobial properties, making it a popular choice in healthcare, natural remedies, and food preservation, serving as a natural substitute for synthetic antimicrobial agents (Galovicova *et al*. 2021).

Although the general antimicrobial properties of above-mentioned spice EOs are well described in the literature (De Guzman & Siemonsma 1999; Oyen & Dung 1999), the data on their inhibitory effects against *B, cereus* in vapour phase are missing.

3. Aims of the Thesis

The main objective of the thesis was to evaluate the *in vitro* growth-inhibitory activity of EOs hydro-distilled from plant species used as spices and food condiments against *B. cereus* using the broth microdilution volatilization method in liquid and vapor phase.

4. Material and Methods

4.1. Plant material

Twelve spice plants, namely *Amomum subulatum, Carum carvi, Cinnamomum cassia, Curcuma longa, Laurus nobilis, Myristica fragrans, Pimenta dioica*, *Piper nigrum*, *Rosa x damascena* 'Amadeus', *Sesamum indicum*, *Syzygium aromaticum* and *Thymus vulgaris*, were chosen based on their traditional culinary and food processing application, with special emphasis on species used in traditional spice mixtures. The plant material was purchased from a local spice store (U Salvatora, Prague, CZ). The plant botanical name, family, common name, plant part, moisture content, yield, and colour of the extracted EOs were determined. A comprehensive overview of the collected plant samples is provided in Table 1.

Table 1. Botanical data and physical characteristics of spice essential oils

Botanical name	Family	Common name	Plant Part	Moisture content (%)	Yield % (v/w)	Colour
Amomum subulatum Roxb.	Zingiberaceae	Black cardamom	Seed pod	14.49±0.72	1.884	Transparent
Carum carvi L.	Apiaceae	Caraway	Fruit	10.85±0.58	2.867	Transparent
Cinnamomum cassia (L.) J. Presl	Lauraceae	Chinese cassia	Bark	16.06±1.05	1.276	Yellow
Curcuma longa L.	Zingiberaceae	Turmeric	Rhizome	12.66±0.66	0.728	Pale yellow
Laurus nobilis L.	Lauraceae	Bay laurel	Leaf	13.55±0.25	0.984	Yellow
Myristica fragrans Houtt.	Myristicaceae	Nutmeg	Seed	11.6±0.46	4.063	Yellow
Pimenta dioica (L.) Merr.	Myrtaceae	Allspice	Fruit	13.74±0.84	1.074	Transparent
Piper nigrum L.	Piperaceae	Black pepper	Fruit	13.17±1.17	2.473	Transparent
Rosa x damascena Herrm. 'Amadeus'	Rosaceae	Damask rose	Petal	17.14±4.54	0.0135	Yellow
Sesamum indicum L.	Pedaliaceae	Sesame	Seed	6.15±0.31	0.058	Transparent
Syzygium aromaticum (L.) Perr. & L.M.Perry	Myrtaceae	Clove	Flower bud	27±1.6	2.25	Transparent
Thymus vulgaris L.	Lamiaceae	Common thyme	Leaf	14.55±0.74	0.381	Yellow

4.2. Distillation of spice essential oils

The dried plant material (30 g for *C. carvi*, *M. fragrans*, *P. nigrum* and *S. aromaticum*; 50 g for *A. subulatum*, *C. cassia*, *C. longa*, *L. nobilis*, *P. dioica* and *S. indicum*; 400 g for *R. x damascena* ‘Amadeus’ and *T. vulgaris*) was grounded into powder using a Grindomix apparatus (GM100 Retsch, Haan, Germany). Each sample was mixed with 1 L of distilled water and subjected to hydro-distillation for 3 h using Clevenger-type apparatus (Merci, Prague, Czech Republic) (Figure 3). Following distillation, the EOs (Figure 4) were collected and stored in sealed glass vials at 4°C.

4.3. Moisture determination

The moisture content of each grinded plant sample (1 g) was measured using a moisture analyser (SMO 01, Scaltec Instruments, Germany). This assessment was conducted three times, and the average values were subsequently utilized to calculate on dry matter content.

4.4. Media and microorganisms

The bacterial standard strains used in this study were *B. cereus* ATCC 11778 obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and *B. cereus* CCM 2010 obtained from the German collection of Microorganisms and Cell Cultures (CCM, Leibniz Institute, Braunschweig, Germany). Bacterial strains from stock cultures were grown in broth medium at 25°C for 24 hours before testing. Both the cultivation and assay medium (broth/agar) used were Mueller–Hinton (MH). The broth's pH was adjusted to 7.6 using Trizma base (Sigma-Aldrich, Praha, Czech Republic). The microbial strains and cultivation media were procured from Oxoid (Basingstoke, UK). The bacterial suspension's turbidity was adjusted to 0.5 McFarland standard using a Densi-LaMeter II (Lachema, Brno, Czech Republic) to prepare the inoculum, aiming for a 10^8 CFU/mL concentration.

4.5. Chemicals

The chemicals used for the positive antibiotic control were chloramphenicol (98%, CAS 56-75-7) diluted in ethanol (96%, CAS 64-17-5). Other chemicals utilized in the antimicrobial susceptibility testing included: dimethyl sulfoxide – DMSO (Penta, Prague, Czech Republic), distilled water, Mueller-Hinton (MH) agar and MH broth (Oxoid, Basingstoke, Hampshire, UK), Trizma base (Sigma-Aldrich, Prague, Czech Republic), potassium chloride - KCl (Sigma-Aldrich, Prague, Czech Republic), sodium chloride - NaCl (Sigma-Aldrich, Prague, Czech Republic) and thiazolyl blue tetrazolium bromide dye - MTT (Sigma-Aldrich, Prague, Czech Republic).

4.6. Antimicrobial assay

The antimicrobial effectiveness of SEOs in the vapor phase was examined using a broth microdilution volatilization method (Houdkova *et al*. 2017) (Figure 1; 2). In the assay, 96-well microtiter plates with a tightly fitting lid featuring flanges (SPL Life Sciences, Naechon-Myeon, Republic of Korea) were used. Initially, all wells, excluding the outermost ones, were filled with MH buffer broth (100 μL). Subsequently, each EO sample was dissolved in DMSO at a maximum concentration of 1% and diluted in MH buffer broth medium. Serial dilutions ranging from 16 to 1024 μg/mL were prepared for all EOs. Bacterial suspensions (10^8 CFU/mL) were then inoculated into the microtiter plates. Following this, 30 μL of agar was deposited into each flange of the lid, except for the outermost ones, and inoculated with 5 μL of bacterial suspension once solidified. The outermost wells and flanges were left vacant to minimize edge effects. The plates were fastened together with stainless steel clamps (Lux Tool, Prague, CZ) and wooden pads for additional stability and safety (Figure 5). Incubation of the closed microtiter plates was carried out at 25°C for 24 hours. Subsequently, all wells and flanges were coloured using MTT (25 μL) at a concentration of 600 μg/mL to visualize metabolically active bacterial colonies. MICs were determined by visually examining bacterial growth post-incubation (30 minutes minimum), with varying colours indicating microbial activity, ranging from yellow (indicating none or minimal activity) to deep purple (indicating significant activity) in both broth (Figure 6) and agar (Figure 7). The MIC values, reflecting the minimal concentrations that inhibited bacterial growth in comparison to the compound-free control, were expressed in µg/mL. Each experiment was replicated three times independently, with the results reported as median and modal MIC values. According to the widely accepted norm in MIC testing, the mode and median were used for the final value calculation when the triplicate endpoints were within the two- and three-dilution range, respectively.

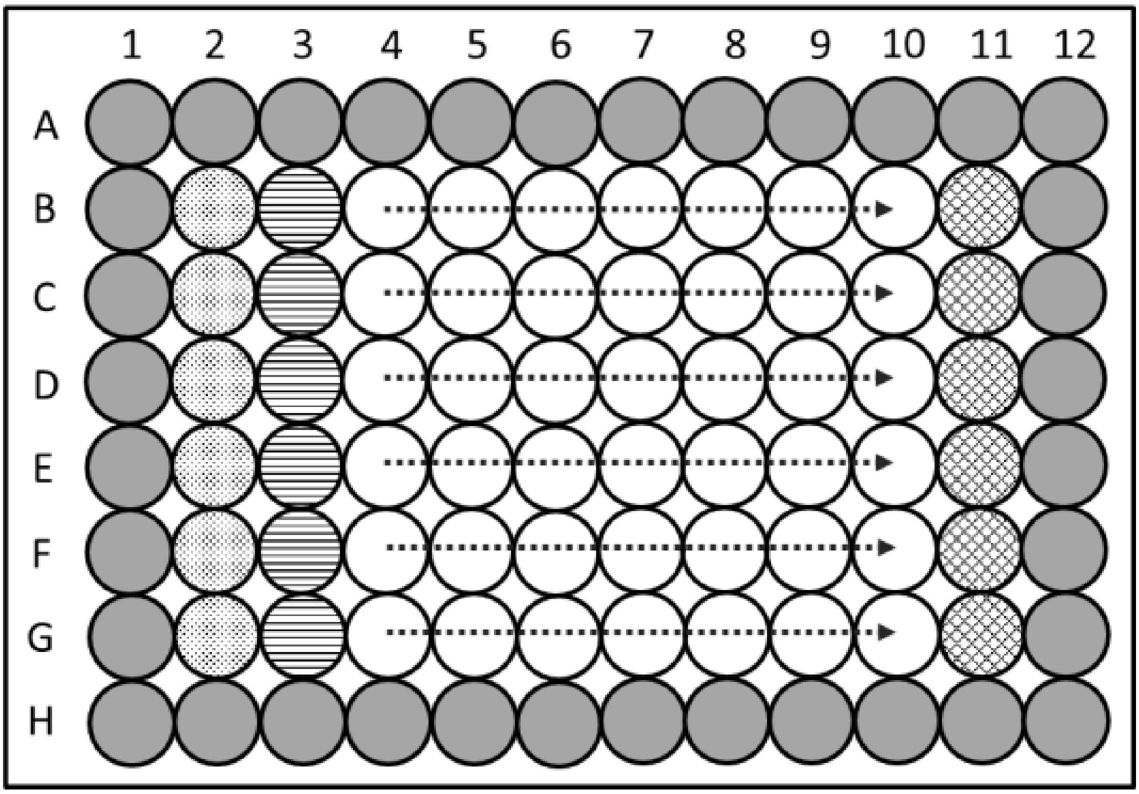

Figure 1. The 96-well microtiter plate: experimental setup with flat-bottom wells. Grey-coloured wells: empty wells, not utilized due to evaporation; Dotted wells: purity control indicating 0% bacterial growth; Striped wells: growth controls showing 100% bacterial growth; White-coloured wells: serial two-fold dilutions of tested volatile compounds; Gridded wells: serial two-fold dilutions of the positive antibiotic control (Houdkova *et al*. 2017).

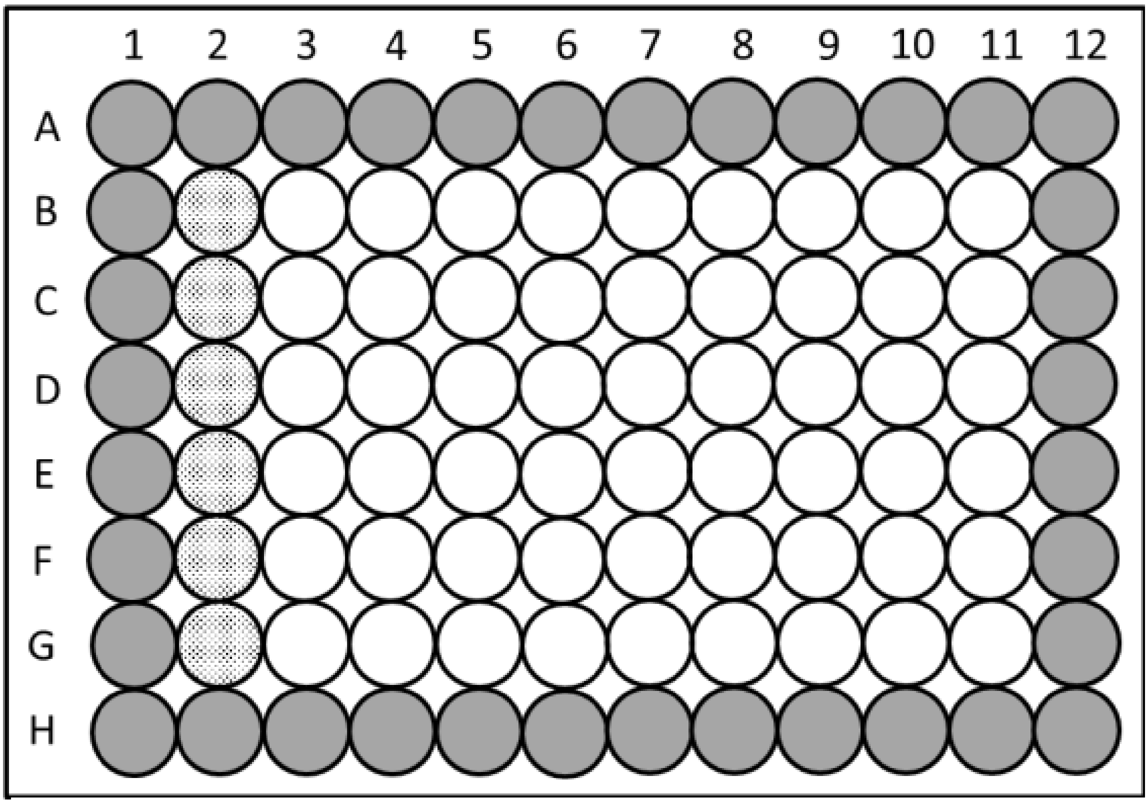

Figure 2. The 96-well microtiter plate lid: experimental setup with flat-bottom flanges. Grey-coloured wells: empty wells, not utilized; Dotted wells: purity control indicating 0% bacterial growth; White-coloured wells: bacterial suspension with agar (Houdkova *et al*. 2017).

5. Results and Discussion

This study evaluated the growth-inhibiting potential of 12 EOs derived from different plant botanical components (bark, flower buds, fruits, leaves, petals, seeds, seed pods, and rhizomes) obtained by hydro-distillation and tested against two strains of *B. cereus* (ATCC 11778; CCM 2010). Among all EOs tested, *C. cassia* (bark), *T. vulgaris* (leaves), *R. x damascena* 'Amadeus' (petals), *S. aromaticum* (flower buds), *M. fragrans* (seeds), and *S. indicum* (seeds) exhibited a certain degree of activity in either liquid or vapor phase against at least one strain of *B. cereus*. The detailed results of the *in vitro* growth-inhibitory effect of EOs from spices against *B. cereus* are summarised in Table 2.

Among all samples tested, EO from bark of *C. cassia* showed the strongest growth-inhibitory effect against both strains of *B. cereus* in liquid phase (MIC = 128 µg/mL) as well as in vapor phase with MIC values 128 and 256 µg/mL for *B. cereus* ATCC 11778 and CCM 2010, respectively. According to Vihanova *et al*. (2021), EO hydro-distilled from the bark of *C. cassia* purchased from a commercial provider exhibited significant effectiveness against *B. cereus* ATCC 11778 in both liquid and vapor phases (MIC = 512 µg/mL). Since both studies used same methodology of antibacterial susceptibility testing, the variability of MIC results can be caused by different origin of plant material tested. This hypothesis can be supported by data reviewed by De Guzman & Siemonsma (1999) showing geographical variations in the chemical composition of *C. cassia* bark EO. According to their book, Australian samples were containing primarily cinnamic aldehyde (87.0%) and benzaldehyde (4.7%), while the major constituents of samples from China were (E)-cinnamic aldehyde (65.5%) and coumarin (8.7%).

As the second most potent sample, *T. vulgaris* demonstrated moderate growth-inhibitory effect against *B. cereus* in the vapor phase, with MIC value of 512 µg/mL for both strains as well as in the liquid phase with MIC values of 256 and 128 µg/mL for *B. cereus* ATCC 11778 and CCM 2010, respectively. Regarding to the liquid phase, our results are well corresponding with findings of Kang *et al*. (2018), who observed moderate inhibitory activity of *T. vulgaris* EO against *B. cereus* ATCC 14579 (MIC = 250 µg/mL) when assayed using broth microdilution method. However, according to the best of our knowledge, this is the first study showing growth-inhibitory effect of *T. vulgaris* EO against *B. cereus* in the vapor phase.

Subsequently, *R. x damascena* ‘Amadeus’ exhibited moderate growth-inhibitory effect against both *B. cereus* strains (MIC = 512 µg/mL) in the vapor phase as well as in the liquid phase with MIC values 512 and 256 µg/mL for *B. cereus* ATCC 11778 and CCM 2010, respectively. Even though, there are numerous studies on the antibacterial properties of the *Rosa* genus (Elhawary *et al*. 2021, Ghavam *et al*. 2021), only few of them are mentioning *R. x damascena* ‘Amadeus’. Although several studies are dealing with nutritional and chemical composition of *R. x damascena* 'Amadeus' (Kalisz *et al*. 2023; Schmitzer *et al*. 2019), according to our best knowledge, its growth-inhibitory activity in the vapor phase against *B. cereus* was tested for first time in this study.

S. aromaticum exhibited moderate growth-inhibitory activity against both strains of *B. cereus* (MIC = 512 µg/mL) in the liquid phase and low or no effect in the vapor phase against *B. cereus* ATCC 11778 (MIC = 1024 µg/mL) and *B. cereus* CCM 2010 (MIC >1024 µg/mL). According to a study by Barakat (2014), EO hydro-distilled from *S. aromaticum* exhibited against *B. cereus* considerably higher MIC (2500 µg/mL) when assayed using the agar dilution method. The variations in the measured MIC values could be attributed to the different methodologies of antimicrobial susceptibility testing. According to the best of our knowledge, no other studies have investigated the vapor phase growth-inhibitory effects of *S. aromaticum* EO from buds against *B. cereus*.

As for *M. fragrans*, only moderate or low growth-inhibitory effect was observed with MIC values ranging from 1024 to 512 µg/mL in the liquid phase against *B. cereus* ATCC 11778 and CCM 2010, respectively. According to Purkait *et al*. (2018), *M. fragrans* EO obtained by hydro-distillation showed very promising MIC values against *B. cereus* MTCC 1272 (MIC = 83.33 µg/mL) when tested by broth microdilution method. The variances between the resulting MIC values may be caused by susceptibility of tested bacterial strains to the EO. Regarding the vapor phase, *M. fragrans* EO exhibited no growth-inhibiting effect against *B. cereus* (MIC >1024 µg/mL). According to the best of our knowledge, there are currently no studies on growth-inhibitory effect of *M. fragrans* EO in the vapor phase.

Lastly, EO from *S. indicum* showed low growth-inhibitory effect against both strains of *B. cereus* (MIC = 1024 µg/mL) in the liquid phase and no activity against both strains (MIC > 1024 µg/mL) in the vapor phase. While there are some studies which assess the growth-inhibitory activity of *S. indicum* against *B. cereus* (Mahendra Kumar & Singh 2015; Ogwuche *et al*. 2014), there are no available studies on the antimicrobial activity of the *S. indicum* EO.

In comparison of the two bacterial strains tested, the *B. cereus* ATCC 11778 was found to be the more susceptible with MIC values ranging from 128 µg/mL to 1024 µg/mL. Lastly, EOs from *A. subulatum*, *C. carvi*, *C. longa*, *L. nobilis*, *P. dioica*, and *P. nigrum* showed no notable growth-inhibitory activity in both liquid and in vapor phase when tested testing against *B. cereus*.

Table 2. *In vitro* growth-inhibitory effect of essential oils from spices against *Bacillus cereus* in liquid and vapor phase

Botanical name	**Bacterial strain/Growth medium/Minimum inhibitory concentration (µg/mL)**					
	***B. cereus* (ATCC 11778)**		***B. cereus* (CCM 2010)**		**Chloramphenicol (µg/mL)[a]**	
	Broth	**Agar**	**Broth**	**Agar**	**Broth**	**Agar**
Amomum subulatum	>1024	>1024	>1024	>1024	1	ND[b]
Carum carvi	>1024	>1024	>1024	>1024	1	ND
Cinnamomum cassia	128	128	128	256	1	ND
Curcuma longa	>1024	>1024	>1024	>1024	1	ND
Laurus nobilis	>1024	>1024	>1024	>1024	1	ND
Myristica fragrans	1024	>1024	512	>1024	1	ND
Pimenta dioica	>1024	>1024	>1024	>1024	1	ND
Piper nigrum	>1024	>1024	>1024	>1024	1	ND
Rosa x damascena 'Amadeus'	512	512	256	512	1	ND
Sesamum indicum	1024	>1024	1024	>1024	1	ND
Syzygium aromaticum	512	1024	512	>1024	1	ND
Thymus vulgaris	256	512	128	512	1	ND

[a]Positive antibiotic control, [b]Not determined

6. Conclusion

In summary, *C. cassia*, *M. fragrans*, *R. x damascena* 'Amadeus', *S. indicum*, *S. aromaticum*, and *T. vulgaris* exhibited certain degree of *in vitro* growth-inhibitory effect in liquid or vapor phase against at least one of both strains of *B. cereus* tested. Among all EOs tested, *C. cassia* was the only sample to exhibit significant antibacterial activity against both strains in the vapor phase. In comparison with *C. cassia*, *S. aromaticum*, *T. vulgaris*, *S. indicum*, and *R. x damascena* 'Amadeus' exhibited lower antibacterial activity in the vapor phase. The remaining EO samples demonstrated weak or no inhibitory activity. *B. cereus* ATCC 11778 was the most susceptible strain. The results of this study suggest potential application of EO from *C. cassia* bark as volatile antimicrobial agent against *B. cereus*. Additional experiments focusing on organoleptic approval and efficacy in suitable food models are necessary to validate the potential application for commercial food preservation.

7. References

Abidin MRZ, Muhammad R, Abdullah KM, Sharif MSM, Talib SA. 2020. Foodways practices among Iranian students in Malaysia. The International Journal of Academic Research in Business & Social Sciences **11**:181–196.

Agrahari P, Singh DK. 2014. A review on the pharmacological aspects of *Carum carvi*. Journal of Biology and Earth Sciences **4**:1–13.

Alam A, Ansari MJ, Alqarni MH, Salkini MA, Raish M. 2023. Antioxidant, antibacterial, and anticancer activity of ultrasonic nanoemulsion of *Cinnamomum Cassia* L. essential oil. Plants 12 (e834) DOI: 10.3390/plants12040834.

Albarracín W, Sánchez IC, Grau R, Barat JM. 2011. Salt in food processing; usage and reduction: A review. International Journal of Food Science & Technology **46**:1329–1336.

ALrashidi AA, Noumi E, Snoussi M, Feo VD. 2022. Chemical composition, antibacterial and anti-quorum sensing activities of *Pimenta dioica* L. essential oil and its major compound (eugenol) against foodborne pathogenic bacteria. Plants 11 (e540) DOI: 10.3390/plants11040540.

Amat S, Baines D, Alexander TW. 2017. A vapour phase assay for evaluating the antimicrobial activities of essential oils against bovine respiratory bacterial pathogens. Letters in Applied Microbiology **65**:489–495.

Aneja KR, Dhiman R, Aggarwal N, Aneja A. 2016. Bacteriocins as potential biopreservatives in foods: An overview. Pages 75–94 in Sharma C, Sharma AK, editors. Frontiers in Food Biotechnology, Nova Science Publishers, New York.

Antunes SA, Robazza WDS, Schittler L, Gomes GDA. 2012. Synergistic and antimicrobial properties of commercial turmeric (*Curcuma longa*) essential oil against pathogenic bacteria. Food Science and Technology **32**:525–530.

Anzano A, de Falco B, Grauso L, Motti R., Lanzotti V. 2022. Laurel, *Laurus nobilis* L.: A review of its botany, traditional uses, phytochemistry and pharmacology. Phytochemistry Reviews **21**:565–615.

Ayoola GA, Lawore FM, Adelowotan T, Aibinu IE, Adenipekun E, Coker HAB, Odugbemi TO. 2008. Chemical analysis and antimicrobial activity of the essential oil of *Syzigium aromaticum* (clove). African Journal of Microbiology Research **2**:162–166.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. 2008. Biological effects of essential eils–A review. Food and Chemical Toxicology **46**:446–475.

Barakat H. 2014. Composition, Antioxidant, Antibacterial Activities and Mode of Action of Clove (*Syzygium aromaticum* L.) buds essential oil. British Journal of Applied Science & Technology **4**:1934–1951.

Baser KHC, Buchbauer G. 2010. Handbook of essential oils: Science, technology, and applications. CRC Press/Taylor & Francis, Boca Raton.

Bassolé IHN, Juliani HR. 2012. Essential oils in combination and their antimicrobial properties. Molecules **17**:3989–4006.

Behbahani BA, Noshad M, Falah F. 2019. Study of chemical structure, antimicrobial, cytotoxic and mechanism of action of *Syzygium aromaticum* essential oil on foodborne pathogens. Potravinarstvo Slovak Journal of Food Sciences **13**:875–883.

Bello RY, Umar SS, Wakil S, Bello AY. 2022. Antibacterial activity of clove extracts (*Syzygium aromaticum*) against *Bacillus cereus*. International Journal of Advanced Academic Research **8**:33–46.

Bottone EJ. 2010. *Bacillus cereus,* a volatile human pathogen. Clinical Microbiology Reviews **23**:382–398.

Caputo L, Nazzaro F, Souza LF, Aliberti L, De Martino L, Fratianni F, Coppola R, De Feo V. 2017. *Laurus nobilis*: Composition of essential oil and its biological activities. Molecules 22 (e930) DOI: 10.3390/molecules22060930.

Chaure A, Houdkova M, Antih J, Urbanova K, Doskocil I, Naik ML, Patel KS, Kokoska L. 2023. Validation of broth macrodilution volatilization method for testing of essential oils in liquid and vapor phase: Chemical composition, cytotoxicity, and antibacterial effect of Indian medicinal plants against pneumonia-causing pathogens. Molecules 28 (e4625) DOI: 10.3390/molecules28124625.

Chawla R, Sivakumar S, Kaur H. 2021. Antimicrobial edible films in food packaging: current scenario and recent nanotechnological advancements - A review. Carbohydrate Polymer Technologies and Applications 2 (e100024) DOI: 10.1016/j.carpta.2020.100024.

Clarke A, Eberhardt CN. 2002. Microscopy techniques for materials science. Woodhead Publishing, Cambridge.

Coburn PS, Miller FC, Enty MA, Land C, LaGrow AL, Mursalin MH, Callegan MC. 2020. Expression of *Bacillus cereus* virulence-related genes in an ocular infection-related environment. Microorganisms 8 (e607) DOI: 10.3390/microorganisms8040607.

Czarra F. 2009. Spices: A global history. Reaktion Books, Clerkenwell.

Dabscheck G, Silverman L, Ullrich NJ. 2015. *Bacillus cereus* cerebral abscess during induction chemotherapy for childhood acute leukemia. Journal of Pediatric Hematology/Oncology **37**:568–569.

de Guzman CC, Siemonsma JS. 1999. Plant resources of south-east Asia. Backhuys Publishers, Leiden.

Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. 2016. Essential oils' chemical characterization and investigation of some biological activities: A critical review. Medicines **3**:2–10.

Djenane D, Ben Miri Y, Ariño A. 2023. Use of Algerian type Ras El-Hanout spices mixture with marination to increase the sensorial quality, shelf life, and safety of whole rabbit carcasses under low-o2 modified atmosphere packaging. Foods 12 (e2931) DOI: 10.3390/foods12152931.

Edwards-Jones V, Buck R, Shawcross SG, Dawson MM, Dunn K. 2004. The effect of essential oils on methicillin-resistant *Staphylococcus aureus* using a dressing model. Burns **30**:772–777.

Ehling-Schulz M, Lereclus D, Koehler TM. 2019. The *Bacillus cereus* group: *Bacillus* species with pathogenic potential. Microbiology Spectrum **7**:10–1128.

El Asbahani A, Miladi K, Badri W, Sala M, Addi EA, Casabianca H, El Mousadik A, Hartmann D, Jilale A, Renaud FNR, Elaissari A. 2015. Essential oils: From extraction to encapsulation. International Journal of Pharmaceutics **483**:220–243.

El Atki Y, Aouam I, El Kamari F, Taroq A, Nayme K, Timinouni M, Lyoussi B, Abdellaoui A. 2019. Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. Journal of Advanced Pharmaceutical Technology & Research **10**:63–67.

Elhawary EA, Mostafa NM, Labib RM, Singab AN. 2021. Metabolomic profiles of essential oils from selected Rosa varieties and their antimicrobial activities. Plants 10 (e1721) DOI: 10.3390/plants10081721.

Ertürk Ö. 2006. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Biologia **61**:275–278.

European Food Safety Authority. 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSa Journal 16 (e05500) DOI: 10.2903/j.efsa.2018.5500.

Fani M, Kohanteb J. 2017. *In vitro* antimicrobial activity of *Thymus vulgaris* essential oil against major oral pathogens. Journal of Evidence-Based Complementary & Alternative Medicine **22**:660–666.

Farkas J. 2006. Irradiation for better foods. Trends in Food Science & Technology **17**:148–152.

Feitosa BDS, Ferreira OO, Franco CDJP, Karakoti H, Kumar R, Cascaes MM, Jawarkar RD, Mali SN, Cruz JN, Menezes IC, Oliveira MS, Aguiar Andrade EH. 2024. Chemical composition of *Piper nigrum* L. cultivar guajarina essential oils and their biological activity. Molecules 29 (e947) DOI: 10.3390/molecules29050947.

Feyaerts AF, Mathe L, Luyten W, Tournu H, van Dyck K, Broekx L, van Dijck P. 2017. Assay and recommendations for the detection of vapour-phasemediated antimicrobial activities. Flavour and Fragrance Journal **32**:347–353.

Finar IL. 1994. Organic Chemistry Vol. II. Longman scientific *et* technical. ELBS, Oxford.

Firmino DF, Cavalcante TTA, Gomes GA, Firmino NCS, Rosa LD, deCarvalho MG, Catunda FEA. 2018. Antibacterial and antibiofilm activities of *Cinnamomum sp*. essential oil and cinnamaldehyde: Antimicrobial activities. The Scientific World Journal 2018 (e7405736) DOI: 10.1155/2018/7405736.

Franco-Vega A, Reyes-Jurado F, Cardoso-Ugarte GA, Sosa-Morales ME, Palou E, López-Malo A. 2016. Essential oils in food preservation flavor and safety. Elsevier, Cambridge.

Gajic I, Kabic J, Kekic D, Jovicevic M, Milenkovic M, Mitic Culafic D, Trudic A, Ranin L, Opavski N. 2022. Antimicrobial susceptibility testing: A comprehensive review of currently used methods. Antibiotics 11 (e427) DOI: 10.3390/antibiotics11040427.

Galovicova L, Borotova P, Valkova V, Vukovic NL, Vukic M, Stefaniková J, Duranova H, Kowalczewski L, Cmikova N, Kacaniova M. 2021. *Thymus vulgaris* essential oil and its biological activity. Plants 10 (e1959) DOI: 10.3390/plants10091959.

Ghavam M, Afzali A, Manconi M, Bacchetta G, Manca ML. 2021. Variability in chemical composition and antimicrobial activity of essential oil of *Rosa* × *damascena* Herrm. from mountainous regions of Iran. Chemical and Biological Technologies in Agriculture **8**:1–16.

Gilani SR, Shahid I, Javed M, Mehmud S, Ahmed R. 2006. Antimicrobial activities and physico-chemical properties of the essential oil from *Amomum subulatum*. International Journal of Applied Chemistry **2**:81–86.

Goñi P, López P, Sánchez C, Gómez-Lus R, Becerril R, Nerín C. 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chemistry **116**:982–989.

Granum PE, Lindbäck T. 2012. *Bacillus cereus*. Pages 491–502 in Doyle MP, Buchanan RL, editors. Food microbiology: Fundamentals and frontiers. ASM Press, Washington D.C.

Gupta A, Dubey M, Parmar M, Mahajan S, Sharma R. 2011. Evaluation of antimicrobial activity of *Carum carvi* (seeds) extract against *E. coli* and *Aspergillus niger*. Drug Invention Today **3**:211–213.

Gutman I, Cyvin SJ. 2012. Introduction to the theory of benzenoid hydrocarbons. Springer Science & Business Media, Heidelberg.

Hammerbacher A, Coutinho TA, Gershenzon J. 2019. Roles of plant volatiles in defence against microbial pathogens and microbial exploitation of volatiles. Plant, Cell & Environment **42**:2827–2843.

Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H. 2021. Clove essential oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. Molecules 26 (e6387) DOI: 10.3390/molecules26216387.

Houdkova M, Chaure A, Doskocil I, Havlik J, Kokoska L. 2021. New broth microdilution volatilization method for antibacterial susceptibility testing of volatile agents and evaluation of their toxicity using modified MTT assay *in vitro*. Molecules 26 (e4179) DOI: 10.3390/molecules26144179.

Houdkova M, Kokoska L. 2020. Volatile antimicrobial agents and *in vitro* methods for evaluating their activity in the vapour phase: A review. Planta Medica **86**:822–857.

Houdkova M, Rondevaldova J, Doskocil I, Kokoska L. 2017. Evaluation of antimicrobial potential and toxicity of plant volatile compounds using new broth microdilution volatilization method and modified MTT assay. Fitoterapia **118**:56–62.

Huang DF, Xu JG, Liu JX, Zhang H, Hu QP. 2014. Chemical constituents, antibacterial activity and mechanism of action of the essential oil from *Cinnamomum cassia* bark against four food-related bacteria. Microbiology **83**:357-365.

Hwang KW, Son D, Jo HW, Kim CH, Seong KC, Moon JK. 2016. Levels of curcuminoid and essential oil compositions in turmerics (*Curcuma longa L.*) grown in Korea. Applied Biological Chemistry **59**:209–215.

Inouye S, Uchida K, Maruyama N, Yamaguchi H, Abe S. 2006. A novel method to estimate the contribution of the vapour activity of essential oils in agar diffusion assay. Japanese Journal of Medical Mycology **47**:91–98.

Iskandar K, Molinier L, Hallit S, Sartelli M, Hardcastle TC, Haque M, Lugova H, Dhingra S, Sharma P, Islam S, Mohammed I, Mohamed IN, Hanna PA, El Hajj S, Jamaluddin NAH, Salameh P, Roques C. 2021. Surveillance of antimicrobial resistance in low- and middle-income countries: A scattered picture. Antimicrobial Resistance & Infection Control 10 (e63) DOI: 10.1186/s13756-021-00931-w.

Jafri MA, Javed K, Singh S. 2001. Evaluation of the gastric antiulcerogenic effect of large cardamom (fruits of *Amomum subulatum* Roxb). Journal of Ethnopharmacology **75**:89–94.

Jaiswal P, Kumar P, Singh VK, Singh DK. 2009. Biological effects of *Myristica fragrans*. Annual Review of Biomedical Sciences **11**:21–29.

Jarquín-Enríquez L, Ibarra-Torres P, Jiménez-Islas H, Flores-Martínez NL. 2021. *Pimenta dioica*: A review on its composition, phytochemistry, and applications in food technology. International Food Research Journal **28**:873.

Jessberger N, Dietrich R, Granum PE, Märtlbauer E. 2020. The *Bacillus cereus* food infection as multifactorial process. Toxins 12 (e701) DOI: 10.3390/toxins12110701.

Jiang ZT, Sun ML, Li R, Wang Y. 2011. Essential oil composition of Chinese caraway (*Carum carvi L.*). Journal of Essential Oil Bearing Plants **14**:379–382.

Jorgensen JH, Turnidge JD, Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. 2007. Antibacterial susceptibility tests: Dilution and disk diffusion methods. Manual of Clinical Microbiology **9**:1152–1172.

Jose AJ, Leela NK, Zachariah TJ, Rema J. 2019. Evaluation of coumarin content and essential oil constituents in *Cinnamomum cassia* (Nees & T. Nees) J. Presl. Journal of Spices and Aromatic Crops **28**:43–51.

Joshi R, Sharma P, Sharma V, Prasad R, Sud RK, Gulati A. 2013. Analysis of the essential oil of large cardamom (*Amomum subulatum* Roxb.) growing in different agro-climatic zones of Himachal Pradesh, India. Journal of the Science of Food and Agriculture **93**:1303–1309.

Ju J, Chen X, Xie Y, Yu H, Guo Y, Cheng Y, Qian H, Yao W. 2019. Application of essential oil as a sustained release preparation in food packaging. Trends in Food Science & Technology **92**:22–32.

Ju J, Xu X, Xie Y, Guo Y, Cheng Y, Qian H, Yao W. 2018. Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods. Food Chemistry **240**:850–855.

Juneja VK, Golden CE, Mishra A, Harrison MA, Mohr T, Silverman M. 2019. Predictive model for growth of *Bacillus cereus* during cooling of cooked rice. International Journal of Food Microbiology **290**:49–58.

Juteau F, Jerkovic I, Masotti V, Milos M, Mastelic J, Bessière JM, Viano J. 2003. Composition and antimicrobial activity of the essential oil of *Artemisia absinthium* from Croatia and France. Planta Medica **69**:158–161.

Kalia VC. 2013. Quorum sensing inhibitors: An overview. Biotechnology Advances **31**:224–245.

Kalisz A, Włodarczyk Z, Bieniasz M, Smoleń S, Neugebauerová J, Szewczyk-Taranek B, Pawłowska B. 2023. Petals of different ornamental rose cultivars as a rich source of bioactive compounds for functional foods. Scientia Horticulturae 321 (e112240) DOI: 10.1016/j.scienta.2023.112240.

Kang J, Liu L, Wu X, Sun Y, Liu Z. 2018. Effect of thyme essential oil against *Bacillus cereus* planktonic growth and biofilm formation. Applied Microbiology and Biotechnology **102**:10209–10218.

Karsha PV, Lakshmi OB. 2010. Antibacterial activity of black pepper (*Piper nigrum* Linn.) with special reference to its mode of action on bacteria. Indian Journal of Natural Products and Resources **1**:213–215.

Karunakaran E, Biggs CA. 2011. Mechanisms of *Bacillus cereus* biofilm formation: An investigation of the physicochemical characteristics of cell surfaces and extracellular proteins. Applied Microbiology and Biotechnology **89**:1161–1175.

Khalil M, Caponio GR, Diab F, Shanmugam H, Di Ciaula A, Khalifeh H, Vergani L, Calasso M, De Angelis M, Portincasa P. 2022. Unraveling the beneficial effects of herbal Lebanese mixture “Za’atar”. History, studies, and properties of a potential healthy food ingredient. Journal of Functional Foods 90 (e104993) DOI: 10.1016/j.jff.2022.104993.

Khan AF, Mujeerb F, Aha F, Farooqui A. 2015. Effect of plant growth regulators on growth and essential oil content in Palmarosa (*Cymbopogon martini*). Asian Journal of Pharmaceutical and Clinical Research **8**:373–376.

Kokoska L. 2003. Spices, aromatic and medicinal plants of tropics and subtropics. Czech University of Agriculture, Institute of Tropics and Subtropics, Prague.

Kotiranta A, Lounatmaa K, Haapasalo M. 2000. Epidemiology and pathogenesis of *Bacillus cereus* infections. Microbes and Infection **2**:189–198.

Kramer JM, Gilbert RJ. 1989. *Bacillus cereus* and other *Bacillus* species. Foodborne Bacterial Pathogens **19**:21–70.

Krumal K, Kubatkova N, Vecera Z, Mikuska P. 2015. Antimicrobial properties and chemical composition of liquid and gaseous phases of essential oils. Chemical Papers **69**:1084–1092.

Leclercq R, Cantón R, Brown DFJ, Giske CG, Heisig P, MacGowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G. 2013. EUCAST expert rules in antimicrobial susceptibility testing. Clinical Microbiology and Infection **19**:141–160.

Li YX, Erhunmwunsee F, Liu M, Yang K, Zheng W, Tian J. 2022. Antimicrobial mechanisms of spice essential oils and application in food industry. Food Chemistry 382 (e132312) DOI: 10.1016/j.foodchem.2022.132312.

Liu C, Long H, Wu X, Hou J, Gao L, Yao S, Lei M, Zhang Z, Guo D, Wu W. 2021. Quantitative and fingerprint analysis of proanthocyanidins and phenylpropanoids in *Cinnamomum verum* bark, *Cinnamomum cassia* bark, and Cassia twig by UPLC combined with chemometrics. European Food Research and Technology **247**:2687–2698.

Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. 2017. Antibacterial and antifungal activities of spices. International Journal of Molecular Sciences 18 (e1283) DOI: 10.3390/ijms18061283.

Liu Y, Lai Q, Göker M, Meier-Kolthoff JP, Wang M, Sun Y, Wang L, Shao Z. 2015. Genomic insights into the taxonomic status of the *Bacillus cereus* group. Scientific Reports 5 (e14082) DOI: 10.1038/srep14082.

Loghmani-Khouzani H. 2007. Essential oil composition of *Rosa damascena* Mill. cultivated in central Iran. Scientia Iranica **14**:316–319.

Macit E, Çağlar A, Bakirci A. 2017. The possibilities of using some spice essential oils in ice cream production. Alinteri Journal of Agriculture Sciences **32**:63–68.

Maffei ME, Gertsch J, Appendino G. 2011. Plant volatiles: Production, function and pharmacology. Natural Product Reports **28**:1359–1380.

Mahmoudi R, Kaboudari A, Pakbin B. 2020. Applications of medicinal herbs and essential oils in food safety. IntechOpen, London.

Majed R, Faille C, Kallassy M, Gohar M. 2016. *Bacillus cereus* biofilms—Same, only different. Frontiers in Microbiology 7 (e1054) DOI: 10.3389/fmicb.2016.01054.

McDowell RH, Sands EM, Friedman H. 2023. *Bacillus cereus*. StatPearls Publishing, St. Petersburg, Florida. Available from https://www.ncbi.nlm.nih.gov/books/NBK459121/ (accessed February 12, 2024).

Meghwal M, Goswami TK. 2013. *Piper nigrum* and piperine: An update. Phytotherapy Research **27**:1121–1130.

Mili A, Das S, Nandakumar K, Lobo R. 2021. A comprehensive review on *Sesamum indicum* L.: Botanical, ethnopharmacological, phytochemical, and pharmacological aspects. Journal of Ethnopharmacology 281 (e114503) DOI: 10.1016/j.jep.2021.114503.

Mittal M, Gupta N, Parashar P, Mehra V, Khatri M. 2014. Phytochemical evaluation and pharmacological activity of *Syzygium aromaticum*: A comprehensive review. International Journal of Pharmacy and Pharmaceutical Sciences **6**:67–72.

Mukurumbira AR, Shellie RA, Keast R, Palombo EA, Jadhav SR. 2022. Encapsulation of essential oils and their application in antimicrobial active packaging. Food Control 136 (e108883) DOI: 10.1016/j.foodcont.2022.108883.

Murbach Teles Andrade BF, Nunes Barbosa L, da Silva Probst I, Fernandes Júnior A. 2014. Antimicrobial activity of essential oils. Journal of Essential Oil Research **26**:34–40.

Nabila B, Piras A, Fouzia B, Falconieri D, Kheira G, Fedoul FF, Majda SR. 2022. Chemical composition and antibacterial activity of the essential oil of *Laurus nobilis* leaves. Natural Product Research **36**:989–993.

Naz S, Jabeen S, Ilyas S, Manzoor F, Aslam F, Ali A. 2010. Antibacterial activity of *Curcuma longa* varieties against different strains of bacteria. Pakistan Journal of Botany **42**:455–62.

Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals **6**:1451–1474.

Ngow HA & Khairina WW. 2013. *Bacillus cereus* endocarditis in native aortic valve. Journal of Infection and Chemotherapy **19**:154–157.

Ni ZJ, Wang X, Shen Y, Thakur K, Han J, Zhang JG, Hu F, Wei ZJ. 2021. Recent updates on the chemistry, bioactivities, mode of action, and industrial applications of plant essential oils. Trends in Food Science & Technology **110**:78–89.

Nikolic V, Nikolic L, Dinic A, Gajic I, Urosevic M, Stanojevic L, Urosevic M, Danilovic B. 2021. Chemical composition, antioxidant and antimicrobial activity of nutmeg (*Myristica fragrans* Houtt.) seed essential oil. Journal of Essential Oil Bearing Plants **24**:218–227.

Norman J. 2021. Spices of the world. Shepheard Walwyn Publishers, London.

O'Day DM, Smith RS, Gregg CR, Turnbull PC, Head WS, Ives JA, Ho PC. 1981. The Problem of *Bacillus* species infection with special emphasis on the virulence of *Bacillus cereus*. Ophthalmology **88**:833–838.

Ogunwande IA, Olawore NO, Adeleke KA, Ekundayo O. 2003. Chemical composition of essential oil of *Myristica fragrans* Houtt. (nutmeg) from Nigeria. Journal of Essential Oil Bearing Plants **6**:21–26.

Ogwuche CE, Amupitan JO, Ndukwe GI. 2014. Evaluation of antimicrobial activities of isolated compounds from the leaf of the white specie of *Sesamum Indicum* from Benue state, Nigeria. Journal of Applied Sciences and Environmental Management **18**:568–574.

Omoruyi IM, Emefo OT. 2012. *In vitro* evaluation of the antibiogramic activities of the seeds of *Myristica fragrans* on food borne pathogens. Malaysian Journal of Microbiology **8**:253–258.

Oulkheir S, Aghrouch M, El Mourabit F, Dalha F, Graich H, Amouch F, Ouzaid K, Moukale A, Chadli S. 2017. Antibacterial activity of essential oils extracts from cinnamon, thyme, clove and geranium against a gram negative and gram positive pathogenic bacteria. Journal of Diseases and Medicinal Plants **3**:1–5.

Oyen LPA, Dung NX. 1999. Plant resources of south-east Asia: Essential oil plants. Backhuys Publishers, Leiden.

Pandey AK, Kumar P, Singh P, Tripathi NN, Bajpai VK. 2017. Essential oils: Sources of antimicrobials and food preservatives. Frontiers in Microbiology **7**:1–15.

Papadochristopoulos A, Kerry J, Fegan N, Burgess C, Duffy G. 2021. Natural anti-microbials for enhanced microbial safety and shelf-life of processed packaged meat. Foods 10 (e1598) DOI: 10.3390/foods10071598.

Paparella A, Nawade B, Shaltiel-Harpaz L, Ibdah M. 2022. A review of the botany, volatile composition, biochemical and molecular aspects, and traditional uses of *Laurus nobilis*. Plants 11 (e1209) DOI: 10.3390/plants11091209.

Pexara A, Govaris A. 2018. *Bacillus cereus*: An important foodborne pathogen. Journal of the Hellenic Veterinary Medical Society **61**:127–133.

Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. 2009. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and *Dermatophyte* species. Journal of Medical Microbiology **58**:1454–1462.

Popović-Djordjević J, Cengiz M, Ozer MS, Sarikurkcu C. 2019. *Calamintha incana*: essential oil composition and biological activity. Industrial Crops and Products **128**:162–166.

Pulido MR, García-Quintanilla M, Martín-Peña R, Cisneros JM, McConnell MJ. 2013. Progress on the development of rapid methods for antimicrobial susceptibility testing. Journal of Antimicrobial Chemotherapy **68**:2710–2717.

Purkait S, Bhattacharya A, Bag A, Chattopadhyay RR. 2018. Antibacterial and antioxidant potential of essential oils of five spices. Journal of Food Quality and Hazards Control **5**:61–71.

Rahman NAA, Fazilah A, Effarizah ME. 2015. Toxicity of nutmeg (myristicin): A review. International Journal on Advanced Science, Engineering and Information Technology **5**:61–64.

Rangan C, Barceloux D. 2008. Medical toxicology of natural substances: Foods, fungi, medicinal herbs, plants, and venomous animals. Wiley & Sons, Hoboken, New Jersey.

Rao PS, Navinchandra S, Jayaveera KN. 2012. An important spice, *Pimenta dioica* (Linn.) Merill: A review. International Current Pharmaceutical Journal **1**:221–225.

Rawson A, Patras A, Tiwari BK, Noci F, Koutchma T, Brunton N. 2011. Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. Foodservice Research International **44**:1875–1887.

Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. 2009. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clinical Infectious Diseases **49**:1749–1755.

Reyes-Jurado F, Franco-Vega A, Ramírez-Corona N, Palou E, López-Malo A. 2015. Essential oils: Antimicrobial activities, extraction methods, and their modeling. Food Engineering Reviews **7**:275–297.

Reyes-Jurado F, Navarro-Cruz AR, Ochoa-Velasco CE, Palou E, López-Malo A, Ávila-Sosa R. 2020. Essential oils in vapor phase as alternative antimicrobials: A review. Critical Reviews in Food Science and Nutrition **60**:1641–1650.

Rivera D, Toledo V, Reyes-Jara A, Navarrete P, Tamplin M, Kimura B, Wiedmann M, Silva P, Switt AIM. 2018. Approaches to empower the implementation of new tools to detect and prevent foodborne pathogens in food processing. Food Microbiology **75**:126–132.

Ryu JH, Beuchat LR. 2005. Biofilm formation and sporulation by *Bacillus cereus* on a stainless-steel surface and subsequent resistance of vegetative cells and spores to chlorine, chlorine dioxide, and a peroxyacetic acid–based sanitizer. Journal of Food Protection **68**:2614–2622.

Sabulal N, Baby S. 2021. Chemistry of Amomum essential oils. Journal of Essential Oil Research **33**:427–441.

Sachan AK, Das DR, Kumar M. 2016. *Carum carvi*-An important medicinal plant. Journal of Chemical and Pharmaceutical Research **8**:529–533.

Safaei-Ghomi J, Akhoondi S, Batooli H, Dackhili M. 2009. Chemical variability of essential oil components of two *Rosa x damascena* genotypes growing in Iran. Chemistry of Natural Compounds **45**:262–264.

Sakkas H, Papadopoulou C. 2017. Antimicrobial activity of basil, oregano, and thyme essential oils. Journal of Microbiology and Biotechnology **27**:429–438.

Salmieri S, Islam F, Khan RA, Hossain FM, Ibrahim H, Miao C, Hamad WY, Lecroix M. 2014. Antimicrobial nanocompositefilms made of poly(lactic acid)–cellulose nanocrystals (PLA–CNC) in food applications—Part B: Effect of oregano essential oilrelease on the inactivation of *Listeria monocytogenes* in mixed vegetables. Cellulose **21**:4271–4285.

Satyal P, Dosoky NS, Kincer BL, Setzer WN. 2012. Chemical compositions and biological activities of *Amomum subulatum* essential oils from Nepal. Natural Product Communications **7**:1233–1236.

Satyal P, Murray BL, McFeeters RL, Setzer WN. 2016. Essential oil characterization of *Thymus vulgaris* from various geographical locations. Foods 5 (e70) DOI: 10.3390/foods5040070.

Schmitzer V, Mikulic-Petkovsek M, Stampar F. 2019. Traditional rose liqueur–A pink delight rich in phenolics. Food Chemistry **272**:434–440.

Schneider KR, Schneider RG, Silverberg R, Kurdmongkoltham P, Bertoldi B. 2017. Preventing foodborne illness: *Bacillus cereus*. Institute of Food and Agricultural Sciences **14**:2–5.

Seervi P, Singhal H, Ingale S, Bhati A, Prazapati P. 2014. Study of traditional methods of food preservation. Indian Institute of Technology, Mandi.

Sekiyama Y, Mizukami Y, Takada A, Oosono M, Nishimura T. 1996. Effect of mustard extract vapour on fungi and spore-forming bacteria. Journal of Antibacterial and Antifungal Agents **24**:171–178.

Selmi S, Rtibi K, Hosni K, Sebai H. 2022. Essential oils: Advances in extractions and biological applications. IntechOpen, London.

Shaaban HAE, El-Ghorab AH, Shibamoto T. 2012. Bioactivity of essential oils and their volatile aroma components: Review. Journal of Essential Oil Research **24**:203–212.

Shahbazi Y, Karami N, Shavisi N. 2018. Effect of *Ziziphora clinopodioides* essential oil on shelf life and fate of *Listeria monocytogenes* and *Staphylococcus aureus* in refrigerated chicken meatballs. Journal of Food Safety 38 (e12394) DOI: 10.1111/jfs.12394.

Sırıken B, Yavuz C, Güler A. 2018. Antibacterial activity of *Laurus nobilis*: A review of literature. Medical Science and Discovery **5**:374–379.

Tisserand R, Young R. 2013. Essential oil safety: A guide for health care professionals. Elsevier Health Sciences, London.

Todd EC. 1996. Worldwide surveillance of foodborne disease: The need to improve. Journal of Food Protection **59**:82–92.

Tongnuanchan P, Benjakul S. 2014. Essential oils: Extraction, bioactivities, and their uses for food preservation. Journal of Food Science **79**:R1231–R1249.

Trinh NTT, Dumas E, Thanh ML, Degraeve P, Amara CB, Gharsallaoui A, Oulahal N. 2015. Effect of a Vietnamese *Cinnamomum cassia* essential oil and its major component trans-cinnamaldehyde on the cell viability, membrane integrity, membrane fluidity, and proton motive force of *Listeria innocua*. Canadian Journal of Microbiology **61**:263–271.

Valková V, Ďúranová H, Galovičová L, Vukovic NL, Vukic M, Kačániová, M. 2021. *In vitro* antimicrobial activity of lavender, mint, and rosemary essential oils and the effect of their vapours on growth of *Penicillium* spp. in a bread model system. Molecules 26 (e3859) DOI: 10.3390/molecules26133859.

van Schie CC, Haring MA, Schuurink RC. 2006. Regulation of terpenoid and benzenoid production in flowers. Current Opinion in Plant Biology **9**:203–208.

Vasavada MN, Dwivedi S, Cornforth D. 2006. Evaluation of garam masala spices and phosphates as antioxidants in cooked ground beef. Journal of Food Science **71**:C292–C297.

Verma RK, Kumari P, Maurya RK, Kumar V, Verma RB, Singh RK. 2018. Medicinal properties of turmeric (*Curcuma longa L.*): A review. International Journal of Chemical Studies **6**:1354–1357.

Véronique COMA. 2008. Bioactive packaging technologies for extended shelf life of meat-based products. Meat Science **78**:90–103.

Veysseyre F, Fourcade C, Lavigne JP, Sotto A. 2015. *Bacillus cereus* infection: 57 case patients and a literature review. Médecine et Maladies Infectieuses **45**:436–440.

Vidic J, Chaix C, Manzano M, Heyndrickx M. 2020. Food sensing: Detection of *Bacillus cereus* spores in dairy products. Biosensors 10 (e15) DOI: 10.3390/bios10030015.

Vihanova K, Houdkova M, Promgool T, Urbanova K, Kanokmedhakul S, Kokoska L. 2021. *In vitro* growth-inhibitory effect of essential oils and supercritical carbon dioxide extracts from *Cinnamomum* spp. barks and fruits against food bacterial pathogens in liquid and vapor phase. Journal of Food Safety 41 (e12900) DOI: 10.1111/jfs.12900.

Warburton D. 1984. Miscellaneous compounds. Pages 471–495 in Peters W, Richards WHG, editors. Antimalarial drug II: Current antimalarial and new drug developments. Springer, Heidelberg.

World Health Organization. 2000. Surveillance programme for control of foodborne infections and intoxications in Europe. Seventh report 1993–1998. Available from http://www.who.int (accessed January 10, 2024).

Yang S, Wang Y, Liu Y, Jia K, Zhang Z, Dong Q. 2023. Cereulide and emetic *Bacillus cereus*: Characterizations, impacts and public precautions. Foods 12 (e833) DOI: 10.3390/foods12040833.

Yousefi S, Weisany W, Hosseini SE, Ghasemlou M. 2023. Mechanisms of nanoencapsulation to boost the antimicrobial efficacy of essential oils: A review. Food Hydrocolloids 150 (e109655) DOI: 10.1016/j.foodhyd.2023.109655.

Zabka M, Pavela R, Slezakova L. 2009. Antifungal effect of *Pimenta dioica* essential oil against dangerous pathogenic and toxinogenic fungi. Industrial Crops and Products **30**:250–253.

Zech-Matterne V, Tengberg M, Van Andringa W. 2015. *Sesamum indicum* L.(sesame) in 2nd century BC Pompeii, southwest Italy, and a review of early sesame finds in Asia and Europe. Vegetation History and Archaeobotany **24**:673–681.

Zeuthen P, Bøgh-Sørensen L. 2003. Food preservation techniques. Elsevier, Woodhead Publishing, Cambridge.

Zhang C, Fan L, Fan S, Wang J, Luo T, Tang Y, Chen Z, Yu L. 2019. *Cinnamomum cassia* Presl: A review of its traditional uses, phytochemistry, pharmacology and toxicology. Molecules 24 (e3473) DOI: 10.3390/molecules24193473.

Zhang C, Zhao J, Famous E, Pan S, Peng X, Tian J. 2021. Antioxidant, hepatoprotective and antifungal activities of black pepper (*Piper nigrum* L.) essential oil. Food Chemistry 346 (e128845) DOI: 10.1016/j.foodchem.2020.128845.

Zhang L, Lokeshwar B. 2012. Medicinal properties of the Jamaican pepper plant *Pimenta dioica* and allspice. Current Drug Targets **13**:1900–1906.

Zielińska-Błajet M, Feder-Kubis J. 2020. Monoterpenes and their derivatives—Recent development in biological and medical applications. International Journal of Molecular Sciences 21 (e7078) DOI: 10.3390/ijms21197078.

Appendices

List of the Appendices:

Appendix 1: Photographs of plant material distillation……….…………………...…II

Appendix 2: Photographs of antimicrobial assay……………….………………. III

Appendix 1: Photographs of plant material distillation

Figure 3. The Clevenger type apparatus (Merci, Prague, Czech Republic) (Gábor 2023)

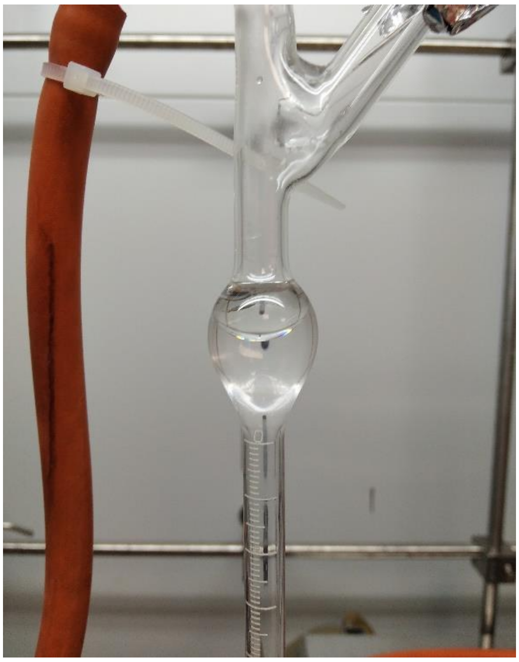

Figure 4. Sample of distilled spice essential oil in the Clevenger type apparatus (Merci, Prague, Czech Republic) (Gábor 2023)

Appendix 2: Photographs of antimicrobial assay

Figure 5. Plates and lids fastened together using clamps and wooden pads (Gábor 2023)

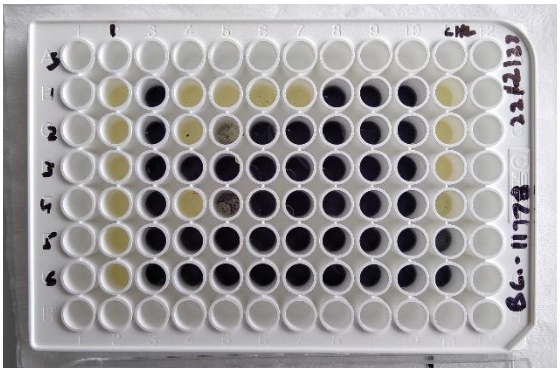

Figure 6. Determination of MIC in the 96-well microtiter plate (Gábor 2023)

Figure 7. Determination of MIC on the lid of the 96-well microtiter plate (Gábor 2023)