الجمهورية الجزائرية الديمقراطية الشعبية

وزارة التعليم العالي والبحث العلمي

Setif 1 University-Ferhat ABBAS
Faculty of Nature and Life Sciences

جامعة سطيف 1 فرحات عباس
كلية علوم الطبيعة والحياة

DEPARTEMENT OF MICROBIOLOGY

N°……/SNV/**2024**

THESIS

Presented by

Amel BOUDECHICHA

For the fulfillment of the requirements for the degree of

DOCTORATE 3RD CYCLE IN BIOLOGICAL SCIENCES

Special filed: **Applied Microbiology**

TOPIC

In vitro Control of Multidrug-resistant Bacterial Isolates and Fungi by Essential Oils and their Nanoformulations

Presented publicly in: 30/09/2024

Jury:

President	**Pr. Aicha DJIDEL**	University of Setif 1
Supervisor	**Pr. Abdelhakim AOUF**	University of Setif 1
Examinators	**Pr. Naouel BOUSSOUALIM**	University of Setif 1
	Pr. Lotfi LOUCIF	University of Batna 2
	Pr. Mohamed SIFOUR	University of Jijel

Laboratory of Applied Microbiology

ACKNOWLEDGMENTS

I would like to express my gratitude to all the members of my thesis jury, who honored me by agreeing to evaluate my work:

I would like to extend my most sincere and distinguished thanks to my thesis supervisor, Professor **Abdelhakim AOUF**, for guiding me in this work.

Professor **Aicha DJIDEL**, for the honor of accepting the presidency of the jury.

Professor **Naouel BOUSSOUALIM**, Professor **Lotfi LOUCIF**, and Professor **Mohamed SIFOUR** readily accepted to be part of my thesis jury as examiners.

I would like to express my utmost gratitude and appreciation to Professor **Amr Farouk** from the Flavor and Aroma Chemistry Department at the National Research Centre in Cairo, Egypt, for his collaboration on this work.

I would also like to thank my PhD and doctoral colleagues for the unforgettable moments we spent together, for their spontaneity, and for their friendly contributions.

Finally, I wish to express my sincere gratitude and appreciation to my family, friends, and everyone who assisted and supported me throughout the course of this work.

*

DEDICATION

I humbly dedicate this work, first and foremost, to **Allah,** whose guidance and blessings have made this journey possible

I would like to express my deepest gratitude to my family, especially to my beloved parents, **Dalila BOUGRINA and Taher BOUDECHICHA**, who have always believed in me and encouraged me to strive for the best. I would not be where I am today without your unwavering support and guidance.

This thesis is dedicated to you—it is as much yours as it is mine!

In loving memory of my dear friend and sister, Doctor **Lina ABED**, whose brilliance and kindness touched the lives of all who knew her. Though she is no longer with us, her spirit and legacy continue to inspire me every day. This thesis is dedicated to her enduring memory and the profound impact she had on my life. Forever in our hearts.

A heartfelt thank you to my sisters and brothers, especially Hadil, for their unwavering support, guidance, and encouragement throughout my journey. They have always been there for me, surrounding me with love and wisdom.

A special dedication to my uncle Tayeb CHELKIA for his caring, encouragement and support, you have been like a second father to me.

I am greatly appreciative of all the encouragement from my friends, who over the years became family.

And last but not least, as the wise Snoop Dogg once said:

"*I wanna thank me for believing in me. I wanna thank me for doing all this hard work. I wanna thank me for never quitting…*"

This would not have been possible without the blissful support of each one of you.

THANK YOU!

Finally, at the end of this research project, it is only fitting to acknowledge all those who have contributed, directly or indirectly, to the development of this thesis. I extend my sincere thanks and profound gratitude to each of you.

Amel BOUDECHICHA

List of Publications

Peer-reviewed Article

1. **Boudechicha, A**., Aouf, A., Farouk, A., Ali, H. S., Elkhadragy, M. F., Yehia, H. M., & Badr, A. N. **(2023)**. Microfluidizing technique application for Algerian Cymbopogon citratus (DC.) stapf effects enhanced volatile content, antimicrobial, and anti-mycotoxigenic properties. *Molecules*, 28(14), 5367. DOI: 10.3390/molecules28145367
2. **Boudechicha, A**., Aouf, A., Ali, H., Alsulami, T., Badr, A. N., Ban, Z., & Farouk, A. **(2024)**. Effect of Microfluidization on the Volatiles and Antibacterial, Antifungal, and Cytotoxic Activities of Algerian Satureja hortensis L. Oil-Loaded Nanoemulsions: In Vitro and In Silico Study. *ACS Omega*. DOI: 10.1021/acsomega.4c00315

Communications

1. **Boudechicha, A**., Beldi, H., Bellouche, D. Huile essentielle des feuilles de *Saccocalyx satureioides* Coss. et Durieu : Composition chimique, activité antibacterienne et activité antibiofilm. 1st International Days of Natural and Life Sciences (INDNS), Normal Higher School of Ouargla, Algeria on Mars 1st-2nd, 2022.
2. **Boudechicha, A**., Bellouche, D. Composition chimique et activité antibacterienne de l'huile essentielle de *Cymbopogon citratus*. 1er seminaire National sur : la Valorisation des Ressources Naturelles et de l'Environnement (VRNE2022), Université Ferhat ABBAS Setif, Algeria on Mars 30th, 2022.
3. **Boudechicha, A**., Beldi, H., Bellouche, D. Chemical Composition, Antibacterial Activity and Antibiofilm Activity of the Essential Oil of *Satureja hortensis* L. from Algeria. International Online Conference on Valuation of Alternative Plants, Degraded & Marginal Lands, Echahid Hamma Lakhdar University, El-oued, Algeria on May 10-11th, 2022.
4. **Boudechicha, A**., Aouf, A. L'effet des huiles essentielles d'*Origanum* sp. et *Rosmarinus* sp., ainsi que leurs nanoformulations sur la biopréservation des pleurotes. 1st International Webinar of Animal Biodiversity Protection and Environment (WIBAPE2022), National Higher School of Agronomy, Algiers, Algeria on May 26-27th, 2022.
5. **Boudechicha, A**., Aouf, A. Cytotoxicité, Caractérisation Phytochimique et Evaluation des Activités Biologiques de l'Huile Essentielle de *Cymbopogon citratus* récolté en Algérie. International Microbiology webinar, Bejaia, Algeria on June 4th, 2022.
6. **Boudechicha, A**., Aouf, A. Activité Antioxydante et Cytotoxicité de l'huile essentielle de *Thymus algeriensis*. Le Premier Séminaire National sur la Biodiversité de la Faune et la Flore en Algérie (SNBFFA-1), Université Fréres Mentouri, Constantine, Algeria on November 29-30th, 2022.
7. **Boudechicha, A**., Yamoun, N., Aouf, A. Activité antibactérienne et composition chimique de l'huile essentielle de *Thymus algeriensis*. Journée nationale sur l'antibiorésistance et environnement, Université Fréres Mentouri, Constantine, Algeria on December 12th, 2022.

ملخص

تمثل مقاومة المضادات الحيوية تهديدًا كبيرًا للصحة العامة العالمية، حيث يؤدي الإفراط في استخدام المضادات الحيوية وظهور البكتيريا المقاومة إلى تقويض العلاجات التقليدية. تقدم الجزيئات الطبيعية المستخلصة من النباتات الطبية بديلًا واعدًا في مكافحة هذه المقاومة، إذ تعمل تقنية النانوتشكيل على تحسين استقرارها وفعاليتها العلاجية. أهداف هذه الدراسة هي تقديم تحليل شامل لمقاومة المضادات الحيوية في بيئة سريرية، واستكشاف إمكانيات الزيوت الأساسية الطبيعية كعوامل مضادة للميكروبات بديلة، وتقييم فعالية تَركِيبَاتها النانوية في تعزيز الأنشطة البيولوجية. كشفت دراسة استعادية أجريت في قسم الأمراض المعدية بمستشفى سطيف الجامعي خلال المرحلة الأولى من تحقيقنا عن معدلات مقلقة لمقاومة الأموكسيسيلين (100%)، السيفازولين(70% <) ، والسيفوتاكسيم (58.06%). وكانت أكثر سلالة بكتيرية تم تحديدها هي *Escherichia coli*المنتجة لإنزيم البيتا لاكتاماز واسع الطيف(BLSE) ، والتي مثلت 29.03% من العزلات .في الجزء الثاني، تناولت الدراسة استخلاص الزيوت الأساسية (HE) من نبات *Satureja hortensis* L. (ASHEO) ونبات *Cymbopogon citratus* (DC.) Stapf (LGEO)في الجزائر باستخدام تقنية التقطير بالبخار، بالإضافة إلى استخدام تقنية الميكروفلويدايزيشن للحصول على نانوتشكيلات هذه الزيوت. تم استخدام جهاز GC–MS لإجراء مقارنة بين ASHEO و LGEOمن حيث محتواهما من المركبات الطيارة في مستحلبات النانو (MF–ASHEO و MF–LGEO). أظهرت نتائج MF–ASHEOوجود 8 مركبات (99.56%) مقارنةً بـ 26 مركبًا في ASHEO (95.46%). أما المركبات التي تم تحديدها في MF–LGEO فقد شكلت 97.53% من إجمالي محتوى الزيت في مستحلب النانو، وهو ما يشبه LGEO المستخلص بالتقطير بالبخار(97.73%). أظهرت الزيوت الأساسية ونانوتشكيلاتها خصائص مضادة للبكتيريا والبيوفيلم، حيث أظهر ASHEO و LGEO فعالية كبيرة ضد عزلات بكتيرية ممرضة من الجراثيم الموجبة والسالبة لصبغة جرام. أظهر ASHEO نشاطًا ضد جميع السلالات البكتيرية المختبرة، مع مناطق تثبيط تتراوح بين 55.66 مم و29.66 مم، بينما تم تثبيط تكوين البيوفيلم بواسطة *E. coli* و*P. aeruginosa* بنسبة تزيد عن 60%، وأظهر LGEO تثبيطًا كاملًا لسلالة *Bacillus subtilis*.تم التحقق من التأثيرات المبيدة للبكتيريا والمثبطة لنموها لمركب الكارفاكرول على البروتينات المستهدفة باستخدام تحليل ADME والنمذجة *in silico* .بالإضافة إلى ذلك، أظهرت الزيوت الأساسية، وخاصةMF–LGEO ، خصائص مضادة للفطريات ضد الفطريات المفرزة للسموم مثل سلالات *Aspergillus*، *Penicillium*، وFusarium، مما يؤثر على معدلات نموها ويقترح إمكانية استخدامها كعلاج للتلوث الفطري السام. كما كشفت الدراسة أن الزيوت الأساسية ونانوتشكيلاتها أظهرت سمية خلوية واعدة ضد ثلاث سلالات خلوية، مع تعزيز تأثيرات مضادة للالتهابات بواسطة تقنية الميكروفلويدايزيشن، حيث ارتبطت هذه النتائج بتعديلات في مكونات النانوتشكيل. أظهرت الزيوت الأساسية التي تم دراستها تأثيرات مضادة للأكسدة معتدلة في تثبيط الجذور الحرة، مع قيمة IC50 بلغت 536.47 ± 21.99 ميكروغرام/مل لـ ASHEO، وقيمة IC50 بلغت 82.87 ± 2.15 ميكروغرام/مل لـ .LGEO كان لتقنية الميكروفلويدايزيشن تأثير عميق على محتوى المركبات الطيارة والنشاط البيولوجي للزيت .تدعم نتائجنا استخدام نباتي *S. hortensis* و*C. citratus* لاستخراج زيوتهما الأساسية الثمينة، خاصةً للوقاية من الأمراض الفطرية السامة والالتهابات البكتيرية المقاومة للمضادات الحيوية.

كلمات مفتاحية: *Satureja hortensis* L. ؛ *Cymbopogon citratus* (DC.) Stapf؛ الزيوت الأساسية؛ التشكيل النانوي؛ مضاد للبكتيريا؛ مضاد للفطريات؛ مضاد للأكسدة؛ سمية للخلايا؛ *in silico*.

Abstract

Antibiotic resistance is one of the biggest global public health threats. The abuse of antibiotics and the emergence of resistant strains of bacteria undermine the effectiveness of conventional treatments. Natural molecules extracted from medicinal plants offer a promising alternative to combating this resistance, with nanoformulation improving their bioavailability and therapeutic effectiveness. A retrospective study conducted at the department of infection diseases at CHU Setif during the initial phase of our investigation revealed alarmingly high rates of resistance to amoxicillin (100%), cefazoline (>70%), and cefotaxime (58.06%). *Escherichia coli* ESBL represented the most common bacterial strain identified (29.03%). In the second part, the study examined the extraction by hydrodistillation of essential oils (EOs) from Algerian *Satureja hortensis* L. (ASHEO) and *Cymbopogon citratus* (DC.) Stapf (LGEO), and the microfluidization technique was used to get their nanoformulations. The GC-MS apparatus was utilized for a comparative examination of ASHEO and LGEO with their microfluidization nanoemulsions (MF-ASHEO and MF-LGEO) volatile content. MF-ASHO showed 8 compounds (99.56%) vs ASHEO's 26 compounds (95.46%). The identified components in MF-LGEO represented 97.53% of the total nanoemulsion oil, which was similar to the hydrodistilled LGEO (97.73%). The essential oils and nanoformulations showed antibacterial and antibiofilm properties, while ASHEO and LGEO showed superior efficacy against pathogenic isolates of Gram-positive and Gram-negative bacteria. ASHEO effectively exhibited activity against all tested bacterial strains with inhibition zones measured between 55.66 mm and 29.66 mm, and the biofilm formation of *E. coli* and *P. aeruginosa* was suppressed by over 60%, while LGEO demonstrated complete inhibition of *B. subtilis*. The bactericidal and bacteriostatic effects of carvacrol on the target proteins were validated by ADME and *in silico* analyses. Additionally, essential oils, especially MF-LGEO, showed antifungal properties against mycotoxigenic fungi including Aspergillus, Penicillium and Fusarium strains by influencing their growth rates and suggesting potential treatment for toxigenic fungal contamination. The study also found that essential oils and nanoformulations showed promising cytotoxicity against three cell line strains, with microfluidization enhancing anti-inflammatory effects, and these findings were linked to alterations in nanoformulation components. Studied essential oils revealed moderate antioxidant effects in radical scavenging, with an IC50 value of 536.47 ± 21.99 $\mu g.mL^{-1}$ for ASHEO and an IC50 value equal to (82.87 ± 2.15 $\mu g.mL^{-1}$). The microfluidization procedure has a profound impact on both the volatile content and biological activity of the oil. Our findings reassure the use of *S. hortensis* and *C. citratus* for their valuable essential oils and to prevent fungal toxigenic diseases and pathogenic-resistant bacteria.

Key words: *Satureja hortensis* L.; *Cymbopogon citratus* (DC.) Stapf; essential oils microfluidization; antibacterial; antifungal; antioxidant; cytotoxicity; *in silico.*

Résumé

La résistance aux antibiotiques constitue une menace majeure pour la santé publique mondiale, l'abus d'antibiotiques et l'émergence de bactéries résistantes compromettant les traitements conventionnels. Les molécules naturelles extraites de plantes médicinales offrent une alternative prometteuse dans la lutte contre cette résistance, et la nanoformulation permet d'améliorer leur stabilité et leur efficacité thérapeutique. Les objectifs de cette étude sont de fournir une analyse complète de la résistance aux antibiotiques en milieu clinique, d'explorer le potentiel des huiles essentielles naturelles en tant qu'agents antimicrobiens alternatifs, et d'évaluer l'efficacité de leurs nanoformulations pour améliorer les activités biologiques. Une étude rétrospective menée au département des maladies infectieuses du CHU de Sétif au cours de la phase initiale de notre enquête a révélé des taux alarmants de résistance à l'amoxicilline (100 %), à la céfazoline (> 70 %), et à la cefotaxime (58,06 %). La souche bactérienne la plus fréquemment identifiée était *Escherichia coli* productrice de BLSE, représentant 29,03 % des isolats. Dans la deuxième partie, l'étude a porté sur l'extraction par hydrodistillation des huiles essentielles (HE) de *Satureja hortensis* L. (ASHEO) et de *Cymbopogon citratus* (DC.) Stapf (LGEO) d'Algérie, avec l'utilisation de la technique de microfluidisation pour obtenir leurs nanoformulations. L'appareil GC-MS a permis de réaliser un examen comparatif des ASHEO et LGEO avec leur teneur en composés volatils dans les nanoémulsions issues de la microfluidisation (MF-ASHEO et MF-LGEO). MF-ASHEO a révélé 8 composés (99,56 %) contre les 26 composés identifiés dans ASHEO (95,46 %). Les composants identifiés dans MF-LGEO représentaient 97,53 % du total de l'huile dans la nanoémulsion, un résultat similaire à celui de l'huile hydrodistillée LGEO (97,73 %). Les huiles essentielles et leurs nanoformulations ont démontré des propriétés antibactériennes et antibiofilm, avec ASHEO et LGEO montrant une efficacité supérieure contre des isolats pathogènes de bactéries Gram-positives et Gram-négatives. ASHEO a montré une activité contre toutes les souches de bactéries testées, avec des zones d'inhibition mesurées entre 55,66 mm et 29,66 mm, et la formation de biofilms par *E. coli* et *P. aeruginosa* a été inhibée à plus de 60 %, tandis que LGEO a montré une inhibition totale de *Bacillus subtilis*. Les effets bactéricides et bactériostatiques du carvacrol sur des protéines cibles ont été validés par des analyses ADME et *in silico*. De plus, les huiles essentielles, en particulier MF-LGEO, ont démontré des propriétés antifongiques contre des champignons mycotoxigènes, y compris des souches d'*Aspergillus*, *Penicillium* et *Fusarium*, influençant leur taux de croissance et suggérant un potentiel thérapeutique pour le traitement de la contamination fongique toxique. L'étude a également révélé que les huiles essentielles et leurs nanoformulations avaient une cytotoxicité prometteuse contre trois lignées cellulaires, la microfluidisation renforçant les effets anti-inflammatoires, ces résultats étant liés à des modifications des composants de la nanoformulation. Les huiles essentielles étudiées ont montré des effets antioxydants modérés dans la capture des radicaux libres, avec une valeur IC50 de 536,47 ± 21,99 $\mu g.mL^{-1}$ pour ASHEO et une valeur IC50 de 82,87 ± 2,15 $\mu g.mL^{-1}$ pour LGEO. La procédure de microfluidisation a un impact profond sur la teneur en composés volatils et l'activité biologique des huiles. Nos résultats soutiennent l'utilisation de *S. hortensis* et *C. citratus* pour leurs huiles essentielles précieuses, notamment pour la prévention des maladies fongiques et des infections causées par les bactéries multirésistantes.

Mots clés : *Satureja hortensis* L. ; *Cymbopogon citratus* (DC.) Stapf ; huiles essentielles ; microfluidisation ; antibactérien ; antifongique ; antioxydant ; cytotoxicité ; *in silico.*

Table of content

Literature Review

Experimental Work

I. Material and Methods

II. Results and Discussion

List of Abbreviations

ADME: Absorption, Distribution, Metabolism, and Excretion

AFNOR : Association Française de la Normalisation

AMR: Antimicrobial Resistance

ARGs: Antibiotic Resistance Genes

ASHEO: Algerian *S. hortensis* essential oil

ATCC: American Type Culture Collection

BHT: Butylated hydroxyanisole

CLSI: Clinical Laboratory Standards Institute

CMC: Carboxymethyl cellulose

DMEM: Dulbecco's Modified Eagle Medium

DMSO: Dimethyl sulphoxide

DPPH: 2,2′-diphenyl-1-picrylhydrazyl

EOs: Essential Oils

EPR: Enhanced permeation and retention

ESBL: Extended Spectrum Beta-lactamases

GC-MS: Gas Chromatography-Mass Spectrometry

HPLC: High-Performance Liquid Chromatography

HUVECs: Human umbilical vein endothelial cells

IC50: Half-maximal inhibitory concentration

LC-MS: liquid chromatography coupled to mass spectrometry

LG: Lemon grass

LGEO: Lemon grass essential oil

MBC: Minimum Bactericidal Concentration

MDR: Multidrug Resistance

MF-ASHEO: Microfluidized Algerian *S. hortensis* essential oil

MFC: Minimal Antifungal Concentration

MF-LGEO: Microfluidized Lemon grass essential oil

MIC: Minimum Inhibitory Concentration

MRSA: Methicillin-resistant *Staphylococcus aureus*

MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

OM: Outer membrane

PDI: polydispersity index

PK: Pitted keratolysis

QS: Quorum sensing

RI: Retention indices

ROS: Reactive Oxygen Species

SD: Standard deviation

TEM: Transmission Electron Microscopy

TM: Traditional Medicines

TPs: Transformation products

TTC: 2,3,5-triphenyl-2H-tetrazolium chloride

VRE: Vancomycin-resistant enterococci

VRSA: Vancomycin-resistant *S. aureus*

WHO: World Health Organization

List of Figures

List of Tables

Introduction

Introduction

"*I did not invent penicillin. Nature did that. I only discovered it by accident* " is a quote attributed to Alexander Fleming, the renowned Scottish bacteriologist best known for his discovery of penicillin. The accidental discovery of this antibiotic underscores its significant influence, as it stands as one of the pivotal medical breakthroughs of the 20th century. Although Fleming was not the originator of penicillin, his fortunate discovery sparked a revolutionary period in medicine, ultimately leading to the saving of numerous lives. Pathogenic germs have posed an enduring danger throughout history, leading to multiple outbreaks and worldwide pandemics, necessitating this requirement. A series of scientific investigations and observations, including those of Pasteur, Joubert, and Duchesne, have fueled this pursuit, which has ultimately resulted in the discovery of numerous antibiotics (Shama, 2016).

The promising landscape that these anti-infectives presented has gradually reduced in less than a half of century since their first use. Over the past 40 years, microbial infections have become increasingly recurrent due to the progressive emergence of antimicrobial resistance (AMR). When this resistance occurs to multiple drugs of more than three classes, it is known as multidrug resistance (MDR) (Mancuso *et al*., 2021). The overuse of antibiotics, mutations, the exchange of resistance genes, the propagation of resistant clones, and other factors are the primary drivers of MDR development (Catalano *et al*., 2022). Presently, antimicrobial resistance is responsible for at least 700,000 deaths annually worldwide. Without the development of new and improved treatments, the World Health Organization (WHO) (2018) warns that this figure could skyrocket to 10 million by 2050, underscoring a significant global health threat.

The recent advancement in the treatment of AMR applies various options, such as combinatorial drug approaches, plant-derived products, bio-nanotechnology approaches, and

many others. In recent years, there has been an increasing interest in natural product-based medicines from plant origin. In contemporary times, there has been a notable surge in scholarly attention towards medicinal remedies derived from natural products of botanical provenance. Plants represent a significant reservoir of biologically active secondary metabolites, harboring substantial therapeutic promise (Atanasov *et al.*, 2021). Estimates from the World Health Organization (WHO, 2018) indicate that almost 80% of the population in Africa and 40% of China's population depend on traditional plant-based medicines as an essential component of their basic healthcare routine.

Medicinal plants are a valuable source of diverse bioactive secondary metabolites, which can be utilized in the creation of groundbreaking medicinal medicines that offer unique health advantages. Algeria is a rich reservoir of diverse medicinal and aromatic plants. The chemo-diversity and therapeutic effects of Algerian plants vary from those of the same plants found in other regions and climates due to the unique geographical position of the country (Selwal *et al*., 2023). Various aromatic plants cultivated in Algeria have been identified as potential sources for the production of essential oils (EOs) (Benziane *et al*., 2023). Therefore, EOs have emerged as a compelling resource for discovering novel, potent, and secure bioactive compounds that offer a wide range of therapeutic advantages, particularly in terms of antioxidant and antibacterial properties (Mohamed and Alotaibi, 2023).

Plants belonging to groups such as Boraginaceae, Lamiaceae, and Poaceae are grown worldwide for their bioactive components, including EOs, alkaloids, and polyphenols (Llinares *et al*., 2021). These oils, such as *Satureja hortensis* (Summer savory) and *Cymbopogon citratus* (Lemongrass), which are commonly used, serve as natural defenses against predators and environmental stressors. These plants possess abundant EOs including biologically active compounds like thymol and geranial, which have antibacterial effects (Rezende *et al*., 2022).

In this context, we were interested in studying two plants of the Algerian flora, represented by *Satureja hortensis* L. and *Cymbopogon citratus* (DC.) Stapf, collected in Boussaada, M'Sila.

The objectives of this study are to provide a comprehensive analysis of antibiotic resistance within a clinical setting, explore the potential of natural EOs as alternative antimicrobial agents, and assess the efficacy of their nanoformulations in enhancing biological activity. By examining the antibacterial and antifungal properties of these formulations, the study aims to identify promising natural alternatives that could contribute to addressing the growing threat of multidrug-resistant pathogens.

Literature Review

Literature Review

1. Antibiotics

1.1. Antibiotic's History and Classification

Antibiotics are an important class of medications that are extensively used in healthcare. These antimicrobial medicines have dual functions, serving as preventive measures (prophylactic) and as treatments for diseases caused by various microorganisms, such as bacteria and fungi. The word "antibiotic" usually refers to drugs that have the ability to either kill or inhibit the growth of bacteria (Yang *et al*., 2021).

In the annals of medical history, the advent of the first antibiotic, Salvarsan, in 1910, heralded a transformative epoch. Over a span slightly exceeding a century, antibiotics have exerted a profound influence on modern medicine, contributing to a remarkable extension of the average human lifespan by 23 years. The watershed moment occurred with the discovery of penicillin in 1928 by the English Bacteriologist Sir Alexander Fleming, instigating a golden age of natural product antibiotic exploration that reached its zenith in the mid-1950s. Subsequent years have witnessed a gradual wane in the discovery and development of antibiotics, accompanied by the emergence of drug resistance in numerous human pathogens. Virtually all classes of antibiotics were discovered during a "golden age" that extended from 1936 to 1962 (Hutchings *et al*., 2019) (Figure 01).

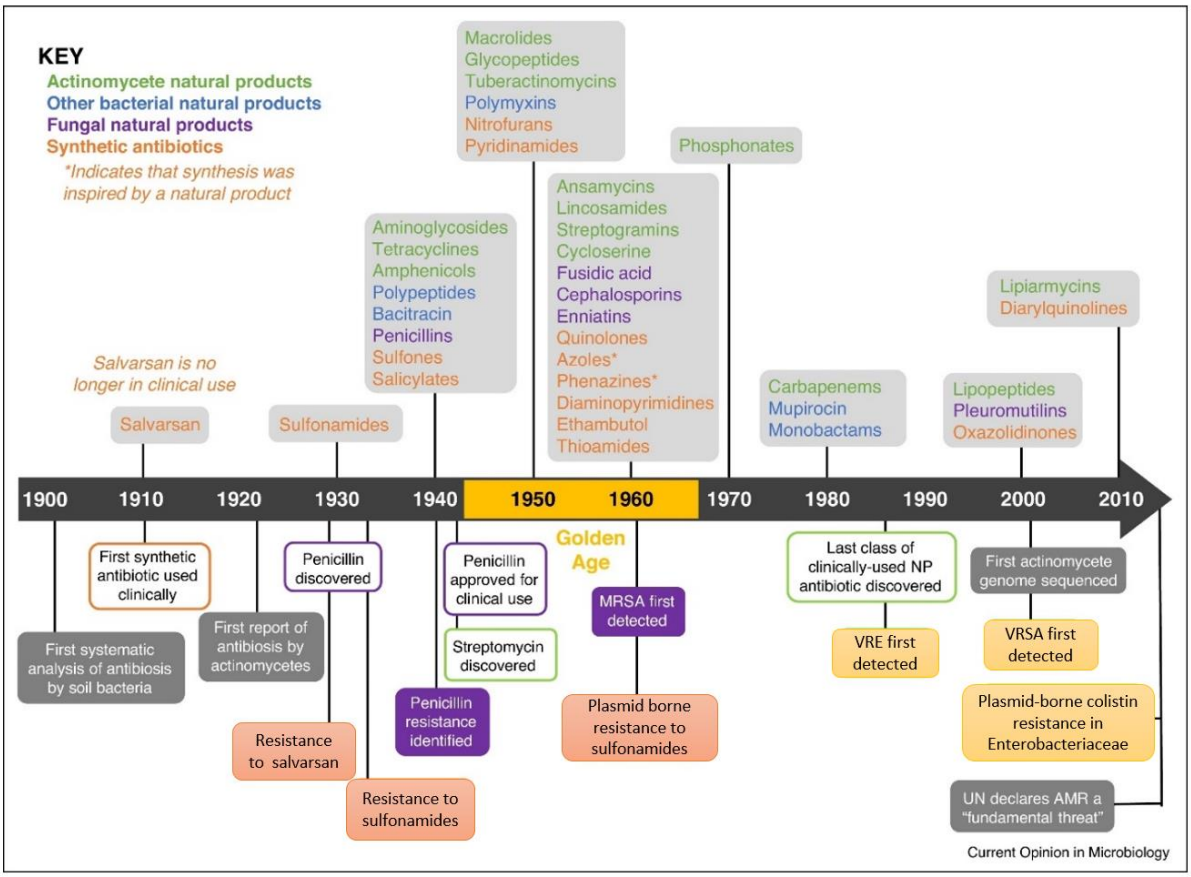


Figure 01: Timeline showing the decade new classes of antibiotic reached the clinic (Hutchings *et al*., 2019). The antibiotics are colored per their source: green = actinomycetes, blue = other bacteria, purple = fungi and orange = synthetic. At the bottom of the timeline are key dates relating to antibiotic discovery and antimicrobial resistance, including the first reports of drug resistant strains methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), vancomycin-resistant *S. aureus* (VRSA) and plasmid-borne colistin resistance in Enterobacteriaceae.

Antibiotics can be classified in various ways, but the most commonly used schemes center around their molecular structures, mode of action, and spectrum of activity (Calderón and Sabundayo, 2007). Another classification criterion is the route of administration, which includes injectable, oral, and topical antibiotics.

1.1.1. Antibiotics' Classification Based on Source and Mechanism of Action

Considering the origin of antibiotics, they can be categorized into three main groups: (i) natural compounds derived from microorganisms, (ii) semi-synthetic variants that involve structural modifications of natural products, and (iii) entirely synthetic products. While natural antibiotics such as benzylpenicillin, cephalosporins, and gentamicin present a significant drawback due to their high toxicity, semi-synthetic antibiotics like ampicillin and amikacin, as well as synthetic antibiotics such as moxifloxacin and norfloxacin, demonstrate an enhanced therapeutic effect and lower toxicity when compared to their natural counterparts (Pancu *et al*., 2021). Several antibiotics, such as Chloramphenicol, are classified simultaneously as natural, semi-synthetic, and synthetic (Rai and Kosalec, 2022).

The various structures of antibiotics are closely related to distinct mechanisms of action (figure 02). Earlier research identified primary bacterial targets for antibiotics, including cell wall synthesis, protein synthesis, cell membrane function, and nucleic acid synthesis. (Ullah *et al*., 2017).

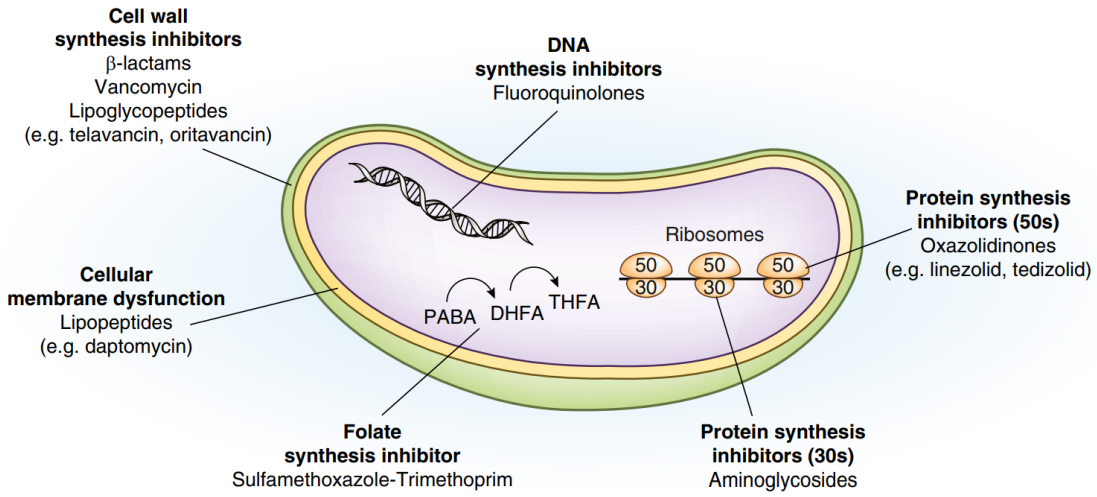


Figure 02: Bacterial targets and action mechanisms of different antibiotics (Eyler and Shvets, 2019).

It is essential to consider the comprehensive overview provided in the (Table 01).

Table 01: All classes of clinically used antibiotics and their source (Hutchings *et al*., 2019).

Class[a]	**Discovery reported**[b]	**Introduced clinically**	**Example (and producing organism)**	**Molecular Target**
Antibiotics from Actinomycetes				
Aminoglycosides	1944	1946	Kanamycin A (*Streptomyces kanamyceticus*)	Protein synthesis: 30S ribosomal subunit
Tetracyclines	1948	1948	Tetracycline (*Streptomyces aureofaciens*)	Protein synthesis: 30S ribosomal subunit
Amphenicols	1947	1949	Chloramphenicol (*Streptomyces venezuelae*)	Protein synthesis: 50S ribosomal subunit
Macrolides	1952	1952	Erythromycin (*Saccharopolyspora erythraea*)	Protein synthesis: 50S ribosomal subunit
Tuberactinomycins	1951	1953	Viomycin (*Streptomyces puniceus*)	Protein synthesis: 30S and 50S ribosomal subunits (binds to the Intersubunit bridge B2a)
Glycopeptides	1954	1958	Vancomycin (*Amycolatopsis orientalis*)	Cell wall synthesis: D-Ala-D-Ala termini of lipid II
Lincosamides	1962	1963	Clindamycin (Semi-synthetic derivative of lincomycin (*Streptomyces lincolnensis*)	Protein synthesis: 50S ribosomal subunit
Ansamycins	1959	1963	Rifamycin SV Semi-synthetic derivative of rifamycin (*Amycolatopsis rifamycinica*)	Nucleic acid synthesis: RNA polymerase

Cycloserines	1954	1958	Seromycin (*Streptomyces orchidaceus*)	Cell wall synthesis: inhibition of alanine racemase and D-alanine-D-alanine ligase
Streptogramins	1953	1965	Pristinamycin (*Streptomyces pristinaespiralis*)	Protein synthesis: 50S ribosomal subunit
Phosphonates	1969	1971	Fosfomycin (*Streptomyces fradiae*)	Cell wall synthesis: MurA (UDP-GlcNAc3-enolpyruvyltransferase) inhibition
Carbapenems	1976	1985	Meropenem Synthetic molecule based on thienamycin (*Streptomyces cattleya*)	Cell wall synthesis: penicillin-binding proteins
Lipopeptides	1987	2003	Daptomycin (*Streptomyces roseosporus*)	Cell wall: cell membrane disruption
Lipiarmycins	1975	2011	Fidaxomicin (*Dactylosporangium aurantiacum* subsp. *hamdenesis*)	Nucleic acid synthesis: RNA polymerase
Antibiotics from other bacteria				
Polypeptides	1939	1941	Gramicidin A (*Bacillus brevis*)	Cell wall: forms ion channels that increase the permeability of the bacterial cell membrane
Bacitracin	1945	1948	Bacitracin A (*Bacillus subtilis*)	Cell wall synthesis: inhibition of dephosphorylation of C_{55}-isoprenyl pyrophosphate
Polymyxins	1950	1959	Colistin (*Paenibacillus polymyxa*)	Cell wall: cell membrane disruption
Mupirocin	1971	1985	Mupirocin (*Pseudomonas fluorescens*)	Protein synthesis: isoleucyl t-RNA synthetase

Monobactams	1981	1986	Aztreonam Synthetic molecule based on SQ 26,180 (*Chromobacterium violaceum*)	Cell wall synthesis: penicillin-binding proteins
Antibiotics from fungi				
Penicillins	1929	1943	Amoxicillin Semi-synthetic derivative of penicillin (*Penicillium chrysogenum*)	Cell wall synthesis: penicillin-binding proteins
Fusidic acid	1958	1962	Fusidic acid (*Fusidium coccineum*)	Protein synthesis: elongation factor G
Enniatins[c]	1953	1963	Fusafungine (*Fusarium lateritium*)	Cell wall: cell membrane disruption
Cephalosporins	1948	1964	Cefacetrile Semi-synthetic derivative of cephalosporin C (*Acremonium chrysogenum*)	Cell wall synthesis: penicillin-binding proteins
Pleuromutilins	1951	2007	Retapamulin Semi-synthetic derivative of pleuromutilin (*Pleurotus mutilus*)	Protein synthesis: 50S ribosomal subunit
Synthetic antibiotics				
Arsphenamines[d]			Salvarsan	Not known
Sulfonamides	1907	1910	Mafenide	Folate synthesis: inhibition of dihydropteroate synthetase
Salicylates[e]	1931	1936	4-Aminosalicylic acid	Folate synthesis: prodrug that inhibits dihydrofolate reductase
Sulfones	1908	1945	Dapsone	Folate synthesis: inhibition of

				dihydropteroate synthetase
Pyridinamides	1952	1952	Isoniazid	Cell wall: prodrug that inhibits the synthesis of mycolic acids
Nitrofurans	1945	1953	Nitrofurantoin	DNA synthesis: DNA damage
Azoles[f]	1959	1960	Metronidazole	DNA synthesis: DNA damage
(Fluoro) quinolones	1962	1962	Ciprofloxacin	DNA synthesis: inhibition of DNA gyrase, and topoisomerase IV
Diaminopyrimidines	1950	1962	Trimethroprim	Folate synthesis: inhibition of dihydrofolate reductase
Ethambutol	1962	1962	Ethambutol	Cell wall: arabinosyl transferase inhibition
Thioamides	1956	1965	Ethionamide	Cell wall: prodrug that inhibits the synthesis of mycolic acids
Phenazines[f]	1954	1969	Clofazimine	DNA synthesis: binds to guanine bases
Oxazolidinones	1987	2000	Linezolid	Protein synthesis: 50S ribosomal subunit
Diarylquinolines	2004	2012	Bedaquiline	ATP synthesis: proton pump inhibition

[a] Classes are defined by origin, structure and/or mechanism of action, which distinguishes between bacitracin, colistin and daptomycin, for example.

[b] Year reported refers to first report in literature.

[c] The European Medicines Agency recommended the withdrawal of fusafungine from the market in February 2016.

[d] Salvarsan is no longer in clinical use.

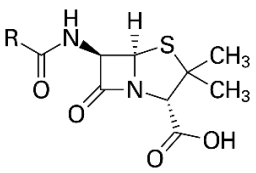
[e] Salicylic acids are found in nature, but this was not the source of this class of antibiotic.

[f] Compound synthesis was inspired by natural antibiotic classes**.**

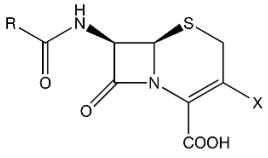
1.1.2. Antibiotics' Classification Based on Chemical Structure

Antibiotics belonging to identical structural classifications commonly exhibit analogous profiles regarding efficacy, toxicity, and potential allergic reactions. Notable categories delineated by chemical or molecular architectures encompass beta-lactams, macrolides, tetracyclines, quinolones, aminoglycosides, sulphonamides, glycopeptides, and oxazolidinones (Frank and Tacconelli, 2012) ; Adzitey, 2015).

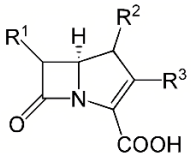
The fundamental structural element common to all 56 beta-lactam antibiotics is the four-membered beta-lactam ring. Bacterial beta-lactamases catalyze the cleavage of this ring, leading to the inactivation of the antibiotic. The primary basis for the antibacterial efficacy of these compounds lies in the intactness of the beta-lactam ring (Chambers *et al*. 1995). (Figure 03) presents the general chemical structures of the main antibiotic classes.



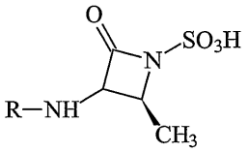
Penicillins



Cephalosporins

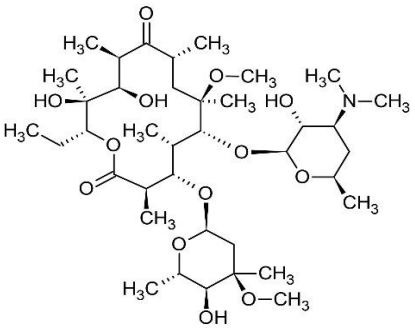


Carbapenems

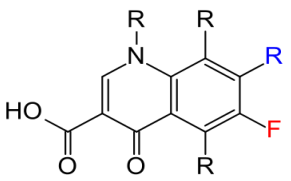


Monobactams

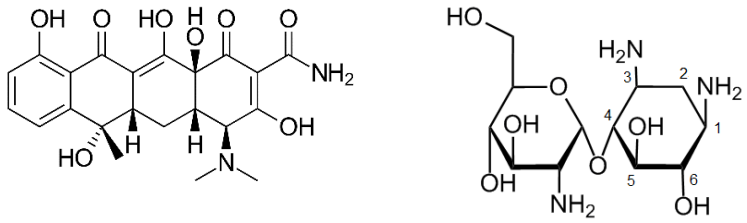
β-lactams



Macrolides



Quinolones



Tetracyclines Aminoglycosides

Figure 03: Chemical Structures of Main Antibiotic Classes (de Souza Mendes and de Souza Antunes, 2013).

1.1.3. Antibiotics' Classification by the Type of Pharmacological Effects

Antibiotics exhibit either bactericidal or bacteriostatic effects, serving as a basis for their classification. Bactericidal compounds induce bacterial cell death by impeding processes such as cell wall synthesis, cell membrane function, or protein synthesis (Walsh, 2003). Examples within this category encompass β-lactams, aminoglycosides, glycopeptides, ansamycins, quinolones, streptogramins, lipopeptides, and macrolides. In contrast, bacteriostatic agents hinder bacterial cellular activity and growth without causing cell death. This category includes sulfonamides, tetracyclines, chloramphenicol, oxazolidinones, and macrolides (Ullah *et al*., 2017).

1.1.4. Antibiotics' Classification Based on the Spectrum of Activity

Antibiotics vary in their spectrum of activity, influencing the range of microorganisms they target. Extended spectrum antibiotics, like tetracyclines, combat bacteria, rickettsiae, and protozoa. Broad spectrum antibiotics, exemplified by quinolones, are effective against both Gram-positive and Gram-negative bacteria. On the other hand, narrow spectrum antibiotics, such as macrolides, specifically target either Gram-positive or Gram-negative bacterial

organisms (Pancu *et al*., 2021). This spectrum concept guides clinicians in selecting antibiotics based on the precise nature of the infection.

1.1.5. Antibiotics' Classification Based on Route of Administration

Antibacterial agents are also classified based on the mode of administration. Topical antibiotics are applied directly to body surfaces, parenteral antibiotics are administered via injection, and oral antibiotics are taken orally (Zhou *et al*., 2020). The selection of administration routes is influenced by factors like convenience, patient compliance, as well as the pharmacokinetics and pharmacodynamic properties of the drug.

1.2. Antibiotics' Resistance

1.2.1. Types of Resistance

The ability of microorganisms to oppose the effects of antimicrobial drugs is known as antibiotic resistance, and it arises when an antibiotic becomes less effective at preventing bacterial growth (Nadeem *et al*., 2020). The four principal forms of antibiotic resistance evolve as:

A. Natural Resistance (Intrinsic, Structural)

In this form of resistance, the development of resistance is not linked to antibiotic use but is instead attributed to the structural properties of bacteria (Kadhum Abu Gulel and Hasan, 2019). This phenomenon arises due to intrinsic resistance, wherein microorganisms deviate from the target antibiotic structure, or when antibiotics, due to their specific characteristics, fail to interact with their intended targets (Waglechner and Wright, 2017). An illustrative example is observed with Gram-negative bacteria and vancomycin; the outer membrane hinders the movement of vancomycin antibiotics, rendering these Gram-negative bacteria naturally resistant to vancomycin (Antonoplis *et al*., 2019).

B. Acquired Resistance

Apart from the resistance that arises from modifications in the genetic characteristics of bacteria, another kind of resistance occurs when bacteria lose their ability to respond to antibiotics that they were previously susceptible to. This acquired resistance can arise from modifications in the main chromosome or additional chromosome structures such as plasmids and transposons (Andersson *et al*., 2020).

Chromosomal resistance is a consequence of mutations occurring randomly in the bacterial chromosome, which can be induced by various physical and chemical factors. These mutations may lead to changes in the composition of bacterial cells, resulting in decreased drug permeability or alterations to the drug's target within the cell (Majeed and Aljanaby, 2019).

Extrachromosomal genetic materials are necessary for extrachromosomal resistance and can be transferred by integrons, transposons, and plasmids. Plasmids are DNA segments that can replicate independently of chromosomal DNA (Thomas and Frost, 2021). Typically, plasmids are responsible for the production of antibiotic-inactive enzymes.

Resistance to antibiotics like streptomycin, aminoglycosides, erythromycin, and lincomycin can develop in response to these forms of chromosomal resistance (Krause *et al*., 2016).

C. Cross-resistance

It refers to the resistance exhibited by specific microorganisms against a particular antibiotic, often operating through identical or related mechanisms and extending to resistance against other antibiotics. This phenomenon is commonly observed when antibiotics share structural similarities, such as resistance to erythromycin, neomycin, kanamycin, or resistance to cephalosporins and penicillins (Etebu and Arikekpar, 2016). However, cross-resistance can also manifest between entirely different drug groups, as seen in cases like erythromycin-

lincomycin, and this resistance may have a chromosomal origin or not (Hasan and AL-Harmoosh, 2020).

D. Multi-drug Resistance (MDR)

Multidrug-resistant organisms possess the ability to resist the impact of various antimicrobial medications belonging to different chemical classes or subclasses simultaneously, employing a range of strategies. Various bacterial species, isolated from diverse clinical specimens, exhibited indications of employing one or more strategies to resist antimicrobial agents as described in (figure 04) (Fair and Tor, 2014).

Either one or both of these mechanisms can induce multidrug resistance in bacteria. Initially, bacteria may accumulate multiple genes, each encoding drug resistance, within a single cell, typically on resistance (R) plasmids. Alternatively, increased expression of genes responsible for multidrug efflux pumps, expelling a variety of medicines, can lead to multidrug resistance. Antibiotic-resistant bacteria have the ability to transfer copies of DNA specifying defense mechanisms to other bacteria, even those of closely related species. Subsequently, these recipient bacteria can transmit the resistant genes, giving rise to new generations of antibiotic-resistant bacteria. This phenomenon is referred to as horizontal gene transfer (Okoye *et al*., 2022).

Bacterial strains are classified as multidrug-resistant (MDR) if they demonstrate resistance to three or more types of antimicrobials. Species that exhibit resistance to all but one or two classes of antibiotics are considered highly resistant to medicines. If a species is resistant to all available antibiotics, it is termed pan-drug resistant (Stanford *et al*., 2020).

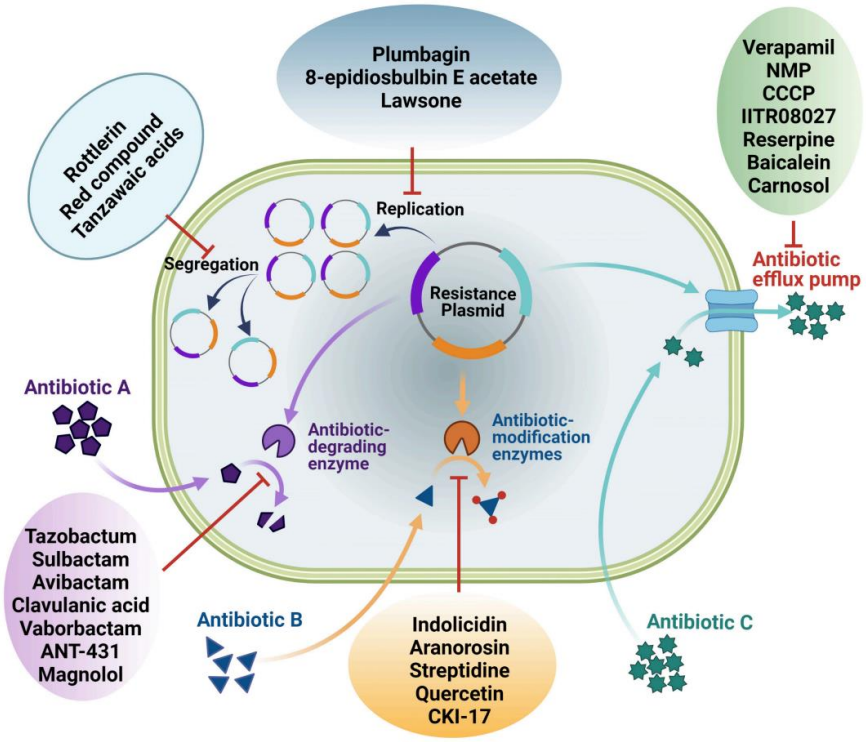


Figure 04 : Illustration depicts multidrug resistance (MDR) mechanisms bacteria (Chawla *et al*., 2022).

1.2.2. Mechanisms of Antibiotics Resistance

Four main categories can be used to classify antimicrobial resistance mechanisms: (1) prevention of cell penetration; (2) expulsion via efflux pumps; (3) degradation or modification (inactivating proteins); (4) modification of drug targets. Gram-negative bacteria employ all four main mechanisms, while gram-positive bacteria less frequently employ the strategy of limiting drug uptake (due to the absence of an LPS outer membrane) and lack certain types of drug efflux mechanisms (Reygaert, 2018) (as elaborated on in later sections of this manuscript). (Figure 05) illustrates a comprehensive overview of the molecular mechanisms causing antibiotic resistance.

A. Prevention of Cell Penetration

This mechanism arises from alterations in the permeability of both the internal and external membranes, leading to reduced drug uptake into the cell or swift expulsion through pump systems (Hasan and AL-Harmoosh, 2020). This is especially important for Gram-negative bacteria because of their double membrane structure, which makes the cellular envelope relatively impermeable. Because of this intrinsic resistance, it is difficult to develop new antimicrobials that can penetrate the cell envelope, in contrast antibiotics, which are effective against Gram-positive pathogens (Darby *et al*., 2023). A decrease in membrane permeability can occur due to mutations in porin proteins found in resistant strains. For instance, a mutation in specific porins, such as *Opr*D, can confer resistance to carbapenems in *Pseudomonas aeruginosa* strains (Li *et al*., 2012).

B. Expulsion via Efflux Pumps

Furthermore, even in the case where an antibiotic is able to pass through the outer membrane (OM), it can be swiftly expelled out of the cell by a variety of broad-spectrum efflux pumps, which may result in resistance. These pumps use the energy from the hydrolysis of ATP or the proton motive force to pump out chemicals from the cell (Bhowmik *et al*., 2023). The active pump systems in the tetracycline class of antibiotics are the most typical route via which resistance develops. Tetracyclines are expended and unable to concentrate inside the cell by an energy-dependent active pumping system. (Li *et al*., 2020).

This mechanism of resistance is in plasmid and chromosomal control. For instance, active pumping systems are efficient in countering quinolones, 14-membered macrolides, chloramphenicol, and beta-lactams (Guo *et al*., 2020).

C. Degradation or Modification (inactivating proteins)

Bacteria employ two primary methods to render drugs inactive: through the modification of the drug or by transferring a chemical group to the drug. Since most bacteria produce enzymes that break down antibiotics, one of the most significant pathways for resistance to antibiotics is enzymatic inactivation (Schaenzer and Wright, 2020). The β-lactamases constitute a vast category of enzymes that hydrolyze drugs. Tetracycline is another drug susceptible to inactivation through hydrolysis, facilitated by the *tetX* gene (Tooke *et al*., 2019).

Additionally, there are many different transferases identified; acetylation is the most commonly used mechanism. Acetylation is used against a variety of drugs, such as aminoglycosides, chloramphenicol, streptogramins, and fluoroquinolones. Phosphorylation and adenylation are mainly utilized against aminoglycosides (Murina *et al*., 2018) .

D. Modification of Drug Targets

Antimicrobial agents can target various components within bacterial cells, and bacteria, in turn, can modify an equal number of these targets to develop resistance against the drugs. The target regions of the interaction with the antibiotics and the modifications that occur in the drug-related receptor are different; these target regions may involve complex enzymes and ribosomes (Martinez, 2014).

Primarily, one mechanism through which gram-positive bacteria, develop resistance to β-lactam drugs involves modifications in the structure and/or quantity of penicillin-binding proteins (PBPs). PBPs serve as transpeptidases crucial in the formation of peptidoglycan within the cell wall. Variations in the number of PBPs, such as an increase in those with reduced drug-binding capability or a decrease in PBPs with normal drug binding, can influence the effectiveness of drug binding to the target. Structural changes, exemplified by the acquisition

of the *mecA* gene leading to PBP2a alteration in *S. aureus*, can diminish the drug's ability to bind or entirely prevent drug binding (Larsen *et al*., 2022).

Drug resistance targeting ribosomal subunits can arise through ribosomal mutation (e.g., aminoglycosides, oxazolidinones), methylation of ribosomal subunits (e.g., aminoglycosides, macrolides in gram-positive bacteria, oxazolidinones, streptogramins), often associated with erm genes, or ribosomal protection (e.g., tetracyclines). These mechanisms disrupt the drug's capacity to bind to the ribosome. The extent of drug interference significantly differs across these mechanisms (Roberts, 2004 ; Giuliodori *et al*., 2018).

In the case of drugs aimed at inhibiting nucleic acid synthesis, such as fluoroquinolones, resistance occurs through alterations in DNA gyrase (e.g., *gyr A* in gram-negative bacteria) or topoisomerase IV (e.g., *grl A* in gram-positive bacteria). These mutations induce structural changes in gyrase and topoisomerase, diminishing or completely preventing the drug's capacity to bind to these components (Mathur *et al*., 2021; Bhatt and Chatterjee, 2022).

Resistance to drugs that target metabolic pathways typically arises through mutations occurring in enzymes critical to the folate biosynthesis pathway, such as DHPS (dihydropteroate synthase) and DHFR (dihydrofolate reductase), or via the overproduction of resistant forms of these enzymes (e.g., sulfonamides-resistant DHPS, trimethoprim-resistant DHFR). Sulfonamides and trimethoprim exert their effects by binding to these enzymes, effectively mimicking the structures of the natural substrates (sulfonamides mimic p-amino-benzoic acid, trimethoprim mimics dihydrofolate). These drugs act through competitive inhibition by binding to the active site of the enzymes. Mutations in these enzymes are most often located in or around the active site, leading to structural alterations that impede drug binding while still permitting the natural substrate to bind (Smilack, 1999; Huovinen, 2001).

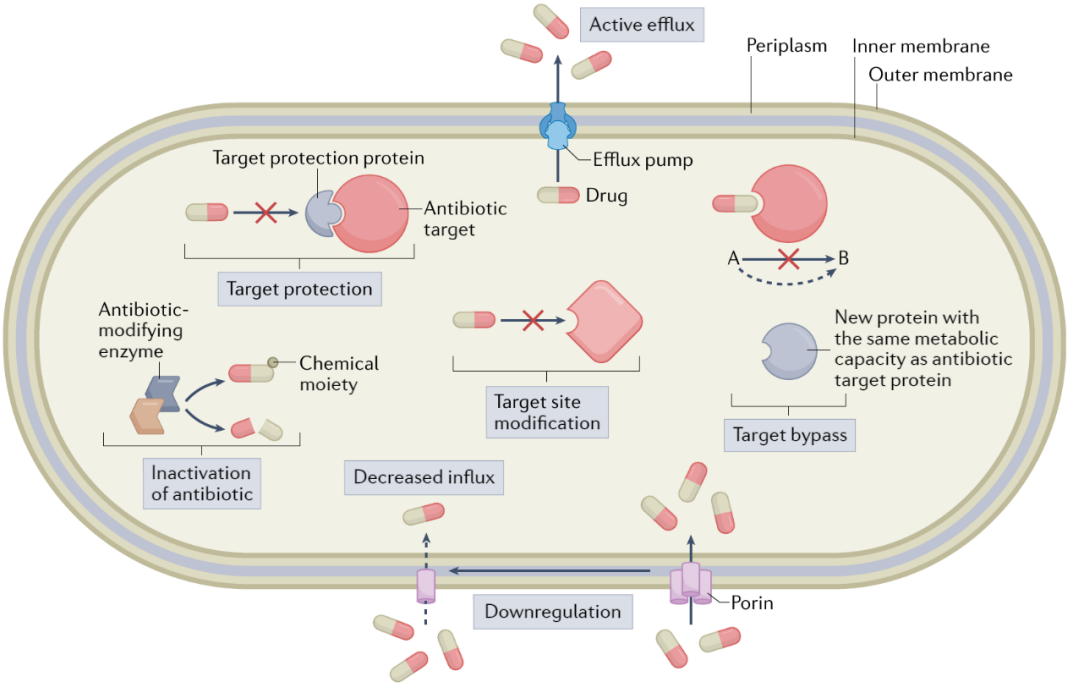


Figure 05: Overview of the molecular mechanisms of antibiotic resistance (Darby *et al*., 2023).

1.3. Antibiotic's Resistance from Biofilms

Bacterial biofilms refer to collective gatherings of microbes enclosed within a polymeric matrix, typically situated on a surface (Davies, 2003). These biofilms, accounting for a significant portion (65%) of infections and contributing to antibiotic treatment failures, are associated with diverse infections. They play a role in various conditions such as medical device and implant-related infections (Khatoon *et al*., 2018), chronic infections, as well as ailments affecting the lungs, bladder, wounds, dental structures, skin, ears, nose, throat, sinus, and orthopedic regions (Hancock *et al*., 2021).

Formation of bacterial biofilms occurs in five main phases as shown in (Figure 06): (1) adherence to a surface by free-moving (planktonic) cells, (2) essentially irreversible surface attachment and the creation of a protective polymeric extracellular matrix, (3) development of cell clusters embedded within the biofilm matrix (maturation I stage), (4) growth and maturation

of microcolonies (maturation II stage), and (5) detachment and dispersal of some bacterial cells permitting new biofilm foci to form (Hancock *et al*., 2021; Sauer *et al*., 2022).

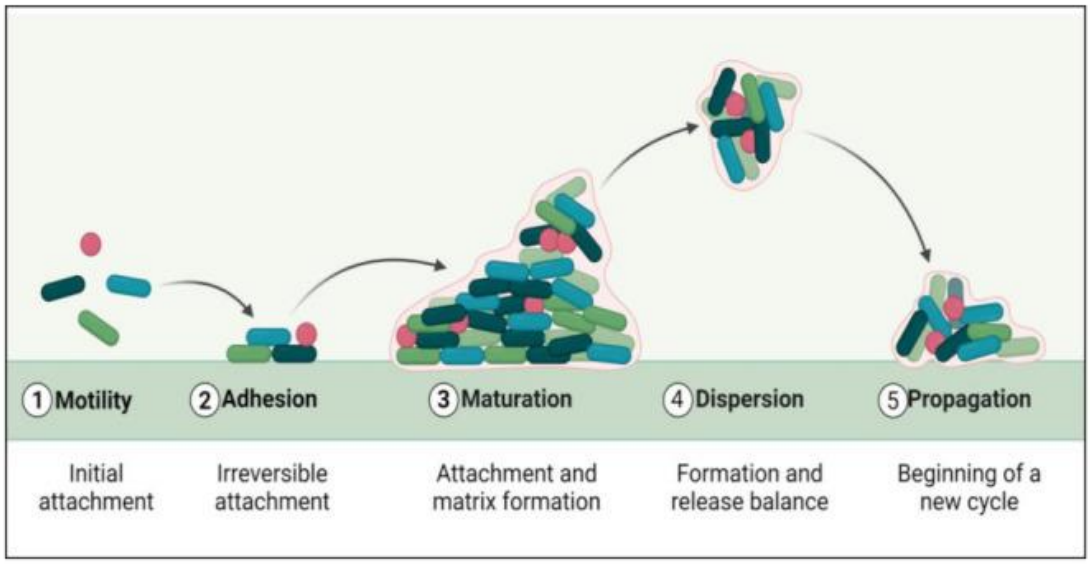


Figure 06: Phases of biofilm formation (Asma *et al*., 2022).

Hundreds of genes, including dozens of regulators, control the substantial changes in growth state and cellular metabolism that occur during these phases. Biofilm cells, which are considered a stress adaptation, have distinctive transcriptional and proteomic profiles that allow them to withstand harsh environmental conditions and demonstrate resilience against a variety of stressors, including antibiotics (Hancock *et al*., 2021).

Moreover, biofilms elude the host's defense mechanisms, enhancing their capacity to endure and instigate chronic infections. In addition to the inherent genetic resistance carried by specific bacteria, biofilms exhibit an adaptive resistance that is approximately 10–1000 times greater compared to their planktonic counterparts against most antibiotics. Upon dispersal from biofilms, bacteria revert to their usual susceptibility (Davies, 2003).

2. Essential Oils (EOs)

2.1. Definition

Aromatherapy got its name from the combination of "aroma," referring to fragrance or smell, and "therapy," indicating treatment. This boasts a rich history spanning thousands of years. Even Hippocrates, considered the father of modern medicine, endorsed aromatherapy, emphasizing the importance of aromatic baths and scented massages for overall well-being. Over time, influential figures in the realm of EOs, which are volatile, concentrated, and aromatic extracts obtained from plant materials (flowers, roots, stems, leaves, seeds, bark, wood), defended aromatherapy as a legitimate therapy for enhancing the health of the mind, body, and spirit (Farrar and Farrar, 2020).

A definition of EOs was proposed in October 1987 by AFNOR (Association Française de la Normalisation), it designates "*A product obtained from plant material, either by steam distillation, mechanical processes from the peel of Citrus fruits, or by dry distillation. The essential oil is then separated from the aqueous phase through physical processes for the first two methods, and it may undergo physical treatments that do not result in significant changes to its composition*".

Essential oils exhibit solubility in alcohol, ether, and fixed oils, while remaining insoluble in water. Typically, these volatile oils are in a liquid state and colorless at room temperature, possessing a distinctive scent. They commonly have a density lower than unity, except for certain instances such as cinnamon, sassafras, and vetiver. Particularly, these oils demonstrate a refractive index and exceptionally high optical activity. Found in various herbs, these volatile oils contribute to the diverse scents emitted by plants. Their applications extend to the cosmetics industry, perfumery, and aromatherapy (Dhifi *et al*., 2016).

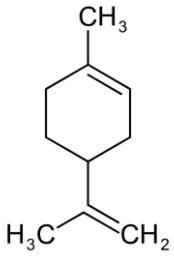
2.2. Chemical Composition of EOs

Essential oils, which are substances produced from aromatic plants, are characterized by a chemical composition that is often complex and highly diverse yet analyzable. Some of its compounds are consistently present, while others are secreted when the plant undergoes stress, such as infection, damage, encounters with predators, or changes in weather conditions. The components in EOs can exhibit variability based on factors like harvest timing, cultivar, geographical origin, and even the extraction method (Angane *et al*., 2022).

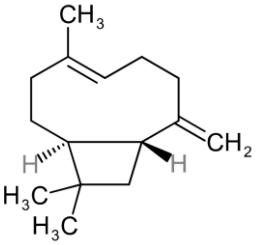
Determining the most potent compounds within EOs can be a challenging task. The most commonly utilized techniques for identifying the chemical composition of EOs include gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) , high-performance liquid chromatography (HPLC) (Ambrosio *et al*., 2019) ; Deng *et al*., 2020), and liquid chromatography coupled to mass spectrometry (LC-MS) (Turek and Stintzing, 2013).

The main chemical constituents of EOs are terpenes and polyphenols. They have specific origins in primary metabolic precursors and are generated via diverse biosynthetic pathways (de Sousa *et al*., 2023). (Figure 07) shows the structural formula of some of the major components of EOs. These chemical compounds have been reported to have biological properties and their mechanisms of action are discussed later.

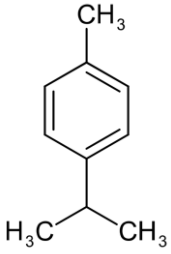
Terpenes and terpenoids



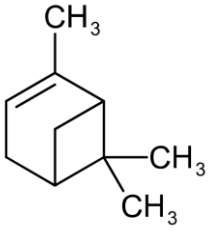
Limonene



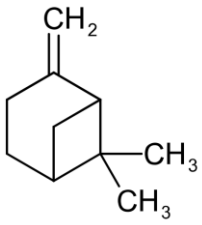
β-caryophyllene



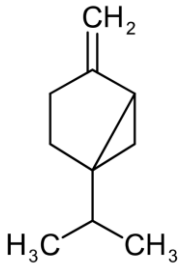
ρ-cymene



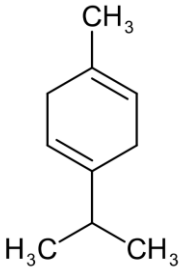
α-pinene



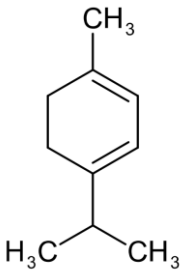
β-pinene



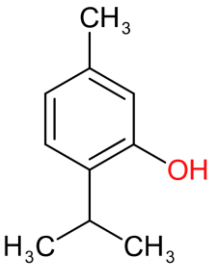
Sabinene



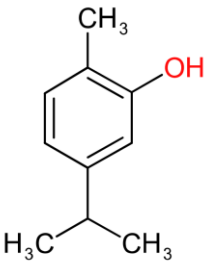
γ-terpinene



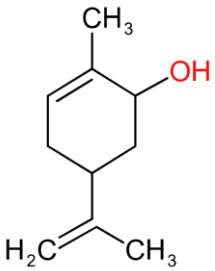
α-terpinene



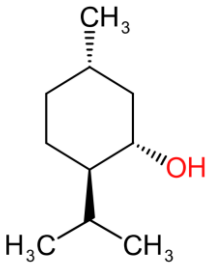
Thymol



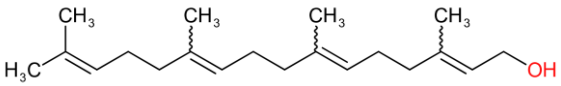
Carvacrol



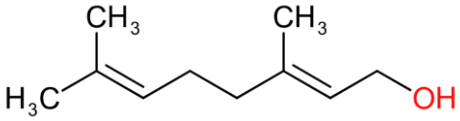
Carveol



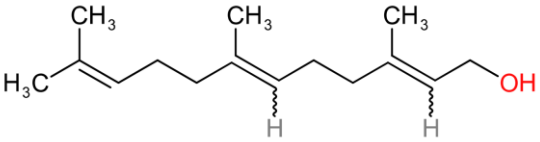
Menthol



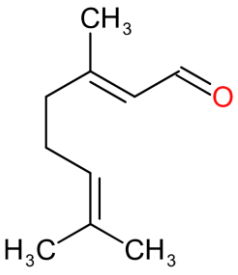
Geranylgeraniol



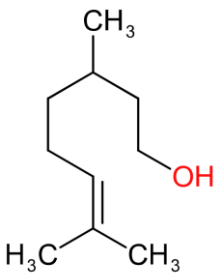
Geraniol



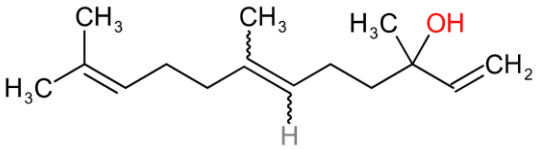
Farnesol



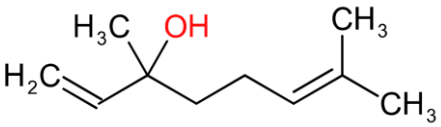
Citral



Citronellol

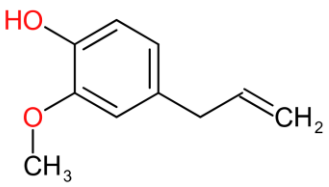


Nerolidol

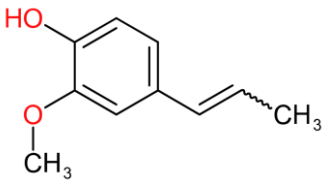


Linalool

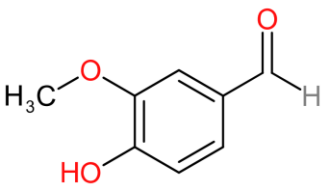
Phenylpropenes



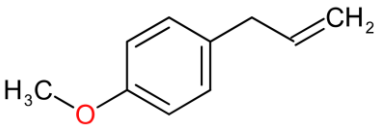
Eugenol



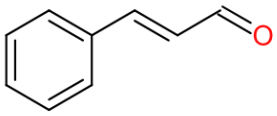
Isoeugenol



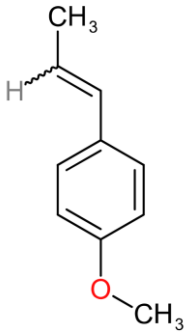
Vanillin



Estragole

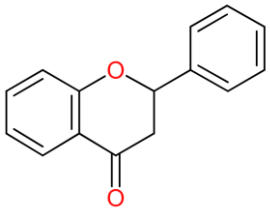


Cinnamaldehyde

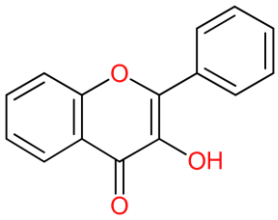


Anethole

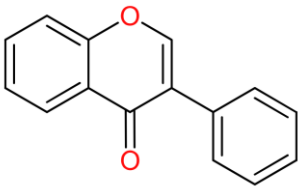
Flavonoids



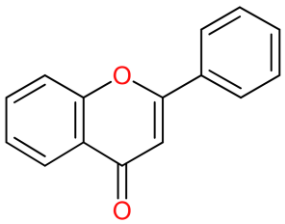
Flavanones



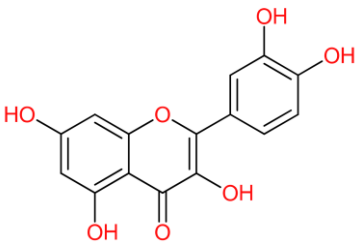
Flavonols



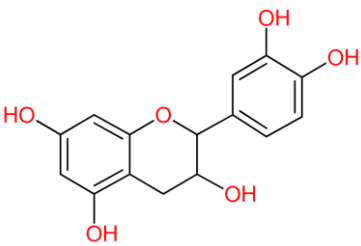
Isoflavones



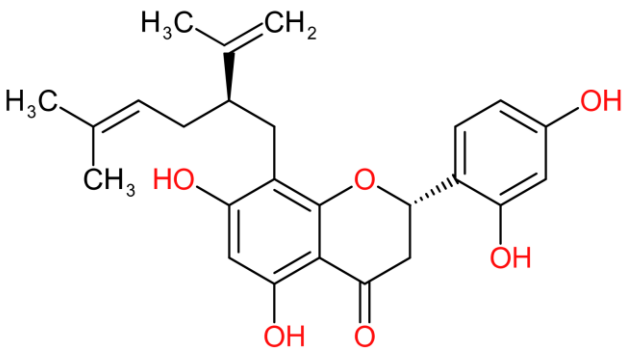
Flavones



Quercetin



Catechins



Sophoraflavanone G

Figure 07: Structural formula of some of the major components of EOs (Angane *et al*., 2022).

- **Terpenes and Terpenoids**

Terpenes are hydrocarbons, whereas terpenoids represent a modified category of terpenes featuring functional groups containing oxygen, such as ketone, hydroxy, aldehyde, ether, or carboxylic moieties. The chemical structures of terpenes can vary from linear to mono- or polycyclic compounds, with their backbone formed by the condensation of two to numerous 5-carbon-base (C5) units, known as isoprene units. Monoterpenes (from the Latin mono, or one) are the most representative and simple terpenes (Tian *et al*., 2019). Examples of terpenoids include linalool, menthol, geraniol, carvacrol, citronellal, linalyl acetate, piperitone, and thymol (Masyita *et al*., 2022). These biologically active compounds exhibit diverse effects, such as anticancer (Kamran *et al*., 2022), antibacterial (Guimarães *et al*., 2019), and antioxidant properties (Gutiérrez-del-Río *et al*., 2021).

- **Phenylpropenes**

Phenylpropanoids form a category of organic compounds synthesized by plants through the shikimate pathway, with their biogenetic precursor being the aromatic amino acid L-phenylalanine. The core structure of phenylpropanoids consists of a phenyl ring connected to a C3 propane moiety. Although present in the plant kingdom, they are less common compared to terpenes. Instances of phenylpropanoids, including compounds like eugenol, estragole,

isoeugenol, myristicin, cinnamaldehyde, and vanillin, serve as examples in this context (Masyita *et al*., 2022). Recent research points to the anticancer properties of anethole (Contant *et al*., 2021). Additionally, myristicin has been recognized for its antiproliferative and anti-inflammatory effects (Abdullah *et al*., 2018). Furthermore, vanillin has been associated with diverse biological activities, encompassing antidiabetic, antimicrobial, analgesic, and antifungal effects (Olatunde *et al*., 2022).

- **Flavonoids**

The majority of flavonoids have been classified as therapeutic agents. They are naturally produced through the phenylpropanoid pathway, and their bioactivity relies on the absorption mechanism and bioavailability (Shkondrov *et al*., 2017). Flavonoids are categorized into various subgroups, such as flavones, flavonols, flavanones, and others, based on their degree of oxidation. They find applications in various domains, including natural dyes, cosmetics, and skincare products, as well as anti-wrinkle skin agents (Ullah *et al*., 2020).

- **Other Constituents**

Essential oils comprise various amino acid derivatives, including alanine, isoleucine, leucine, valine, and methionine. While polyketides, lipids, and sulfur derivatives are infrequently present in EOs, examples include jasmonic acid, methyl jasmonate, cis-jasmone, (Z)-3-hexenal, and allicin (Dajic Stevanovic *et al*., 2020).

2.3. Biological Activities of EOs

2.3.1. Antibacterial Activity

The antibacterial impact of EOs may be observed through either inhibiting cell growth or inducing cell-killing, though discerning between these mechanisms is difficult. The effectiveness of EOs against microorganisms relies on factors such as their chemical

composition, environmental conditions, and the structures of the target bacteria, whether Gram-positive or Gram-negative (Fancello *et al*., 2016). Various *in vitro* techniques, including determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) through methods like broth macro dilution/microdilution or agar disk/well diffusion, are employed to assess the efficacy of antimicrobial compounds. Agar disk/well diffusion and broth macro dilution/microdilution, which are frequently used in clinical microbiology laboratories, have been recognized as useful methods for assessing the antibacterial activity of EOs (Balouiri *et al*., 2016).

Essential oils have surfaced as a promising alternative for addressing microbial infections, sparking interest in the exploration of novel therapeutic compounds that disrupt quorum sensing (QS) mechanisms and inhibit biofilm formation (Martínez *et al*., 2021).

2.3.2. Antifungal Activity

The use of EOs emerges as a potential direction in addressing the serious challenges encountered in the treatment of fungal infections, marked by increased resistance due to the extensive application of antifungal agents. This recognition has prompted the need for alternative, nonconventional approaches to formulate effective antifungal treatment strategies (Abd Rashed *et al*., 2021). Several research investigations have demonstrated the effectiveness of EOs in treating fungal infections; however, not all studies have delved into the fundamental mechanisms of their actions. The predominant parameter utilized in assessing antimicrobial properties is the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), representing the lowest concentration of antifungal agents required to inhibit fungal growth or lethally affect mycetes, respectively (Natu and Tatke, 2019); (Sharifi-Rad *et al*., 2017).

2.3.3. Antioxidant Activity

Over the past few years, there have been reports on the considerable effectiveness of herbal antioxidant products in treating various diseases. EOs have various modes of action as antioxidants, such as combining with free radicals generated by the damaged mitochondrial membrane to produce reactive phenoxy radicals. These radicals' further combine with reactive oxygen species (ROS), preventing additional damage (Tit and Bungau, 2023). The use of numerous EOs with antioxidant properties as natural antioxidants has become an area of significant interest, particularly in the fields of food science and medicine. For example, EOs of citrus and salvia exhibit significant antioxidant potentials (Agarwal *et al*., 2022 ; Mot *et al*., 2022).

2.3.4. Anti-inflammatory Activity

Inflammation is a normal protective response induced by the body in the presence of harm or infection to eliminate damaged or dead cells. During this process, there is a cascade activation of enzymes, adhesion molecules, and pro-inflammatory cytokines. In addition to their antioxidant and antimicrobial properties, EOs also exhibit anti-inflammatory activity by inhibiting pro-inflammatory mediators. Their interactions are associated with signaling cascades involving cytokines, transcription factors, and the expression of pro-inflammatory genes (Spisni *et al*., 2020).

Several investigations have been carried out to examine the influence of EOs on inflammation in the colon (Zhang *et al*., 2017); (Rezayat *et al*., 2018).

2.3.5. Antiproliferative Activity

The exploration of plants used in different forms of traditional medicine has unveiled several valuable drugs, including taxol, camptothecin, vincristine, and vinblastine, as revealed by

scientific studies (Loizzo *et al*., 2008). Numerous studies indicate that specific EOs can influence the activity of carcinogen-metabolizing enzymes (Blowman *et al*., 2018). Moreover, these oils demonstrate potential in chemoprevention (Magalhães *et al*., 2021), exhibit antitumor properties (Machado *et al*., 2022), and have the capacity to induce apoptosis in various cancer cell lines (Mohamed Abdoul-Latif *et al*., 2023).

2.4.Methods for Extracting EOs

Several techniques are used to isolate EOs from various plant materials, including hydrodistillation, solvent extraction, cold expression, and enfleurage. Each method present unique characteristics and drawbacks in terms of quality, efficiency, and cost.

2.4.1. Hydrodistillation

Hydrodistillation is the simplest and most common method of extracting EOs, first described by Avicenna (980-1037) when he extracted pure essential oil from rose using the alembic. It can be used to extract EOs from whole plants or specific plant parts such as flowers, roots, leaves, or stems. This hydrodistillation technique, equipped with a Clevenger-type apparatus (figure 08), involves immersing the plant material in water and then heating it in a flask. The water vapor, directed over the plants in a distillation chamber, flows towards a condenser until the release of volatile aromatic compounds. The steam and EOs are then collected in the condenser, where they naturally separate. The steam is recovered for reuse, while the EOs can be collected in an alcoholic solution ready for bottling (Khan *et al*., 2023).

However, this procedure is characterized by significant energy consumption and high temperatures (100° C). Therefore, due to its increasing interest in recent years, several modules have been developed and improved, such as microwave-assisted accelerated hydrodistillation, microwave-assisted compressed hydrodistillation, microwave-assisted hydrodistillation, and vacuum microwave hydrodistillation (Singh Chouhan *et al*., 2019).

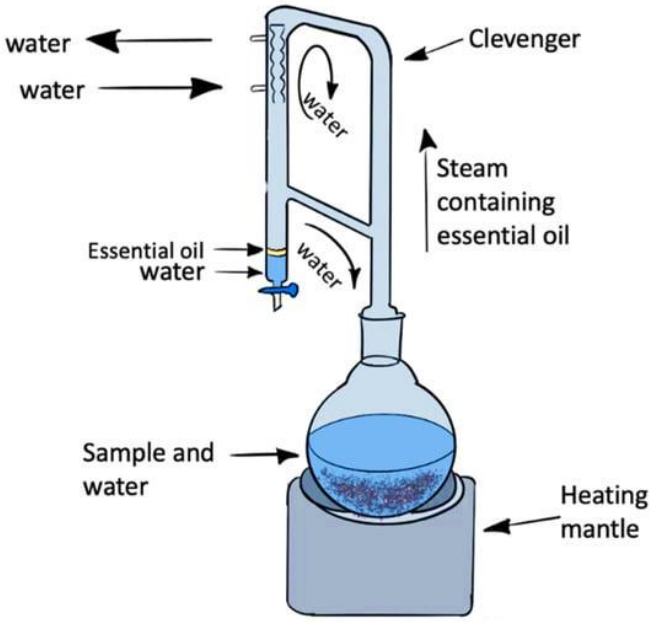


Figure 08: Schematic representation of hydro distillation (Teresa-Martínez *et al*., 2022)**.**

2.4.2. Solvent Extraction

Solvent extraction, also known as solid-liquid extraction, is an ancient technique that underwent development in the 11th century by modifying the conditions during the blending process with a solvent. The techniques involved in this process include maceration, infusion digestion, decoction, and percolation. Soxhlet extraction emerged in the 18th century as a further advancement. In maceration, powdered plant materials are combined with a solvent; if the solvent is cold or boiling water, it is referred to as an infusion. In digestion, powdered plant materials are mixed with a solvent similarly to maceration but with gentle heating as shown in (Figure 09). Decoction is an extraction method where powdered plant materials are mixed with water and boiled. Percolation involves mixing powdered plant materials with a solvent in a percolator (Zhang *et al*., 2018).

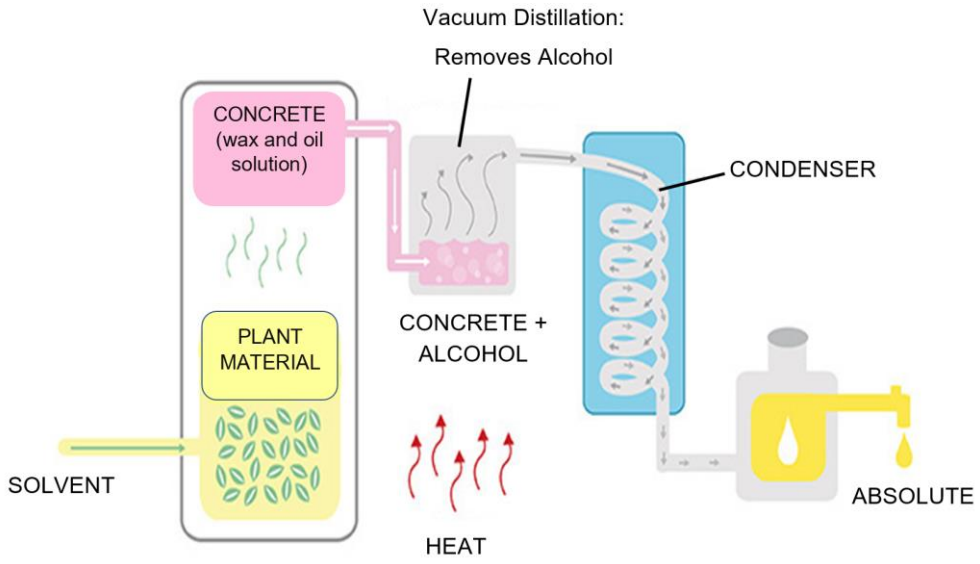


Figure 09: Schematic of solvent extraction method (Asfaw, 2022).

2.4.3. Cold Pressing or Mechanical Pressing

Cold extraction, also known as cold pressing, is a technique suitable for plants that contain significant amounts of EOs in their peels, such as citrus fruits (orange, grapefruit, and lemon). Its operating principle involves pressing the citrus peels, which are torn by needles, creating compressed areas in the skin that encourage the EOs to emerge and be collected by a collector (Çakaloğlu Ebcim *et al*., 2018). The EOs is then separated by centrifugation, dried with anhydrous sodium sulfate, and stored in the dark at 4°C until analysis time (Figure 10).

The use of significant amounts of water in this technique can alter the qualities of EOs through hydrolysis, the dissolution of oxygenated compounds, and the transportation of microorganisms. Consequently, manufacturers are actively exploring methods to eliminate the reliance on water in such extractions. Thus, to avoid these alterations, new conventional physical processes have emerged. They are based on the rupture of oil sacs through bursting under the influence of a vacuum, or the use of the principle of abrasion of fresh bark (Durazzo *et al*., 2022).

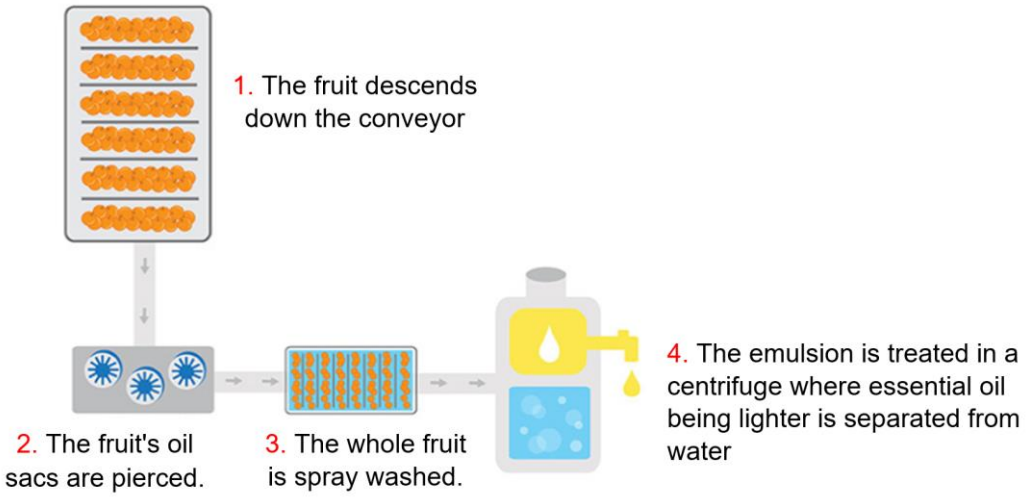


Figure 10: Schematic diagram of the cold press oil extraction setup (Asfaw, 2022).

2.4.4. Efleurage

Enfleurage stands as another traditional extraction technique with ancient origins, primarily employed for extracting EOs from flowers like jasmine. In this method, a purified and odorless cold fat is applied to plant material, such as flowers as exposed in (Figure 11). The fragrances emitted by the flowers are then absorbed by the fat. The process involves replacing old flowers with new ones, and this cycle is repeated over extended periods until the fat reaches saturation. Subsequently, the saturated fat is collected and subjected to extraction using alcohol. In contemporary terms, this method is characterized as time-consuming, labor-intensive, and expensive. It lacks practical applications for EOs in the food industry and is essentially considered obsolete today (Stratakos and Koidis, 2016).

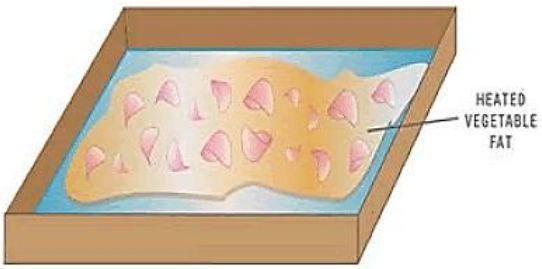


Figure 11: Schematic representation of efleurage extraction method (Asfaw, 2022).

2.4.5. Supercritical CO_2 Extraction

The extraction of EOs using carbon dioxide is an extraction process that employs supercritical gases to extract volatile compounds from plants. The uniqueness of this technique lies in the high quality of EOs coupled with a short extraction duration, as it does not require the use of chemical solvents, leaving no residues in the EOs. This technique is widely regarded as the most efficient and environmentally friendly among various extraction methods. Supercritical CO_2 is a fluid with properties of both a liquid and a gas, providing excellent extraction capabilities with specific pressure and temperature. The carbon dioxide penetrates plant cells to extract aromatic compounds without damaging the plant and without leaving residues in the elimination phase (Yang and Hu, 2014). The schematic representation of supercritical CO2 extraction is illustrated in (Figure 12).

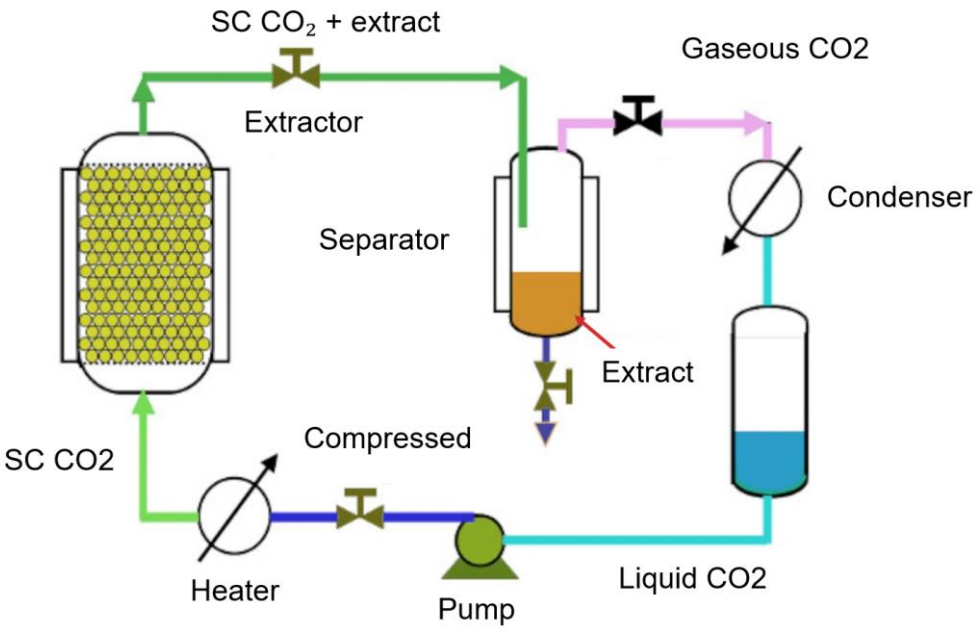


Figure 12: Schematic representation of supercritical CO_2 extraction (Asfaw, 2022).

2.5. Nanoformulations of EOs

Essential oils show promising properties, but before considering them as an alternative disease control system, we must address challenges related to their volatility in the environment, low water solubility, and susceptibility to oxidation. The application of nanoformulation to EOs could address these issues by safeguarding them against degradation and evaporation losses. This approach enables a controlled release of the products and facilitates their handling (Sedaghat Doost *et al*., 2020).

Nanoemulsions play a crucial role in improving drug delivery owing to their nanometer-sized particles (ranging from 50 to 1000 nm), extensive surface area, enhanced stability, optical transparency, controlled release, and favorable flow properties. Researchers have successfully formulated various nanoemulsions based on EOs, demonstrating heightened antimicrobial and antibiofilm potential (Ullah *et al*., 2022).

Nevertheless, Nanoemulsions can be generated through different emulsification methods, encompassing oil-in-water (O/W) or water-in-oil (W/O), as well as multiple emulsions like water-in-oil–water (W/O/W) or oil-in-water-in-oil (O/W/O), as illustrated in (Figure 13). The creation of nanoemulsions containing EOs is achievable through high-energy processes such as high pressure, microfluidization, or sonication, and low-energy processes including spontaneous emulsion, phase inversion, and emulsion inversion point (Hunde *et al*., 2023).

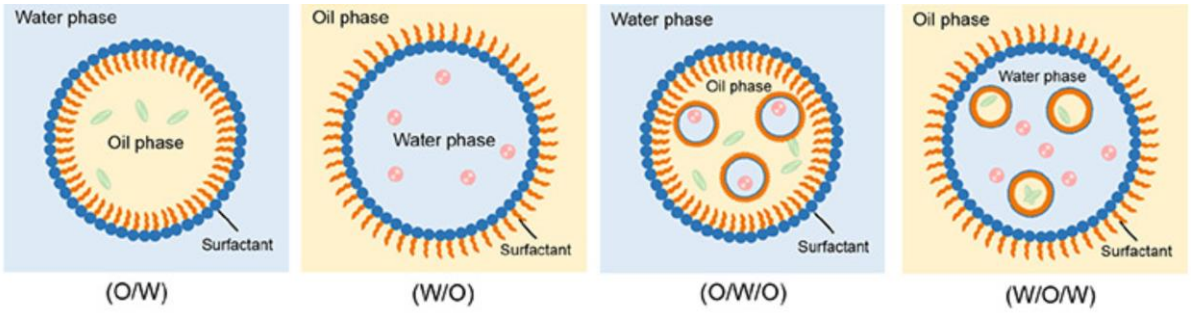


Figure 13: Schematic illustrating the various emulsification techniques (Koshani and Jafari, 2019).

2.5.1. High Energy Methods

The synthesis of oil nanoemulsions is mainly achieved through high-energy emulsification, a technique widely utilized. High-energy approaches are preferred over traditional emulsions for nanoemulsion formation, primarily due to their kinetic stability, enhanced bioavailability, and optical transparency. Ultrasound generators, high-pressure homogenizers, and high-shear stirring, as illustrated in (Figure 14), are common high-energy methods, selected for their ease of formation and the ability to yield large-scale production (Mei *et al.*, 2011); (Espitia *et al.*, 2019).

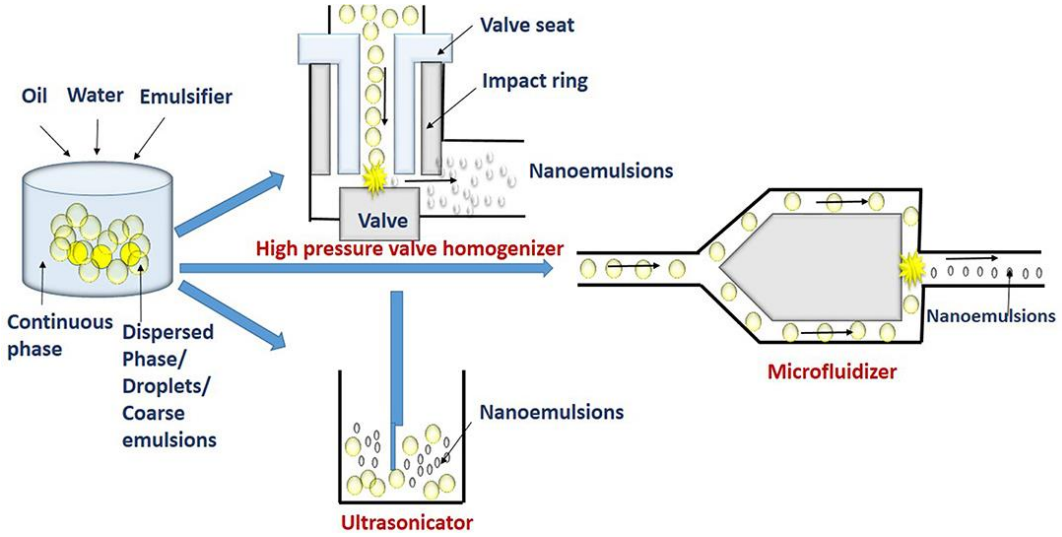


Figure 14: High energy methods such as high pressure homogenization (HPH), microfluidizer, and ultrasonication break macroemulsions into smaller droplets (Aswathanarayan and Vittal, 2019).

2.5.2. Low-Energy Methods

Methods with low energy input for preparing nanoemulsions include phase inversion temperature, phase transition, and self-emulsification methods. The control and efficacy of low-energy emulsification methods primarily rely on the physicochemical properties of surfactants during the nanomaterial preparation process (Nuchuchua *et al.*, 2009). Many studies have

focused on creating stable nanoemulsions using low-energy input methods. (Pavoni *et al*., 2020). A schematic representation of a low-energy method for generating nanoemulsions is represented in (Figure 15).

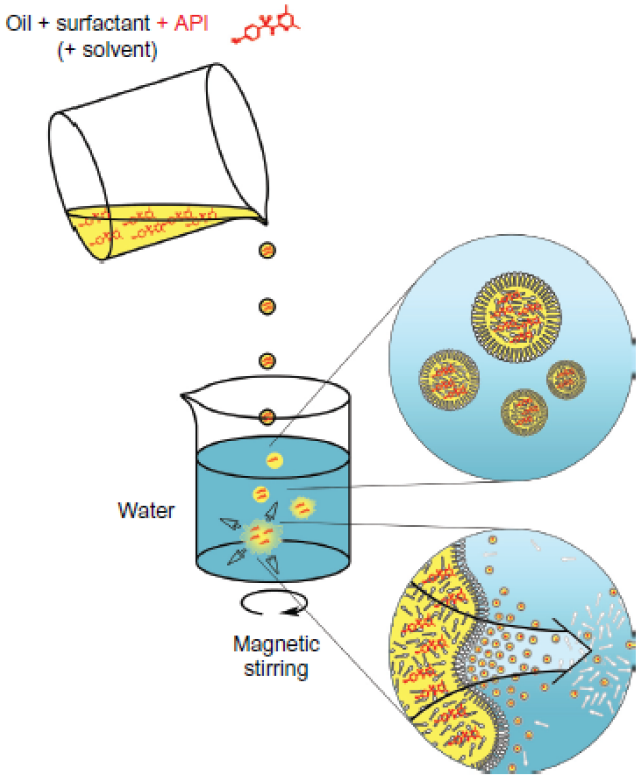


Figure 15: Schematic illustration of a low-energy technique to produce nanoemulsions (API: Active principle ingredient) (Vandamme and Anton, 2010).

3. Description of the Studied Medicinal Plants

3.1. *Satureja hortensis* L.

3.1.1. Botanical Description and Taxonomy

Summer savory, scientifically known as *Satureja hortensis* L., in Arabic as "الصعتر الصيفي", is an annual herbaceous plant characterized by a robust branching structure and linear leaves. This plant belongs to the Lamiaceae family. This plant, a yearly herb, reaches a height of 10–25cm with branched stems. It features a highly branched stem (10–35cm) adorned with short, backward-pointing white hairs. The leaves are linear or linear-lanceolate, approximately obtuse, emucronate, slightly stalked, arranged in opposite pairs, and have entire margins (10–30mm×1–4mm). These leaves are relatively thick, have a fringed edge, and contain glandular punctuation on their surfaces. Its flowers, found in clusters at the upper nodes of the branches, exhibit hues ranging from purple to violet-white, featuring red peels on the inside (Hassanzadeh *et al*., 2016 ; Fierascu *et al*., 2018).

Present across the Mediterranean region, this genus includes over 91 species. Originating from North Africa, the Middle East, Southern and Southeastern Europe, and Central Asia, Iran is particularly rich in biodiversity, hosting more than 8 species within this diversified genus (Bimbiraitė-Survilienė *et al*., 2021 ; Peiri and Fazeli, 2022). The aerial part and the full classification of *S. hortensis* L. are shown in (Figure 16) and (Table 02), respectively, bellow.

Figure 16: Aerial part of *Satureja hortensis* L. (www.sekrety-zdrowia.org).

Table 02: Taxonomy of *Satureja hortensis* L. ("Catalog Record Search," n.d.).

Botanical name	***Satureja hortensis* L.**
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	*Satureja*
Species	*hortensis*

3.1.2. Chemical Composition and Bioactive Compounds

Examining the overall composition, the moisture content in fresh leaves is 72%, with protein at 4.2%, fat at 1.65%, sugar at 4.45%, fiber at 8.60%, and ash at 2.11%. On a dry weight basis, the primary provider of bioactive compounds includes the volatile oil (up to 5%), triterpenic acids, tannins (up to 8%), mucilage, resins, sugars, mineral salts, and other constituents (Fierascu *et al*., 2018).

Several studies detail the composition of the EOs extracted from *S. hortensis*. The EOs components are influenced by the plant's growth environment, leading to anticipated variations in the EOs composition across different regions where *S. hortensis* is cultivated. The plant itself contains a volatile oil ranging from 0.2% to 3% (Hassanzadeh *et al*., 2016). The major constituents of the volatile oil obtained include carvacrol, thymol, phenols, and flavonoids. Various studies have identified γ-terpinene (15.30–39%), carvacrol (11–67%), thymol (0.3–28.2%), and p-cymene (3.5–19.6%) as the primary components in these volatile oils (Hamidpour *et al*., 2014 ; Katar *et al*., 2017 ; Mohtashami *et al*., 2018).

Despite variations in their findings, all studies generally report similar components in the volatile oils, with some detecting additional elements such as α-phellandrene, α- and β-pinene, sabinene, terpineol, α-thujene, among others (Estaji *et al*., 2018). Therefore, when exploring the potential application of EOs or extracts derived from *S. hortensis*, it is crucial to include information about the harvesting area and general composition. This ensures a comprehensive understanding of the reviewed results.

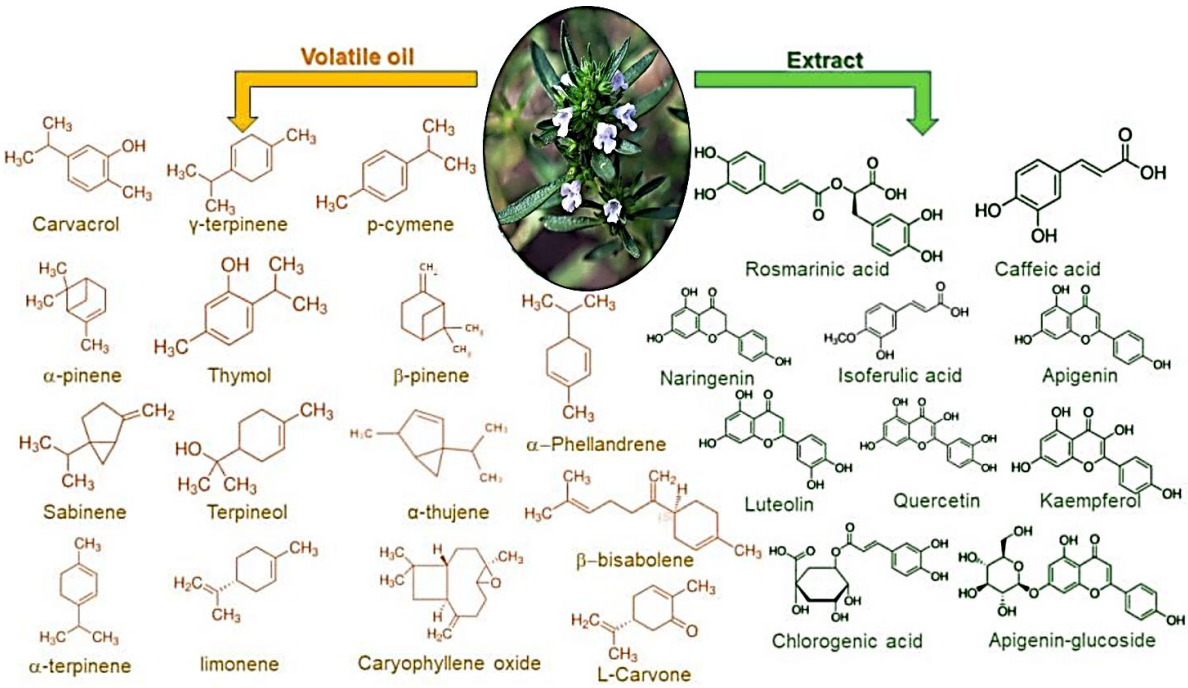


Figure 17 : Main bioactive compounds of *S. hortensis* (Fierascu *et al*., 2018).

3.1.3. Biological Activities

The presence of monoterpenes, such as γ-terpinene, carvacrol, and thymol in the EOs, strongly suggests the potential for effective biological activities. The evaluation of the EOs extracted from Iranian *S. hortensis* demonstrated significant antimicrobial efficacy against various microorganisms. Minimum inhibitory concentration (MIC) values ranged 0.07 to 0.15 µl/ml for *E. coli*, and 0.31 to 0.62 µl/ml for *Salmonella* sp (Seyedtaghiya *et al*., 2021). Another study regarding the effect of EOs obtained from an Algerian S. hortensis revealed a good antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus mirabilis*, and *Streptococcus enterococcus* (Jafari *et al*., 2016).

The commercial EOs (from Iran) has demonstrated significant antioxidant effects through various assays, such as 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS), ferric thiocyanate, and β-carotene bleaching (Fathi *et al*., 2013). *In vitro* studies have confirmed the antioxidant activity of *S. hortensis* essential oil, attributed to the presence of polyphenolic compounds (Rodríguez-Yoldi, 2021). The addition of *S. hortensis* essential oil (ASHEO), obtained through hydro-distillation from Turkish vegetal material, endowed chitosan nanoparticles with antioxidant properties (ranging from 43.66% to 56.99%, as determined by the DPPH assay (Feyzioglu and Tornuk, 2016).

Several studies have explored the anti-inflammatory effects of *S. hortensis.* A study conducted by (Hajhashemi *et al*., 2002) presents the effects of various natural products derived from the roots of Iranian *S. hortensis*, including EOs, hydroalcoholic extract, and polyphenolic extract. (Bimbiraitė-Survilienė *et al*., 2021) enhanced the efficacy of *S. hortensis* extracts in

their study, demonstrating their ability to combat lipid peroxidation in human epidermal cells, including melanocytes and melanoma.

In the past decade, various review papers have highlighted additional activities of *S. hortensis* natural products. These include antiviral properties (evaluated against HIV virus) (Costa *et al*., 2015) (Hassanzadeh *et al*., 2016), inhibitory effects of methanol extracts on the adhesion of activated human platelets to laminin-coated plates (Hamidpour *et al*., 2014) (Jafari *et al*., 2016) as well as antispasmodic and diuretic effects (Lesjak *et al*., 2018).

3.2. *Cymbopogon citratus* (DC.) Stapf

3.2.1. Botanical Description and Taxonomy

The term Cymbopogon originates from the Greek terms "kymbe" (meaning boat) and "pogon" (meaning beard), describing the arrangement of flower spikes. Cymbopogon comprises approximately 55 species that are native to tropical and semi-tropical regions of Asia. These plants are also grown in South and Central America, Africa, and various other tropical nations (Shah *et al*., 2011). *Cymbopogon citratus* (DC.) Stapf (Lemon grass), (Figure 18), is a perennial aromatic herb without branching, emitting a lemon fragrance and growing in dense clumps. Its leaves can reach a length of 90 cm and a width of 1.25 cm. They are isolated, light green, highly fragrant, long, tapered, and gathered in sheaths over a certain portion of their lengths. Additionally, the edges of the leaves are hyaline, formed by numerous small teeth directed towards the top of the plant. The underground part of *C. citratus* consists of a bulbous rhizome. Although this plant rarely blooms, it has a floral stalk that can reach a length of 60 cm with numerous branches ending in clustered, greenish spikes. *C. citratus* reproduces through rhizomes and thrives in semi-tropical and tropical regions (Mathieu *et al*., 2015).

The common names of *C. citratus* are citronnelle or herbe citron (French), lemon grass or West Indian lemongrass (English), Pasto limón (Spanish), Erva-cidreira (Portuguese),

Westindisches zitrongrasen (German), Magnérin or Amagnérin (Ivory Coast), ce kala (Mali), nche awuta or ahihia tii (Nigeria), عشب الليمون (Arabic) (Shah *et al*., 2011). The complete classification and aerial part of *C. citratus* are illustrated in (Figure 18) and (Table 03), respectively, in the following part.

Figure 18: Aerial part of *Cymbopogon citratus* (DC.) Stapf (anonymous).

Table 03: Taxonomy of *Cymbopogon citratus* (DC.) Stapf (Shah *et al*., 2011).

Botanical name	*Cymbopogon citratus* (DC.) Stapf
Kingdom	Plantae
Phylum	Tracheophyta
Class	Liliopsida
Order	Poales
Family	Poaceae
Genus	*Cymbopogon* Spreng
Species	*citratus*

3.2.2. Chemical Composition and Bioactive Compounds

Cymbopogon citratus has a low moisture content of 5.7%, contributing to its significant antimicrobial activities and storage capability. It also contains crude fiber at 9.28%, facilitating

food digestion and enhancing food absorption by the body. The plant includes crude fat, crude ash, crude protein, and 5% carbohydrates, serving as an energy source or booster. It's worth noting that the crude fiber content in lemongrass is notably higher compared to other conventional plants (Oladeji *et al*., 2019). Several essential mineral components were identified, including potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), zinc (Zn), phytate, and phosphorus (P) (Boukhatem *et al*., 2014). Additionally, other minerals present in C. citratus encompass chromium (Cr), nickel (Ni), copper (Cu), arsenic (As), cadmium (Cd), and lead (Pb) (Tibenda *et al*., 2022).

The qualitative and quantitative phytochemical screening of *C. citratus* has revealed important bioactive chemical compounds, potentially linked to the plant's therapeutic potency. Various bioactive constituents, including ketones, alcohols, phenols, terpenes, flavonoids, saponins, steroids, tannins, alkaloids, geranial, terpenoids, polyphenols, esters, aldehyde, and fatty acids, have been isolated and analyzed (Roriz *et al*., 2014).

The literature states that the key components in *C. citratus* include EOs and flavonoids, which play a substantial role in the plant's notable medicinal and pharmacological effects. Citral, the main component of *C. citratus* EOs, is a mixture of two isomeric acyclic monoterpene aldehydes: trans-citral, known as geranial, and cis-citral, known as neral. Geranial boasts a strong lemon odor, while neral has a milder and sweeter floral-herbal aroma (Oladeji *et al*., 2019). The EOs of *C. citratus* primarily consists of monoterpene fractions, with citral making up 23.6% to 91.8% in the aerial parts (leaves and stalks). Additionally, myrcene (up to 16.2%), geraniol (up to 41.2%), and geranyl acetate (up to 4.1%). It's worth noting that citral holds industrial importance as it serves as a raw material for the production of ionone, vitamin A, and β-carotene (Ranitha. *et al*., 2014).

3.2.3. Biological Activities

The efficiency of *C. citratus* has been documented in numerous researches. Researchers has identified various effects of LG (lemon grass), encompassing antibacterial, neuroprotective, anti-diabetic, antioxidant, free radical scavenging, anti-tumor, and immunomodulatory properties (Figure 19).

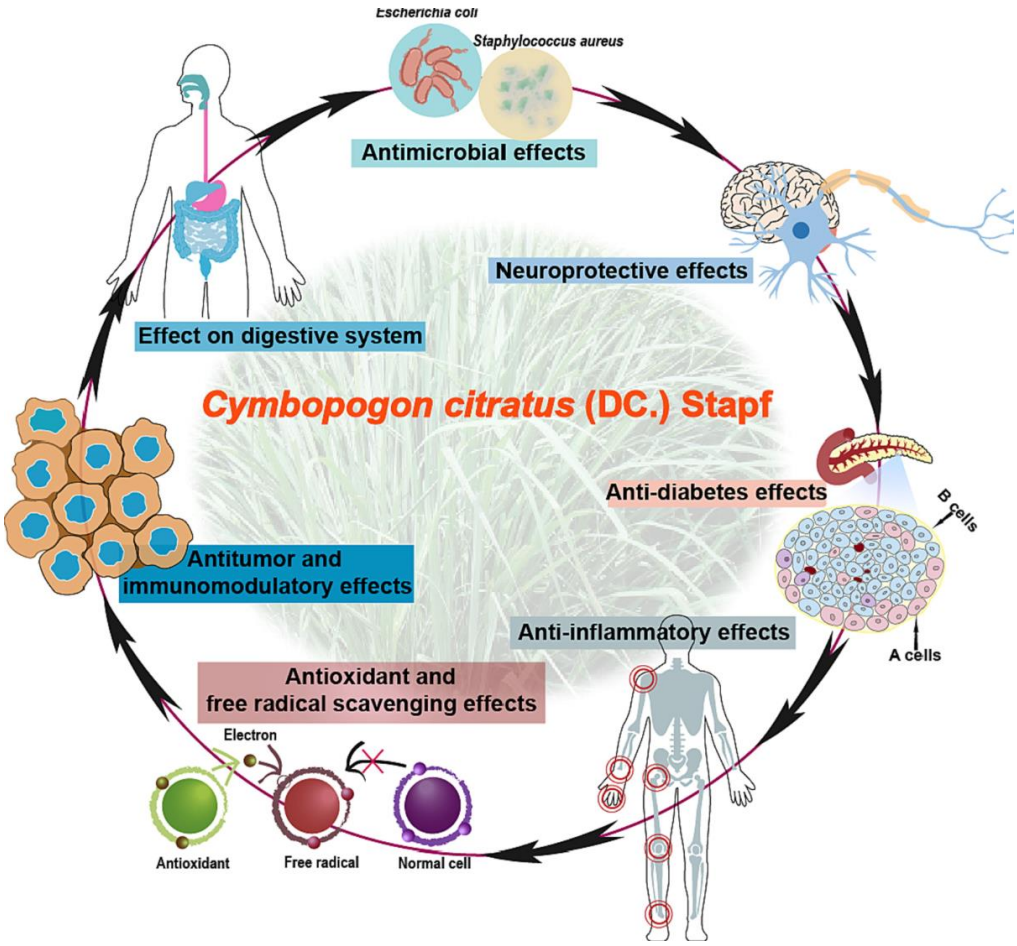


Figure 19 : Modern pharmacological effects of *C. citratus* (Du *et al*., 2023).

C. citratus essential oil comprises bioactive compounds that have the potential to disrupt the bacterial cell membrane, leading to the leakage of intracellular contents and subsequent cell death. The presumed bactericidal effectiveness against a broad spectrum of bacteria is attributed to this mechanism of action (Yap *et al*., 2021). A study conducted by Boudechicha *et al*. (2023) revealed noteworthy findings regarding the antifungal activity of LG EOs and its

microfluidization oil. The results demonstrated significant antifungal effects against mycotoxigenic strains of fungi. Furthermore, when evaluating the antimicrobial impact, the study highlighted the heightened potency of microfluidization oil in comparison to LG essential oil when tested against pathogenic strains of both Gram-positive and Gram-negative bacteria.

Reactive Oxygen Species (ROS) can induce oxidative stress in the body, leading to cellular damage associated with various disorders like heart and neurodegenerative diseases, cancer, and general inflammation when present in elevated and sustained levels. To mitigate such damages, the consumption of exogenous antioxidants proves beneficial. Citral, which is the main component of its EOs *C. citratus*, is suggested to play a crucial role in alleviating endothelial dysfunctions linked to oxidative stress. This is evidenced by their ability to reduce ROS production in human umbilical vein endothelial cells (HUVECs) and mitigate the vasoconstriction induced by thromboxane A2 (Figueirinha *et al*., 2008; Balakrishnan *et al*., 2014 ; Campos *et al*., 2014).

The presence of citral in the EOs of this plant reduces inflammation in the mouse ileum and edema in the Wistar rat's paw aponeurosis. The mechanism of action involves reducing lymphocyte migration through the inhibition of β7-expression. This explains the use of this plant in cases of colic and gastrointestinal disorders in both humans and animals (Watanabe *et al*., 2010). Citral, geranial, neral, and carvone as the primary constituents of *C. citratus* have been identified as having the capacity to hinder the production of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) (Boukhatem *et al*., 2014).

It was revealed that lemongrass (LG) exhibited anti-tumor properties and demonstrated a noticeable inhibitory impact on various cancer cells. This effect was primarily ascribed to the presence of its ethanolic compounds. Lemongrass (LG) exhibited a potent anti-colon cancer effect, as evidenced by the ethanol extract inducing apoptosis in colon cancer cells in a time-

and dose-dependent manner in vitro. Notably, this effect did not cause harm to healthy cells. Moreover, in mice xenotransplanted with colon cancer, LG effectively inhibited tumor growth at a dosage of 16 mg/kg (Ruvinov *et al*., 2019).

Experimental part

Material and Methods

I. Material and Methods

1. Material

1.1. Chemicals and Microorganisms

All chemicals used in this study were of HPLC-grade and were procured from Sigma-Aldrich, Saint Louis, MO, USA. The HepG2 cell line, Vero normal cell line (derived from the African green monkey kidney), and WI-38 normal lung cells were acquired from the American Type Culture Collection (ATCC)® through VACSERA (Cairo, Egypt). Dimethyl sulphoxide (DMSO) was sourced from Merck, Darmstadt, Germany. Fetal calf serum (FCS) and antibiotic (ciprofloxacin) were obtained from Hyclone, Logan, UT, USA, while Dulbecco's Modified Eagle Medium (DMEM) was purchased from Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA.

This study investigated the following bacterial strains from the laboratory of applied microbiology from Setif 1 University: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883), *Proteus mirabilis* (clinical isolate), *Acinetobacter baumannii* (clinical isolate), *Bacillus subtilus* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), and *Methicillin-resistant Staphylococcus aureus* (MRSA) (clinical isolate). These strains pose a potential threat to human health and exhibit fast-emerging resistance to antibacterial substances. The clinical isolates are multi-drug-resistant strains.

Different types of fungi from the ITEM (agro-food microbial culture collection, ISPA, CNR, Bari, Italy) were used in the antifungal test. These included *Aspergillus flavus* ITEM 698, *Aspergillus parasiticus* ITEM 11, *Aspergillus carbonarius* ITEM 5010, *Aspergillus ochraceus* ITEM 5117, *Aspergillus oryzae* ITEM B5, *Penicillium verrucosum*

NRRL 695, *Penicillium chrysogenum* ATCC 48271, *Fusarium graminearum* ATCC 56091, *Fusarium moniliforme* ITEM 52539, and *Fusarium oxysporum* ITEM 12591.

1.2. Plant Material

The aerial parts of Algerian Summer savory (*Satureja hortensis* L.) and lemongrass (*Cymbopogon citratus* (DC.) Stapf) were harvested from Bousaada, M'Sila Province, located between the Saharan Atlas Mountains and the El-Hodna Salt Lake in north-central Algeria in Marsh 2021. The taxonomic identification and deposition of plant materials, carried out by Dr Saouli Nassira from the Department of Biology and Plant Ecology (Faculty of Life Sciences and Nature, Ferhat Abbas University, Setif 1, Algeria), resulted in the assignment of a voucher specimen marked with the Algerian number CAS28/06/21 for *Cymbopogon citratus* (DC.) Stapf and SAS28/03/21 for *Satureja hortensis* L.

Following harvesting, the plant specimens underwent drying at room temperature in a dark and arid environment.

2. Methods

2.1. Retrospective Study

2.1.1. Location of the Study

This study, conducted retrospectively, aimed to describe the bacterial isolates collected from diagnostic samples obtained from hospitalized patients at the Infectious Diseases service of the University Hospital Center (UHC) of Setif. The study spanned eight months, from January 1, 2023, to August 31, 2023, inclusive.

2.1.2. Data Collection

It involved extracting the results of all antibiograms conducted during the study period recorded in the register of the infectious diseases service. These results included the

identification number, date of isolation, identified microorganism, type of specimen, as well as the tested antibiotics with their susceptibility profile (S, I, R). This data was supplemented with specimen source information obtained from laboratory records.

2.2. Plant Extraction

The EOs were obtained through the hydrodistillation technique employing the Clevenger apparatus. A 100g sample was heated with distilled water for a duration of 3-4 hours. Subsequently, the oily phase was condensed, and the resulting EOs were extracted, dehydrated using anhydrous sodium sulfate, and stored in airtight glass vials sealed with aluminum foil at a temperature of +4 °C until analysis. This procedure was replicated thrice for consistency and reliability of results.

2.3. Nanoformulation Preparation

The initial step involved the preparation of a coarse emulsion by blending a solution of carboxymethyl cellulose (CMC) at a concentration of 2%, EOs at 1% volume/volume (v/v), and Tween 80 at 1% v/v with a magnetic stirrer for a duration of 30 minutes. Following this, the emulsion was introduced into a microfluidization system (M110P, Microfluidics, Westwood, MA, USA) and subjected to a pressure of 150 MPa for five cycles to achieve the nanoemulsion state. Subsequently, the resulting emulsion was stored under refrigeration conditions until it underwent further investigation (Salvia-Trujillo *et al*., 2013).

2.4. Nanoemulsion Characterizations

Particle size distribution, polydispersity index (PDI), and ξ-potential were assessed utilizing a Zetasizer Nano ZS instrument (Nano-S90, Malvern Panalytical Ltd., UK) under controlled conditions at a temperature of 25 ± 0.1° C. The nanoemulsion was stored in a sealed, 50 mL graduated bottle at 25° C for a period of five days (Boudechicha *et al*., 2023).

2.5. Gas Chromatography-Mass Spectrometry (GC–MS)

The objective of this investigation was to assess the influence of the microfluidization technique on the volatile components of ASHEO and LGEO through GC-MS analysis. Following the formation of the nanoemulsion, it was combined with diethyl ether using a vortex mixer and then transferred to a 2 mL screw-cap vial for subsequent analysis after settling and drying with anhydrous sodium sulfate. This extraction procedure was replicated three times to ensure accuracy. The volatile compounds present in LGEO) and ASHEO as well as their respective nanoemulsions were analyzed using gas chromatography. (Agilent 8890 GC System) coupled with a mass spectrometer (Agilent 5977B GC/MSD), employing an HP-5MS fused silica capillary column (30 m length, 0.25 mm inner diameter, 0.25 mm film thickness). The temperature of the oven was initially set at 50°C and then ramped from 50°C to 200°C at a rate of 5°C/min, followed by an increase from 200°C to 280°C at a rate of 10°C/min, and held isothermal for 7 minutes. Helium gas was utilized as the carrier at a flow rate of 1.0 mL/min. Injection of 1 µL of the sample occurred at 230°C with a split ratio of 1:50. Mass spectra in electron impact mode (EI) were recorded at 70 eV over a scanning m/z range from 39 to 500 amu. Peaks were identified by comparison with NIST databases, standards, and published literature. The percentages of detected compounds were calculated based on GC peak areas, and the Kovats index of each compound was determined by referencing retention times to those of C6-C26 n-alkanes and comparing them to established literature values.

2.6. Evaluation of Biological Activities

2.6.1. Antibacterial Activity

A. Disc Diffusion

The antimicrobial properties of ASHEO, MF-ASHEO, LGEO, and MF-LGEO were assessed using the agar diffusion method described by Santos *et al*. (2019). Eight bacterial species were subjected to testing, comprising MDR bacteria. Following a 24-hour incubation period at 37°C, the diameters of inhibition zones were measured. The obtained results were compared against positive controls using cefazolin KZ (30µg, UG LF9015, Liofilchem, Italy) and a negative control comprising DMSO. To ensure statistical reliability, all experiments were conducted in triplicate.

B. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) values of ASHEO, MF-ASHEO, LGEO, and MF-LGEO were determined in triplicate using the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018). Each strain's overnight bacterial suspension was adjusted to 0.5 McFarland. Subsequently, 50 µL of this suspension was combined with 50 µL of Muller Hinton Broth (MHB), supplemented with concentrations ranging from 0.007 to 1 mg/mL of EOs and there nanoemulsions, along with dimethyl sulfoxide (DMSO) at 0.1% and 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) at 0.05% (added after incubation). The inclusion of DMSO aids in enhancing oil solubility, while TTC facilitates the determination of bacterial growth (Figure 20A). A well devoid of bacterial suspension served as the negative control. Bacterial growth was assessed following an incubation period of 18-24 hours at 37°C.

The minimum bactericidal concentration (MBC) is the lowest concentration of EOs capable of killing more than 99.9% of the initial bacterial inoculum (i.e. less than 0.01% of survivors). 20 µL are taken from wells where no change of color is observed, seeds in spot form on Muller Hinton Agar (MHA) and incubated at 37C for 24h (Figure 20B).

A **B**

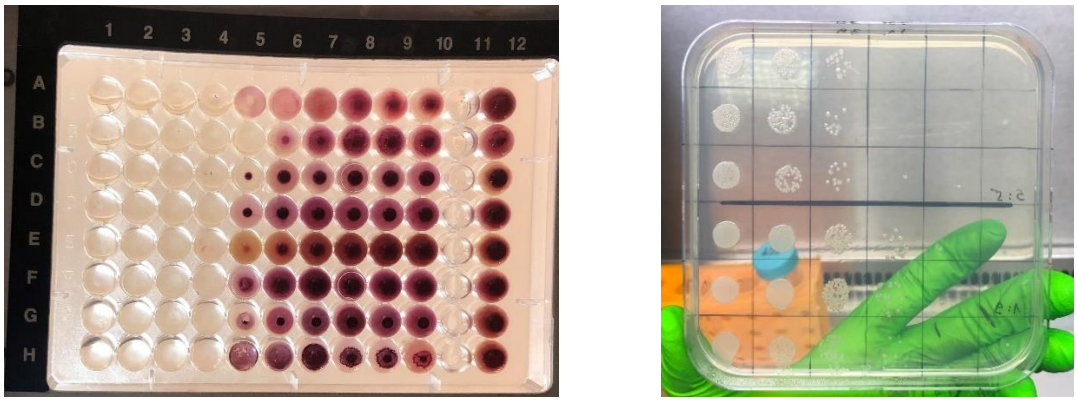


Figure 20: Reading of the MIC (A) and MBC (B) following incubation.

2.6.2. Antibiofilm Activity

The impact of our molecules on bacterial biofilm formation was assessed utilizing the Cristal violet assay as detailed by Gómez-Sequeda *et al*. (2020). Sterile 96-well polystyrene plates were used for inoculation. Each well received 100 µL of TSB containing EOs at varying concentrations, combined with 100 µL of bacterial inoculum adjusted to a concentration of 10^9 CFU/mL. A well devoid of EOs served as the positive control, while another without bacterial suspension acted as the negative control. Following a 48-hour incubation period at 37°C, the medium from each well was meticulously aspirated, and the plate was washed thrice with sterile physiological saline (0.9%). Subsequently, the plate was subjected to drying at 60°C for 45 minutes, after which 200 µL of crystal violet solution (0.4%) was added to each well and allowed to incubate at room temperature for 15 minutes. The plate was then rinsed thrice with saline. Finally, 200 µL of 30% acetic acid solution,

prepared with ultra-pure water, was introduced to each well to dissolve the crystal violet stain. Absorbance readings were obtained at 595 nm using the microplate reader (Perkin Elmer, BioTek®), and the percentage of inhibition was determined using the subsequent formula:

$$\text{Biofilm Inhibition \%} = \frac{(\text{Abs negative control} - \text{Abs test})}{\text{Abs negative control}} \times 100$$

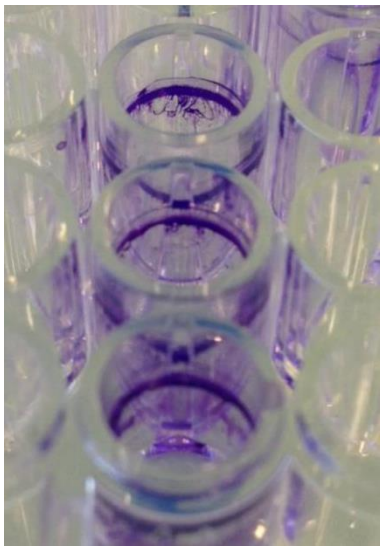


Figure 21: Detection of biofilm formation using the CV staining method.

2.6.3. Antifungal Activity

A. Preparation of Spore Suspension

Ten strains of toxigenic fungi were utilized in this study. These strains were obtained from the Food Toxicology and Contaminant Department, National Research Centre, Cairo, Egypt. For the purpose of antifungal assessment and evaluation of the impact of oils and its microfluidized solutions on fungal growth inhibition, all ten strains were utilized. Additionally, two of these strains were specifically chosen to evaluate anti-aflatoxigenic efficiency (Shehata *et al*., 2019).

To prepare the spore suspension, toxigenic fungal cultures were cultivated on Czapek-Dox slants agar at 22 ± 1°C for 5 days. A sterile solution of Tween 80 at a concentration of 0.01% (v/v) was then applied over the culture slant agar to facilitate the extraction of conidia. The surface of the slant was gently scraped using a loop to aid in the release of spores. The resulting inoculum concentration ranged from 1.22 to 1.41 × 10^3 colony-forming units per milliliter (CFU/mL), as determined using a Burker-Turk counting chamber (Hemocytometer).

Determination of the EOs and their Nanoformulations Activity Against Toxigenic Fungi

In this study, wells on Czapek-Dox agar plates were loaded with 100 µL of either crude or microfluidized EOs, following the application of spore suspensions from each fungal species. The inhibition impact of the crude or microfluidized oil was measured by measuring the apparent zone diameter in millimeters surrounding each well for every fungus. A larger inhibition zone diameter indicated a greater antifungal effect for each extract. Antifungal susceptibility testing was conducted using the broth microdilution method, following the guidelines established by the Clinical Laboratory Standards Institute (CLSI) as per approved standard M38-A2, specifically recommended for toxigenic fungal strains under investigation. The minimal antifungal concentration (MFC) was determined following the method outlined by Badr *et al*. (2017). An antifungal standard material (fluconazole) was utilized as a positive control in this study.

2.6.4. Cytotoxicity Assay

In this study, we subjected HepG2, WI-38, and Vero cell lines to treatment with both oil and nanoemulsion, followed by assessment using the MTT assay. The cells were initially seeded at a density of 1 × 10^5 cells/well, and cultured in DMEM supplemented with 10% PBS serum and antibiotics. Concentrations of both oils and nanoemulsions were serially

diluted, ranging from 0.097 to 1000 µg/mL for HepG2 cells and 31.25 to 1000 µg/mL for Vero and WI-38 cells. As a positive control, cisplatin was employed.

To evaluate cell viability, the absorbance at 540 nm was measured using a microplate reader (BMG LABTECH-FLUOstar Omega microplate reader, Ortenberg, Germany) after dissolving the formazan crystals in DMSO for a duration of 20 minutes. This allowed for the quantification of cellular metabolic activity and assessment of the impact of oil and nanoemulsion treatment on the viability of the tested cell lines. The proportion of surviving cells and IC50 were calculated using the formula:

$$\text{Cell viability } \% = \frac{\text{OD}_{\text{ sample}} - \text{OD}_{\text{ blank}}}{\text{OD}_{\text{ control}} - \text{OD}_{\text{ blank}}} \text{ x } 100$$

Where OD sample is the optical density of the sample, OD blank is the optical density of the blank (DMSO), and OD control is the optical density of the control.

Cell viability was evaluated utilizing the WST-1 assay, employing the Abcam® kit (ab155902 WST-1 Cell Proliferation Reagent). Initially, 3×10^3 cells were seeded into 96-well plates and allowed to incubate in complete media for 24 hours. Following this, the cells were subjected to treatment with oil or nanoemulsion at varying concentrations for a duration of 48 hours. Subsequently, they were exposed to 10 µL of the WST-1 reagent. Cell viability was then quantified using a microplate reader.

Furthermore, morphological alterations in cell lines resulting from exposure to various concentrations of ASHEO, LGEO and there nanoemulsions were examined. This evaluation was conducted using a Zeiss Axio Vert A1 microscope (Carl Zeiss Microscopy Gmbh, 07745 Jena, Germany) and compared to untreated controls. Such observations provided insights into the effects of the treatments on the cellular structure and morphology, aiding in the comprehensive assessment of their impact on cell behavior and health.

2.6.5. Antioxidant Activity

The antioxidative capacity of ASHEO, ALGO and there nanoemulsions were evaluated by assessing their ability to scavenge free radicals, employing the DPPH assay as described by Hatano *et al*. (1988). Briefly, our samples or BHT were introduced into a DPPH solution and incubated in darkness for 30 minutes. When a DPPH solution is combined with an antioxidant, the color of the matching hydrazine changes from purple to yellow (Figure 22). Subsequently, the absorbance of the solution was quantified at 517 nm utilizing a UV-Vis spectrophotometer (JASCO V-730 Spectrophotometer, MD 21601, USA). Each experiment was conducted in triplicate, and the resultant data were averaged for analysis.

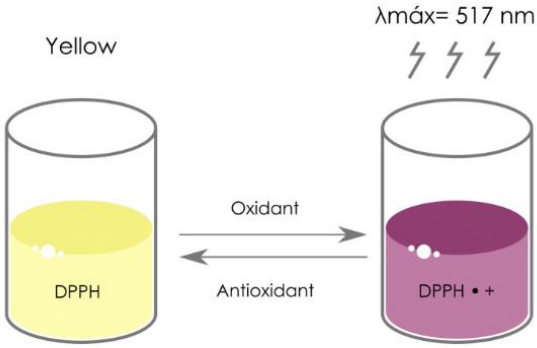


Figure 22: Color shift from purple to yellow during antioxidant-induced transformation of DPPH solution (www.libios.fr).

The inhibition percentage was calculated using the following formula:

$$I(\%)=\left(\frac{Abs_c\text{-}Abs_s}{Abs_C}\right)\times 100$$

Abs_C represents the absorbance of the control used in the study, while AbsS indicates the absorbance of the test sample. The concentration at which 50% inhibition (IC50) occurs was determined by plotting the percentage of inhibition against the concentration of the extract.

2.7. Molecular Docking

The crystal structures of enzymes associated with bactericidal/bacteriostatic effects (isoleucyl-tRNA synthetase, DNA gyrase, dihydropteroate synthase, D-alanine: D-alanine ligase, IV topoisomerase, dihydrofolate reductase, and penicillin-binding protein 1a) were obtained from the Protein Data Bank (https://www.rcsb.org/, accessed on 17 August 2022) with the following PDB IDs: 1JZQ, 1KZN, 2VEG, 2ZDQ, 3RAE, 3SRW, and 3UDI. Carvacrol as a ligand was downloaded from the PubChem database and accessed on 3 May 2023 via http://pubchem.ncbi.nlm.nih.gov/. The enzymes were prepared as receptors by removing water and co-crystallized ligands and ions. Pymol software ver. 2.5.1 protonated them, and Avogadro Software ver. 1.2.0 optimized the ligands' 3D structure via the MMFF94 force field. CB-DOCK2, a web-based program, performed blind docking (accessed on 3 May 2023 via http://clab.labshare.cn/cb-dock/php/). It converted input files to pdbqt format using OpenBabel and MGL Tools, predicted the protein cavities, and calculated their centers and sizes. The top N cavities (n = 5 by default) were submitted to AutoDock Vina for docking. The final results are displayed after N rounds of computation. The success rates for top-ranking poses with RMSDs less than 2 Å from their location in the X-ray crystal structure were shown by the benchmarks of Liu *et al*. (2022). Best-docked complexes are analyzed using Discovery Studio software (Ver. 21.1.0.20298), as described by Farouk *et al*. (2023).

2.8. *In silico* ADME Study

In our study, we analyzed the *in silico* ADME profiles of carvacrol utilizing the Swiss ADME server, which is operated by the Swiss Institute of Bioinformatics (Daina *et al*.,

2017). As part of the ligand preparation process, we generated and submitted SMILES notations for evaluation.

2.9. Statistical Analysis

All experiments were conducted in three replicates to ensure robustness and reliability. The gathered data is presented as the mean value alongside its standard deviation (SD). Statistical analyses were conducted utilizing GraphPad Prism 5 software, provided by GraphPad Software Inc., located in La Jolla, USA.

Results and Discussion

II. Results and Discussion

1. Retrospective Study

During the observational period of the study, 325 records were analyzed, with 31 originating from hospitalized patients who underwent antibiogram testing. The bacterial strains primarily originated from urinary tract samples (37%), followed by wound pus samples (29.03%) and blood culture samples (12.90%) as presented in (Figure 23), respectively. Among the major antibiotic classes tested, cephalosporins antibiotics were the most frequently assessed, comprising 31.51% of the total, followed by penicillin A at 23.35%, quinolones at 18.42%, and other antibiotic classes. Carbapenems and polypetides like colistin were the least tested antibiotic class, representing only 2.43% and 1.94% of the total, respectively. as showed in the (Figure 24).

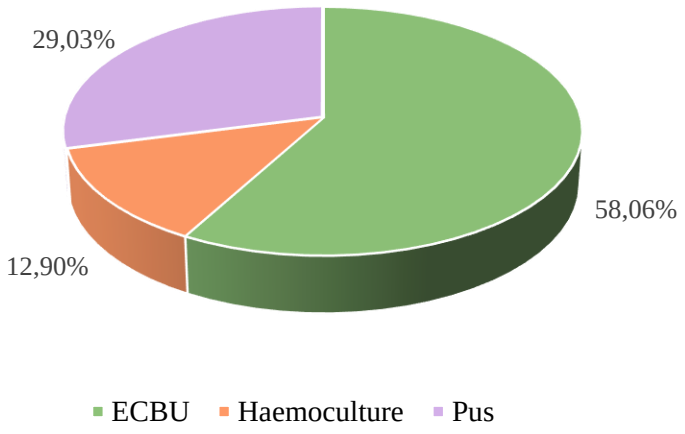


Figure 23: Main sites of bacteriological sampling in the infectious diseases service.

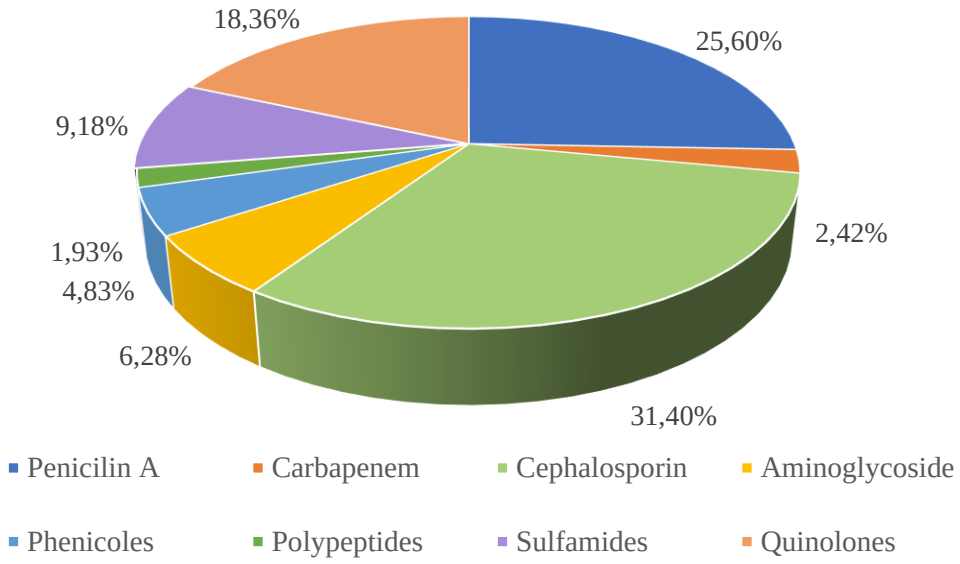


Figure 24: Rates of the major antibiotic classes tested in the infectious diseases service.

The study findings regarding antibiotic sensitivity in the examined service exhibit diverse patterns of resistance among the isolated strains. The data presented in the (Figure 25) depict varying levels of resistance to commonly tested antibiotics over the study period. Notably, amoxicillin resistance peaked at 100%, indicating a significant challenge in treating infections with this antibiotic. Similarly, cefazoline resistance surpassed 70%, suggesting diminished effectiveness in combating bacterial pathogens. Cefotaxime resistance reached 58.06%, underscoring the complexity of addressing bacterial infections with this antibiotic. Resistance rates to ciprofloxacin and gentamicin were observed at 54.84% and 32.26%, respectively, indicating a concerning trend in declining susceptibility to these agents.

Conversely, resistance to imipenem, amikacin, colistin, and fosfomycin remained relatively low throughout the study period, with average rates of 16.13%, 9.68%, 12.90%, and 19.35%, respectively. This suggests that these antibiotics may still be effective treatment options for certain infections. However, continuous surveillance is crucial to monitor for any emerging resistance trends that could compromise their efficacy.

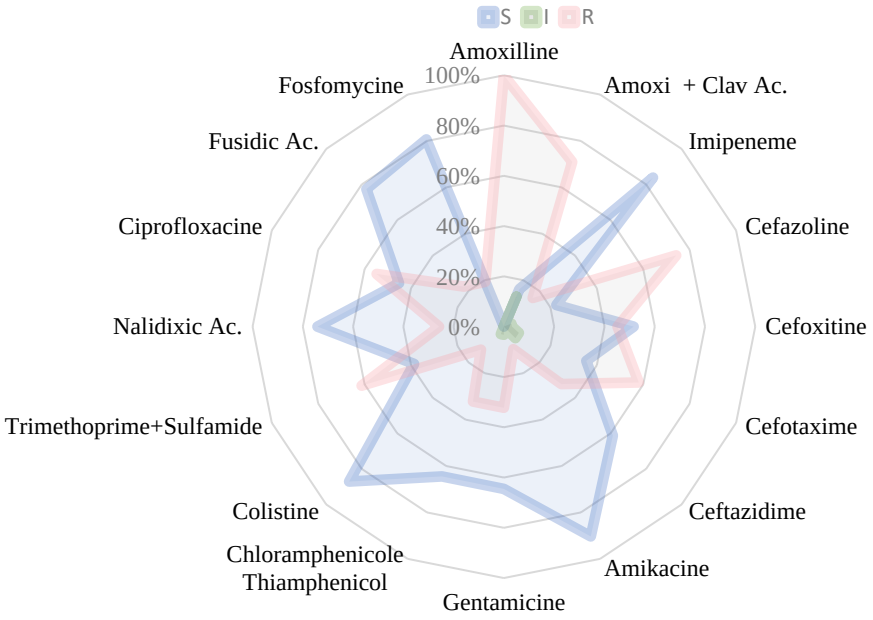


Figure 25: Proportion of antibiotic sensitivity within the infectious diseases service.

Several studies have documented increasing rates of resistance to commonly used antibiotics in healthcare settings, mirroring the trends observed in our analysis. For example, a study by (Smith and Coast, 2013) demonstrated a significant rise in resistance to ciprofloxacin among Gram-negative bacteria, consistent with our findings. Similarly, the emergence of multidrug-resistant strains, as evidenced by the high rates of resistance to amoxicillin and cefazoline in our study, has been well-documented in the literature (Ventola, 2015).

Furthermore, the persistence of low resistance rates to certain antibiotics, such as imipenem, amikacin, colistin, and fosfomycin, highlights the ongoing importance of these agents in the treatment of bacterial infections. While resistance to these antibiotics remains relatively low, continued monitoring is essential to detect any emerging resistance trends and ensure their continued effectiveness (Paterson and Bonomo, 2005) ; (Falagas and Kasiakou, 2005).

The identification of isolated bacteria was carried out at the central laboratory of the Setif University Hospital Center (Figure 26). *Escherichia coli* ESBL was the most frequently isolated species (29.03%), followed by *Klebsiella pneumoniae*, *E. coli* (16.13%), and *Klebsiella pneumoniae* ESBL (9.68%). In addition to the commonly encountered genus in infections (Proteus, Citrobacter, Serratia, and Staphylococcus), rare species were also isolated in very small proportions, such as *Klebsiella oxytoca* (1 strain from hemoculture), *Citrobacter freundii* (1 strain from urine), and *Acinetobacter calcoaceticus* (1 strain from pus).

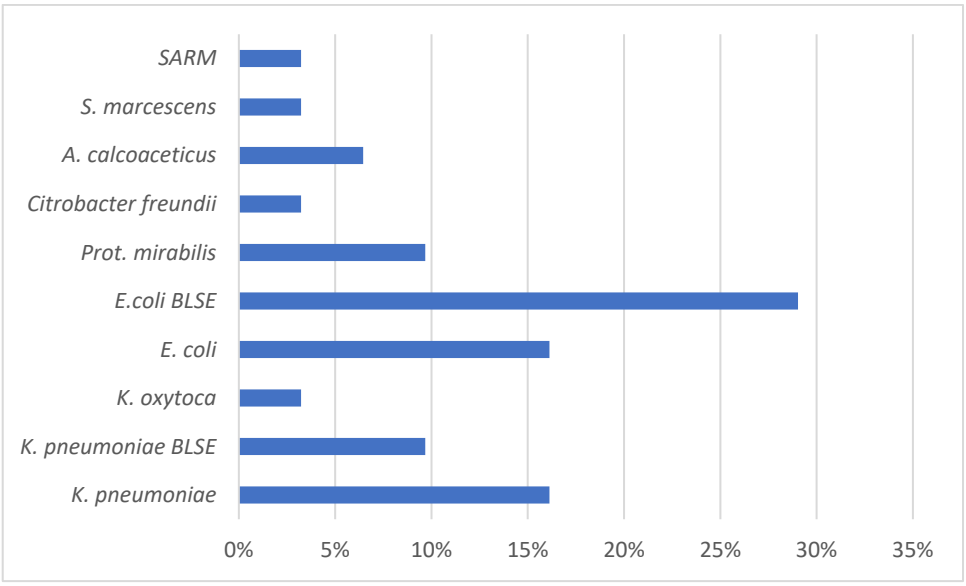


Figure 26: Proportion of the identified bacteria in the infectious diseases service.

Our findings align with previous research on antibiotic resistance, highlighting the importance of ongoing surveillance and stewardship efforts to combat the emergence and spread of multidrug-resistant pathogens (World Health Organization, 2022). The prevalence of ESBL-producing strains underscores the need for effective infection control measures and judicious antibiotic use to prevent further dissemination of resistant organisms (Barlam *et al.*, 2016); (Tinker *et al.*, 2021). In addition, identifying uncommon species, even in tiny

numbers, emphasizes the significance of thorough microbiological surveillance to identify and control new infections promptly (Arthur *et al*., 2011).

To summarize, the study's results emphasize the ever-changing characteristics of antibiotic resistance and the worries it presents in medical practice. To minimize the effects of antibiotic resistance and maintain the effectiveness of current treatments, it is crucial to maintain ongoing surveillance, stewardship activities, and infection control measures.

2. Chemical Composition of Extracted EOs and their Nanoformulation

The extraction process undertaken in this study yielded 2.78 ± 0.05% for *S. hortensis*, surpassing yields reported in other Algerian regions like Tizi-Ouzou (0.06%), Bordj-Ménaïel (2.2%), and Jedioua Relizane (0.68%), according to (Djenane *et al*., 2019) and (Chouitah *et al*., 2018). However, there is a lack of data in the literature regarding the extraction yield of ASHEO collected from Bousaada, M'Sila Province in Algeria. Various factors, including age, genotype, agronomic and climatic conditions, and harvesting period, influence oil yield and plant productivity, as noted by (Djenane *et al*., 2019).

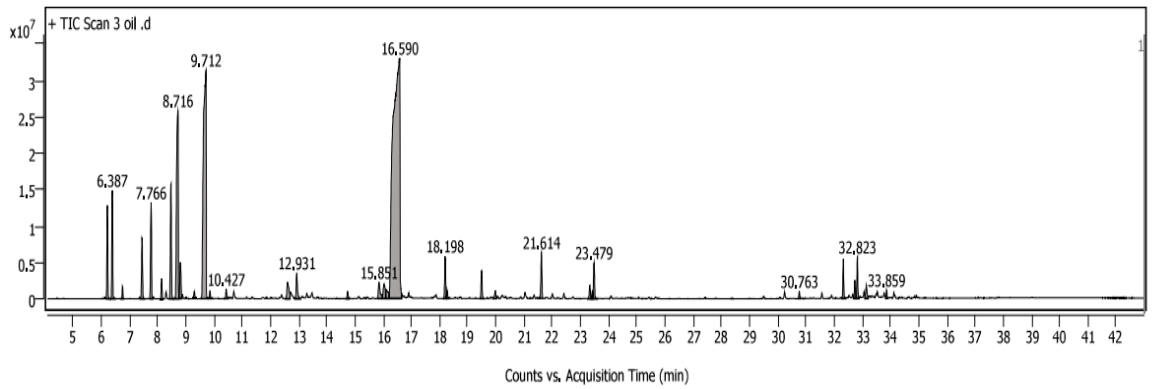
The principal constituents identified in the obtained oil, termed ASHEO, were carvacrol (45.15%) and γ-terpinene (17.72%), as detailed in (Table 04) and (Figure 27A). Subsequent formulation of a nanoemulsion using microfluidization (MF-ASHEO) led to a significant enhancement in the concentration of carvacrol (94.51%), while diminishing the levels of γ-terpinene (0.43%) and ρ-cymene (undetected) in comparison to the original sample (ASHEO), as depicted in (Table 04) and (Figure 27B).

Table 04: Percentage of volatile components of ASHEO and MF-ASHEO identified by GC-MS analysis.

S/N	Compound	RI [a]	LRI [b]	Area%		Identification Method [c]
				ASHEO	MF-ASHEO	
1	α -Thujene	933	930	2.30	-	RI, MS, STD
2	α-Pinene	942	939	**2.74**	-	RI, MS, STD
3	Camphene	955	954	0.29	-	RI, MS
4	β-Pinene	981	979	1.64	-	RI, MS, STD
5	β-Myrcene	993	990	**2.54**	-	RI, MS, STD
6	α-Phellandrene	1004	1002	0.50	-	RI, MS
7	α-Terpinene	1018	1017	**3.38**	-	RI, MS, STD
8	*p*-Cymene	1023	1024	**9.01**	-	RI, MS, STD
9	Limonene	1031	1029	1.19	-	RI, MS, STD
10	*E*-β-Ocimene	1049	1050	0.22	-	RI, MS
11	γ-Terpinene	1061	1059	**17.72**	0.43	RI, MS, STD
12	*cis*-Sabinene hydrate	1073	1070	0.18	-	RI, MS
13	Terpinolene	1092	1088	0.27	-	RI, MS
14	Borneol	1170	1169	0.92	-	RI, MS
15	Terpinen-4-ol	1181	1177	0.93	0.51	RI, MS
16	Isothymol methyl ether	1240	1244	0.19	-	RI, MS
17	Bornyl acetate	1285	1288	-	0.44	
18	Thymol	1289	1290	2.16	1.48	RI, MS, STD
19	Carvacrol	1299	1298	**45.15**	94.51	RI, MS, STD
20	Carvacryl acetate	1337	1340	1.14	0.87	RI, MS
21	β-Cubebene	1390	1388	0.20	-	RI, MS
22	β-Caryophyllene	1415	1419	0.80	-	RI, MS
23	Aromandendrene	1440	1439	0.28	-	RI, MS
24	β-Bisabolene	1507	1505	1.32	-	RI, MS
25	Spathulenol	1577	1578	0.39	-	RI, MS
26	Caryophyllene oxide	1584	1582	1.30	0.82	RI, MS
27	α-Cadinol	1655	1654	0.95	0.50	RI, MS
	Total	**-**	**-**	**95.46**	**99.56**	-

RI a: Retention indices calculated on DB-5 column using alkanes standards. LRI b: Retention indices according to the literature. c: Confirmed by comparison with the retention indices, the mass spectrum of the authentic compounds, and the NIST mass spectra library data. ASHEO: Algerian *Satureja hortensis* L. essential oil; MF-ASHEO: microfluidizing emulsion of Algerian *Satureja hortensis* L. essential oil

A



B

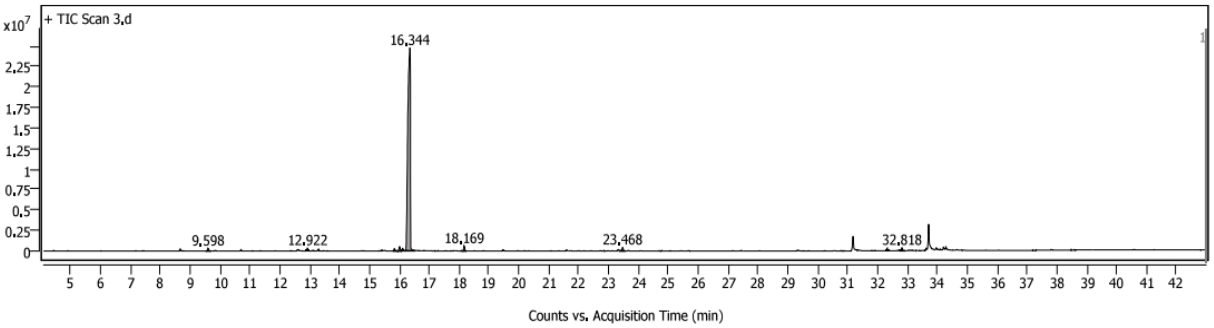


Figure 27: Volatile chromatograms for (A) ASHEO and (B) MF-ASHEO.

In contrast to previous literature on *S. hortensis*, our study aligns with (Djenane *et al*., 2019) in identifying carvacrol, γ-terpinene, and p-cymene as the dominant compounds. However, (Chouitah *et al*., 2018). presented a different *S. hortensis* profile, with cadinol, myrtenal, himachalene, selinene as primary constituents. Our study revealed a higher concentration of monoterpenes, consistent with (Chambre *et al*., 2020a) findings in Romania. Typically, *S. hortensis* L. EOs from various regions exhibit higher levels of carvacrol than thymol, as observed in Turkey and other locations ((Tozlu *et al*., 2011)

(Bimbiraitė-Survilienė *et al*., 2021). However, specific chemotypes in Turkey (KIZIL *et al*., 2009) and Libya (Giweli *et al*., 2012) have shown thymol as the major component. Environmental factors such as area, climate, and seasonality can influence oil composition, as seen in (Baher *et al*., 2002) research where water stress altered carvacrol and γ-terpinene contents.

High-pressure homogenization methods, such as high-pressure homogenization and microfluidization, have the capability to modify the composition of EOs, augmenting certain components while diminishing others. For instance, in Algerian *Saccocalyx satureioides* oil nanoemulsion, thymol and carvacrol were found to be more prevalent, while borneol and α-terpineol concentrations decreased compared to hydrodistilled oil (Aouf *et al*., 2020). Similarly, employing microfluidization in our study led to a significant rise in carvacrol content (94.51%), at the expense of γ-terpinene and p-cymene, as demonstrated in (Table 04). (Chambre *et al*., 2020a) conducted heating treatments on *S. hortensis* L. oil, observing effects on γ-terpinene and carvacrol concentrations under different temperatures and durations. Heating resulted in decreased γ-terpinene content while increasing carvacrol concentration, suggesting γ-terpinene as a precursor to carvacrol, converted via aromatization and hydroxylation processes during storage (Mohtashami *et al*., 2018). Our findings support this hypothesis, showing an inverse relationship between carvacrol and its precursor, γ-terpinene, during microfluidization. Carvacrol's bioactivity as an antioxidant, antimicrobial, antiparasitic, anti-inflammatory, antinociceptive, hepatoprotective, anticancer, and pain management agent underscores its significance in various sectors like food and pharmaceuticals (Chambre *et al*., 2020). Nonetheless, further research is imperative to elucidate the mechanisms involved in volatile compound transfer during intensive-energy processes.

Using the same extraction technique, the yield of *C. citratus* obtained is 1.76 ± 0.05%, surpassing the yields reported by (Boukhatem *et al*., 2014) and (Benoudjit *et al*., 2022) in the Blida region of northern Algeria, which were 0.6% and 0.8% (v/w) respectively. To our knowledge, there is no existing literature regarding the LGEO of Bousaada, M'Sila Province, Algeria.

The nanoemulsion chemical analysis of the LGEO produced by the microfluidization technique showed a quantitative difference compared to the content of the raw LGEO volatile oil (Table 05). However, some compounds of minor content were found to be absent from the LGEO volatile content by nanoformation technique (camphor, citronellal, bergamotene, α-farnesene, and caryophyllene oxide) and also in new components detected in MF-LGEO (decanal) (Table 05; Figure 28). Identified components in the nanoemulsion were represented in 97.53% of the total nanoemulsion oil. Similar to the hydrodistilled LGEO, geranial (35.48%) and neral (28.95%) were predominant, followed by geraniol and geranyl acetate (7.97% and 4.55%, respectively). Several mono and sesquiterpenes, such as camphor, citronellal, bergamotene, α-farnesene, and caryophyllene oxide, were found in the nanoemulsion LGEO when compared to the hydrodistilled oil.

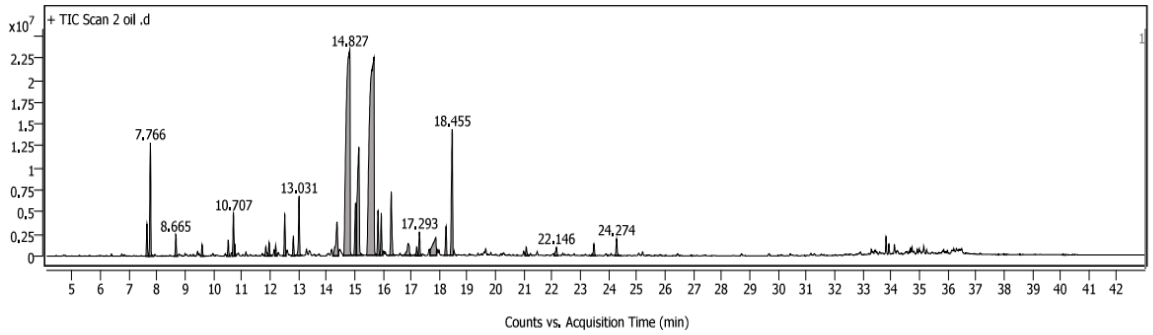
Table 05: Percentage of volatile components of LGEO and MF-LGEO identified by GC-MS analysis.

S/N	Compound	RI [a]	LRI [b]	Area%		Identification Method [c]
				LGEO	MF-LGEO	
1	6-Methyl-5-heptene-2-one	983	985	0.94	0.59	RI, MS
2	***β*-Myrcene**	992	991	**3.61**	2.76	RI, MS, STD
3	*Z*-*β*-Ocimene	1040	1037	0.64	0.52	RI, MS
4	*E*-*β*-Ocimene	1051	1050	0.34	0.70	RI, MS

5	*γ*-Terpinene	1063	1059	0.48	0.81	RI, MS
6	Linalool	1100	1096	1.42	0.98	RI, MS, STD
7	Perillene	1105	1103	0.31	-	RI, MS
8	*trans*-Pinocarveol	1140	1139	0.42	0.42	RI, MS
9	Camphor	1145	1146	0.47	-	RI, MS
10	Citronellal	1156	1153	0.34	-	RI, MS
11	Isoneral	1171	1170	1.35	0.85	RI, MS
12	Rose furan oxide	1180	1177	0.65	0.36	RI, MS
13	Isogeranial	1189	1185	2.07	1.44	RI, MS
14	Decanal	1204	1201	-	1.57	RI, MS
15	Citronellol	1228	1225	2.10	1.42	RI, MS, STD
16	**Neral**	1240	1238	**26.91**	28.95	RI, MS, STD
17	Geraniol	1258	1255	9.69	7.97	RI, MS, STD
18	**Geranial**	1270	1267	**30.73**	35.48	RI, MS, STD
19	Dihydrolinalool acetate	1279	1275	1.41	1.88	RI, MS
20	**Carvacrol**	1298	1299	**2.61**	3.95	RI, MS, STD
21	Nerolic acid	1337	1340	0.97	-	RI, MS
22	Geranic acid	1351	1355	1.14	0.51	RI, MS
23	Neryl acetate	1365	1361	2.19	1.36	RI, MS
24	**Geranyl acetate**	1384	1383	**5.06**	4.55	RI, MS
25	Bergamotene (*α*-trans-)	1438	1434	0.33	-	RI, MS
26	*α*-Farnesene	1508	1505	0.43	-	RI, MS
27	Caryophyllene oxide	1582	1583	0.47	-	RI, MS
28	Selin-6-en-4α-ol	1633	1636	0.65	0.46	RI, MS
	Total	**-**	**-**	**97.73**	**97.53**	-

RI a: Retention indices calculated on DB-5 column using alkanes standards. LRI b: Retention indices according to the literature. c: Confirmed by comparison with the retention indices, the mass spectrum of the authentic compounds, and the NIST mass spectra library data. LGEO: lemongrass emulsion of essential oil; MF-LGEO: microfluidizing emulsion of lemongrass essential oil.

A



B

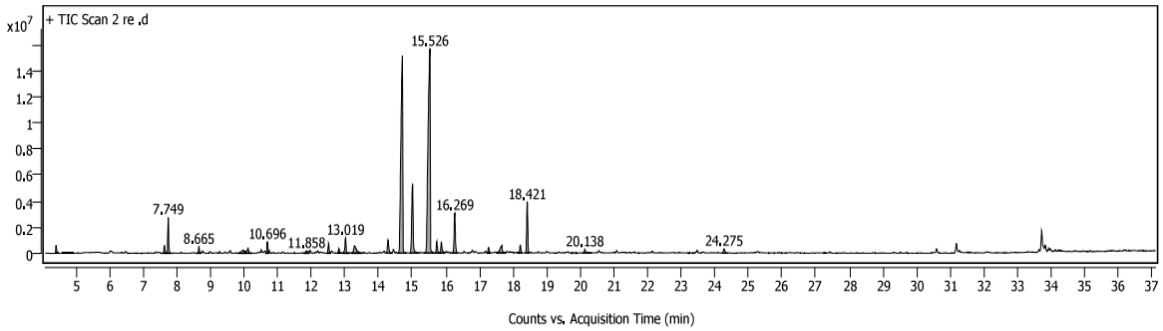


Figure 28: Volatile chromatograms for (A) LGEO and (B) MF-LGEO.

From a qualitative perspective, our findings are consistent with previous research (Benoudjit *et al*., 2022), which also identified neral and geranial as key components of LGEO. However, there are quantitative discrepancies. Notably, in our study, geraniol was found to be significantly more abundant compared to β-myrcene, ranking second in concentration after geranial and neral in Blida LGEO (Boukhatem *et al*., 2014).

According to our study, the qualitative volatile profile of LGEO remained consistent after formulating a nanoemulsion using the microfluidization technique, although there were quantitative variations. However, the predominant components remained unchanged. Additionally, both oil and citral content increased in the microfluidized nanoemulsion. These compounds have been previously associated with high antifungal activity (Zheng *et al*.,

2021). Therefore, this technological process may suggest enhancements in oil application for ensuring microbial safety in food products.

In light of our study findings, Pilong *et al*. (2022) observed a notable increase in eugenol content (70.69%) following the microfluidization of clove EOs into nanoemulsion compared to the untreated oil (60.11%). This enhancement is likely due to the substantial increase in the surface area/volume ratio of eugenol facilitated by the numerous fine droplets of clove EOs. Conversely, the microfluidization process led to a reduction in other constituents of clove oil, such as benzyl alcohol and caryophyllene. The authors underscored the importance of conducting further mechanistic investigations in this domain. Additionally, various studies have suggested that the physical stability and biological activity of emulsions may undergo alterations due to phenomena like Ostwald ripening, flocculation, or coalescence (Chang *et al*., 2012).

3. Characterization of Nanoparticles

In the nanoemulsion MF-ASHEO, the average particle size measures 41.72 ± 12.72 nm, showcasing a monomodal distribution pattern indicative of uniformity. Notably, this size falls within the ultra-fine range, being less than 100 nm, which is particularly significant for its potential applications. Additionally, the nanoemulsion exhibits a mean zeta potential of -39.4 ± 3.75 mV, as illustrated in (Figures 29A and 29B). This zeta potential value suggests a high degree of stability, crucial for the sustained dispersion of particles. Furthermore, the polydispersity index (PDI) of 0.291 ± 0.04 underscores the uniformity and consistency in particle size distribution within the nanoemulsion.

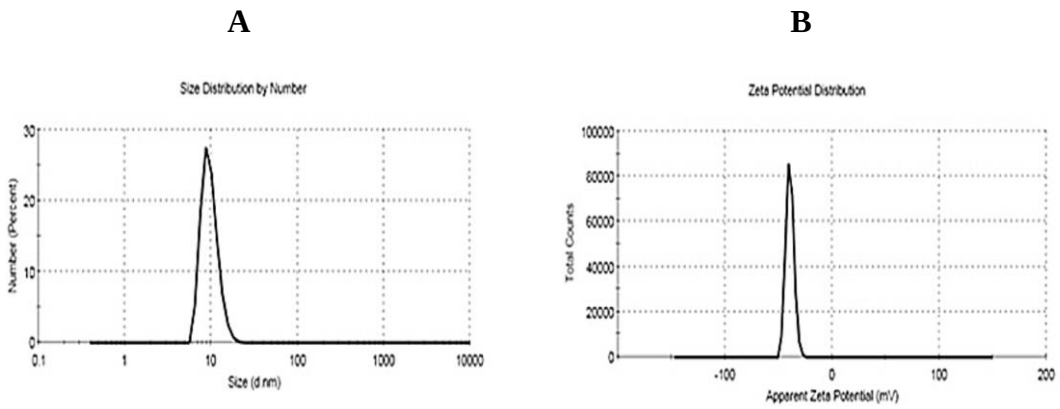


Figure 29: (A) Particle size distribution and (B) zeta-potential of MF-ASHEO nanoemulsion.

The operational pressure has a notable impact on both the average droplet size and the Polydispersity Index (PDI) of nanoemulsions. Consistent with our study's findings, higher pressures tend to yield smaller droplets, with the smallest observed at 150 MPa (Salvia-Trujillo *et al*., 2014). However, during production, droplet size may increase due to collision and coalescence, induced by vigorous Brownian motion and insufficient surfactant adsorption at higher pressures. For instance, nanoemulsions containing D-limonene and terpenes, treated at 300 MPa for ten cycles, exhibited droplet sizes ranging from 74.4 to 356.7 nm (Donsì *et al*., 2011). Additionally, apart from operational pressure, interactions between carvacrol and surfactant, as well as the adsorption of Tween80 into the MF-ASHEO nanoemulsion, played a role in reducing droplet size (Karsli *et al*., 2022). It is hypothesized that the phenol group of carvacrol, the primary component in MF-ASHEO constituting 94.51%, is positioned at the oil droplet's surface, oriented towards the water phase. This arrangement enhances the flexibility of the interphase, facilitating stronger compaction of the surfactant blend. The diverse sizes of amphiphilic molecules may lead to improved packaging and more effective stabilization of the interfaces.

The process of microfluidization resulted in a significant decrease in the zeta potential of MF-ASHEO droplets, reaching -39.4 mV, indicating an increase in their overall electric charge. When the zeta potential of droplets falls below ±30 mV, it weakens the repulsive forces between them within the nanoemulsion, potentially leading to instability (Salvia-Trujillo *et al*., 2015). The non-ionic emulsifier/surfactant Tween 80 imparts a negative charge to oil droplets by selectively absorbing hydroxyl ions from the aqueous phase or anionic impurities like free fatty acids present in the oil or surfactant phases (McClements and Rao, 2011). Furthermore, the anionic hydrocolloid CMC contributes to the negative zeta potential of nanoemulsions, aiding in their stabilization. The stability of the nanoemulsion depends on interactions and competition with species that have already been adsorbed onto the droplet surfaces (Arancibia *et al*., 2016). Discrepancies in the zeta potential between emulsions and nanoemulsions containing different EOs may stem from variations in ionizable oil compounds (Guerra-Rosas *et al*., 2016). Notably, the MF-ASHEO nanoemulsion exhibits a lower negative zeta potential compared to Algerian lemongrass oil, which contains citral isomers as the major constituents (Boudechicha *et al*., 2023).

The particle size, potential and Polydispersity Index (PDI) of the LGEO nanoemulsions produced through microfluidization were measured. The findings indicated that the average droplet diameter of the nanoemulsions was 20.76 ± 0.36 nm, with a PDI of 0.179 ± 0.03 (refer to Figure 30A). Additionally, the interfacial electrical charge of the droplets following microfluidization processing was recorded as -23.9 mV (Figure 30B).

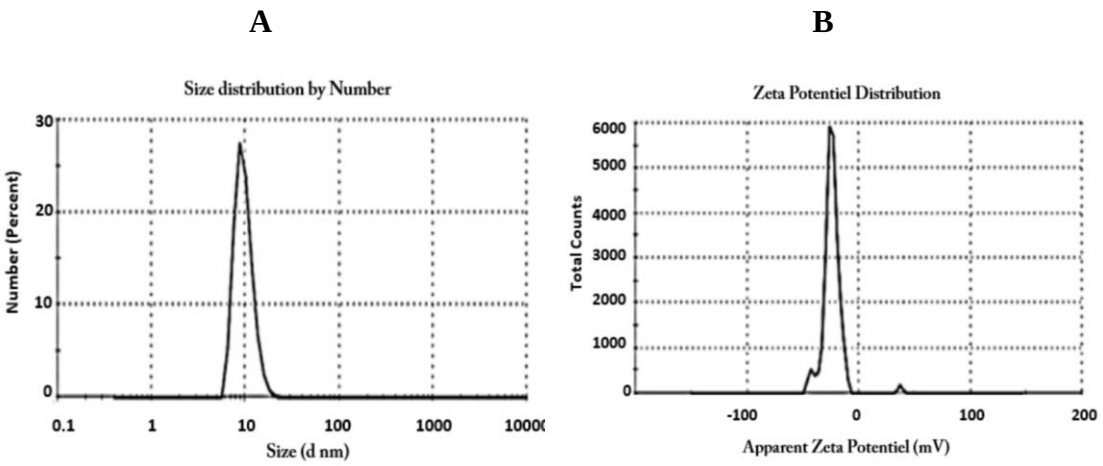


Figure 30: (A) Particle size distribution and (B) zeta-potential of MF-LGEO nanoemulsion.

In comparison to the droplet sizes observed in eugenol and clove nanoemulsions, the size of LGEO generated through microfluidization was found to be smaller. This reduction can be ascribed to the greater polarity of eugenol, which enhances its solubility in the aqueous phase compared to citral isomers (Gago *et al*., 2019). Various factors such as molecular structure, volatile compound content, interfacial tension, or surfactant affinity of different EOs or their primary compound-loaded nanoemulsions may contribute to the discrepancies in droplet size (Salvia-Trujillo *et al*., 2015). Notably, Qian and McClements, (2011) reported a significant reduction in the average droplet size of corn oil nanoemulsions processed once through microfluidization at different pressures, down to 165 nm. Subsequent cycles through the microfluidizer had minimal impact. Recent studies have focused on creating essential oil-infused nanoemulsions using microfluidization. Donsì *et al*. (2011) observed that nanoemulsions containing D-limonene, combined with terpenes and treated for ten cycles at 300 MPa, exhibited droplet diameters ranging from 74.4 to 356.7 nm. However, it's noteworthy that increased operating pressure may lead to collision and coalescence of droplets during nanoemulsion production, resulting in larger droplet sizes due to Brownian motion and sluggish surfactant adsorption (Jafari *et al*., 2007).

4. Antibacterial Activity

4.1. Agar Diffusion

The findings of the antibacterial efficacy using the disc diffusion technique, revealed the robust activity and broad spectrum of ASHEO. Primarily, their antibacterial activities against Gram-positive strains of pathogenic bacteria were found to be more potent compared to Gram-negative strains (see figure 31), even surpassing those of standard antibiotics. This particular EOs exhibited activity against all tested bacterial strains; with inhibition zones varying from 55.66 mm to 29.66 mm. According to the statistical analysis, MRSA and *Bacillus subtilis* demonstrated the highest sensitivity, followed by *E. coli*, *S. aureus*, *P. mirabilis*, and *A. baumannii*, while *P. aeruginosa* and *K. pneumoniae* exhibited lower sensitivity. Conversely, MF-ASHEO did not exhibit any inhibitory activity.

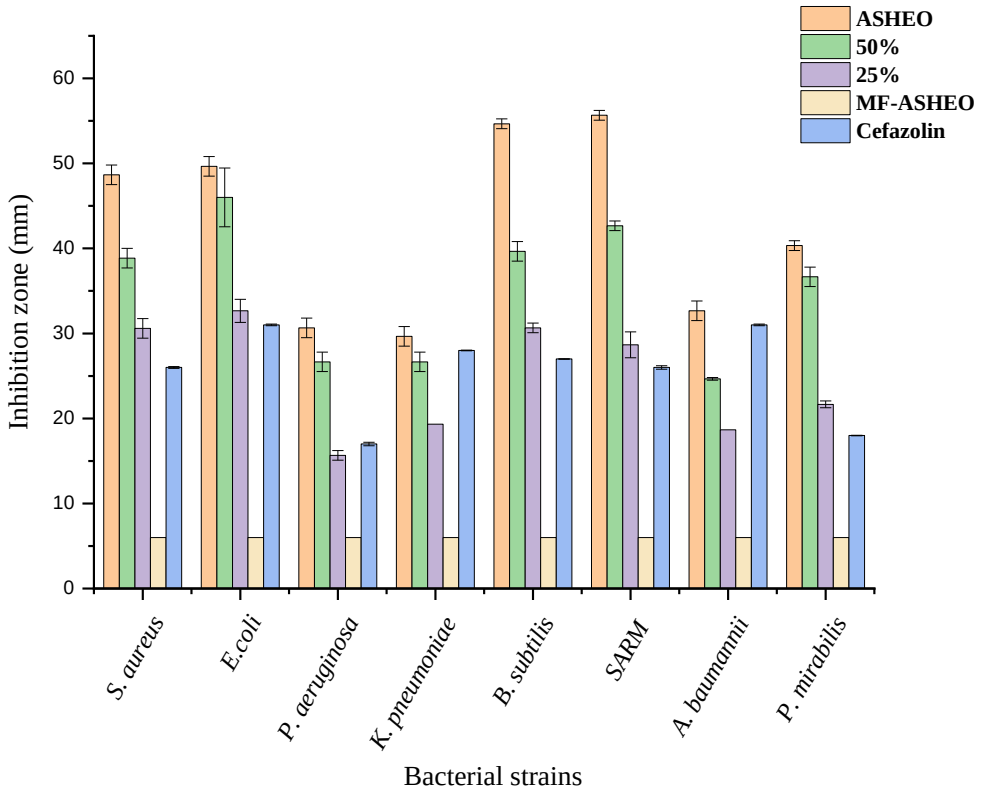


Figure 31: Antibacterial activity of ASHEO, MF-ASHEO and cefazolin using the disc diffusion method.

The inherent hydrophobic nature of EOs components is recognized for their ability to infiltrate the lipids present in bacterial cell membranes, thereby disrupting their organization and augmenting membrane permeability (Abou Baker *et al*., 2020). In our investigation, all tested strains exhibited sensitivity to ASHEO, a phenomenon largely attributed to the elevated concentration of carvacrol, a principal active constituent within EOs (Friedman, 2014). These results are consistent with previous findings regarding the antimicrobial efficacy of EOs rich in carvacrol, as reported for *S. hortensis* and *S. montana*. Notably, EOs abundant in phenolic compounds, such as carvacrol and thymol, as found in *S. montana* hydrolate, have been extensively documented for their potent antimicrobial properties (Pino-Otín *et al*., 2022). Reliable with the findings of (Sharma *et al*., 2020) who reported the lack of inhibitory effects in nanoemulsions containing Tween 80 and *Eucalyptus globulus* EOs, our study did not observe any antibacterial activity for MF-ASHEO. This absence of activity might stem from several factors, including the low oil concentration in the nanoemulsion formulation (1%), potential influences of physicochemical properties on the antimicrobial efficacy of nanoemulsions, and the short duration of incubation. Consequently, there is a need for further enhancements to enhance the antimicrobial efficacy of the developed nanoemulsion.

Regarding the activities differentiation between LGEO and MF-LGEO, all the concentrations of the tested EO exhibited significant activity against all tested bacterial strains, producing inhibition zones ranging from 16.66 mm to a total inhibition (see figure 32). Statistical analysis indicated that *Bacillus subtilis*, *S. aureus* and MRSA were the most susceptible strains with total inhibition, 48.66 and 49.66 mm, respectively, followed by *E. coli*, *P. mirabilis*, and *P. aeruginosa*. Conversely, *A. baumannii* and *K. pneumoniae* exhibited lower sensitivity. Interestingly, MF-LGEO demonstrated no inhibitory activity.

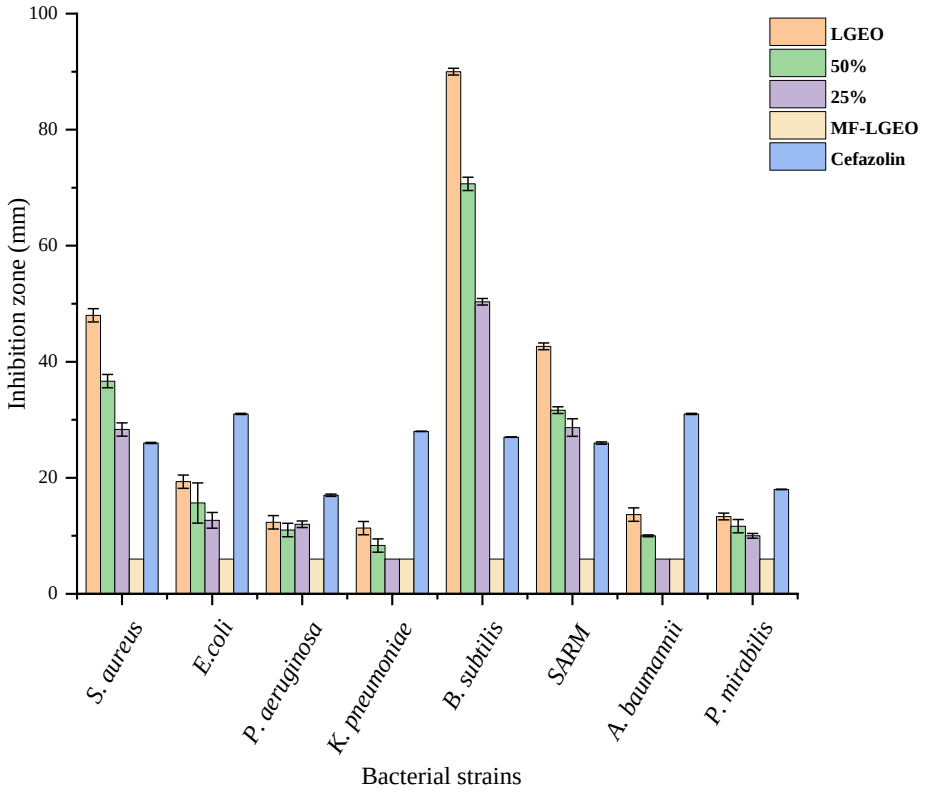


Figure 32: Antibacterial activity of LGEO, MF-LGEO and cefazolin using the disc diffusion method.

The high susceptibility of the tested bacteria to LGEO may be attributed to its elevated citral content, particularly geranial (30.73%) and neral (26.91%). Previous studies by Kumar Bharti (2013) have highlighted that *C. citratus* EO primarily comprises oxygenated monoterpenoids, such as geranial and neral, which exhibit antibacterial properties against pathogens like *S. aureus* and *Salmonella enterica*. Additionally, Subramaniam *et al*. (2020) have documented the potential of volatile oil from *C. citratus* against 13 gram-positive and 9 gram-negative isolates. This aligns with findings from Schweitzer *et al*., (2022), who demonstrated the efficacy of *C. citratus* EO against pathogens associated with pitted keratolysis (PK), including *Kytococcus sedentarius*, *Dermatophilus congolensis*, and *Bacillus thuringiensis*.

According to the findings by Ziani *et al*. (2011), oil droplets were observed to potentially reduce the effectiveness of surfactant-based antimicrobials. This result holds significant implications for developing efficient antimicrobial agents suitable for emulsion-based food and beverage products. Our own observations align with these collective findings.

4.2. MICs and MBCs

To fully exploit the antibacterial potential of our EOs and their nanoformulations, it was paramount for our research to meticulously evaluate both minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs).

The EOs extracted from *S. hortennsis* displayed remarkable antibacterial efficacy, effectively inhibiting the variety of pathogenic bacteria within a concentration range of 0.031 to 0.125 $mg.mL^{-1}$ (Table 06). Particularly noteworthy was its pronounced effect on *B. subtilis* and *S. aureus*, as previously observed with the paper disk diffusion method, both showing heightened sensitivity with MIC and MBC values as low as 0.031 $mg.mL^{-1}$. Conversely, *P. aeruginosa* and *A. baumannii* exhibited the highest MIC and MBC values at 0.125 $mg.mL^{-1}$ and 0.061 $mg.mL^{-1}$, respectively. Interestingly, *E. coli*, *K. pneumoniae*, MRSA, and *P. mirabilis* demonstrated comparable MICs of 0.062 $mg.mL^{-1}$.

Table 06: MICs and MBCs of ASHEO against the bacterial strains.

Bacterial Strains	**MIC ($mg.mL^{-1}$)**	**MBC ($mg.mL^{-1}$)**
S. aureus	0.031	0.062
E. coli	0.062	0.125
P. aeruginosa	0.125	0.125
K. pneumoniae	0.062	0.062
B. subtilis	0.031	0.031

MRSA	0.062	0.031
A. baumannii	0.125	0.062
P. mirabilis	0.062	0.062

The present study reaffirmed the antibacterial effectiveness of ASHEO against both reference and clinical isolates. These findings are consistent with another investigation involving EOs from *S. hortensis* and *S. montana*, which reported similar minimum inhibitory concentrations (MICs) (Khoury *et al*., 2016). ASHEO exhibited potent activity with low MIC values, measuring approximately 0.125 $mg.mL^{-1}$ against *P. aeruginosa* and 0.031 $mg.mL^{-1}$ against *S. aureus* and *B. subtilis* (Djenane *et al*., 2011). In contrast, its efficacy was comparatively weaker in the study conducted by (Abou Baker *et al*., 2020), where the EOs from *S. hortensis* demonstrated MIC values of 3 $mg.mL^{-1}$ against *P. aeruginosa* and 2 $mg.mL^{-1}$ against *S. aureus*.

MICs and MBCs assessment of LGEO against tested bacteria are presented in the (Table 07). LGEO showed action mainly against the Gram-positive pathogens, among which *B. subtilis* and *S. aureus* was the most affected revealed a relatively low MIC (0.031 $mg.mL^{-1}$) and MBC (0.031 $mg.mL^{-1}$). Among Gram-negative bacteria, *K. pneumonia*, *A. baumannii* and *P. mirabilis* demonstrated identical MIC and MBC values (0.062 $mg.mL^{-1}$), whereas the highest MIC values were observed against *P. aeruginosa* and *E. coli* (0.125 $mg.mL^{-1}$).

Table 07: MICs and MBCs of LGEO against the bacterial strains.

Bacterial Strains	**MIC ($mg.mL^{-1}$)**	**MBC ($mg.mL^{-1}$)**
S. aureus	0.031	0.031
E. coli	0.125	0.125
P. aeruginosa	0.125	0.125
K. pneumoniae	0.062	0.062
B. subtilis	0.031	0.031
MRSA	0.062	0.031
A. baumannii	0.062	0.062
P. mirabilis	0.062	0.062

Further investigations are needed to elucidate the roles lemongrass EOs. Moreover, the low inhibitory concentration (0.078 $mg.mL^{-1}$) of lemongrass EO against a *S. aureus*, aligns with our findings (Aiemsaard *et al*., 2011). Another study by De Silva *et al*. (2017) demonstrated the susceptibility of lemongrass oil against pathogenic bacteria isolated from pet turtles including *S. enterica*, *P. aeruginosa* and *P. mirabilis*. However, the MIC and MBC values of the citronella oil ranged from 0.244 $\mu g.mL^{-1}$ to 0.977 $\mu g.mL^{-1}$ when tested against the bacterial isolates reported by Wei and Wee, (2013) were significantly higher compared to those observed in the present study for essential oil.

5. Antibiofilm Activity

In the CV assay, ASHEO exhibited substantial inhibition of biofilm formation across all tested bacterial strains, contrasting with the limited effect observed with MF-ASHEO, which corroborates the previously mentioned antibacterial activity results. Notably, MF-ASHEO demonstrated negligible impact on biofilm formation, explaining its absence in (Figure 33).

Furthermore, biofilm development showed a dose-dependent reduction in the presence of ASHEO. Specifically, ASHEO effectively suppressed more than 60% biofilm formation of *E. coli* and *P. aeruginosa* at concentrations of MIC/2 (0.062 $mg.mL^{-1}$), MIC/4 (0.031 $mg.mL^{-1}$), and MIC/8 (0.015 $mg.mL^{-1}$) as depicted in (Figure 34). Similarly, each concentration of ASHEO (MIC, MIC/2, MIC/4, and MIC/8) effectively inhibited biofilm development in *B. subtilis*. Noteworthy reductions in biofilm formation were observed for *A. baumannii* and *S. aureus*, with reductions of 52.8%, and 32.08%, respectively, at a concentration of 0.015 $mg.mL^{-1}$ (sub-MICs). While the reduction was less pronounced for MRSA, *K. pneumoniae*, and *P. mirabilis* at the lowest ASHEO concentration, it still surpassed 50%, reaching maximum efficacy at 0.125 $mg.mL^{-1}$.

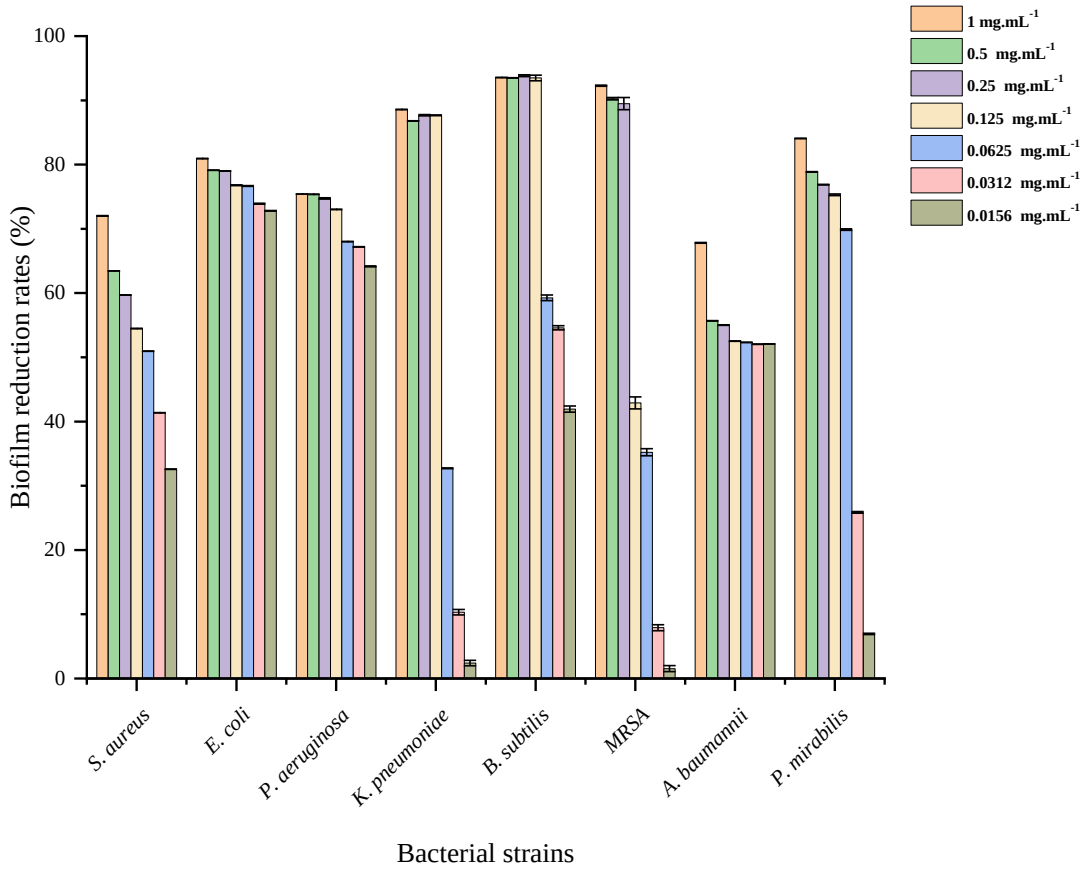


Figure 33: Biofilm reduction rates produced by ASHEO.

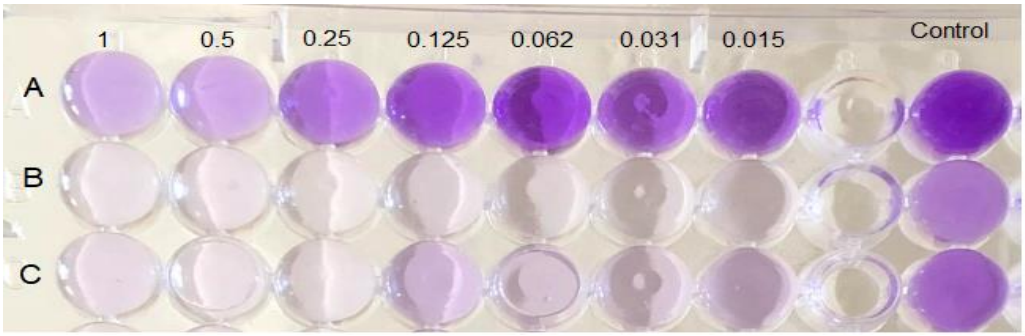


Figure 34: Antibiofilm activity of the ASHEO against (A) *P. aeruginosa*, (B) *A. baumannii*, and (C) *P. mirabilis*. (Biofilm formation is visually indicated by the varying intensities of violet color on the test plate, progressing from left to right (wells 1 to 7), with concentrations in $mg.mL^{-1}$. The 9^{th} well serves as the control).

Bacterial biofilms represent an enduring hazard of contamination within the food sector, constituting a notable peril to human health. Concerningly, numerous bacteria proficient in biofilm formation are evolving resistance against sanitizing agents, emphasizing the pressing requirement for innovative approaches to treatment. (Rossi *et al*., 2022).

Our study found that ASHEO exhibited significant antibiofilm activity against all tested biofilm-producing strains. Previous investigations have highlighted the potential of *Satureja spp*. in combating biofilm formation by both bacterial and fungal strains (Miladi *et al*., 2016). In a study carried out by Sharifi *et al*. (2018) demonstrated a notable effect of sub-MIC *Satureja hortensis* essential oil in preventing biofilm formation by *S. aureus* and disrupting existing biofilms. They suggested that compounds like thymol and terpinene in *Satureja hortensis* essential oil play a pivotal role in its anti-biofilm and anti-adhesive properties. In a comparable investigation, Seyedtaghiya *et al*. (2021) documented the effect of *S. hortensis* essential oil to inhibit biofilm formation in *E. coli* and *Salmonella* isolate obtained from poultry-related infections.

The findings regarding the antibiofilm efficacy of LGEO are summarized in the accompanying (Figure 35). According to the data presented, a concentration of 1 mg/mL demonstrated a noteworthy 50% reduction in antibiofilm activity across all bacterial strains. Particularly notable was the impact of LGEO on biofilms formed by *B. subtilis* and *S. aureus*, where a concentration as low as 0.062 $mg.mL^{-1}$ yielded a reduction exceeding 30%. In contrast, other bacterial strains exhibited reductions of less than 20% at this concentration.

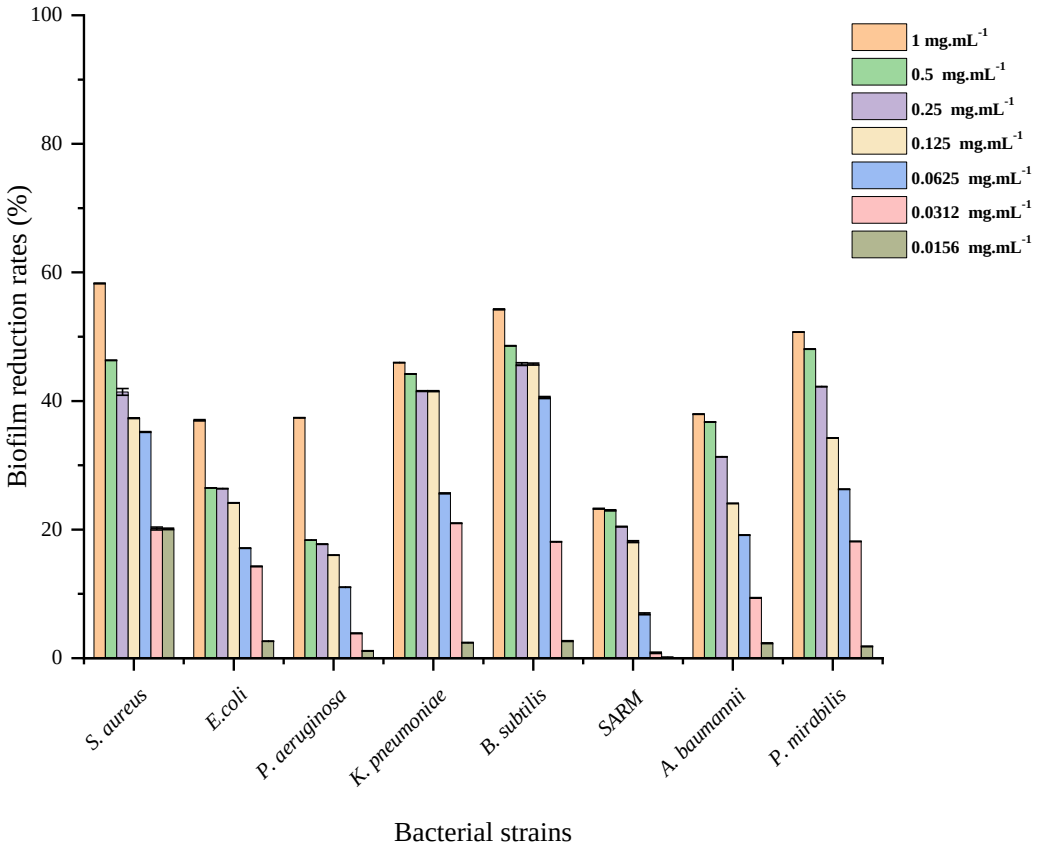


Figure 35: Biofilm reduction rates produced by LGEO.

Several research studies have illustrated the bactericidal impact of lemongrass on the formation of biofilms. In a prior investigation focusing on the EOs of *C. citratus*, biofilm formation by *K. pneumoniae*, *P. aeruginosa*, and *S. epidermidis* was examined, revealing

increasing efficacy in proportion to concentration, ultimately achieving total eradication at a concentration of 0.2% (v/v) (Khosakueng *et al*., 2024). Additionally, Adukwu *et al*. (2012) found that *S. aureus* biofilms exhibited complete loss of viability when treated with lemongrass essential oil concentrations ranging from 0.125% to 4% (v/v) over 24 hours. Our own observations align with these collective findings. Moreover, Martínez *et al*. (2021) observed that certain EOs significantly impacted biofilms, even in cases where they did not demonstrate direct antibacterial activity.

6. Antifungal Activity

The antifungal efficacy of ASHEO and MF-ASHEO was assessed against ten strains of toxigenic fungi (refer to Figure 36). It was observed that the application of the microfluidized technique augmented the antifungal potency. Relative to the standard antifungal agent (fluconazole), the fungi inhibition zones were approximately half the size of the standard effect. Furthermore, the antifungal effectiveness against toxigenic fungal strains followed an ascending order: *Aspergillus* < *Fusarium* < *Penicillium*. The results in (Figure 36) revealed that the diameter of inhibition zones against *Penicillium* fungi ranged from more than 50% (14.01±0.34 to 16.11±0.55 mm) compared to fluconazole (26.51±0.16 to 26.66±0.21 mm). Additionally, certain Aspergillus strains (*A. niger* and *A. carbonarius*) exhibited larger inhibition zones than others (*A. flavus*, *A. parasiticus*, and *A. ochraceous*).

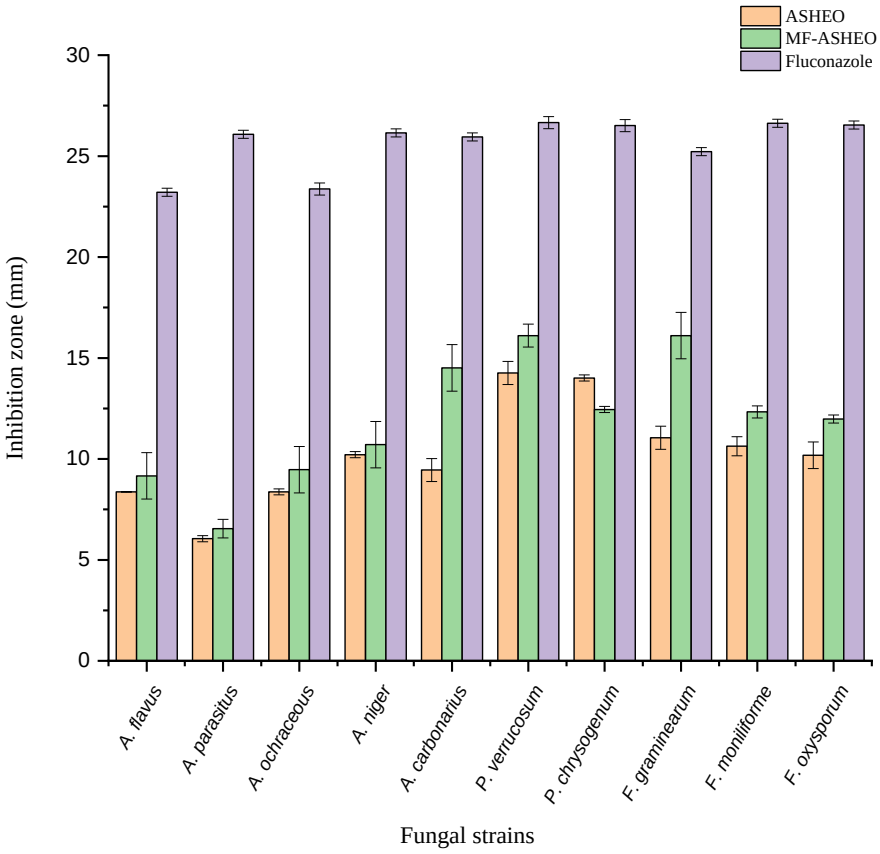


Figure 36: Antifungal activity of ASHEO, MF-ASHEO and fluconazole.

Our findings align with prior research conducted by various authors, illustrating the efficacy of *S. hortensis* essential oil on a range of fungal strains including *Rhizopus stolonifer* and *Rhizopus oryzae* (Tančinová *et al*., 2022), *R. stolonifer, P. digitatum*, *Aspergillus niger and Botrytis cinerea* (Nabigol, 2011), and Aspergillus species (García-Díaz *et al*., 2020). These results indicate a potential avenue for the development of natural antifungals for post-harvest disease management.

Additionally, Kambiz *et al*. (2013) provide clear evidence of the antifungal properties present in the alcoholic extract of *S. hortensis*. This extract has demonstrated effectiveness against both phytopathogenic and food spoilage fungi, suggesting its potential use as a protective agent in various food products based on previous research (Felšöciová *et al*., 2020).

The potent antifungal effect of savory species is likely linked to their high concentrations of phenolic compounds, notably carvacrol and thymol, both major monoterpene constituents. Recent studies have highlighted carvacrol's efficacy in combating fungal proliferation: it disrupts the cell membranes of *Botrytis cinerea* mycelia (Zhang *et al*., 2019) and inhibits the growth of *A. flavus* when used in vapor form (Duan *et al*., 2024). These findings underscore carvacrol's potential as a promising biofumigant for controlling post-harvest grain fungal infestations.

The current study observed a heightening in the antifungal properties of *S. hortensis* essential oil through the utilization of microfluidization technique. These findings are consistent with previous research indicating that transforming solutions of carvacrol and thymol into nanoform leads to enhanced efficacy (Hajibonabi *et al*., 2023). Precisely, the nanoemulsion derived from carvacrol and thymol demonstrated improved antimicrobial characteristics. Our investigation further strengthens the link between microfluidization, utilized for nanoemulsion preparation, and the enhanced properties of Algerian *S. hortensis* essential oil, particularly in terms of biological activities.

In relation to the antifungal properties of LGEO and MF-LGEO, the findings illustrate their effectiveness against a range of toxigenic fungal strains. Particularly noteworthy is their pronounced efficacy against *Fusarium* strains. It is notable that MF-LGEO exhibits superior inhibition against these fungal strains compared to LGEO, as depicted in (Figure 37). This enhanced effect may be attributed to the preservation mechanism inherent in the encapsulation process, which helps maintain the bioactive components of the oil, thereby protecting them from degradation. Moreover, both LGEO and MF-LGEO exhibit notable effectiveness in inhibiting the growth of several Aspergillus species, such as *A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. oryzae*, and *A. carbonarius*. The

inhibition zones observed range from 26±0.15 mm to 28±0.94 mm for LGEO and from 28±0.33 mm to 34±0.66 mm for MF-LGEO, underscoring their potential as agents for controlling fungal proliferation. The inhibition zone diameters against Penicillium fungi surpassed 50%, ranging from 34.01±0.57 to 34.26±0.55 mm, whereas those of fluconazole ranged from 26.51±0.16 to 26.66±0.21 mm. The antifungal effectiveness of MF-LGEO surpasses that of the conventional antifungal medication Fluconazole and approaches the efficacy of LGEO.

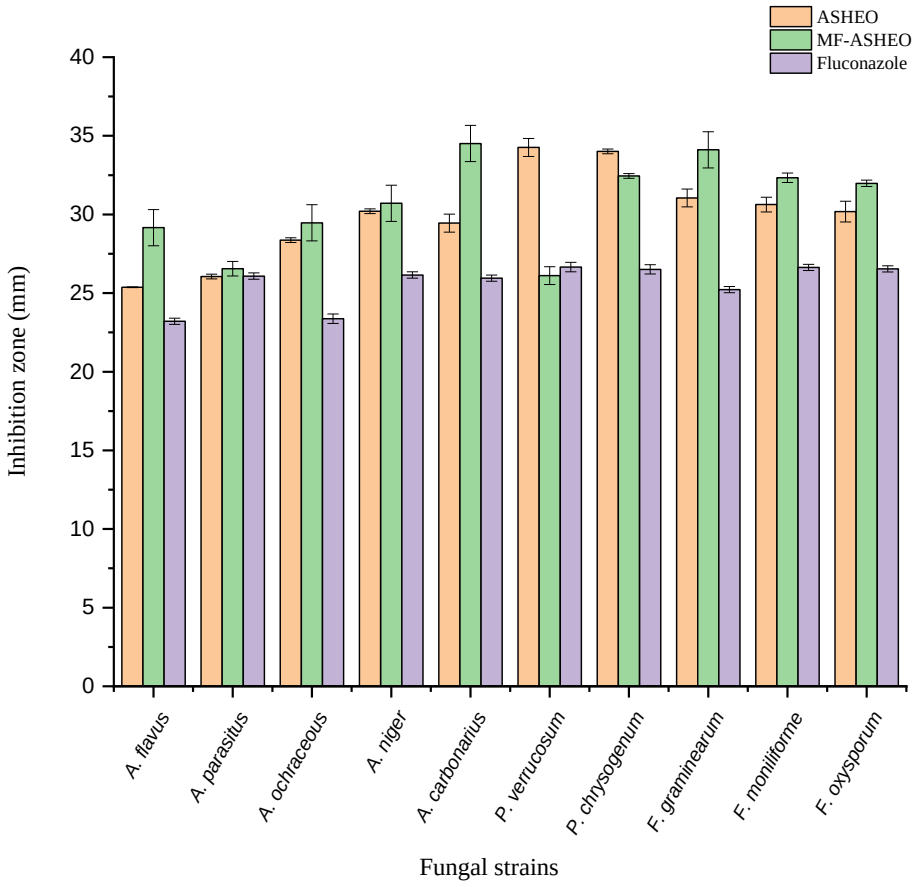


Figure 37: Antifungal activity of LGEO, LGEO and fluconazole.

The highest fungicidal efficacy of *C. citratus* may be attributed primarily to the presence of citral and geraniol as major constituents (Scariot *et al*., 2021) (Sampaio *et al*., 2020). Even so, other components such as citronellol, α-ocimene, α-pinene, and linalool

might also contribute to its antifungal properties (Victoria *et al*., 2012). The EOs derived from *C. citratus* has demonstrated antifungal activity against various fungi, including *Raffaelea quercus-mongolicae* and *Rhizoctonia solani* (Lee *et al*., 2020); *Aspergillus flavus* and *Aspergillus parasiticus* (Sawadogo *et al*., 2022); and *Colletotrichum musae*, *Fusarium incarnatum*, and *Fusarium verticillioides* (Kamsu *et al*., 2019).

Additional research has demonstrated that incorporating EOs into colloidal systems like nanoformulations results in increased antimicrobial efficacy compared to using the EO alone (Ahmed *et al*., 2021) (Jayari *et al*., 2022). As outlined by Donsì *et al*. (2011), nanoformulations comprising small particles with a hydrophilic surface can traverse the cell membrane. This process involves the fusion of emulsifier droplets with the phospholipid bilayer of microorganisms, enhancing their accessibility across the cell membrane and ultimately causing cell death by membrane rupture.

7. Cytotoxicity

This study aimed to assess the cytotoxic effects of ASHEO and MF-ASHEO on HepG2, Vero, and WI-38 cells using MTT and WST-1 viability assays, with cisplatin as the standard reference drug. The findings revealed that both ASHEO and its nanoemulsions significantly reduced the viability of HepG2 cells compared to Vero and WI-38 cells. Notably, ASHEO exhibited the most potent growth-inhibitory activity against HepG2 cells, with the lowest IC50 values (16.69 and 23.92 $\mu g.mL^{-1}$) compared to cisplatin (IC50 20.71 and 40.95 $\mu g.mL^{-1}$) in MTT and WST-1 assays, respectively. The lower IC50 values observed in HepG2 cells compared to Vero and WI-38 cells suggest the selective cytotoxicity of ASHEO and its microfluidized nanoemulsion, as summarized in (Table 08).

Table 08: Cytotoxic assessment of ASHEO and its microfluidized nanoemulsion against HepG2, Vero, and WI-38 cell lines using MTT and WST-1 assays.

Cell line	ASHEO (µg.mL^{-1})		MF-ASHEO (IC_{50} µg.mL^{-1})		Cisplatin (Control) (IC_{50} µg.mL^{-1})	
	MTT	WST-1	MTT	WST-1	MTT	WST-1
HepG2	16.69±0.5*	23.92±2.0	126.0±1.82	249.08±2.77	20.71±1.15	40.95±1.88
Vero	88.34±5.0	89.94±4.91	-	-	142.33±4.12	287.6±3.43
WI-38	-	-	207.15±2.61	648.71±4.11	277.6±4.5	401.2±3.66

*The data were expressed as means ±SEM (where n = 3, $p \leq 0.05$); SD: standard division

In (Figure 38), the morphology of HepG2 cells exposed to ASHEO and MF-ASHEO was examined at concentrations of 15.62 and 125 µg.mL^{-1} for 24 hours, in comparison to the control cell line. These concentrations were chosen based on their demonstrated cell viability below 50% in the MTT assay. The results revealed that the alterations in HepG2 cell morphology were dose-dependent. Cells exposed to ASHEO and MF-ASHEO exhibited significant changes in morphology, with a reduced cell count per field, suggesting cell detachment during exposure. This indicates a potential impact of ASHEO and MF-ASHEO on cell membranes. Additionally, a notable accumulation of lipid vacuoles within the cells was observed. The HepG2 cells displayed morphological changes, appearing shriveled and smaller, with increased intercellular spaces and an irregular, rounded shape compared to the control group. Furthermore, some cells exhibited shrinkage at concentrations below the IC50 and showed signs of apoptotic cell death, including membrane rupture.

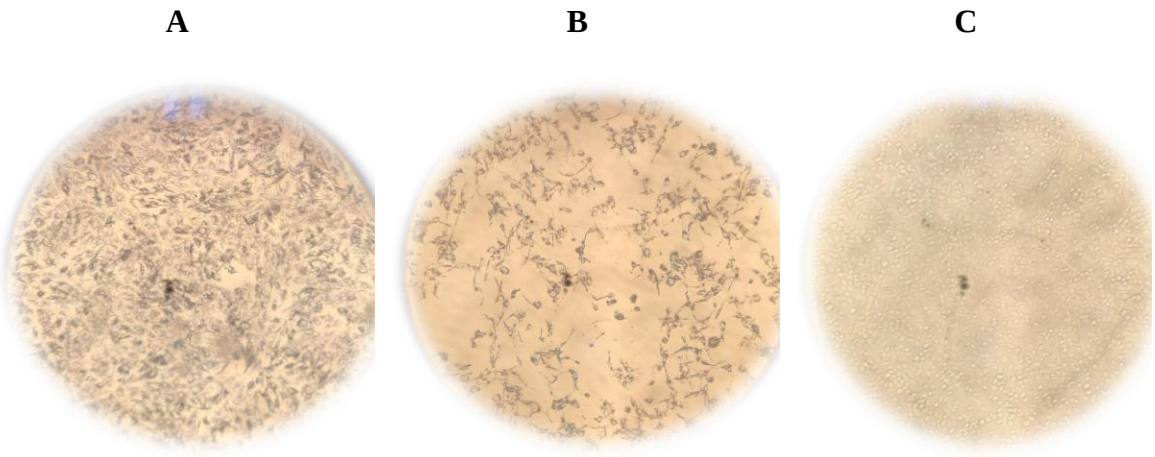


Figure 38: Morphology of HepG2 Cells Following 24-Hour Cultivation with Treatments: (A) ASHEO, (B) MF-ASHEO, Compared to (C) Control Cell Line.

To our knowledge, there have been limited studies investigating the cytotoxicity of *S. hortensis* volatile oil, particularly with regard to Algerian oils and nanoemulsion activity. The cytotoxic effects observed for the current ASHEO) align with findings by (Popovici *et al.*, 2019), where the oil exhibited concentration-dependent activity and demonstrated greater efficacy than hydro-ethanolic extract on two tumor cell lines, A375 (IC_{50} 22.27 µM) and B164A5 (IC_{50}, 34.16 µM), as determined by the MTT assay.

It is noteworthy that MF-ASHEO exhibited lower cytotoxicity than ASHEO after 24 hours of exposure in our study. This difference in cytotoxicity between ASHEO and MF-ASHEO *in vitro* may be attributed to the entrapment of bioactive compounds in nanostructures, altering their route of association or internalization to the targeted cell (Ding and Ma, 2015), consistent with findings by Milhomem-Paixão *et al*. (2017) and da Silva Gündel *et al*. (2018) on the cytotoxicity of andiroba and basil oils nanoemulsions.

As suggested by Mansour *et al*. (2023), an effective drug delivery system should maximize therapeutic benefits while minimizing toxicity. Our study indicates that utilizing

MF-ASHEO as a delivery system enhances drug efficacy, reducing the required concentration while maintaining effectiveness and minimizing side effects. Importantly, low cytotoxicity in normal cells does not necessarily imply reduced bioavailability of bioactive compounds *in vivo*. Nanostructures can selectively accumulate in the desired target tissues, capitalizing on the unique characteristics of pathological processes for therapeutic purposes. For instance, nanoparticles can preferentially accumulate in inflammatory lesions due to abnormal blood vessels and reduced lymphatic drainage, a phenomenon known as the enhanced permeation and retention (EPR) effect, thereby increasing the concentration of bioactive compounds at the desired target site (Watanabe *et al*., 2010 ; J. Liu *et al*., 2022).

As illustrated in (Table 09), treatment with LGEO and its nanoemulsions led to a decrease in cell viability percentage in HepG2 cells compared to Vero and WI-38 cells. Notably, LGEO demonstrated the highest growth inhibitory activity against the HepG2 cell line, exhibiting the lowest IC_{50} values (1.78 and 28.54 µg.mL^{-1}) compared to cisplatin (IC_{50} 20.71 and 40.95 µg.mL^{-1}) in both MTT and WST-1 assays, respectively. The lower IC_{50} values observed in HepG2 cells compared to Vero and WI-38 cells underscore the selectivity of the investigated LGEO, as depicted in (Table 09).

Table 09: Cytotoxic assessment of LGEO and its microfluidized nanoemulsion against HepG2, Vero, and WI-38 cell lines using MTT and WST-1 assays.

Cell line	**LGEO (µg.mL^{-1})**		**MF-LGEO (IC_{50} µg.mL^{-1})**		**Cisplatin (Control) (IC_{50} µg.mL^{-1})**	
	MTT	WST-1	MTT	WST-1	MTT	WST-1
HepG2	1.78 ± 0.08	28.54 ± 2.26	230.77 ± 3.12	249.08 ± 2.77	20.71±1.15	40.95±1.88
Vero	236.91 ± 5.2	111.04 ± 6.76	-	-	142.33±4.12	287.6±3.43
WI-38	-	-	618.65 ± 5.61	957.41 ± 7.11	277.6±4.5	401.2±3.66

The observed toxic effects of the current MF-LGEO are in line with the results reported by (Trang *et al*., 2020), who demonstrated the cytotoxic activity of *C. citratus* oils from various regions in Vietnam against lung cancer cells, with an IC50 range of 4.25–8.93 $\mu g.mL^{-1}$. Citral, the main component of LGEO consisting of neral and geranial, has been found to possess cytotoxic activity against several human leukemia cell lines, prompting apoptosis in leukemia cells via procaspase-3 activation (Nordin *et al*., 2019). Moreover, geraniol, the second most prevalent compound, exhibits notable anticancer properties through various signaling pathways (Cho *et al*., 2016). A comparison between the cytotoxic potential of LGEO and its nanoemulsion indicates a reduced efficacy in nanoemulsions due to the lower concentration of LGEO within the formulation. This observation is consistent with the findings of (Verma and Preet, 2021), where exposure of HEK293 cell lines to 100 ppm of LGEO nanoemulsion for 48 hours resulted in a maximum cell survivability of 61%. The alterations observed in the volatile constituents following the microfluidizing procedure could be associated with the safety attributes indicated by the cytotoxicity findings. With the quantitative rise in neral and geranial, previous studies have documented anti-inflammatory properties (Liao *et al*., 2015).

8. Antioxidant Activity

Employing the DPPH method, the antiradical activity is presented as the inhibition percentage at different concentrations of the samples (Figure 39). The inhibition percentage increased with increasing the concentration for ASHEO, LGEO and BHT. In contrast, MF-ASHEO and MF-LGEO showed no reductant activity in the DPPH assay.

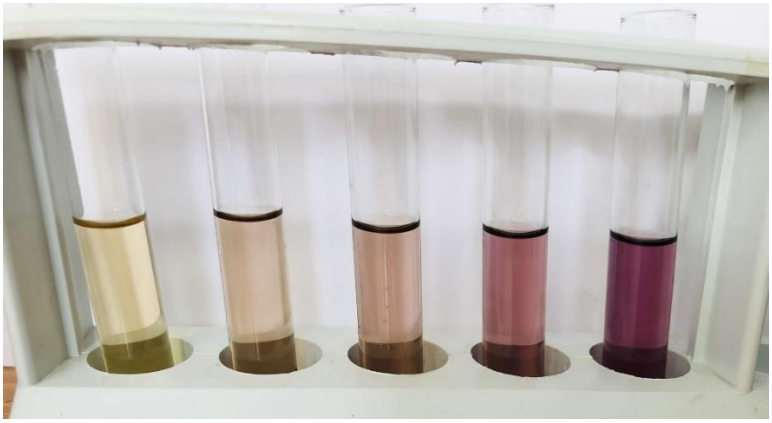


Figure 39: Color transition from purple to yellow seen during the transformation of DPPH solution by different concentration of samples.

As presented in the (Figure 40), the ASHEO inhibited 46.70 ± 1.51% at a 500 µg.mL^{-1} concentration. However, the BHT showed 85.00 ± 0.02% at only 125 µg.mL^{-1}. The half-maximum inhibitory concentration (IC_{50}) of both samples is presented in (Figure 41). The t-test revealed significant differences between ASHEO and BHT ($p \leq 0.0001$). The IC_{50} was 536.47 ± 21.99 µg.mL^{-1} and 15.48 ± 0.06 µg.mL^{-1} for ASHEO and BHT, respectively.

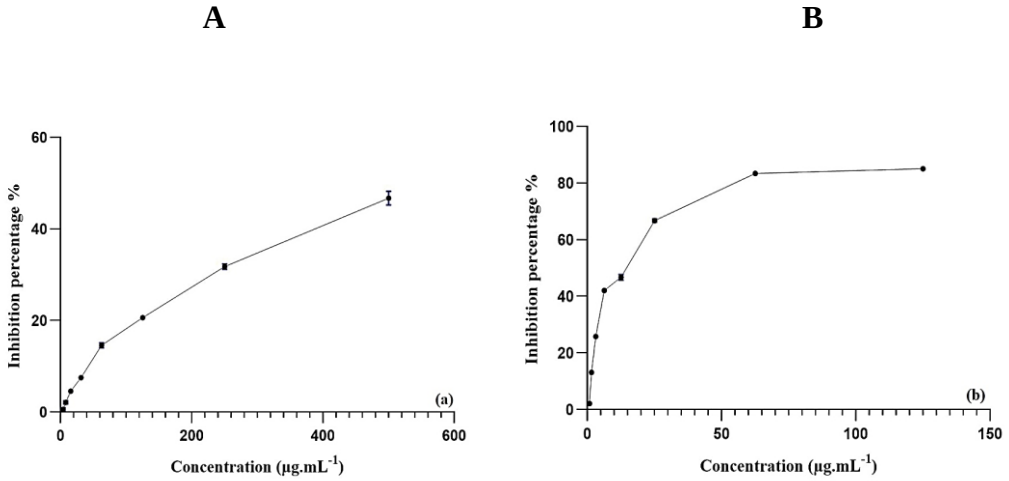


Figure 40: The free radical scavenging activity of ASHEO (A) and BHT (B).

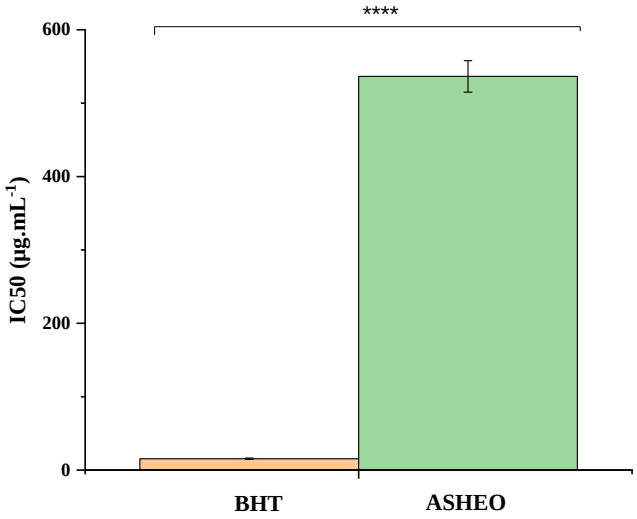


Figure 41: The IC50 of ASHEO and BHT.

The ASHEO and BHT standards demonstrated effective scavenging of DPPH radicals, with the IC_{50} values indicating a lower antioxidant activity for ASHEO compared to BHT. The antioxidant activity of ASHEO could be attributed to its content of terpenes, particularly monoterpene hydrocarbons and oxygenated monoterpenes, as previously determined (Martignago *et al*., 2023). These compounds have the ability to donate protons, thereby stabilizing free radicals. The findings of this study are consistent with research conducted on *S. hortensis* EO by (Abou Baker *et al*., 2020). *S. montana* EO also exhibited notable radical scavenging capacity (Djordjevic *et al*., 2021).

The antioxidant capacity assays of *C. citratus* essential oil, illustrated in the (Figure 42), reveal comparatively lower activity when compared to BHT. While LGEO exhibited a 72% inhibition at a concentration of 500 µg.mL^{-1}, BHT demonstrated a slightly higher inhibition of 70% at a significantly lower concentration of only 20 µg.mL^{-1}. Moreover, LGEO displayed a notably higher IC50 value (82.87 ± 2.15 µg.mL^{-1}) compared to BHT, as showed in the (Figure 43), with an IC50 of 15.48 ± 0.06 µg.mL^{-1}, indicating a less potent antioxidant activity in comparison.

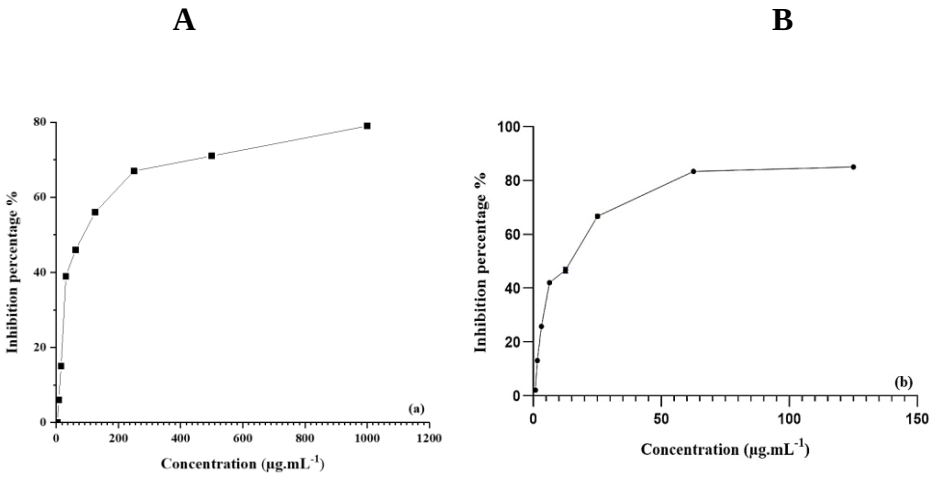


Figure 42: The free radical scavenging activity of ASHEO (A) and BHT (B).

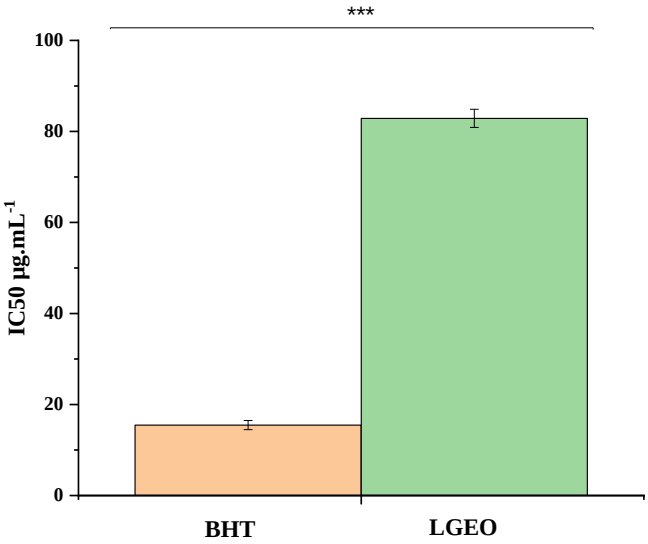


Figure 43: The IC50 of LGEO and BHT.

Fatunmibi *et al*., (2023) observed an IC50 value of 71.82 µg.mL^{-1} for the antioxidant activity of the essential oil extracted in Nigeria from *C. citratus*. Similarly, (Bhatnagar, 2020)'s study on *C. flexuosus* revealed an IC50 value of 43.67 µg/mL, aligning with our own findings. Various plants contain antioxidant compounds that counteract oxidation through diverse mechanisms, thus averting oxidative stress. These compounds, such as free radical

scavengers, reducing agents, and chelating agents, among others, have been identified (Júnior *et al*., 2024).

The absence of reductive activity in the DPPH assay for MF-ASHEO and MF-LGEO may be attributed to the encapsulation of antioxidant compounds within the nanoemulsions, which hinders their release and interaction with the chemical agent. Although the DPPH test did not reveal reductive activity in the nanoemulsions, they may still possess antioxidant properties (Borges *et al*., 2018) ; (Ha *et al*., 2015) found that the release of encapsulated compounds in some nanoemulsions can be influenced by the incubation period of the sample with the reagent. In the present study, the relatively short incubation period may have limited the release of active principles in the nanoemulsions, as evidenced by the expression values for reductive activity.

9. Molecular Docking and ADME study

The antibacterial properties of carvacrol, the main volatile component of the *S. hortensis* under investigation, were examined using a molecular docking method. The study's findings indicate that carvacrol forms bonds with crucial enzymes essential for producing and restoring cell walls, proteins, and nucleic acids. Enzyme crystal structures (isoleucyl-tRNA synthetase, DNA gyrase, dihydropteroatesynthase, D-alanine: D-alanine ligase, IV topoisomerase, dihydrofolate reductase, and penicillin-binding protein 1a) were obtained from Protein DataBank (https://www.rcsb.org/, accessed on August 17, 2022) with the following PDB IDs:1JZQ, 1KZN, 2VEG, 2ZDQ, 3RAE, 3SRW, and 3UDI.

(Figure 44) presents the binding free energies (ΔG). The molecular docking analysis yielded the optimal orientations or locations of the ligand within the receptor site. A lower ΔG signifies a stronger connection between the receptor and the ligand. The binding

affinities of carvacrol ranged from -5.2 to -8.1 kcal/mol. The docking scores for 2zdq were the highest, as shown in (Figure 44).

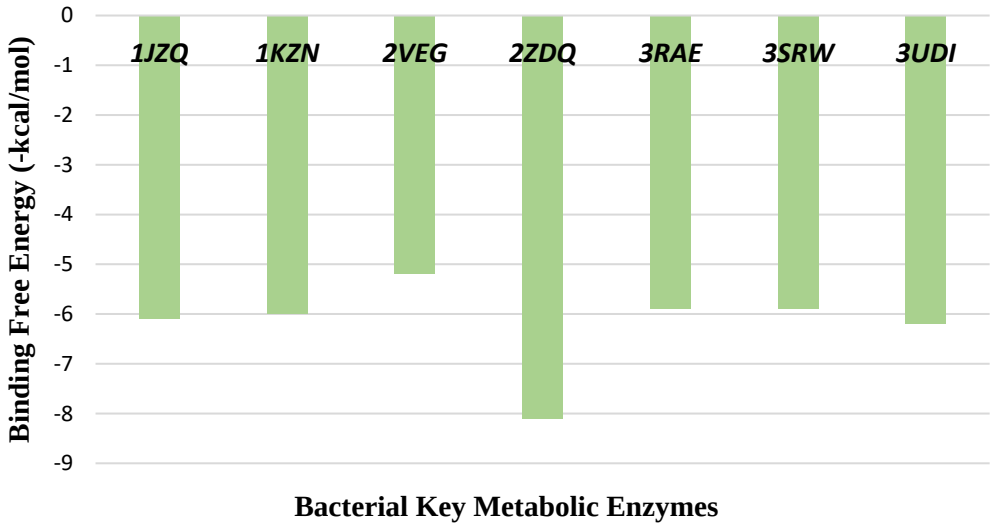


Figure 44: Binding free energy values were computed by molecular docking carvacrol to bacterial metabolic.

(Figure 45) illustrate the interaction between carvacrol and the crystal structure of D-alanine: D-alanine ligase (PDB: 2ZDQ). This interaction exhibits the most elevated docking score. The greater binding affinity of carvacrol with 2ZDQ (-8.1 kcal/mol) is due to the traditional hydrogen bonding between carvacrol (acting as a hydrogen donor) and the O atom of GLU A:197, as well as the OH group of TYR A:218 (acting as hydrogen acceptors), as illustrated in (Figure 45). The study discovered hydrogen bonding with lengths ranging from 2.4 to 2.7Å. In addition to hydrogen bonds, there are other interactions found between the residues of 2ZDQ and carvacrol. The interactions mentioned are π-anion, π-σ, π-π stacking, and π-alkyl electrostatic and hydrophobic contacts. A π-anion type electrostatic bond is exclusively established between the negatively charged O atom of GLU A:197 and the π-orbitals of carvacrol. The hydrophobic interaction between carvacrol and PHE A:272 involves the interaction between the C-H group of carvacrol and the π-orbitals of PHE

A:272. The study also found that carvacrol alkyl groups form interactions with aryl-containing amino acids such as PHE A:151, 222, and 272 through their π-orbitals, as shown in (Figure 45). Moreover, a π-π stacked interaction refers to the attractive force that occurs between aromatic rings as a result of the presence of π-electron clouds, such as the π-orbitals of PHE A:151 and 272, and carvacrol.

Hydrogen bonds within biological complexes are crucial for ensuring the specificity of molecular recognition. Their corresponding free energy varies between -1.5 and -4.7 kcal/mol (Ferreira de Freitas and Schapira, 2017). Weak hydrogen bonds, such as C-H....π-interactions, play a significant role in maintaining the stability of proteins (Brandl *et al*., 2001). The alkyl groups of carvacrol frequently interact with amino acids containing aryl groups, such as phenylalanine, through their π-orbitals. The predominant type of interactions observed in protein-ligand complexes are hydrophobic interactions involving aliphatic and aromatic carbons. Among the aromatic systems, benzene rings are the most frequently encountered (Ferreira de Freitas and Schapira, 2017).

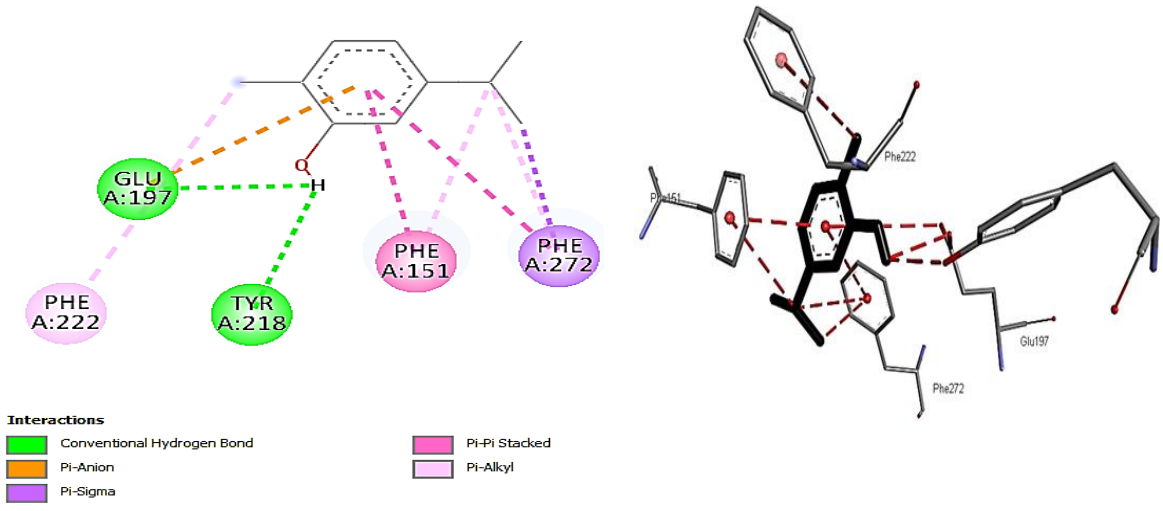


Figure 45: Interactions of carvacrol with 2ZDQ.

As far as we know, there have been no previous studies on the computer-based analysis of carvacrol and its impact on enzymes related to the killing or inhibiting of bacteria, as well as the production of aflatoxins. This topic is explored in the present work. Nevertheless, a comparable in-silico investigation of carvacrol, the primary constituent of *Origanum compactum* Benth oil, demonstrated similar effectiveness against nicotinamide adenine dinucleotide phosphate oxidase (2CDU) and *S. aureus* nucleoside diphosphate kinase (3Q8U) with a glide score of -6.082 and -6.039 kcal/mol, respectively (El Abdali *et al*., 2023). The experimental data revealed that carvacrol has a strong and stable binding affinity (-5.5 to -5.6 kcal/mol) with *E. coli* ESBL, including TEM-72, SHV-2, and CTXM-9. Hydrogen bonding between amino acids in ESBL proteins serves to attach the protein residues and enhance their interaction with carvacrol. In addition, hydrophobic interactions play a significant role in the interactions between ligands and proteins. Carvacrol demonstrated a total of five hydrophobic interactions with TEM-72, two with SHV-2, and three with CTXM-9 (Khan *et al*., 2020).

Carvacrol was analysed by SwissADME for its ADME characteristics, revealing a molecular weight below 500. Additionally, it possesses fewer than five hydrogen bond donors, acceptors, and Log p values. The value of Log p determines the hydrophobicity and lipophilicity of a substance, which in turn affects its transportation. The values of the topological polar surface area (TPSA) and the number of rotatable bonds also meet the acceptable criteria. Carvacrol possesses sufficient hydrophobic and mild lipophilic characteristics, enabling it to rapidly traverse the membrane surface and reach the receptor site. Furthermore, the Log S value of the substance falls within a suitable range, indicating that it can be readily absorbed in the gastrointestinal tract. The solubility (Log S) has a substantial impact on the absorption and distribution characteristics of a substance. In

addition, carvacrol has pharmacological characteristics and adheres to Lipinski's rule of 5, as depicted in (Figure 46).

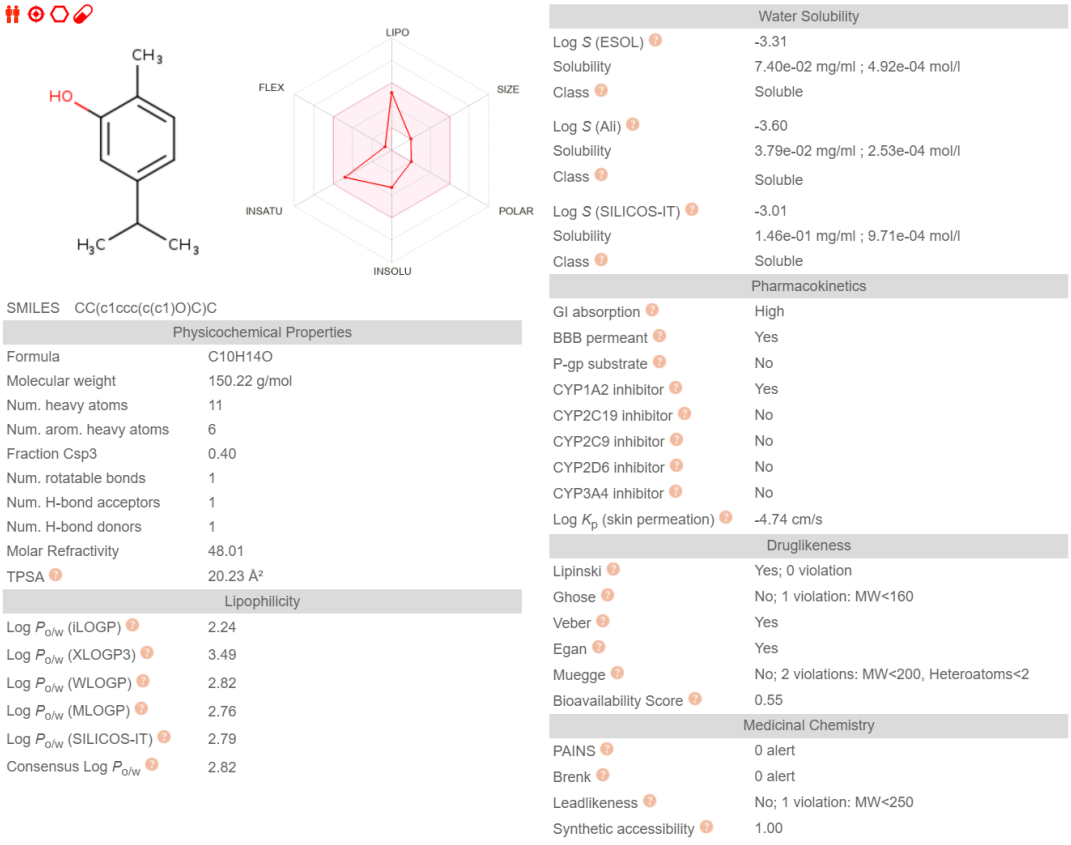


Figure 46: *In silico* ADME Properties of carvacrol using SwissADME.

Predicting drug-likeness filters at an early stage is crucial for optimizing small compounds for therapeutic purposes and enhancing their prospect of being developed into effective drugs. These filters rely on empirical principles that prioritise important pharmacokinetic indices, offering vital information for drug discovery. (Figure 46) displays the crucial drug-likeness filters for carvacrol, a compound suggested by SwissADME as a promising option for the development of cancer drugs. Based on Lipinski's rule of five, which permits a maximum of one violation, the tested chemical is suitable for oral administration as a medication in humans. Moreover, carvacrol has significant absorption in the human intestines, making it a suitable choice for oral delivery. The ADME analysis of

our study aligns with prior research conducted by Herrera-Calderon *et al*. (2020) and Fatima *et al*. (2022) who investigated the mechanism of action of carvacrol on several receptors implicated in the advancement of breast cancer and its potential as an anticancer drug. Considering these pharmacokinetic features, carvacrol shows potential as a viable therapeutic option for antibacterial or antifungal treatments.

Conclusion

Conclusion

Antibiotic resistance is one of the biggest global public health threats. The abuse of antibiotics and the emergence of resistant strains of bacteria undermine the effectiveness of conventional treatments. In this context, the use of natural molecules extracted from medicinal plants offers a promising alternative to combating this resistance, due to their chemical diversity and multiple mechanisms of action. Furthermore, the nanoformulation of these molecules can improve their bioavailability and therapeutic effectiveness.

The objective of our study in the initial section was to conduct a retrospective investigation in the service of Infectious Diseases at Setif Hospital. The data demonstrate a wide range of resistance patterns among the isolated isolates, with different levels of resistance detected for routinely tested drugs. The resistance rates for amoxicillin, cefazoline, and cefotaxime are particularly alarming since they reach 70%. This indicates that there are major difficulties in effectively treating infections with these medicines. The decreasing vulnerability to ciprofloxacin and gentamicin highlights the pressing need to address the increasing patterns of resistance. In addition, the identification of individual bacteria emphasizes the high occurrence of *E. coli* ESBL (29.03%) as the most commonly detected species, followed by *K. pneumoniae*, *E. coli* (16.13%), and *K. pneumoniae* ESBL (9.68%).

Overall, our study highlights the importance of continuous research and monitoring to guide the implementation of effective procedures for managing antibiotics and addressing the increasing problem of antibiotic resistance in healthcare settings.

The second section of our study mainly examined the extraction of EOs from *S. hortensis* and *C. citratus*. Additionally, it involved preparing the nanoformulations of these oils using microfluidization and investigating their biological activities. Microfluidization, a high-pressure homogenization technique, has significantly impacted the volatile composition of

ASHEO and LGEO when loaded into nanosystems. This includes changes in carvacrol, thymol, for *S. hortensis* and γ-Terpinene, neral, and geranial for *C. citratus*, as well as a notable increase in decanal content, setting a new record. The generated microfluidization emulsion of MF-ASHEO and MF-LGEO exhibited unique droplet diameters of 20.76 ± 0.36 nm and 41.72 ± 12.72 nm, with a PDI of 0.291±0.04 and 0.179 ± 0.03, respectively. The ξ-potential nanoemulsion value highlighted the emulsion stability of both plants under investigation.

The cytotoxicity of the EOs and nanoformulations under investigation exhibited encouraging values in two assays (MTT and WST-1) when tested against three distinct cell line strains. Furthermore, the cell line experiment also documents the ameliorative effect of the microfluidization process, which results in an increase in the anti-inflammatory effect. The findings are depicted and associated with the alterations in components identified through the GC-MS analysis of the nanoformulations.

Upon measuring the antibacterial and antibiofilm properties, it was determined that ASHEO and LGEO exhibited superior efficacy against pathogenic isolates of Gram-positive and Gram-negative bacteria compared to MF-ASHEO and MF-LGEO. Significantly, LGEO demonstrated complete inhibition of *B. subtilis*, while ASHEO effectively exhibited activity against all bacterial strains that were tested. The inhibition zones measured between 55.66 mm and 29.66 mm, and the biofilm formation of *E. coli* and *P. aeruginosa* was suppressed by over 60% at concentrations of MIC/2 (0.062 $mg.mL^{-1}$), MIC/4 (0.031 $mg.mL^{-1}$), and MIC/8 (0.015 $mg.mL^{-1}$). Additionally, the examined EOs, along with their microfluidization oils, showed antifungal properties against mycotoxigenic strains of fungi. Among these, MF-LGEO exhibited exceptional antifungal activity against five strains of the Aspergillus genus, three strains of the Penicillium genus and two strains of Fusarium genus, influencing their growth rates. The results

mentioned above suggest that MF-LGEO may serve as a viable treatment for toxigenic fungal contamination, as it inhibits the production of toxins by said fungi.

Studied EOs revealed moderate antioxidant effects in radical scavenging, with an IC50 value of 536.47 ± 21.99 µg.mL^{-1} for ASHEO and an IC50 value equal to 82.87 ± 2.15 µg.mL^{-1}.

The bactericidal and bacteriostatic effects of carvacrol on the target proteins were validated by ADME and *in silico* analyses. This abundant molecule found in *S. hortensis* and comprising *C. citratus* molecules, was found to be an intriguing possibility for the development of cancer medications. Furthermore, due to its substantial absorption capacity in the human intestines, carvacrol is a suitable selection for oral administration. Considering these pharmacokinetic features, carvacrol shows potential as a viable therapeutic option for antibacterial or antifungal treatments.

Satureja hortensis L. and *Cymbopogon citratus* (DC.) Stapf has been historically used for their valuable EOs and to prevent fungal toxigenic diseases and pathogenic resistant bacteria infections. These findings corroborate these traditional uses. The microfluidization procedure has a profound impact on both the volatile content and biological activity of the oil.

Exploring the identities, purifications, and isolations of the molecules associated with the aforementioned activities would be appealing in the context of the present research.

In view of this study:

- It will be interesting to expand the retrospective study to numerous hospital structures to determine the frequencies and levels of current resistance and characterize the genes and resistance mechanisms of MDRs bacteria.

- Evaluate *in vivo* activities to ensure the clinical use of studied EOs.

- Conduct a molecular-scale analysis to determine the antivirulence potential of the examined plants against MDR strains at the structural level.

References

Bibliography

Abd Rashed, A., Rathi, D.-N.G., Ahmad Nasir, N.A.H., Abd Rahman, A.Z., 2021. Antifungal Properties of Essential Oils and Their Compounds for Application in Skin Fungal Infections: Conventional and Nonconventional Approaches. Molecules 26, 1093. https://doi.org/10.3390/molecules26041093

Abdullah, M.L., Hafez, M.M., Al-Hoshani, A., Al-Shabanah, O., 2018. Anti-metastatic and anti-proliferative activity of eugenol against triple negative and HER2 positive breast cancer cells. BMC Complement. Altern. Med. 18, 321. https://doi.org/10.1186/s12906-018-2392-5

Abou Baker, D.H., Al-Moghazy, M., ElSayed, A.A.A., 2020. The *in vitro* cytotoxicity, antioxidant and antibacterial potential of *Satureja hortensis* L. essential oil cultivated in Egypt. Bioorganic Chem. 95, 103559. https://doi.org/10.1016/j.bioorg.2019.103559

Adukwu, E.C., Allen, S.C.H., Phillips, C.A., 2012. The anti-biofilm activity of lemongrass (Cymbopogon flexuosus) and grapefruit (Citrus paradisi) essential oils against five strains of Staphylococcus aureus. J. Appl. Microbiol. 113, 1217–1227. https://doi.org/10.1111/j.1365-2672.2012.05418.x

Adzitey, F., 2015. Antibiotic Classes and Antibiotic Susceptibility of Bacterial Isolatesfrom Selected Poultry; A Mini Review, in: World s Veterinary Journal. p. 36. https://doi.org/10.5455/wvj.20150853

Agarwal, P., Sebghatollahi, Z., Kamal, M., Dhyani, A., Shrivastava, A., Singh, K.K., Sinha, M., Mahato, N., Mishra, A.K., Baek, K.-H., 2022. Citrus Essential Oils in Aromatherapy: Therapeutic Effects and Mechanisms. Antioxid. Basel Switz. 11, 2374. https://doi.org/10.3390/antiox11122374

Ahmed, H.A., Aboul-Enein, A.M., Abou-Elella, F., Salem, S.H.E., Aly, H.F., Nassrallah, A., Salama, Z., 2021. Nano-Formulations of Hesperidin and Essential Oil Extracted from Sweet Orange Peel: Chemical Properties and Biological Activities. Egypt. J. Chem. 64, 5373–5385. https://doi.org/10.21608/ejchem.2021.84783.4139

Aiemsaard, J., Aiumlamai, S., Aromdee, C., Taweechaisupapong, S., Khunkitti, W., 2011. The effect of lemongrass oil and its major components on clinical isolate mastitis pathogens and their mechanisms of action on *Staphylococcus aureus* DMST 4745. Res. Vet. Sci. 91, e31–e37. https://doi.org/10.1016/j.rvsc.2011.01.012

Ambrosio, C.M.S., Ikeda, N.Y., Miano, A.C., Saldaña, E., Moreno, A.M., Stashenko, E., Contreras-Castillo, C.J., Da Gloria, E.M., 2019. Unraveling the selective antibacterial activity and chemical composition of citrus essential oils. Sci. Rep. 9, 17719. https://doi.org/10.1038/s41598-019-54084-3

Andersson, D.I., Balaban, N.Q., Baquero, F., Courvalin, P., Glaser, P., Gophna, U., Kishony, R., Molin, S., Tønjum, T., 2020. Antibiotic resistance: turning evolutionary principles into clinical reality. FEMS Microbiol. Rev. 44, 171–188. https://doi.org/10.1093/femsre/fuaa001

Angane, M., Swift, S., Huang, K., Butts, C.A., Quek, S.Y., 2022a. Essential Oils and Their Major Components: An Updated Review on Antimicrobial Activities, Mechanism of Action and Their Potential Application in the Food Industry. Foods 11, 464. https://doi.org/10.3390/foods11030464

Angane, M., Swift, S., Huang, K., Butts, C.A., Quek, S.Y., 2022b. Essential Oils and Their Major Components: An Updated Review on Antimicrobial Activities, Mechanism of

Action and Their Potential Application in the Food Industry. Foods 11, 464. https://doi.org/10.3390/foods11030464

Antonoplis, A., Zang, X., Wegner, T., Wender, P.A., Cegelski, L., 2019. A Vancomycin-Arginine Conjugate Inhibits Growth of Carbapenem-resistant *E. coli* and Targets Cell-Wall Synthesis. ACS Chem. Biol. 14, 2065–2070. https://doi.org/10.1021/acschembio.9b00565

Aouf, A., Ali, H., Al-Khalifa, A.R., Mahmoud, K.F., Farouk, A., 2020. Influence of Nanoencapsulation Using High-Pressure Homogenization on the Volatile Constituents and Anticancer and Antioxidant Activities of Algerian Saccocalyx satureioides Coss. et Durieu. Mol. Basel Switz. 25, 4756. https://doi.org/10.3390/molecules25204756

Arancibia, C., Navarro-Lisboa, R., Zúñiga, R.N., Matiacevich, S., 2016. Application of CMC as Thickener on Nanoemulsions Based on Olive Oil: Physical Properties and Stability. Int. J. Polym. Sci. 2016, 1–10. https://doi.org/10.1155/2016/6280581

Arthur, R.R., LeDuc, J.W., Hughes, J.M., 2011. Global Surveillance for Emerging Infectious Diseases. Trop. Infect. Dis. Princ. Pathog. Pract. 105–109. https://doi.org/10.1016/B978-0-7020-3935-5.00014-8

Asfaw, M.D., 2022. Basic Essential Oil Extraction Techniques and Procedures from Aromatic Plants. J. Chromatogr. Sep. Tech. 13, 1–5. https://doi.org/10.35248/2157-7064.22.13.489

Asma, S.T., Imre, K., Morar, A., Herman, V., Acaroz, U., Mukhtar, H., Arslan-Acaroz, D., Shah, S.R.A., Gerlach, R., 2022. An Overview of Biofilm Formation–Combating Strategies and Mechanisms of Action of Antibiofilm Agents. Life 12, 1110. https://doi.org/10.3390/life12081110

Aswathanarayan, J.B., Vittal, R.R., 2019. Nanoemulsions and Their Potential Applications in Food Industry. Front. Sustain. Food Syst. 3. https://doi.org/10.3389/fsufs.2019.00095

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., Supuran, C.T., 2021. Natural products in drug discovery: advances and opportunities. Nat. Rev. Drug Discov. 20, 200–216. https://doi.org/10.1038/s41573-020-00114-z

Badr, A.N., Shehata, M.G., Abdel-Raze, A.G., 2017. Antioxidant Activities and Potential Impacts to Reduce Aflatoxins Utilizing Jojoba and Jatropha Oils and Extracts. Int. J. Pharmacol. 13, 1103–1114. https://doi.org/10.3923/ijp.2017.1103.1114

Baher, Z.F., Mirza, M., Ghorbanli, M., Bagher Rezaii, M., 2002. The influence of water stress on plant height, herbal and essential oil yield and composition inSatureja hortensis L. Flavour Fragr. J. 17, 275–277. https://doi.org/10.1002/ffj.1097

Balakrishnan, B., Paramasivam, S., Arulkumar, A., 2014. Evaluation of the lemongrass plant (Cymbopogon citratus) extracted in different solvents for antioxidant and antibacterial activity against human pathogens. Asian Pac. J. Trop. Dis. 4, S134–S139. https://doi.org/10.1016/S2222-1808(14)60428-X

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity: A review. J. Pharm. Anal. 6, 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Barlam, T.F., Cosgrove, S.E., Abbo, L.M., MacDougall, C., Schuetz, A.N., Septimus, E.J., Srinivasan, A., Dellit, T.H., Falck-Ytter, Y.T., Fishman, N.O., Hamilton, C.W., Jenkins, T.C., Lipsett, P.A., Malani, P.N., May, L.S., Moran, G.J., Neuhauser, M.M., Newland, J.G., Ohl, C.A., Samore, M.H., Seo, S.K., Trivedi, K.K., 2016. Implementing an Antibiotic Stewardship Program: Guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of

America. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 62, e51-77. https://doi.org/10.1093/cid/ciw118

Ben, Y., Fu, C., Hu, M., Liu, L., Wong, M.H., Zheng, C., 2019. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environ. Res. 169, 483–493. https://doi.org/10.1016/j.envres.2018.11.040

Benoudjit, F., Hamoudi, I., Aboulouz, A., 2022. Extraction and characterization of Essential Oil and Hydrolate obtained from an Algerian Lemongrass (Cymbopogon citratus). Algerian J. Environ. Sci. Technol. 8.

Benziane, M.Y., Bendahou, M., Benbelaïd, F., Khadir, A., Belhadef, H., Benaissa, A., Ouslimani, S., Mahdi, F., Muselli, A., 2023. Efficacy of endemic Algerian essential oils against single and mixed biofilms of *Candida albicans* and *Candida glabrata*. Arch. Oral Biol. 145, 105584. https://doi.org/10.1016/j.archoralbio.2022.105584

Bhatnagar, A., 2020. Chemical composition and antioxidant activity of essential oil of Cymbopogon flexuosus. J. Appl. Nat. Sci. 12, 25–29. https://doi.org/10.31018/jans.v12i1.2207

Bhatt, S., Chatterjee, S., 2022. Fluoroquinolone antibiotics: Occurrence, mode of action, resistance, environmental detection, and remediation - A comprehensive review. Environ. Pollut. Barking Essex 1987 315, 120440. https://doi.org/10.1016/j.envpol.2022.120440

Bhowmik, P., Modi, B., Roy, P., Chowdhury, A., 2023. Strategies to combat Gram-negative bacterial resistance to conventional antibacterial drugs: a review. Osong Public Health Res. Perspect. 14, 333–346. https://doi.org/10.24171/j.phrp.2022.0323

Bimbiraitė-Survilienė, K., Stankevičius, M., Šuštauskaitė, S., Gęgotek, A., Maruska, A., Skrzydlewska, E., Barsteigienė, Z., Akuneca, I., Ragazinskiene, O., Lukošius, A., 2021. Evaluation of Chemical Composition, Radical Scavenging and Antitumor Activities of Satureja hortensis L. Herb Extracts. Antioxidants 10, 53. https://doi.org/10.3390/antiox10010053

Blowman, K., Magalhães, M., Lemos, M.F.L., Cabral, C., Pires, I.M., 2018. Anticancer Properties of Essential Oils and Other Natural Products. Evid.-Based Complement. Altern. Med. ECAM 2018, 3149362. https://doi.org/10.1155/2018/3149362

Borges, R.S., Keita, H., Ortiz, B.L.S., Dos Santos Sampaio, T.I., Ferreira, I.M., Lima, E.S., de Jesus Amazonas da Silva, M., Fernandes, C.P., de Faria Mota Oliveira, A.E.M., da Conceição, E.C., Rodrigues, A.B.L., Filho, A.C.M.P., Castro, A.N., Carvalho, J.C.T., 2018. Anti-inflammatory activity of nanoemulsions of essential oil from Rosmarinus officinalis L.: in vitro and in zebrafish studies. Inflammopharmacology 26, 1057–1080. https://doi.org/10.1007/s10787-017-0438-9

Boudechicha, A., Aouf, A., Farouk, A., Ali, H.S., Elkhadragy, M.F., Yehia, H.M., Badr, A.N., 2023. Microfluidizing Technique Application for Algerian Cymbopogon citratus (DC.) Stapf Effects Enhanced Volatile Content, Antimicrobial, and Anti-Mycotoxigenic Properties. Molecules 28, 5367. https://doi.org/10.3390/molecules28145367

Boukhatem, M.N., Ferhat, M.A., Kameli, A., Saidi, F., Kebir, H.T., 2014. Lemon grass (Cymbopogon citratus) essential oil as a potent anti-inflammatory and antifungal drugs. Libyan J. Med. 9, 10.3402/ljm.v9.25431. https://doi.org/10.3402/ljm.v9.25431

Brandl, M., Weiss, M.S., Jabs, A., Sühnel, J., Hilgenfeld, R., 2001. C-H...pi-interactions in proteins. J. Mol. Biol. 307, 357–377. https://doi.org/10.1006/jmbi.2000.4473

Çakaloğlu Ebcim, B., Ozyurt, V., Otles, S., 2018. Cold press in oil extraction. A review. Ukr. Food J. 7, 640–654. https://doi.org/10.24263/2304-974X-2018-7-4-9

Calderón, C.B., Sabundayo, B.P., 2007. Antimicrobial classifications: Drugs for bugs, in: Antimicrobial Susceptibility Testing Protocols. CRC Press, pp. 7–52.

Campos, J., Schmeda-Hirschmann, G., Leiva, E., Guzmán, L., Orrego, R., Fernández, P., González, M., Radojkovic, C., Zuñiga, F.A., Lamperti, L., Pastene, E., Aguayo, C., 2014. Lemon grass (Cymbopogon citratus (D.C) Stapf) polyphenols protect human umbilical vein endothelial cell (HUVECs) from oxidative damage induced by high glucose, hydrogen peroxide and oxidised low-density lipoprotein. Food Chem. 151, 175–181. https://doi.org/10.1016/j.foodchem.2013.11.018

Catalano, A., Iacopetta, D., Ceramella, J., Scumaci, D., Giuzio, F., Saturnino, C., Aquaro, S., Rosano, C., Sinicropi, M.S., 2022. Multidrug Resistance (MDR): A Widespread Phenomenon in Pharmacological Therapies. Molecules 27, 616. https://doi.org/10.3390/molecules27030616

Catalog Record Search [WWW Document], n.d. URL https://arctos.database.museum/ (accessed 2.2.24).

Chambre, D.R., Moisa, C., Lupitu, A., Copolovici, L., Pop, G., Copolovici, D.-M., 2020a. Chemical composition, antioxidant capacity, and thermal behavior of Satureja hortensis essential oil. Sci. Rep. 10, 21322. https://doi.org/10.1038/s41598-020-78263-9

Chambre, D.R., Moisa, C., Lupitu, A., Copolovici, L., Pop, G., Copolovici, D.-M., 2020b. Chemical composition, antioxidant capacity, and thermal behavior of Satureja hortensis essential oil. Sci. Rep. 10, 21322. https://doi.org/10.1038/s41598-020-78263-9

Chang, Y., McLandsborough, L., McClements, D.J., 2012. Physical Properties and Antimicrobial Efficacy of Thyme Oil Nanoemulsions: Influence of Ripening Inhibitors. J. Agric. Food Chem. 60, 12056–12063. https://doi.org/10.1021/jf304045a

Chawla, M., Verma, J., Gupta, R., Das, B., 2022. Antibiotic Potentiators Against Multidrug-Resistant Bacteria: Discovery, Development, and Clinical Relevance. Front. Microbiol. 13.

Cho, M., So, I., Chun, J.N., Jeon, J.-H., 2016. The antitumor effects of geraniol: Modulation of cancer hallmark pathways (Review). Int. J. Oncol. 48, 1772–1782. https://doi.org/10.3892/ijo.2016.3427

Chouitah, O., Meddah, B., Sonnet, P., 2018. Chemical Composition and Antimicrobial Activity OF Essential Oil from the Leaves of Satureja hortensis L. Algerian J. Nat. Prod. 6, 639–644. https://doi.org/10.5281/zenodo.2577838

Contant, C., Rouabhia, M., Loubaki, L., Chandad, F., Semlali, A., 2021. Anethole induces anti-oral cancer activity by triggering apoptosis, autophagy and oxidative stress and by modulation of multiple signaling pathways. Sci. Rep. 11, 13087. https://doi.org/10.1038/s41598-021-92456-w

Costa, D.C., Costa, H.S., Albuquerque, T.G., Ramos, F., Castilho, M.C., Sanches-Silva, A., 2015. Advances in phenolic compounds analysis of aromatic plants and their potential applications. Trends Food Sci. Technol. 45, 336–354. https://doi.org/10.1016/j.tifs.2015.06.009

da Silva Gündel, S., Velho, M.C., Diefenthaler, M.K., Favarin, F.R., Copetti, P.M., de Oliveira Fogaça, A., Klein, B., Wagner, R., Gündel, A., Sagrillo, M.R., Ourique, A.F., 2018. Basil oil-nanoemulsions: Development, cytotoxicity and evaluation of antioxidant and antimicrobial potential. J. Drug Deliv. Sci. Technol. 46, 378–383. https://doi.org/10.1016/j.jddst.2018.05.038

Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7, 42717. https://doi.org/10.1038/srep42717

Dajic Stevanovic, Z., Sieniawska, E., Glowniak, K., Obradovic, N., Pajic-Lijakovic, I., 2020. Natural Macromolecules as Carriers for Essential Oils: From Extraction to Biomedical Application. Front. Bioeng. Biotechnol. 8.

Darby, E.M., Trampari, E., Siasat, P., Gaya, M.S., Alav, I., Webber, M.A., Blair, J.M.A., 2023. Molecular mechanisms of antibiotic resistance revisited. Nat. Rev. Microbiol. 21, 280–295. https://doi.org/10.1038/s41579-022-00820-y

Davies, D., 2003. Understanding biofilm resistance to antibacterial agents. Nat. Rev. Drug Discov. 2, 114–122. https://doi.org/10.1038/nrd1008

De Silva, B.C.J., Jung, W.-G., Hossain, S., Wimalasena, S.H.M.P., Pathirana, H.N.K.S., Heo, G.-J., 2017. Antimicrobial property of lemongrass (Cymbopogon citratus) oil against pathogenic bacteria isolated from pet turtles. Lab. Anim. Res. 33, 84–91. https://doi.org/10.5625/lar.2017.33.2.84

de Sousa, D.P., Damasceno, R.O.S., Amorati, R., Elshabrawy, H.A., de Castro, R.D., Bezerra, D.P., Nunes, V.R.V., Gomes, R.C., Lima, T.C., 2023. Essential Oils: Chemistry and Pharmacological Activities. Biomolecules 13, 1144. https://doi.org/10.3390/biom13071144

Deng, W., Liu, K., Cao, S., Sun, J., Zhong, B., Chun, J., 2020. Chemical Composition, Antimicrobial, Antioxidant, and Antiproliferative Properties of Grapefruit Essential Oil Prepared by Molecular Distillation. Molecules 25, 217. https://doi.org/10.3390/molecules25010217

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, 25. https://doi.org/10.3390/medicines3040025

Ding, H., Ma, Y., 2015. Theoretical and computational investigations of nanoparticle-biomembrane interactions in cellular delivery. Small Weinh. Bergstr. Ger. 11, 1055–1071. https://doi.org/10.1002/smll.201401943

Djenane, D., Aboudaou, M., Ferhat, M., Ouelhadj, A., Ariño, A., 2019. Effect of the aromatisation with summer savory (Satureja hortensis L.) essential oil on the oxidative and microbial stabilities of liquid whole eggs during storage. J. Essent. Oil Res. 31, 1–12. https://doi.org/10.1080/10412905.2019.1610516

Djenane, D., Yangüela, J., Amrouche, T., Boubrit, S., Boussad, N., Roncalés, P., 2011. Chemical composition and antimicrobial effects of essential oils of Eucalyptus globulus, Myrtus communis and Satureja hortensis against Escherichia coli O157:H7 and Staphylococcus aureus in minced beef. Food Sci. Technol. Int. Cienc. Tecnol. Los Aliment. Int. 17, 505–515. https://doi.org/10.1177/1082013211398803

Djordjevic, N., Mancic, S., Karabegovic, I., Cvetkovic, D., Stanojevic, J., Savic, D., Danilovic, B., 2021. Influence of the Isolation Method to the Composition and Antimicrobial and Antioxidative Activity of Winter Savory (Satureja montana L.) Essential Oil. J. Essent. Oil Bear. Plants 24, 386–399. https://doi.org/10.1080/0972060X.2021.1933611

Donsì, F., Annunziata, M., Sessa, M., Ferrari, G., 2011a. Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. LWT - Food Sci. Technol. 44, 1908–1914. https://doi.org/10.1016/j.lwt.2011.03.003

Donsì, F., Annunziata, M., Sessa, M., Ferrari, G., 2011b. Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. LWT - Food Sci. Technol. 44, 1908–1914. https://doi.org/10.1016/j.lwt.2011.03.003

Du, X., Zhang, M., Wang, S., Li, J., Zhang, J., Liu, D., 2023. Ethnopharmacology, chemical composition and functions of Cymbopogon citratus. Chin. Herb. Med. https://doi.org/10.1016/j.chmed.2023.07.002

Duan, W.-Y., Zhu, X.-M., Zhang, S.-B., Lv, Y.-Y., Zhai, H.-C., Wei, S., Ma, P.-A., Hu, Y.-S., 2024. Antifungal effects of carvacrol, the main volatile compound in *Origanum vulgare* L. essential oil, against *Aspergillus flavus* in postharvest wheat. Int. J. Food Microbiol. 410, 110514. https://doi.org/10.1016/j.ijfoodmicro.2023.110514

Durazzo, A., Fawzy Ramadan, M., Lucarini, M., 2022. Editorial: Cold Pressed Oils: A Green Source of Specialty Oils. Front. Nutr. 8, 836651. https://doi.org/10.3389/fnut.2021.836651

d'Urso de Souza Mendes, C., de Souza Antunes, A.M., 2013. Pipeline of Known Chemical Classes of Antibiotics. Antibiotics 2, 500–534. https://doi.org/10.3390/antibiotics2040500

El Abdali, Y., Mahraz, M.A., Beniaich, G., Mssillou, I., Chebaibi, M., Bin Jardan, Y., Lahkimi, A., Nafidi, H.-A., Aboul-Soud, M., Bourhia, M., Bouia, A., 2023. Essential oils of Origanum compactum Benth: Chemical characterization, in vitro, in silico, antioxidant, and antibacterial activities. Open Chem. 21, 20220282. https://doi.org/10.1515/chem-2022-0282

Espitia, P.J.P., Fuenmayor, C.A., Otoni, C.G., 2019. Nanoemulsions: Synthesis, Characterization, and Application in Bio-Based Active Food Packaging. Compr. Rev. Food Sci. Food Saf. 18, 264–285. https://doi.org/10.1111/1541-4337.12405

Estaji, A., Roosta, H.R., Rezaei, S.A., Hosseini, S.S., Niknam, F., 2018. Morphological, physiological and phytochemical response of different Satureja hortensis L. accessions to salinity in a greenhouse experiment. J. Appl. Res. Med. Aromat. Plants 10, 25–33. https://doi.org/10.1016/j.jarmap.2018.04.005

Etebu, E., Arikekpar, I., 2016. Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. Int. J. Appl. Microbiol. Biotechnol. Res. 4, 90–101.

Eyler, R.F., Shvets, K., 2019. Clinical Pharmacology of Antibiotics. Clin. J. Am. Soc. Nephrol. CJASN 14, 1080–1090. https://doi.org/10.2215/CJN.08140718

Fair, R.J., Tor, Y., 2014. Antibiotics and Bacterial Resistance in the 21st Century. Perspect. Med. Chem. 6, PMC.S14459. https://doi.org/10.4137/PMC.S14459

Falagas, M.E., Kasiakou, S.K., 2005. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 40, 1333–1341. https://doi.org/10.1086/429323

Fancello, F., Petretto, G.L., Zara, S., Sanna, M.L., Addis, R., Maldini, M., Foddai, M., Rourke, J.P., Chessa, M., Pintore, G., 2016. Chemical characterization, antioxidant capacity and antimicrobial activity against food related microorganisms of Citrus limon var. pompia leaf essential oil. LWT - Food Sci. Technol. 69, 579–585. https://doi.org/10.1016/j.lwt.2016.02.018

Farouk, A., Alsulami, T., Ali, H., Badr, A., 2023. In-Vitro and In-Silico Investigation for the Spent-Coffee Bioactive Phenolics as a Promising Aflatoxins Production Inhibitor. Toxins 15, 1–20. https://doi.org/10.3390/toxins15030225

Farrar, A.J., Farrar, F.C., 2020. Clinical Aromatherapy. Nurs. Clin. North Am. 55, 489–504. https://doi.org/10.1016/j.cnur.2020.06.015

Fathi, A., Sahari, M.A., Barzegar, M., Naghdi Badi, H., 2013. Antioxidant Activity of Satureja hortensis L. Essential Oil and its Application in Safflower Oil. فصلنامه علمی پژوهشی گیاهان دارویی 12, 51–67.

Fatima, K., Luqman, S., Meena, A., 2022. Carvacrol Arrests the Proliferation of Hypopharyngeal Carcinoma Cells by Suppressing Ornithine Decarboxylase and

Hyaluronidase Activities. Front. Nutr. 9, 857256. https://doi.org/10.3389/fnut.2022.857256

Fatunmibi, O., Njoku, S., Asekun, O., Ogah, J., 2023. Chemical composition, antioxidant and antimicrobial Activity of the essential oil from the leaves of Cymbopogon citratus 1–05.

Felšöciová, S., Vukovic, N., Jeżowski, P., Kačániová, M., 2020. Antifungal activity of selected volatile essential oils against Penicillium sp. Open Life Sci. 15, 511–521. https://doi.org/10.1515/biol-2020-0045

Ferreira de Freitas, R., Schapira, M., 2017. A systematic analysis of atomic protein-ligand interactions in the PDB. MedChemComm 8, 1970–1981. https://doi.org/10.1039/c7md00381a

Feyzioglu, G.C., Tornuk, F., 2016. Development of chitosan nanoparticles loaded with summer savory (Satureja hortensis L.) essential oil for antimicrobial and antioxidant delivery applications. LWT 70, 104–110. https://doi.org/10.1016/j.lwt.2016.02.037

Fierascu, I., Dinu-Pirvu, C.E., Fierascu, R.C., Velescu, B.S., Anuta, V., Ortan, A., Jinga, V., 2018. Phytochemical Profile and Biological Activities of Satureja hortensis L.: A Review of the Last Decade. Mol. J. Synth. Chem. Nat. Prod. Chem. 23, 2458. https://doi.org/10.3390/molecules23102458

Figueirinha, A., Paranhos, A., Pérez-Alonso, J.J., Santos-Buelga, C., Batista, M.T., 2008. Cymbopogon citratus leaves: Characterization of flavonoids by HPLC–PDA–ESI/MS/MS and an approach to their potential as a source of bioactive polyphenols. Food Chem. 110, 718–728. https://doi.org/10.1016/j.foodchem.2008.02.045

Frank, U., Tacconelli, E., 2012. Classification of the Antibiotics, in: Frank, U., Tacconelli, E. (Eds.), The Daschner Guide to In-Hospital Antibiotic Therapy. Springer, Berlin, Heidelberg, pp. 1–4. https://doi.org/10.1007/978-3-642-18402-4_1

Friedman, M., 2014. Chemistry and Multibeneficial Bioactivities of Carvacrol (4-Isopropyl-2-methylphenol), a Component of Essential Oils Produced by Aromatic Plants and Spices. J. Agric. Food Chem. 62, 7652–7670. https://doi.org/10.1021/jf5023862

Frieri, M., Kumar, K., Boutin, A., 2017. Antibiotic resistance. J. Infect. Public Health 10, 369–378. https://doi.org/10.1016/j.jiph.2016.08.007

Gago, C.M.L., Artiga-Artigas, M., Antunes, M.D.C., Faleiro, M.L., Miguel, M.G., Martín-Belloso, O., 2019. Effectiveness of nanoemulsions of clove and lemongrass essential oils and their major components against Escherichia coli and Botrytis cinerea. J. Food Sci. Technol. 56, 2721–2736. https://doi.org/10.1007/s13197-019-03762-1

García-Díaz, M., Gil-Serna, J., Patiño, B., García-Cela, E., Magan, N., Medina, Á., 2020. Assessment of the Effect of Satureja montana and Origanum virens Essential Oils on Aspergillus flavus Growth and Aflatoxin Production at Different Water Activities. Toxins 12, 142. https://doi.org/10.3390/toxins12030142

Geng, J., Liu, X., Wang, J., Li, S., 2022. Accumulation and risk assessment of antibiotics in edible plants grown in contaminated farmlands: A review. Sci. Total Environ. 853, 158616. https://doi.org/10.1016/j.scitotenv.2022.158616

Giuliodori, A.M., Spurio, R., Milón, P., Fabbretti, A., 2018. Antibiotics Targeting the 30S Ribosomal Subunit: A Lesson from Nature to Find and Develop New Drugs. Curr. Top. Med. Chem. 18, 2080–2096. https://doi.org/10.2174/1568026618666181025092546

Giweli, A., Džamić, A.M., Soković, M., Ristić, M.S., Marin, P.D., 2012. Antimicrobial and Antioxidant Activities of Essential Oils of Satureja thymbra Growing Wild in Libya. Molecules 17, 4836–4850. https://doi.org/10.3390/molecules17054836

Gmurek, M., Horn, H., Majewsky, M., 2015. Phototransformation of sulfamethoxazole under simulated sunlight: Transformation products and their antibacterial activity

toward Vibrio fischeri. Sci. Total Environ. 538, 58–63. https://doi.org/10.1016/j.scitotenv.2015.08.014

Gómez-Sequeda, N., Cáceres, M., Stashenko, E.E., Hidalgo, W., Ortiz, C., 2020. Antimicrobial and Antibiofilm Activities of Essential Oils against Escherichia coli O157:H7 and Methicillin-Resistant Staphylococcus aureus (MRSA). Antibiotics 9, 730. https://doi.org/10.3390/antibiotics9110730

Guerra-Rosas, M.I., Morales-Castro, J., Ochoa-Martínez, L.A., Salvia-Trujillo, L., Martín-Belloso, O., 2016. Long-term stability of food-grade nanoemulsions from high methoxyl pectin containing essential oils. Food Hydrocoll. 52, 438–446. https://doi.org/10.1016/j.foodhyd.2015.07.017

Guimarães, A.C., Meireles, L.M., Lemos, M.F., Guimarães, M.C.C., Endringer, D.C., Fronza, M., Scherer, R., 2019. Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. Molecules 24, 2471. https://doi.org/10.3390/molecules24132471

Guo, R., Li, K., Qin, J., Niu, S., Hong, W., 2020. Development of polycationic micelles as an efficient delivery system of antibiotics for overcoming the biological barriers to reverse multidrug resistance in Escherichia coli. Nanoscale 12, 11251–11266. https://doi.org/10.1039/D0NR01366H

Gupta, V.K., Kumar, M.M., Bisht, D., Kaushik, A., 2017. Plants in our combating strategies against Mycobacterium tuberculosis: progress made and obstacles met. Pharm. Biol. 55, 1536–1544. https://doi.org/10.1080/13880209.2017.1309440

Gutiérrez-del-Río, I., López-Ibáñez, S., Magadán-Corpas, P., Fernández-Calleja, L., Pérez-Valero, Á., Tuñón-Granda, M., Miguélez, E.M., Villar, C.J., Lombó, F., 2021. Terpenoids and Polyphenols as Natural Antioxidant Agents in Food Preservation. Antioxidants 10, 1264. https://doi.org/10.3390/antiox10081264

Ha, T.V.A., Kim, S., Choi, Y., Kwak, H.-S., Lee, S.J., Wen, J., Oey, I., Ko, S., 2015. Antioxidant activity and bioaccessibility of size-different nanoemulsions for lycopene-enriched tomato extract. Food Chem. 178, 115–121. https://doi.org/10.1016/j.foodchem.2015.01.048

Hajhashemi, V., Ghannadi, A., Pezeshkian, S.K., 2002. Antinociceptive and anti-inflammatory effects of Satureja hortensis L. extracts and essential oil. J. Ethnopharmacol. 82, 83–87. https://doi.org/10.1016/S0378-8741(02)00137-X

Hajibonabi, A., Yekani, M., Sharifi, S., Nahad, J.S., Dizaj, S.M., Memar, M.Y., 2023. Antimicrobial activity of nanoformulations of carvacrol and thymol: New trend and applications. OpenNano 13, 100170. https://doi.org/10.1016/j.onano.2023.100170

Hamidpour, R., Hamidpour, S., Hamidpour, M., Shahlari, M., Sohraby, M., 2014. Summer Savory: From the Selection of Traditional Applications to the Novel Effect in Relief, Prevention, and Treatment of a Number of Serious Illnesses such as Diabetes, Cardiovascular Disease, Alzheimer's Disease, and Cancer. J. Tradit. Complement. Med. 4, 140–144. https://doi.org/10.4103/2225-4110.136540

Hancock, R.E.W., Alford, M.A., Haney, E.F., 2021. Antibiofilm activity of host defence peptides: complexity provides opportunities. Nat. Rev. Microbiol. 19, 786–797. https://doi.org/10.1038/s41579-021-00585-w

Hasan, T., AL-Harmoosh, R., 2020. Mechanisms of Antibiotics Resistance in Bacteria. Syst. Rev. Pharm. 11, 817–823. https://doi.org/10.31838/srp.2020.6.118

Hassanzadeh, M.K., Tayarani Najaran, Z., Nasery, M., Emami, S.A., 2016. Summer Savory (Satureja hortensis L.) Oils, in: Essential Oils in Food Preservation, Flavor and Safety. Elsevier, pp. 757–764. https://doi.org/10.1016/B978-0-12-416641-7.00086-9

Hatano, T., Kagawa, H., Yasuhara, T., Okuda, T., 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull. (Tokyo) 36, 2090–2097. https://doi.org/10.1248/cpb.36.2090

Herrera-Calderon, O., Yepes-Pérez, A.F., Quintero-Saumeth, J., Rojas-Armas, J.P., Palomino-Pacheco, M., Ortiz-Sánchez, J.M., Cieza-Macedo, E.C., Arroyo-Acevedo, J.L., Figueroa-Salvador, L., Peña-Rojas, G., Andía-Ayme, V., 2020. Carvacrol: An In Silico Approach of a Candidate Drug on HER2, PI3Kα, mTOR, hER-α, PR, and EGFR Receptors in the Breast Cancer. Evid.-Based Complement. Altern. Med. ECAM 2020, 8830665. https://doi.org/10.1155/2020/8830665

Hunde, Y., Bachheti, A., Chaubey, K.K., Husen, A., Bachheti, R.K., 2023. Nanoemulsions from Essential Oils: Preparation, Characterization, and Their Applications, in: Husen, A., Bachheti, R.K., Bachheti, A. (Eds.), Current Trends in Green Nano-Emulsions: Food, Agriculture and Biomedical Sectors, Smart Nanomaterials Technology. Springer Nature, Singapore, pp. 21–38. https://doi.org/10.1007/978-981-99-5398-1_2

Huovinen, P., 2001. Resistance to trimethoprim-sulfamethoxazole. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 32, 1608–1614. https://doi.org/10.1086/320532

Hutchings, M.I., Truman, A.W., Wilkinson, B., 2019a. Antibiotics: past, present and future. Curr. Opin. Microbiol., Antimicrobials 51, 72–80. https://doi.org/10.1016/j.mib.2019.10.008

Hutchings, M.I., Truman, A.W., Wilkinson, B., 2019b. Antibiotics: past, present and future. Curr. Opin. Microbiol., Antimicrobials 51, 72–80. https://doi.org/10.1016/j.mib.2019.10.008

Jafari, F., Ghavidel, F., Zarshenas, M.M., 2016a. A Critical Overview on the Pharmacological and Clinical Aspects of Popular Satureja Species. J. Acupunct. Meridian Stud. 9, 118–127. https://doi.org/10.1016/j.jams.2016.04.003

Jafari, F., Ghavidel, F., Zarshenas, M.M., 2016b. A Critical Overview on the Pharmacological and Clinical Aspects of Popular Satureja Species. J. Acupunct. Meridian Stud. 9, 118–127. https://doi.org/10.1016/j.jams.2016.04.003

Jafari, S.M., He, Y., Bhandari, B., 2007. Optimization of nano-emulsions production by microfluidization. Eur. Food Res. Technol. 225, 733–741. https://doi.org/10.1007/s00217-006-0476-9

Jayari, A., Donsì, F., Ferrari, G., Maaroufi, A., 2022. Nanoencapsulation of Thyme Essential Oils: Formulation, Characterization, Storage Stability, and Biological Activity. Foods 11, 1858. https://doi.org/10.3390/foods11131858

Júnior, A.S.S., Aidar, F.J., Silva, L.A.S., de B. Silva, T., de Almeida, S.F.M., Teles, D.C.S., de L. Junior, W., Schimieguel, D.M., de Souza, D.A., Nascimento, A.C.S., Camargo, E.A., dos Santos, J.L., de O. e Silva, A.M., de S. Nunes, R., Borges, L.P., Lira, A.A.M., 2024. Influence of Lemongrass Essential Oil (Cymbopogon flexuosus) Supplementation on Diabetes in Rat Model. Life 14, 336. https://doi.org/10.3390/life14030336

Kadhum Abu Gulel, H., Hasan, T., 2019. The Study of Bacillus Subtils Antimicrobial Activity on Some of the Pathological Isolates. Int. J. Drug Deliv. Technol. 9. https://doi.org/10.25258/ijddt.9.2.12

Kambiz, D., Kamaleh, G., Behnam, H., Mitra, S., 2013. Antifungal activity of Satureja hortensis alcoholic extract against Aspergillus and Candida species. J. Med. Plants Res. 7, 2271–2274. https://doi.org/10.5897/JMPR12.659

Kamran, S., Sinniah, A., Abdulghani, M.A.M., Alshawsh, M.A., 2022. Therapeutic Potential of Certain Terpenoids as Anticancer Agents: A Scoping Review. Cancers 14, 1100. https://doi.org/10.3390/cancers14051100

Kamsu, N.P., Tchinda, S.E., Tchameni, N.S., Jazet, D.P.M., Madjouko, M.A., Youassi Youassi, O., Sameza, M.L., Tchoumbougnang, F., Menut, C., 2019. Antifungal activities of essential oils of cinnamon (Cinnamomum zeylanicum) and lemongrass (Cymbopogon citratus) on crown rot pathogens of banana. Indian Phytopathol. 72, 131–137. https://doi.org/10.1007/s42360-018-0104-1

Karsli, G.T., Sahin, S., Oztop, M.H., 2022. High-Pressure-Homogenized Clove and Thyme Oil Emulsions: Formulation, Stability, and Antioxidant Capacity. ACS Food Sci. Technol. 2, 1832–1839. https://doi.org/10.1021/acsfoodscitech.2c00231

Katar, D., Kacar, O., Kara, N., Aytaç, Z., Göksu, E., Kara, S., Katar, N., Erbaş, S., Telci, İ., Elmastaş, M., 2017. Ecological variation of yield and aroma components of summer savory (Satureja hortensis L.). J. Appl. Res. Med. Aromat. Plants 7, 131–135. https://doi.org/10.1016/j.jarmap.2017.07.005

Khan, I., Bahuguna, A., Shukla, S., Aziz, F., Chauhan, A.K., Ansari, M.B., Bajpai, V.K., Huh, Y.S., Kang, S.C., 2020. Antimicrobial potential of the food-grade additive carvacrol against uropathogenic *E. coli* based on membrane depolarization, reactive oxygen species generation, and molecular docking analysis. Microb. Pathog. 142, 104046. https://doi.org/10.1016/j.micpath.2020.104046

Khan, S., Sahar, A., Tariq, T., Sameen, A., Tariq, F., 2023. Chapter 1 - Essential oils in plants: Plant physiology, the chemical composition of the oil, and natural variation of the oils (chemotaxonomy and environmental effects, etc.), in: Nayik, G.A., Ansari, M.J. (Eds.), Essential Oils. Academic Press, pp. 1–36. https://doi.org/10.1016/B978-0-323-91740-7.00016-5

Khatoon, Z., McTiernan, C.D., Suuronen, E.J., Mah, T.-F., Alarcon, E.I., 2018. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4, e01067. https://doi.org/10.1016/j.heliyon.2018.e01067

Khosakueng, M., Taweechaisupapong, S., Boonyanugomol, W., Prapatpong, P., Wongkaewkhiaw, S., Kanthawong, S., 2024. Cymbopogon citratus L. essential oil as a potential anti-biofilm agent active against antibiotic-resistant bacteria isolated from chronic rhinosinusitis patients. Biofouling 40, 26–39. https://doi.org/10.1080/08927014.2024.2305387

Khoury, M., Stien, D., Eparvier, V., Ouaini, N., El Beyrouthy, M., 2016. Report on the Medicinal Use of Eleven Lamiaceae Species in Lebanon and Rationalization of Their Antimicrobial Potential by Examination of the Chemical Composition and Antimicrobial Activity of Their Essential Oils. Evid.-Based Complement. Altern. Med. ECAM 2016, 2547169. https://doi.org/10.1155/2016/2547169

KIZIL, S., Turk, M., Özgüven, M., Khawar, K.M., 2009. Full Blooming Stage is Suitable for Herbage Yield and Essential Oil Content of Summer Savory (Satureja hortensis L.). J. Essent. Oil Bear. Plants 12, 620–629. https://doi.org/10.1080/0972060X.2009.10643765

Koshani, R., Jafari, S.M., 2019. Ultrasound-assisted preparation of different nanocarriers loaded with food bioactive ingredients. Adv. Colloid Interface Sci. 270, 123–146. https://doi.org/10.1016/j.cis.2019.06.005

Krause, K.M., Serio, A.W., Kane, T.R., Connolly, L.E., 2016. Aminoglycosides: An Overview. Cold Spring Harb. Perspect. Med. 6, a027029. https://doi.org/10.1101/cshperspect.a027029

Kumar Bharti, S., 2013. Essential Oil of Cymbopogon Citratus Against Diabetes: Validation by In vivo Experiments and Computational Studies. J. Bioanal. Biomed. 05. https://doi.org/10.4172/1948-593X.1000098

Larsen, J., Raisen, C.L., Ba, X., Sadgrove, N.J., Padilla-González, G.F., Simmonds, M.S.J., Loncaric, I., Kerschner, H., Apfalter, P., Hartl, R., Deplano, A., Vandendriessche, S.,

Černá Bolfíková, B., Hulva, P., Arendrup, M.C., Hare, R.K., Barnadas, C., Stegger, M., Sieber, R.N., Skov, R.L., Petersen, A., Angen, Ø., Rasmussen, S.L., Espinosa-Gongora, C., Aarestrup, F.M., Lindholm, L.J., Nykäsenoja, S.M., Laurent, F., Becker, K., Walther, B., Kehrenberg, C., Cuny, C., Layer, F., Werner, G., Witte, W., Stamm, I., Moroni, P., Jørgensen, H.J., de Lencastre, H., Cercenado, E., García-Garrote, F., Börjesson, S., Hæggman, S., Perreten, V., Teale, C.J., Waller, A.S., Pichon, B., Curran, M.D., Ellington, M.J., Welch, J.J., Peacock, S.J., Seilly, D.J., Morgan, F.J.E., Parkhill, J., Hadjirin, N.F., Lindsay, J.A., Holden, M.T.G., Edwards, G.F., Foster, G., Paterson, G.K., Didelot, X., Holmes, M.A., Harrison, E.M., Larsen, A.R., 2022. Emergence of methicillin resistance predates the clinical use of antibiotics. Nature 602, 135–141. https://doi.org/10.1038/s41586-021-04265-w

Lee, J.-E., Seo, S.-M., Huh, M.-J., Lee, S.-C., Park, I.-K., 2020. Reactive oxygen species mediated-antifungal activity of cinnamon bark (*Cinnamomum verum*) and lemongrass (*Cymbopogon citratus*) essential oils and their constituents against two phytopathogenic fungi. Pestic. Biochem. Physiol. 168, 104644. https://doi.org/10.1016/j.pestbp.2020.104644

Lesjak, M., Beara, I., Simin, N., Pintać, D., Majkić, T., Bekvalac, K., Orčić, D., Mimica-Dukić, N., 2018. Antioxidant and anti-inflammatory activities of quercetin and its derivatives. J. Funct. Foods 40, 68–75. https://doi.org/10.1016/j.jff.2017.10.047

Li, H., Luo, Y.-F., Williams, B.J., Blackwell, T.S., Xie, C.-M., 2012. Structure and function of OprD protein in Pseudomonas aeruginosa: From antibiotic resistance to novel therapies. Int. J. Med. Microbiol. IJMM 302, 10.1016/j.ijmm.2011.10.001. https://doi.org/10.1016/j.ijmm.2011.10.001

Li, T., Liu, C., Lu, J., Gaurav, G.K., Chen, W., 2020. Determination of how tetracycline influences nitrogen removal performance, community structure, and functional genes of biofilm systems. J. Taiwan Inst. Chem. Eng. 106, 99–109. https://doi.org/10.1016/j.jtice.2019.10.004

Liao, P.-C., Yang, T.-S., Chou, J.-C., Chen, J., Lee, S.-C., Kuo, Y.-H., Ho, C.-L., Chao, L.K.-P., 2015. Anti-inflammatory activity of neral and geranial isolated from fruits of *Litsea cubeba* Lour. J. Funct. Foods 19, 248–258. https://doi.org/10.1016/j.jff.2015.09.034

Liu, J., Liu, Z., Pang, Y., Zhou, H., 2022. The interaction between nanoparticles and immune system: application in the treatment of inflammatory diseases. J. Nanobiotechnology 20, 127. https://doi.org/10.1186/s12951-022-01343-7

Liu, Y., Yang, X., Gan, J., Chen, S., Xiao, Z.-X., Cao, Y., 2022. CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic Acids Res. 50, W159–W164. https://doi.org/10.1093/nar/gkac394

Llinares, R., Ramírez, P., Carmona, J.A., Trujillo-Cayado, L.A., Muñoz, J., 2021. Assessment of Fennel Oil Microfluidized Nanoemulsions Stabilization by Advanced Performance Xanthan Gum. Foods 10, 693. https://doi.org/10.3390/foods10040693

Loizzo, M.R., Tundis, R., Menichini, F., Saab, A.M., Statti, G.A., Menichini, F., 2008. Antiproliferative effects of essential oils and their major constituents in human renal adenocarcinoma and amelanotic melanoma cells. Cell Prolif. 41, 1002–1012. https://doi.org/10.1111/j.1365-2184.2008.00561.x

M., R., Nour, Abdurahman H., Sulaiman, Z.A., Nour, Azhari H., S., T.R., 2014. A Comparative Study of Lemongrass (Cymbopogon Citratus) Essential Oil Extracted by Microwave-Assisted Hydrodistillation (MAHD) and Conventional Hydrodistillation (HD) Method. Int. J. Chem. Eng. Appl. 5, 104–108. https://doi.org/10.7763/IJCEA.2014.V5.360

Machado, T.Q., da Fonseca, A.C.C., Duarte, A.B.S., Robbs, B.K., de Sousa, D.P., 2022. A Narrative Review of the Antitumor Activity of Monoterpenes from Essential Oils: An Update. BioMed Res. Int. 2022, 6317201. https://doi.org/10.1155/2022/6317201

Magalhães, M., Manadas, B., Efferth, T., Cabral, C., 2021. Chemoprevention and therapeutic role of essential oils and phenolic compounds: Modeling tumor microenvironment in glioblastoma. Pharmacol. Res. 169, 105638. https://doi.org/10.1016/j.phrs.2021.105638

Majeed, H.T., Aljanaby, A.A.J., 2019. Antibiotic Susceptibility Patterns and Prevalence of Some Extended Spectrum Beta-Lactamases Genes in Gram-Negative Bacteria Isolated from Patients Infected with Urinary Tract Infections in Al-Najaf City, Iraq. Avicenna J. Med. Biotechnol. 11, 192–201.

Mancuso, G., Midiri, A., Gerace, E., Biondo, C., 2021. Bacterial Antibiotic Resistance: The Most Critical Pathogens. Pathogens 10, 1310. https://doi.org/10.3390/pathogens10101310

Mansour, A., Romani, M., Acharya, A.B., Rahman, B., Verron, E., Badran, Z., 2023. Drug Delivery Systems in Regenerative Medicine: An Updated Review. Pharmaceutics 15, 695. https://doi.org/10.3390/pharmaceutics15020695

Marques, R.Z., Wistuba, N., Brito, J.C.M., Bernardoni, V., Rocha, D.C., Gomes, M.P., 2021. Crop irrigation (soybean, bean, and corn) with enrofloxacin-contaminated water leads to yield reductions and antibiotic accumulation. Ecotoxicol. Environ. Saf. 216, 112193. https://doi.org/10.1016/j.ecoenv.2021.112193

Martignago, C.C.S., Soares-Silva, B., Parisi, J.R., Silva, L.C.S.E., Granito, R.N., Ribeiro, A.M., Renno, A.C.M., de Sousa, L.R.F., Aguiar, A.C.C., 2023. Terpenes extracted from marine sponges with antioxidant activity: a systematic review. Nat. Prod. Bioprospecting 13, 23. https://doi.org/10.1007/s13659-023-00387-y

Martínez, A., Manrique-Moreno, M., Klaiss-Luna, M.C., Stashenko, E., Zafra, G., Ortiz, C., 2021. Effect of Essential Oils on Growth Inhibition, Biofilm Formation and Membrane Integrity of Escherichia coli and Staphylococcus aureus. Antibiotics 10, 1474. https://doi.org/10.3390/antibiotics10121474

Martinez, J.L., 2014. General principles of antibiotic resistance in bacteria. Drug Discov. Today Technol. 11, 33–39. https://doi.org/10.1016/j.ddtec.2014.02.001

Masyita, A., Mustika Sari, R., Dwi Astuti, A., Yasir, B., Rahma Rumata, N., Emran, T.B., Nainu, F., Simal-Gandara, J., 2022. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chem. X 13, 100217. https://doi.org/10.1016/j.fochx.2022.100217

Mathieu, K., Kamagate, M., Camille, K., Die-Kakou, H., Yao, A., Kakou, A., 2015. Cymbopogon citratus (DC.) Stapf: ethnopharmacology, phytochemical, pharmacological activities and toxicology. Phytothérapie 14, 1–9. https://doi.org/10.1007/s10298-015-1014-3

Mathur, P., Sanyal, D., Callahan, D.L., Conlan, X.A., Pfeffer, F.M., 2021. Treatment technologies to mitigate the harmful effects of recalcitrant fluoroquinolone antibiotics on the environ- ment and human health. Environ. Pollut. Barking Essex 1987 291, 118233. https://doi.org/10.1016/j.envpol.2021.118233

McClements, D.J., Rao, J., 2011. Food-Grade Nanoemulsions: Formulation, Fabrication, Properties, Performance, Biological Fate, and Potential Toxicity. Crit. Rev. Food Sci. Nutr. 51, 285–330. https://doi.org/10.1080/10408398.2011.559558

Mei, Z., Xu, J., Sun, D., 2011. O/W nano-emulsions with tunable PIT induced by inorganic salts. Colloids Surf. Physicochem. Eng. Asp. 375, 102–108. https://doi.org/10.1016/j.colsurfa.2010.11.069

Miladi, H., Mili, D., Ben Slama, R., Zouari, S., Ammar, E., Bakhrouf, A., 2016. Antibiofilm formation and anti-adhesive property of three mediterranean essential oils against a foodborne pathogen Salmonella strain. Microb. Pathog. 93, 22–31. https://doi.org/10.1016/j.micpath.2016.01.017

Milhomem-Paixão, S.S.R., Fascineli, M.L., Muehlmann, L.A., Melo, K.M., Salgado, H.L.C., Joanitti, G.A., Pieczarka, J.C., Azevedo, R.B., Santos, A.S., Grisolia, C.K., 2017. Andiroba Oil (*Carapa guianensis* Aublet) Nanoemulsions: Development and Assessment of Cytotoxicity, Genotoxicity, and Hematotoxicity. J. Nanomater. 2017, 1–11. https://doi.org/10.1155/2017/4362046

Mohamed, A.A., Alotaibi, B.M., 2023. Essential oils of some medicinal plants and their biological activities: a mini review. J. Umm Al-Qura Univ. Appl. Sci. 9, 40–49. https://doi.org/10.1007/s43994-022-00018-1

Mohamed Abdoul-Latif, F., Ainane, A., Houmed Aboubaker, I., Mohamed, J., Ainane, T., 2023. Exploring the Potent Anticancer Activity of Essential Oils and Their Bioactive Compounds: Mechanisms and Prospects for Future Cancer Therapy. Pharmaceuticals 16, 1086. https://doi.org/10.3390/ph16081086

Mohtashami, S., Rowshan, V., Tabrizi, L., Babalar, M., Ghani, A., 2018a. Summer savory (Satureja hortensis L.) essential oil constituent oscillation at different storage conditions. Ind. Crops Prod. 111, 226–231. https://doi.org/10.1016/j.indcrop.2017.09.055

Mohtashami, S., Rowshan, V., Tabrizi, L., Babalar, M., Ghani, A., 2018b. Summer savory (*Satureja hortensis* L.) essential oil constituent oscillation at different storage conditions. Ind. Crops Prod. 111, 226–231. https://doi.org/10.1016/j.indcrop.2017.09.055

Mot, M.-D., Gavrilaș, S., Lupitu, A.I., Moisa, C., Chambre, D., Tit, D.M., Bogdan, M.A., Bodescu, A.-M., Copolovici, L., Copolovici, D.M., Bungau, S.G., 2022. Salvia officinalis L. Essential Oil: Characterization, Antioxidant Properties, and the Effects of Aromatherapy in Adult Patients. Antioxidants 11, 808. https://doi.org/10.3390/antiox11050808

Murina, V., Kasari, M., Hauryliuk, V., Atkinson, G.C., 2018. Antibiotic resistance ABCF proteins reset the peptidyl transferase centre of the ribosome to counter translational arrest. Nucleic Acids Res. 46, 3753–3763. https://doi.org/10.1093/nar/gky050

Nabigol, A., 2011. Chemical composition and anti-fungal activities of three essential oils from Satureja spp. on four post-harvest pathogens of strawberry fruit. J. Hortic. Sci. Biotechnol. 86, 371–376. https://doi.org/10.1080/14620316.2011.11512776

Nadeem, S.F., Gohar, U.F., Tahir, S.F., Mukhtar, H., Pornpukdeewattana, S., Nukthamna, P., Moula Ali, A.M., Bavisetty, S.C.B., Massa, S., 2020. Antimicrobial resistance: more than 70 years of war between humans and bacteria. Crit. Rev. Microbiol. 46, 578–599. https://doi.org/10.1080/1040841X.2020.1813687

Natu, K.N., Tatke, P.A., 2019. Essential oils – prospective candidates for antifungal treatment? J. Essent. Oil Res. 31, 347–360. https://doi.org/10.1080/10412905.2019.1604437

Nordin, N., Yeap, S.K., Rahman, H.S., Zamberi, N.R., Abu, N., Mohamad, N.E., How, C.W., Masarudin, M.J., Abdullah, R., Alitheen, N.B., 2019. In vitro cytotoxicity and anticancer effects of citral nanostructured lipid carrier on MDA MBA-231 human breast cancer cells. Sci. Rep. 9, 1614. https://doi.org/10.1038/s41598-018-38214-x

Nuchuchua, O., Sakulku, U., Uawongyart, N., Puttipipatkhachorn, S., Soottitantawat, A., Ruktanonchai, U., 2009. In vitro characterization and mosquito (Aedes aegypti) repellent activity of essential-oils-loaded nanoemulsions. AAPS PharmSciTech 10, 1234–1242. https://doi.org/10.1208/s12249-009-9323-1

Okoye, E.L., Kemakolam, C., Ugwuoji, E.T., Ogbonna, I., 2022. Multidrug Resistance Tracing by Plasmid Profile Analysis and the Curing of Bacteria from Different Clinical Specimens. Adv. Gut Microbiome Res. 2022, e3170342. https://doi.org/10.1155/2022/3170342

Oladeji, O.S., Adelowo, F.E., Ayodele, D.T., Odelade, K.A., 2019. Phytochemistry and pharmacological activities of Cymbopogon citratus: A review. Sci. Afr. 6, e00137. https://doi.org/10.1016/j.sciaf.2019.e00137

Olatunde, A., Mohammed, A., Ibrahim, M.A., Tajuddeen, N., Shuaibu, M.N., 2022. Vanillin: A food additive with multiple biological activities. Eur. J. Med. Chem. Rep. 5, 100055. https://doi.org/10.1016/j.ejmcr.2022.100055

Pancu, D.F., Scurtu, A., Macasoi, I.G., Marti, D., Mioc, M., Soica, C., Coricovac, D., Horhat, D., Poenaru, M., Dehelean, C., 2021. Antibiotics: Conventional Therapy and Natural Compounds with Antibacterial Activity—A Pharmaco-Toxicological Screening. Antibiotics 10, 401. https://doi.org/10.3390/antibiotics10040401

Parmanik, A., Das, S., Kar, B., Bose, A., Dwivedi, G.R., Pandey, M.M., 2022. Current Treatment Strategies Against Multidrug-Resistant Bacteria: A Review. Curr. Microbiol. 79, 388. https://doi.org/10.1007/s00284-022-03061-7

Paterson, D.L., Bonomo, R.A., 2005. Extended-spectrum beta-lactamases: a clinical update. Clin. Microbiol. Rev. 18, 657–686. https://doi.org/10.1128/CMR.18.4.657-686.2005

Pavoni, L., Perinelli, D.R., Bonacucina, G., Cespi, M., Palmieri, G.F., 2020. An Overview of Micro- and Nanoemulsions as Vehicles for Essential Oils: Formulation, Preparation and Stability. Nanomaterials 10, 135. https://doi.org/10.3390/nano10010135

Peiri, T., Fazeli, A., 2022. Identification of Ecotypes and Different Species of Savory (Satureja spp.) Using DNA Barcoding Genes. J. Veg. Sci. 6, 82–96. https://doi.org/10.22034/iuvs.2022.540180.1179

Pilong, P., Chuesiang, P., Mishra, D.K., Siripatrawan, U., 2022. Characteristics and antimicrobial activity of microfluidized clove essential oil nanoemulsion optimized using response surface methodology. J. Food Process. Preserv. 46, e16886. https://doi.org/10.1111/jfpp.16886

Pino-Otín, M.R., Gan, C., Terrado, E., Sanz, M.A., Ballestero, D., Langa, E., 2022. Antibiotic properties of Satureja montana L. hydrolate in bacteria and fungus of clinical interest and its impact in non-target environmental microorganisms. Sci. Rep. 12, 18460. https://doi.org/10.1038/s41598-022-22419-2

Popovici, R.A., Vaduva, D., Pinzaru, I., Dehelean, C.A., Farcas, C.G., Coricovac, D., Danciu, C., Popescu, I., Alexa, E., Lazureanu, V., Stanca, H.T., 2019. A comparative study on the biological activity of essential oil and total hydro-alcoholic extract of *Satureja hortensis* L. Exp. Ther. Med. 18, 932–942. https://doi.org/10.3892/etm.2019.7635

Qian, C., McClements, D.J., 2011. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. Food Hydrocoll. 25, 1000–1008. https://doi.org/10.1016/j.foodhyd.2010.09.017

Rai, M., Kosalec, I., 2022. Promising Antimicrobials from Natural Products. Springer Nature.

Reygaert, W.C., 2018. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol. 4, 482–501. https://doi.org/10.3934/microbiol.2018.3.482

Rezayat, S.M., Dehpour, A.-R., Motamed, S.M., Yazdanparast, M., Chamanara, M., Sahebgharani, M., Rashidian, A., 2018. Foeniculum vulgare essential oil ameliorates

acetic acid-induced colitis in rats through the inhibition of NF-kB pathway. Inflammopharmacology 26, 851–859. https://doi.org/10.1007/s10787-017-0409-1

Rezende, D.A. d. C.S., Oliveira, C.D., Batista, L.R., Ferreira, V.R.F., Brandão, R.M., Caetano, A.R.S., Alves, M.V.P., Cardoso, M.G., 2022. Bactericidal and antioxidant effects of essential oils from Satureja montana L., Myristica fragrans H. and Cymbopogon flexuosus. Lett. Appl. Microbiol. 74, 741–751. https://doi.org/10.1111/lam.13659

Roberts, M.C., 2004. Resistance to macrolide, lincosamide, streptogramin, ketolide, and oxazolidinone antibiotics. Mol. Biotechnol. 28, 47–62. https://doi.org/10.1385/MB:28:1:47

Rodríguez-Yoldi, M.J., 2021. Anti-Inflammatory and Antioxidant Properties of Plant Extracts. Antioxidants 10, 921. https://doi.org/10.3390/antiox10060921

Roriz, C.L., Barros, L., Carvalho, A.M., Santos-Buelga, C., Ferreira, I.C.F.R., 2014. Pterospartum tridentatum, Gomphrena globosa and Cymbopogon citratus: A phytochemical study focused on antioxidant compounds. Food Res. Int. 62, 684–693. https://doi.org/10.1016/j.foodres.2014.04.036

Rossi, C., Chaves-López, C., Serio, A., Casaccia, M., Maggio, F., Paparella, A., 2022. Effectiveness and mechanisms of essential oils for biofilm control on food-contact surfaces: An updated review. Crit. Rev. Food Sci. Nutr. 62, 2172–2191. https://doi.org/10.1080/10408398.2020.1851169

Ruvinov, I., Nguyen, C., Scaria, B., Vegh, C., Zaitoon, O., Baskaran, K., Mehaidli, A., Nunes, M., Pandey, S., 2019. Lemongrass Extract Possesses Potent Anticancer Activity Against Human Colon Cancers, Inhibits Tumorigenesis, Enhances Efficacy of FOLFOX, and Reduces Its Adverse Effects. Integr. Cancer Ther. 18, 1534735419889150. https://doi.org/10.1177/1534735419889150

Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R., Martín-Belloso, O., 2015. Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils. Food Hydrocoll. 43, 547–556. https://doi.org/10.1016/j.foodhyd.2014.07.012

Salvia-Trujillo, L., Rojas-Graü, M.A., Soliva-Fortuny, R., Martín-Belloso, O., 2014. Formulation of Antimicrobial Edible Nanoemulsions with Pseudo-Ternary Phase Experimental Design. Food Bioprocess Technol. 7, 3022–3032. https://doi.org/10.1007/s11947-014-1314-x

Salvia-Trujillo, L., Rojas-Graü, M.A., Soliva-Fortuny, R., Martín-Belloso, O., 2013. Effect of processing parameters on physicochemical characteristics of microfluidized lemongrass essential oil-alginate nanoemulsions. Food Hydrocoll. 30, 401–407. https://doi.org/10.1016/j.foodhyd.2012.07.004

Sampaio, T.S., Blank, A.F., Gagliardi, P.R., Wisniewski Jr, A., Arrigoni-Blank, M. de F., Nizio, D.A. de C., Alves, M.F., Nascimento Junior, A.F. de, 2020. Antifungal activity of essential oils of Myrcia ovata chemotypes and their major compounds on phytopathogenic fungi. Biosci J Online 364–375.

Santos, J.D.C., Coelho, E., Silva, R., Passos, C.P., Teixeira, P., Henriques, I., Coimbra, M.A., 2019. Chemical composition and antimicrobial activity of Satureja montana byproducts essential oils. Ind. Crops Prod. 137, 541–548. https://doi.org/10.1016/j.indcrop.2019.05.058

Sauer, K., Stoodley, P., Goeres, D.M., Hall-Stoodley, L., Burmølle, M., Stewart, P.S., Bjarnsholt, T., 2022. The biofilm life cycle: expanding the conceptual model of biofilm formation. Nat. Rev. Microbiol. 20, 608–620. https://doi.org/10.1038/s41579-022-00767-0

Sawadogo, I., Paré, A., Kaboré, D., Montet, D., Durand, N., Bouajila, J., Zida, E.P., Sawadogo-Lingani, H., Nikiéma, P.A., Nebié, R.H.C., Bassolé, I.H.N., 2022. Antifungal and Antiaflatoxinogenic Effects of Cymbopogon citratus, Cymbopogon nardus, and Cymbopogon schoenanthus Essential Oils Alone and in Combination. J. Fungi 8, 117. https://doi.org/10.3390/jof8020117

Scariot, F.J., Pansera, M.S., Delamare, A.P.L., Echeverrigaray, S., 2021. Citral and geraniol induce necrotic and apoptotic cell death on Saccharomyces cerevisiae. World J. Microbiol. Biotechnol. 37, 42. https://doi.org/10.1007/s11274-021-03011-8

Schaenzer, A.J., Wright, G.D., 2020. Antibiotic Resistance by Enzymatic Modification of Antibiotic Targets. Trends Mol. Med. 26, 768–782. https://doi.org/10.1016/j.molmed.2020.05.001

Schweitzer, B., Balázs, V.L., Molnár, S., Szögi-Tatár, B., Böszörményi, A., Palkovics, T., Horváth, G., Schneider, G., 2022. Antibacterial Effect of Lemongrass (Cymbopogon citratus) against the Aetiological Agents of Pitted Keratolyis. Molecules 27, 1423. https://doi.org/10.3390/molecules27041423

Sedaghat Doost, A., Nikbakht Nasrabadi, M., Kassozi, V., Nakisozi, H., Van der Meeren, P., 2020. Recent advances in food colloidal delivery systems for essential oils and their main components. Trends Food Sci. Technol. 99, 474–486. https://doi.org/10.1016/j.tifs.2020.03.037

Seyedtaghiya, M.H., Fasaei, B.N., Peighambari, S.M., 2021. Antimicrobial and antibiofilm effects of Satureja hortensis essential oil against Escherichia coli and Salmonella isolated from poultry. Iran. J. Microbiol. 13, 74–80. https://doi.org/10.18502/ijm.v13i1.5495

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., Mann, A.S., 2011a. Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (Lemon grass). J. Adv. Pharm. Technol. Res. 2, 3–8. https://doi.org/10.4103/2231-4040.79796

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., Mann, A.S., 2011b. Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (Lemon grass). J. Adv. Pharm. Technol. Res. 2, 3–8. https://doi.org/10.4103/2231-4040.79796

Shama, G., 2016. La Moisissure et la Bactérie: Deconstructing the fable of the discovery of penicillin by Ernest Duchesne. Endeavour 40, 188–200. https://doi.org/10.1016/j.endeavour.2016.07.005

Sharifi, A., Mohammadzadeh, A., Zahraei Salehi, T., Mahmoodi, P., 2018. Antibacterial, antibiofilm and antiquorum sensing effects of Thymus daenensis and Satureja hortensis essential oils against Staphylococcus aureus isolates. J. Appl. Microbiol. 124, 379–388. https://doi.org/10.1111/jam.13639

Sharifi-Rad, J., Sureda, A., Tenore, G.C., Daglia, M., Sharifi-Rad, Mehdi, Valussi, M., Tundis, R., Sharifi-Rad, Marzieh, Loizzo, M.R., Ademiluyi, A.O., Sharifi-Rad, R., Ayatollahi, S.A., Iriti, M., 2017. Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. Mol. Basel Switz. 22, 70. https://doi.org/10.3390/molecules22010070

Sharma, A.D., Farmaha, M., Kaur, I., 2020. Preparation and characterization of O/W nanoemulsion with eucalyptus essential oil and study of in vitro antibacterial activity, in: Nanomedicine Research Journal. https://doi.org/10.22034/nmrj.2020.04.006

Shkondrov, A., Krasteva, I., Pavlova, D., Zdraveva, P., 2017. Determination of flavonoids in related astragalus species (Sect. Incani) occurring in Bulgaria 70, 363–366.

Singh Chouhan, K.B., Tandey, R., Sen, K.K., Mehta, R., Mandal, V., 2019. Critical analysis of microwave hydrodiffusion and gravity as a green tool for extraction of essential oils: Time to replace traditional distillation. Trends Food Sci. Technol. 92, 12–21. https://doi.org/10.1016/j.tifs.2019.08.006

Smilack, J.D., 1999. Trimethoprim-sulfamethoxazole. Mayo Clin. Proc. 74, 730–734. https://doi.org/10.4065/74.7.730

Smith, R., Coast, J., 2013. The true cost of antimicrobial resistance. BMJ 346, f1493. https://doi.org/10.1136/bmj.f1493

Spisni, E., Petrocelli, G., Imbesi, V., Spigarelli, R., Azzinnari, D., Donati Sarti, M., Campieri, M., Valerii, M.C., 2020. Antioxidant, Anti-Inflammatory, and Microbial-Modulating Activities of Essential Oils: Implications in Colonic Pathophysiology. Int. J. Mol. Sci. 21, 4152. https://doi.org/10.3390/ijms21114152

Stanford, K., Zaheer, R., Klima, C., McAllister, T., Peters, D., Niu, Y.D., Ralston, B., 2020. Antimicrobial Resistance in Members of the Bacterial Bovine Respiratory Disease Complex Isolated from Lung Tissue of Cattle Mortalities Managed with or without the Use of Antimicrobials. Microorganisms 8, 288. https://doi.org/10.3390/microorganisms8020288

Stratakos, A.C., Koidis, A., 2016. Chapter 4 - Methods for Extracting Essential Oils, in: Preedy, V.R. (Ed.), Essential Oils in Food Preservation, Flavor and Safety. Academic Press, San Diego, pp. 31–38. https://doi.org/10.1016/B978-0-12-416641-7.00004-3

Subramaniam, G., Yew, X.Y., Sivasamugham, L.A., 2020. Antibacterial activity of *Cymbopogon citratus* against clinically important bacteria. South Afr. J. Chem. Eng. 34, 26–30. https://doi.org/10.1016/j.sajce.2020.05.010

Tančinová, D., Medo, J., Mašková, Z., Barboráková, Z., Hlebová, M., 2022. SELECTED PLANT ESSENTIAL OILS OF THE LAMIACEEAE AND APIACEAE FAMILY AS THE ANTIFUNGAL AGENTS IN THE VAPOUR PHASE AGAINST RHIZOPUS STOLONIFER AND RHIZOPUS ORYZAE ISOLATED FROM MOULDING BREADS. | Journal of Microbiology, Biotechnology & Food Sciences | EBSCOhost [WWW Document]. https://doi.org/10.55251/jmbfs.9113

Teresa-Martínez, G.D., Cardador-Martínez, A., Téllez-Pérez, C., Allaf, K., Jiménez-Martínez, C., Alonzo-Macías, M., 2022. Effect of the Instant Controlled Pressure Drop Technology in Cardamom (Elettaria cardamomum) Essential Oil Extraction and Antioxidant Activity. Molecules 27, 3433. https://doi.org/10.3390/molecules27113433

Thomas, C.M., Frost, L.S., 2021. Plasmid Genomes, Introduction to, in: Bell, E. (Ed.), Molecular Life Sciences: An Encyclopedic Reference. Springer, New York, NY, pp. 1–20. https://doi.org/10.1007/978-1-4614-6436-5_107-2

Tian, H., Zada, B., Singh, B.H., Wang, C., Kim, S.-W., 2019. Chapter 13 - Synthetic Biology Approaches for the Production of Isoprenoids in Escherichia coli, in: Singh, S.P., Pandey, A., Du, G., Kumar, S. (Eds.), Current Developments in Biotechnology and Bioengineering. Elsevier, pp. 311–329. https://doi.org/10.1016/B978-0-444-64085-7.00013-7

Tibenda, J.J., Yi, Q., Wang, X., Zhao, Q., 2022. Review of phytomedicine, phytochemistry, ethnopharmacology, toxicology, and pharmacological activities of Cymbopogon genus. Front. Pharmacol. 13, 997918. https://doi.org/10.3389/fphar.2022.997918

Tinker, N.J., Foster, R.A., Webb, B.J., Haydoura, S., Buckel, W.R., Stenehjem, E.A., 2021. Interventions to optimize antimicrobial stewardship. Antimicrob. Steward. Healthc. Epidemiol. ASHE 1, e46. https://doi.org/10.1017/ash.2021.210

Tit, D.M., Bungau, S.G., 2023. Antioxidant Activity of Essential Oils. Antioxidants 12, 383. https://doi.org/10.3390/antiox12020383

Tooke, C.L., Hinchliffe, P., Bragginton, E.C., Colenso, C.K., Hirvonen, V.H.A., Takebayashi, Y., Spencer, J., 2019. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. J. Mol. Biol. 431, 3472–3500. https://doi.org/10.1016/j.jmb.2019.04.002

Tozlu, E., Cakir, A., Kordali, S., Tozlu, G., Ozer, H., Aytas Akcin, T., 2011. Chemical compositions and insecticidal effects of essential oils isolated from *Achillea gypsicola*, *Satureja hortensis*, *Origanum acutidens* and *Hypericum scabrum* against broadbean weevil (*Bruchus dentipes*). Sci. Hortic. 130, 9–17. https://doi.org/10.1016/j.scienta.2011.06.019

Trang, D.T., Hoang, T.K.V., Nguyen, T.T.M., Van Cuong, P., Dang, N.H., Dang, H.D., Nguyen Quang, T., Dat, N.T., 2020. Essential Oils of Lemongrass (*Cymbopogon citratus* Stapf) Induces Apoptosis and Cell Cycle Arrest in A549 Lung Cancer Cells. BioMed Res. Int. 2020, e5924856. https://doi.org/10.1155/2020/5924856

Turek, C., Stintzing, F.C., 2013. Stability of Essential Oils: A Review. Compr. Rev. Food Sci. Food Saf. 12, 40–53. https://doi.org/10.1111/1541-4337.12006

Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Emwas, A.-H., Jaremko, M., 2020. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules 25, 5243. https://doi.org/10.3390/molecules25225243

Ullah, H., Ali, S., Ullah, H., Ali, S., 2017. Classification of Anti-Bacterial Agents and Their Functions, in: Antibacterial Agents. IntechOpen. https://doi.org/10.5772/intechopen.68695

Ullah, N., Amin, A., Alamoudi, Rana A., Rasheed, S.A., Alamoudi, Ruaa A., Nawaz, A., Raza, M., Nawaz, T., Ishtiaq, S., Abbas, S.S., 2022. Fabrication and Optimization of Essential-Oil-Loaded Nanoemulsion Using Box–Behnken Design against Staphylococos aureus and Staphylococos epidermidis Isolated from Oral Cavity. Pharmaceutics 14, 1640. https://doi.org/10.3390/pharmaceutics14081640

Vandamme, T., Anton, N., 2010. Low-energy nanoemulsification to design veterinary controlled drug delivery devices. Int. J. Nanomedicine 5, 867–73. https://doi.org/10.2147/IJN.S13273

Ventola, C.L., 2015. The antibiotic resistance crisis: part 1: causes and threats. P T Peer-Rev. J. Formul. Manag. 40, 277–283.

Verma, A., Preet, S., 2021. Larvicidal and antioxidant activity of green silver nanoparticles synthesized using Musa acuminate peel extract against Aedes aegypti. Int. J. Mosq. Res. 8, 01–05. https://doi.org/10.22271/23487941.2021.v8.i2a.512

Victoria, F.N., Lenardão, E.J., Savegnago, L., Perin, G., Jacob, R.G., Alves, D., Silva, W.P. da, Motta, A. de S. da, Nascente, P. da S., 2012. Essential oil of the leaves of *Eugenia uniflora* L.: Antioxidant and antimicrobial properties. Food Chem. Toxicol. 50, 2668–2674. https://doi.org/10.1016/j.fct.2012.05.002

Waglechner, N., Wright, G.D., 2017. Antibiotic resistance: it's bad, but why isn't it worse? BMC Biol. 15, 84. https://doi.org/10.1186/s12915-017-0423-1

Watanabe, C., Hokari, R., Komoto, S., Kurihara, C., Okada, Y., Matsunaga, H., Takebayashi, K., Kawaguchi, A., Nagao, S., Tsuzuki, Y., Yokoyama, H., Hibi, T., Miura, S., 2010. Lemon grass (Cymbopogon citratus) ameliorates murine spontaneous ileitis by decreasing lymphocyte recruitment to the inflamed intestine. Microcirc. N. Y. N 1994 17, 321–332. https://doi.org/10.1111/j.1549-8719.2010.00032.x

Wei, L.S., Wee, W., 2013. Chemical composition and antimicrobial activity of Cymbopogon nardus citronella essential oil against systemic bacteria of aquatic animals. Iran. J. Microbiol. 5, 147–152.

Wu, J., Wang, J., Li, Z., Guo, S., Li, K., Xu, P., Ok, Y.S., Jones, D.L., Zou, J., 2023. Antibiotics and antibiotic resistance genes in agricultural soils: A systematic analysis. Crit. Rev. Environ. Sci. Technol. 53, 847–864. https://doi.org/10.1080/10643389.2022.2094693

Yang, Q., Gao, Y., Ke, J., Show, P.L., Ge, Y., Liu, Y., Guo, R., Chen, J., 2021. Antibiotics: An overview on the environmental occurrence, toxicity, degradation, and removal methods. Bioengineered 12, 7376–7416. https://doi.org/10.1080/21655979.2021.1974657

Yang, Y., Hu, B., 2014. 21 - Bio-based chemicals from biorefining: lipid and wax conversion and utilization, in: Waldron, K. (Ed.), Advances in Biorefineries. Woodhead Publishing, pp. 693–720. https://doi.org/10.1533/9780857097385.2.693

Yap, P.S.X., Yusoff, K., Lim, S.-H.E., Chong, C.-M., Lai, K.-S., 2021. Membrane Disruption Properties of Essential Oils—A Double-Edged Sword? Processes 9, 595. https://doi.org/10.3390/pr9040595

Zhang, J., Ma, S., Du, S., Chen, S., Sun, H., 2019. Antifungal activity of thymol and carvacrol against postharvest pathogens Botrytis cinerea. J. Food Sci. Technol. 56, 2611–2620. https://doi.org/10.1007/s13197-019-03747-0

Zhang, Q.-W., Lin, L.-G., Ye, W.-C., 2018. Techniques for extraction and isolation of natural products: a comprehensive review. Chin. Med. 13, 20. https://doi.org/10.1186/s13020-018-0177-x

Zhang, Y., Wang, X., Yin, X., Shi, M., Dahlgren, R.A., Wang, H., 2016. Toxicity assessment of combined fluoroquinolone and tetracycline exposure in zebrafish (Danio rerio). Environ. Toxicol. 31, 736–750. https://doi.org/10.1002/tox.22087

Zhang, Z., Shen, P., Liu, J., Gu, C., Lu, X., Li, Y., Cao, Y., Liu, B., Fu, Y., Zhang, N., 2017. In Vivo Study of the Efficacy of the Essential Oil of Zanthoxylum bungeanum Pericarp in Dextran Sulfate Sodium-Induced Murine Experimental Colitis. J. Agric. Food Chem. 65, 3311–3319. https://doi.org/10.1021/acs.jafc.7b01323

Zheng, Y., Shang, Y., Li, M., Li, Y., Ouyang, W., 2021. Antifungal Activities of cis-trans Citral Isomers against Trichophyton rubrum with ERG6 as a Potential Target. Molecules 26, 4263. https://doi.org/10.3390/molecules26144263

Zhou, Y., Li, Y., Zhang, L., Wu, Z., Huang, Y., Yan, H., Zhong, J., Wang, L.-J., Abdullah, H.M., Wang, H.H., 2020. Antibiotic Administration Routes and Oral Exposure to Antibiotic Resistant Bacteria as Key Drivers for Gut Microbiota Disruption and Resistome in Poultry. Front. Microbiol. 11, 1319. https://doi.org/10.3389/fmicb.2020.01319

Ziani, K., Chang, Y., McLandsborough, L., McClements, D.J., 2011. Influence of Surfactant Charge on Antimicrobial Efficacy of Surfactant-Stabilized Thyme Oil Nanoemulsions. J. Agric. Food Chem. 59, 6247–6255. https://doi.org/10.1021/jf200450m

Webography

sekrety-zdrowia.org (2024) 'Cząber na problemy żołądka, jelit, nieżyty dróg oddechowych', sekrety-zdrowia.org. Available at: https://sekrety-zdrowia.org/czaber-kuchni-domowej-apteczce/ (Accessed: 30 Mars 2024).

Plants of the World Online (2024) *Satureja hortensis* L. Available at: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:457680-1 (Accessed: 30 Mars 2024).

ملخص

تمثل مقاومة المضادات الحيوية تهديدًا كبيرًا للصحة العامة العالمية، حيث يؤدي الإفراط في استخدام المضادات الحيوية وظهور البكتيريا المقاومة إلى تقويض العلاجات التقليدية. تقدم الجزيئات الطبيعية المستخلصة من النباتات الطبية بديلًا واعدًا في مكافحة هذه المقاومة، إذ تعمل تقنية النانوتشكيل على تحسين استقرارها وفعاليتها العلاجية. أهداف هذه الدراسة هي تقديم تحليل شامل لمقاومة المضادات الحيوية في بيئة سريرية، واستكشاف إمكانيات الزيوت الأساسية الطبيعية كعوامل مضادة للميكروبات بديلة، وتقييم فعالية تَرَكِيبَاتها النانوية في تعزيز الأنشطة البيولوجية. كشفت دراسة استعادية أجريت في قسم الأمراض المعدية بمستشفى سطيف الجامعي خلال المرحلة الأولى من تحقيقنا عن معدلات مقلقة لمقاومة الأموكسيسيلين (100%)، السيفازولين(70% <) ، والسيفوتاكسيم (58.06%). وكانت أكثر سلالة بكتيرية تم تحديدها هي *Escherichia coli* المنتجة لإنزيم البيتا لاكتاماز واسع الطيف(BLSE) ، والتي مثلت 29.03% من العزلات .في الجزء الثاني، تناولت الدراسة استخلاص الزيوت الأساسية (HE) من نبات *Satureja hortensis* L. (ASHEO) ونبات *Cymbopogon citratus* (DC.) Stapf (LGEO)في الجزائر باستخدام تقنية التقطير بالبخار، بالإضافة إلى استخدام تقنية الميكروفلويديزيشن للحصول على نانوتشكيلات هذه الزيوت. تم استخدام جهاز GC–MS لإجراء مقارنة بين ASHEO و LGEO من حيث محتواهما من المركبات الطيارة في مستحلبات النانو (MF–ASHEO و MF–LGEO). أظهرت نتائج MF–ASHEO وجود 8 مركبات (99.56%) مقارنةً بـ 26 مركبًا في ASHEO (95.46%). أما المركبات التي تم تحديدها في MF–LGEO فقد شكلت 97.53% من إجمالي محتوى الزيت في مستحلب النانو، وهو ما يشبه LGEO المستخلص بالتقطير بالبخار(97.73%). أظهرت الزيوت الأساسية ونانوتشكيلاتها خصائص مضادة للبكتيريا والبيوفيلم، حيث أظهر ASHEO و LGEO فعالية كبيرة ضد عزلات بكتيرية ممرضة من الجراثيم الموجبة والسالبة لصبغة جرام. أظهر ASHEO نشاطًا ضد جميع السلالات البكتيرية المختبرة، مع مناطق تثبيط تتراوح بين 55.66 مم و29.66 مم، بينما تم تثبيط تكوين البيوفيلم بواسطة *E. coli* و*P. aeruginosa* بنسبة تزيد عن 60%، وأظهر LGEO تثبيطًا كاملًا لسلالة *Bacillus subtilis*.تم التحقق من التأثيرات المبيدة للبكتيريا والمثبطة لنموها لمركب الكارفاكرول على البروتينات المستهدفة باستخدام تحليل ADME والنمذجة *in silico* .بالإضافة إلى ذلك، أظهرت الزيوت الأساسية، وخاصةMF–LGEO ، خصائص مضادة للفطريات ضد الفطريات المفرزة للسموم مثل سلالات *Aspergillus*، *Penicillium*، وFusarium، مما يؤثر على معدلات نموها ويقترح إمكانية استخدامها كعلاج للتلوث الفطري السام. كما كشفت الدراسة أن الزيوت الأساسية ونانوتشكيلاتها أظهرت سمية خلوية واعدة ضد ثلاث سلالات خلوية، مع تعزيز تأثيرات مضادة للالتهابات بواسطة تقنية الميكروفلويديزيشن، حيث ارتبطت هذه النتائج بتعديلات في مكونات النانوتشكيل. أظهرت الزيوت الأساسية التي تم دراستها تأثيرات مضادة للأكسدة معتدلة في تثبيط الجذور الحرة، مع قيمة IC50 بلغت 536.47 ± 21.99 ميكروغرام/مل لASHEO ، وقيمة IC50 بلغت 82.87 ± 2.15 ميكروغرام/مل لـ .LGEO كان لتقنية الميكروفلويديزيشن تأثير عميق على محتوى المركبات الطيارة والنشاط البيولوجي للزيت .تدعم نتائجنا استخدام نباتي *S. hortensis* و*C. citratus* لاستخراج زيوتهما الأساسية الثمينة، خاصةً للوقاية من الأمراض الفطرية السامة والالتهابات البكتيرية المقاومة للمضادات الحيوية.

كلمات مفتاحية: *Satureja hortensis* L. ؛ *Cymbopogon citratus* (DC.) Stapf؛ الزيوت الأساسية؛ التشكيل النانوي؛ مضاد للبكتيريا؛ مضاد للفطريات؛ مضاد للأكسدة؛ سمية للخلايا؛ *in silico*.

Abstract

Antibiotic resistance is one of the biggest global public health threats. The abuse of antibiotics and the emergence of resistant strains of bacteria undermine the effectiveness of conventional treatments. Natural molecules extracted from medicinal plants offer a promising alternative to combating this resistance, with nanoformulation improving their bioavailability and therapeutic effectiveness. The objectives of this study are to provide a comprehensive analysis of antibiotic resistance within a clinical setting, explore the potential of natural EOs as alternative antimicrobial agents, and assess the efficacy of their nanoformulations in enhancing biological activity. A retrospective study conducted at the service of infection diseases at CHU Setif during the initial phase of our investigation revealed alarmingly high rates of resistance to amoxicillin (100%), cefazoline (>70%), and cefotaxime (58.06%). *Escherichia coli* ESBL represented the most common bacterial strain identified (29.03%). In the second part, the study examined the extraction by hydrodistillation of essential oils (EOs) from Algerian *Satureja hortensis* L. (ASHEO) and *Cymbopogon citratus* (DC.) Stapf (LGEO), and the microfluidization technique was used to get their nanoformulations. The GC-MS apparatus was utilized for a comparative examination of ASHEO and LGEO with their microfluidization nanoemulsions (MF-ASHEO and MF-LGEO) volatile content. MF-ASHO showed 8 compounds (99.56%) vs ASHEO's 26 compounds (95.46%). The identified components in MF-LGEO represented 97.53% of the total nanoemulsion oil, which was similar to the hydrodistilled LGEO (97.73%). The essential oils and nanoformulations showed antibacterial and antibiofilm properties, while ASHEO and LGEO showed superior efficacy against pathogenic isolates of Gram-positive and Gram-negative bacteria. ASHEO effectively exhibited activity against all tested bacterial strains with inhibition zones measured between 55.66 mm and 29.66 mm, and the biofilm formation of *E. coli* and *P. aeruginosa* was suppressed by over 60%, while LGEO demonstrated complete inhibition of *B. subtilis*. The bactericidal and bacteriostatic effects of carvacrol on the target proteins were validated by ADME and *in silico* analyses. Additionally, essential oils, especially MF-LGEO, showed antifungal properties against mycotoxigenic fungi including Aspergillus, Penicillium and Fusarium strains by influencing their growth rates and suggesting potential treatment for toxigenic fungal contamination. The study also found that essential oils and nanoformulations showed promising cytotoxicity against three cell line strains, with microfluidization enhancing anti-inflammatory effects, and these findings were linked to alterations in nanoformulation components. Studied essential oils revealed moderate antioxidant effects in radical scavenging, with an IC50 value of 536.47 ± 21.99 $\mu g.mL^{-1}$ for ASHEO and an IC50 value equal to (82.87 ± 2.15 $\mu g.mL^{-1}$). The microfluidization procedure has a profound impact on both the volatile content and biological activity of the oil. Our findings reassure the use of *S. hortensis* and *C. citratus* for their valuable essential oils and to prevent mycotoxigenic diseases and pathogenic-resistant bacteria.

Key words: *Satureja hortensis* L.; *Cymbopogon citratus* (DC.) Stapf; essential oils, microfluidization; antibacterial; antifungal;