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Valorization of Essential Oils from *Cymbopogon citratus*, *Schinus molle*, and *Teucrium polium*: Chemical Composition and Biological Activities

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ABSTRACT

Essential oils from medicinal plants have attracted considerable scientific attention due to their complex phytochemical profiles and wide-ranging bioactivities. This study aimed to characterize the chemical composition, biological activities, and sensory attributes of essential oils extracted from three medicinal plants—*Cymbopogon citratus* (CCEO), *Schinus molle* (SMEO), and *Teucrium polium* (TPEO)—collected from ecologically distinct regions of Algeria. The (GC–MS) and (GC–FID) analyses identified 52, 50, and 72 constituents in CCEO, SMEO, and TPEO, respectively. Citral isomers were predominant in CCEO (α-citral: 43.36%, β-citral: 36.16%), while SMEO was rich in α-phellandrene (12.70%), limonene (11.90%), and germacrene D (~10.15%%). Notably, TPEO contained a high level of fenchene (16.45%).

Antioxidant potential, assessed via five standard assays (β-carotene–linoleic acid, DPPH, $ABTS^{+}$, CUPRAC, and metal chelation), revealed moderate to low activity, with CCEO demonstrating the strongest effect, followed by TPEO and SMEO. All oils exhibited moderate inhibitory activity against key enzymes including acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-glucosidase, α-amylase, urease, and tyrosinase, though IC_{50} values exceeded 200 µg/mL. Anti-inflammatory activity, evaluated through human red blood cell membrane stabilization and egg albumin denaturation assays, was evident in all samples. Antimicrobial activity was determined against two Gram-positive bacteria, two Gram-negative bacteria, and two *Candida* species, with CCEO showing the lowest minimum inhibitory concentrations (MICs), followed by SMEO and TPEO.

Furthermore, the EOs demonstrated moderate to strong interference with bacterial communication, including biofilm formation, quorum sensing, violacein production, and motility (swimming and swarming). Cytotoxicity assessment on healthy human colon cells (CCD18-Co) confirmed the safety of all tested oils at the evaluated concentrations.

These findings underscore the promising bioactive potential and safety of Algerian *C. citratus*, *S. molle*, and *T. polium* essential oils, supporting their potential applications in food, pharmaceutical, and cosmetic industries.

Keywords: essential oils, *Cymbopogon citratus*, *Schinus molle*, *Teucrium polium*, phytochemistry, antioxidant, anti-inflammatory, enzyme inhibition, quorum sensing, antibiofilm, cytotoxicity.

الملخص

تحظى الزيوت الأساسية المستخلصة من النباتات الطبية باهتمام علمي متزايد نظراً لغناها بالمركبات الكيميائية النباتية وتنوع تطبيقاتها البيولوجية. تهدف هذه الدراسة إلى تحليل التركيب الكيميائي، وتقييم الخصائص البيولوجية، والصفات الحسية لزيوت أساسية مستخرجة من ثلاث نباتات طبية جُمعت من مناطق بيئية مختلفة في الجزائر، وهي عشبة الليمون *Cymbopogon citratus* (CCEO) ، الفلفل البيروفي *Schinus molle* (SMEO) ، والجعدة *Teucrium polium* (TPEO).

من خلال التحليل بواسطة تقنية الكروماتوغرافيا الغازية المقترنة بمطياف الكتلة (GC-MS) وكاشف التأين باللهب (GC-FID)، تم تحديد 52 مركباً في CCEO، و50 في SMEO، و72 في TPEO. سيطر على زيت CCEO مركبا الألفا-سيترال بنسبة 43.36% و البيتا-سيترال بنسبة 36.16%، بينما احتوى SMEO بشكل أساسي على الألفا-فيلاندرين (12.70%)، ليمونين (11.90%)، وجرماكرين D (10.15%) وتميز TPEO بتركيز غير معتاد من الفينشين (16.45%).

تم تقييم الفعالية المضادة للأكسدة باستخدام خمسة اختبارات قياسية (بيتا كاروتين/حمض اللينوليك، DPPH، $ABTS^{+}$، CUPRAC، واختبار خلب المعادن)، وأظهرت النتائج فعالية معتدلة إلى ضعيفة، حيث سجلت CCEO النشاط الأعلى، تليها TPEO و أخيرا SMEO. كما أظهرت جميع الزيوت تثبيطاً معتدلاً للعديد من الإنزيمات الحيوية (AChE، BChE، α-glucosidase، α-amylase، urease، وtyrosinase)، وكانت قيم IC_{50} أعلى من 200 ميكروغرام/مل. وأظهرت الزيوت فعالية مضادة للالتهاب في اختبار تثبيت غشاء كريات الدم الحمراء البشرية واختبار تخثر ألبومين البيض.

تم اختبار النشاط المضاد للميكروبات ضد نوعين من البكتيريا موجبة الغرام، ونوعين سالبة الغرام، ونوعين من خمائر *Candida*، حيث أظهرت CCEO أقل تركيز مثبط، تليها SMEO ثم TPEO . كما بيّنت الزيوت فعالية ملحوظة في تثبيط التواصل البكتيري (مضاد للبُيوفِيلم، كبح الإحساس الجماعي، تثبيط إنتاج الفيولايسين، الحركة السابحة، والحركة الزاحفة). أظهرت اختبارات السمية الخلوية على خط خلوي بشري سليم (CCD18- Co) عدم وجود أي تأثير سام في التراكيز المستخدمة.

تُبرز هذه النتائج الإمكانات الواعدة للزيوت الأساسية المشتقة من *C. citratus* ، *S. molle*، و *T. polium* المزروعة في الجزائر، وتدعم توجه استخدامها في المجالات الغذائية والدوائية والتجميلية.

الكلمات المفتاحية: الزيوت الأساسية، *Cymbopogon citratus*، *Schinus molle*، *Teucrium polium*، التركيب الكيميائي، مضاد أكسدة، مضاد التهاب، تثبيط إنزيمات، كبح الإحساس الجماعي، مضاد للبُيوفِيلم، السمية الخلوية.

RESUME

Les huiles essentielles issues de plantes médicinales suscitent un intérêt scientifique croissant en raison de la richesse de leur profil phytochimique et de la diversité de leurs activités biologiques. Cette étude vise à caractériser la composition chimique, les propriétés biologiques et les caractéristiques sensorielles des huiles essentielles extraites de trois plantes médicinales récoltées dans différentes régions écologiques d'Algérie : *Cymbopogon citratus* (CCEO), *Schinus molle* (SMEO) et *Teucrium polium* (TPEO).

Les analyses par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC–MS) et à la détection par ionisation de flamme (GC–FID) ont permis d'identifier respectivement 52, 50 et 72 composés dans les huiles CCEO, SMEO et TPEO. Les isomères du citral dominent la CCEO (α-citral : 43.36%; β-citral : 36.16%), tandis que la SMEO est riche en α-phéllandrène (12.70%), limonène (11.90% %) et germacrène D (~10.15%%). La TPEO se distingue par une teneur inhabituelle en fenchène (16.45 %). Le potentiel antioxydant, évalué par cinq tests standards (β-carotène/acide linoléique, DPPH, $ABTS^{+}$, CUPRAC et chélation des métaux), a révélé une activité modérée à faible, la CCEO montrant l'effet le plus élevé, suivi par la TPEO et la SMEO. Les trois huiles ont également montré une inhibition modérée de plusieurs enzymes clés, dont l'acétylcholinestérase (AChE), la butyrylcholinestérase (BChE), l'α-glucosidase, l'α-amylase, l'uréase et la tyrosinase, avec des valeurs d'IC_{50} supérieures à 200 µg/mL. L'activité anti-inflammatoire, évaluée par les tests de stabilisation de la membrane des globules rouges humains (HRBC) et de dénaturation de l'albumine d'œuf, s'est révélée significative pour les trois huiles. L'évaluation de l'activité antimicrobienne contre deux bactéries Gram positif, deux Gram négatif et deux levures du genre *Candida* a montré que la CCEO avait les plus faibles concentrations minimales inhibitrices, suivie par la SMEO et la TPEO. Par ailleurs, les HE ont démontré une inhibition modérée à élevée de la communication bactérienne (formation de biofilm, quorum sensing, production de violacéine, mobilité par nage et essaim). Les tests de cytotoxicité sur une lignée cellulaire humaine saine (CCD18-Co) n'ont révélé aucun effet toxique aux concentrations évaluées.

Ces résultats soulignent le potentiel bioactif et la sécurité d'utilisation des huiles essentielles de *C. citratus*, *S. molle* et *T. polium* cultivées en Algérie, en appui à leur valorisation dans les domaines agroalimentaire, pharmaceutique et cosmétique.

Mots-clés : huiles essentielles, *Cymbopogon citratus*, *Schinus molle*, *Teucrium polium*, composition chimique, antioxydant, anti-inflammatoire, inhibition enzymatique, quorum sensing, antibiofilm, cytotoxicité.

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PUBLICATIONS ARISING FROM THIS THESIS

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COLLATERAL RESEARCH

El Amine, K.M. et al. (2025). Recycling Environment and Bioremediation. In: Abdel Latef, A.A.H., Zayed, E.M., Omar, A.A. (eds) Bioremediation and Nanotechnology for Climate Change Mitigation. *Springer*, Singapore. https://doi.org/10.1007/978-981-96-3069-1_12

LIST OF CONFERENCE PRESENTATIONS

International Conferences

"Bioactive Potential of Lemongrass Essential Oils: Chemical Insights and Health Implications" (ISABI)— Oral Presentation at the *1st International Seminar Et Agri-Food and Industrial Biotechnology*, 26-27 June 2024, Khemis Miliana, Algeria.

"The Potential of *Teucrium polium* in Ethnotherapy Practices: Traditional Wisdom for Animal Well-Being" — Poster Presentation at the *3rd International Webinar on Food Security and Animal Health*, 9-10 July 2023, Algiers, Algeria.

"Biological Activities of Essential Oils from *Schinus molle* Trees Growing in Algeria" — Oral Presentation at the *1st International Webinar on Biodiversity and Valorization of Vegetal and Microorganisms* (WIBVVM), 13-15 December 2022, Oran, Algeria.

"Antioxidant and Anti-Inflammatory Activities of *Teucrium polium* L. From North-Central Algeria" — Poster Presentation at the *1st International Congress of Innovations in Chemistry for Therapeutic Aims*, 23-24 October 2022, Oum El Bouaghi, Algeria.

National Conferences

"Essential Oils as Eco-Friendly Innovations in Agriculture"— Oral Presentation at *the 1er Séminaire National sur l'Innovation et la Vulgarisation Agricole* (SNIVA, 2024), 19 December 2024, Adrar, Algeria.

"Promising Future of Natural Antioxydants : Algerian Medicinal Plants As An Example "— Poster Presentation at La *1ere Journée D'étude Sur La Biodiversité Et La Sécurité Alimentaire Face Au Changement Climatique*, 3rd June 2024, Gelizane, Algeria.

"Biological Activities of Algerian Wild Germander (*Teucrium polium* L.)" — Poster Presentation at the *1st National Webinar on Bioactive Substances 'Substances Naturelle Aujourd'hui, Medicament Promoteur Demain!'* (SNAMPD), 13-14 December 2022, M'sila, Algeria.

"A Comparative Study of Pink Peppercorn (*Schinus molle* L.) Essential Oils Growing in Tree Different Regions in Algeria" — Oral Presentation at the *1st National Seminar on Essential Oils and Aromatherapy* (SNHEA 2022), 19-20 September 2022, Khenchela, Algeria.

ABBREVIATIONS

°C	Degrees Celsius
°F	Degrees Fahrenheit
€	Euro
µg	Microgram
µL	Microliter
5-LOX	5-lipoxygenase
ABTS+	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
AChE	Acetylcholinesterase
AD	Alzheimer's Disease
AFNOR	Association Française de Normalisation (French Association for Standardization)
AHL	Acyl-Homoserine Lactones
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
BC	Before Christ
BChE	Butyrylcholinesterase
BHA	Butylated HydroxyAnisole
BHT	Butylated HydroxyToluene
BSA	Bovine Serum Albumin
C6HSL	N-Hexanoyl-L-homoserine lactone

CAT	Catalase
CBD	Convention on Biological Diversity
CCEO	Cymbopogon citratus essential oil
CFU	colony-forming units
CLP	Classification, Labelling, and Packaging
CLSI	Clinical and Laboratory Standards Institute
CO2	Carbon Dioxide
COVID-19	Coronavirus Disease 2019
COX-2	Cyclooxygenase-2
Cu	Copper
CUPRAC	Cupric Reducing Antioxidant Capacity
CV026	Chromobacterium violaceum 026
CV12472	Chromobacterium violaceum 12472
DIY	"Do It Yourself" projects
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DTNB	5,5'-Dithiobis(2-nitrobenzoic acid)
e.g.	Exempli Gratia
EC	European Commission
ECHA	European Chemicals Agency

EDQM	European Directorate for the Quality of Medicines & HealthCare
EDTA	Ethylenediaminetetraacetic Acid
EI	Electron ionization
EO	Essential oil
Eq.	Equation
EU	European Union
eV	Electron Volt
FDA	Food and Drug Administration
FeCl2	Iron (II) Chloride
FID	Flame Ionization Detection
Fig.	Figure
FT-IR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionization Detection
GC-MS	Gas Chromatography-Mass Spectrometry
GRAS	Generally Recognized as Safe
He	Helium
HRBC	Human Red Blood Cells
IAS	International Allelopathy Society
IC50	Half Maximal Inhibitory Concentration
IL-10	Interleukin-10

IL-6	Interleukin-6
IR	Infrared Spectroscopy
ISO	International Organization for Standardization
IUCN	International Union for Conservation of Nature
LB	Luria-Bertani
LBA	Luria Bertani Agar
LDL	Low-Density Lipoprotein
L-DOPA	L-3,4-dihydroxyphenylalanine
LTB4	Leukotriene B4
M	Molar
m/z	Mass-to-Charge Ratio
MAE	Microwave-assisted extraction
MHB	Mueller-Hinton Broth
MIC	The minimum inhibitory concentration
mL	Milliliter
mM	millimolar
MS	Mass Spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na2SO4	Sodium Sulfate
NaCl	Sodium Chloride
NGOs	Non-Governmental Organizations

NH4	Ammonium
NIST14	National Institute of Standards and Technology (NIST) 2014 Mass Spectral Library
nm	Nanometer
NMR	Nuclear Magnetic Resonance
OD	Optical Density
PA01	P. aeruginosa
PBS	Phosphate-Buffered Saline
PGE2	Prostaglandin E2
pH	A measure of the acidity or alkalinity of a solution
PNPG	4-N-nitrophenyl-α-D-glucopyranoside
psi	Pounds per Square Inch
QS	Quorum Sensing
QSI	Quorum Sensing Inhibition
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
ROS	Reactive Oxygen Species
RPMI	Roswell Park Memorial Institute
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SDGs	Sustainable Development Goals
SMEO	Schinus molle essential oil
SOD	Superoxide dismutase

TNF-α	Tumor Necrosis Factor-alpha
TPEO	Teucrium polium essential oil
TRLIB	Terpene Library
TSB	Tryptose-Soy Broth
UAE	Ultrasound-assisted extraction
UK	United Kingdom
USA	United States of America
USD	United States Dollar
USDA	United States Department of Agriculture
v/v	Volume/Volume
w/v	Weight/Volume
WHO	World Health Organization

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INTRODUCTION

GENERAL INTRODUCTION

In a society saturated with synthetic products, the pursuit of natural alternatives has become an essential requirement. Numerous synthetic substances utilized in food, cosmetics, and medications have been linked to considerable health hazards, including hormonal disruption, allergic reactions, and even cancer (Bergman et al., 2013). Synthetic products frequently depend on limited resources and generate waste that may negatively impact the environment. Consumers, growing more distrustful of huge corporations and their utilization of synthetic ingredients—which can be difficult to understand and comprehend—are pursuing natural alternatives that are more friendly for the body and are less likely to induce harmful effects (Bom et al., 2020). The demand is further driven by concerns regarding the ethical and sustainable sourcing of components, as natural products typically line more closely with these ideals (J. Sharifi-Rad et al., 2019). As a result, the need for natural alternatives has encouraged innovation in multiple areas, resulting in the creation of novel products that utilize nature's capabilities (Ames et al., 1990). These revolutionary natural products are safer, more sustainable, and offer compelling opportunities, generating interest and passion among customers.

Natural products, typically biodegradable and renewable, are produced using sustainable methods that minimize environmental impact. They often come with a sense of authenticity, transparency, and connection to nature that resonates with consumers seeking more genuine and trustworthy alternatives. Many natural product companies prioritize fair trade, organic farming, and ethical business practices, appealing to consumers who want to make more conscious choices. Consequently, the demand for natural alternatives has spurred innovation in various industries, leading to the development of new and creative products that harness the power of nature (Chowdhury et al., 2023).

Essential oils, derived from medicinal plants, have become a successful example of natural incorporation across various sectors. These oils have made significant contributions to pharmacotherapy, particularly in the treatment of cancer and infectious diseases. Advances in analytical tools, genome mining, and microbial culturing are addressing challenges in essential oil-based drug discovery, opening new opportunities, especially in combating antimicrobial resistance. In addition to drug discovery, essential oils are being explored for sustainable applications, such as eco-friendly antifungal agents against phytopathogens. These natural compounds are biodegradable, less toxic, and offer sustainable control mechanisms for

agricultural applications. The success of essential oils extends to the consumer market, where natural skincare products using plant-based oils and extracts, and organic food emphasizing whole, unprocessed ingredients, are thriving. Essential oils offer a feeling of genuineness and a natural connection that resonates with consumers who prioritize health and wellness. The natural product market, driven by the benefits and applications of essential oils, continues to grow with innovative solutions that prioritize sustainability and health.

Algeria's rich biodiversity encompasses substantial natural resources distributed across diverse ecosystems and boasts significant floristic diversity. The country is home to over 3,000 plant species, many of which are endemic, presenting considerable potential for the development of natural products. Several authors have produced a variety of publications on traditional phytotherapy and ethnobotany, and ethnobotanical research has revealed that traditional medicine, particularly herbal treatments, is well-developed in Algeria. However, this country remains poorly explored, and the use of conventional medicine has led to the neglect of these ancestral practices, which are at risk of being forgotten (Belhouala & Benarba, 2021; Benarba, 2016; Benarba et al., 2015; Bouasla & Bouasla, 2017; Boudjelal et al., 2013; Madani et al., 2012; Miara et al., 2018).

In conclusion, this thesis seeks to bridge the gap between traditional knowledge and modern scientific exploration by investigating the chemical composition, bioactive potential, and safety profiles of essential oils from lemongrass, Peruvian pepper tree, and felty germander. The findings of this study may offer a foundation for the development of novel, eco-friendly products and contribute to the ongoing discourse on the integration of natural resources in diverse industrial applications.

This thesis follows a traditional structure combining literature review and experimental research.

- *Part 1:* introduces the study's background, problem, objectives, and research questions.
- *Part 2:* reviews essential oils' chemistry, uses, and economic importance, and details the three selected medicinal plants: *Cymbopogon citratus*, *Schinus molle*, and *Teucrium polium*.
- *Part 3:* presents experimental work, mainly essential oil extraction, phytochemical analysis (via GC-MS and GC-FID), biological activity assays, and safety evaluation.
- *Part 4:* discusses the results, comparing them with existing literature.
- *Part 5:* concludes the study, offering recommendations and future research paths.

LITERATURE REVIEW

1. INTRODUCTION TO ESSENTIAL OILS

1.1. Historical Overview

Essential oils, with their aromatic and medicinal properties, have a history that stretches back to the dawn of humanity. The exact origins of their extraction may be shrouded in the mists of time, as ancient writings often lack detailed procedural descriptions (Hanif et al., 2019). However, the practice of extracting these oils from aromatic plants is a testament to the enduring fascination with their properties (Ríos, 2016).

The use of essential oils is not a recent phenomenon. It has been a part of human civilization since ancient times, with cultures like China, Egypt, India, Persia, and Greece all contributing to its development. The Egyptians, for instance, were using aromatic oils as early as 4500 BC for cosmetics, ointments, and medicinal purposes, while the Greeks documented their use between 500 and 400 BC (Elshafie & Camele, 2017). The widespread knowledge and utilization of essential oils encompassed religious ceremonies, healing, and perfuming, connecting us to a rich historical tradition (Ríos, 2016).

The Muslims significantly advanced the distillation of essential oils, developing techniques in the ninth century that they subsequently introduced to Europe (Hanif et al., 2019). One of the key figures in this advancement was Ibn Sina (980–1037 in Bukhara, Uzbekistan), known in the West as Avicenna. He is credited with inventing an apparatus for distillation called an alembic. His influential work, the Canon of Medicine, written in Arabic, included detailed and comprehensive descriptions of the distillation process and the use of essential oils, providing a wealth of information to the readers. This work was translated into Latin and appeared in Europe in the twelfth century (Buckle, 2015).

Following the fall of the Roman Empire, Muslim and Christian civilizations preserved and expanded their knowledge of fragrances. The Crusaders, who were exposed to the advanced distillation techniques of the Arab world during their campaigns, brought this knowledge back to Europe. This exchange of knowledge, facilitated by the Crusades, led to further development of essential oil extraction techniques in European alchemists and monasteries. During the Renaissance, essential oils in perfumery and cosmetics became widespread (Sonwa, 2000).

By the sixteenth century, the separation of essential oils from aromatic waters and their distinction from fatty oils were well understood. This understanding, coupled with the increasing demand for these oils, led to the commercialization of essential oils for industrial,

therapeutic, and cosmetic purposes. This marked a significant milestone in their historical development, as it not only expanded their use but also created a new industry around their production and distribution. By the end of the nineteenth century, chemists had managed to isolate, separate, and reproduce the active molecules of essential oils, broadening their applications in perfumery, therapy, and other industries (Hanif et al., 2019).

1.2.Concept and Definition

Essential oils, a term originating in the sixteenth century from Paracelsus von Hohenheim's concept of Quinta Essentia (Bhavaniramya et al., 2019), are concentrated hydrophobic liquids containing volatile aromatic compounds extracted from plants. These extractions are typically performed through steam distillation or mechanical processes such as cold pressing, especially for Citrus fruits, due to the thermos-sensitivity of their constituents (Council of Europe, 2004; ISO 9235, 2021). Characterized by their flammability, solubility in organic solvents like alcohol and ether, and insolubility in water, essential oils possess unique and intriguing properties, distinguishing them from fixed or fatty oils. They contain volatile compounds and vanish rapidly without leaving any stain, unlike fatty oils, which contain glycerides of fatty acids and leave a permanent stain on filter paper (Dhifi et al., 2016; Loizzo et al., 2015).

Also known as essences, volatile oils, etheric oils, or aetheroleum, essential oils are typically colorless, liquid at room temperature, and possess a density less than unity, with few exceptions such as vetiver, sassafras, and cinnamon (Başer & Demirci, 2007; Hanif et al., 2019; Sangwan et al., 2001). The French Agency for Normalization (AFNOR) defines essential oils as products obtained from vegetable raw material by steam distillation or mechanical processes from the epicarp of Citrus or 'dry' distillation.

The definition of essential oils excludes products obtained through extraction techniques like solvent extraction, supercritical fluid extraction, or microwave-assisted extraction. Essential oils are typically obtained through distillation, a process that often separates them from associated gums and resins (Başer & Demirci, 2007). Their biological activities make them not just effective alternatives or complements to synthetic compounds, but also powerful sources of therapeutic benefits, offering relief without the same secondary effects (Dhifi et al., 2016).

1.3.Sources and Functions of Essential Oils in Plants

Essential oils are predominantly formed as secondary metabolites in plants. Their localization within the plant can vary significantly. They can be found in aerial parts such as

flowers (e.g., chamomile, peppermint, lavender), leaves (e.g., mint, eucalyptus, bay leaf), and stems. Essential oils are also present in bark (e.g., cinnamon), fruits (e.g., anise, fennel, citrus epicarps), seeds (e.g., coriander, nutmeg), and underground parts like roots and rhizomes (e.g., curcuma, ginger, vetiver) (Hanif et al., 2019; Ríos, 2016). In some plants, essential oils are not directly formed but are produced through the hydrolysis of certain compounds, as seen in valeriana and garlic (Evans, 2009; Franz & Novak, 2010). Their production and storage are in specialized secretory structures. These structures, which vary in morphology, function, and distribution, help to minimize the risk of autotoxicity and can be classified as external or internal. External secretory structures include glandular trichomes, epidermal cells, and osmophores, while internal ones consist of secretory cells (often idioblasts), secretory cavities, and secretory ducts (Zuzarte & Salgueiro, 2015).

Essential oils, beyond their storage and formation, serve a multitude of ecological functions. They act as chemical signals, enabling plants to control and regulate their environment. For example, essential oils can repel predators, attract pollinating insects, inhibit seed germination, and facilitate communication between different plants (Hanif et al., 2019). Furthermore, essential oils are believed to possess antioxidant properties, donating hydrogen in oxidative reactions, particularly in the presence of light. Their antifungal and antibacterial properties help protect plants from pathogenic attacks (Evans, 2009; Ríos, 2016).

Essential oils play pivotal roles in plant defense, protecting against microorganisms, insects, and herbivores. They exhibit insecticidal and deterrent activities, and also contribute to water regulation and allelopathic interactions, thereby enhancing the plant's overall defense mechanisms (Hanif et al., 2019; Zuzarte & Salgueiro, 2015).

1.4.Extraction Methods

Essential oils can be obtained by extracting different components of aromatic plants. The selection of the extraction technique is contingent upon the specific attributes and constituents required for the intended objectives. The methods can be classified into two categories: conventional and advanced.

1.4.1. **Convention extraction methods**

Hydrodistillation

Hydrodistillation, an ancient technique for extracting essential oils (*Figure 01)*, is attributed to the Persian scientist Avicenna and is documented in the European Pharmacopeia (Gavahian et al., 2020).

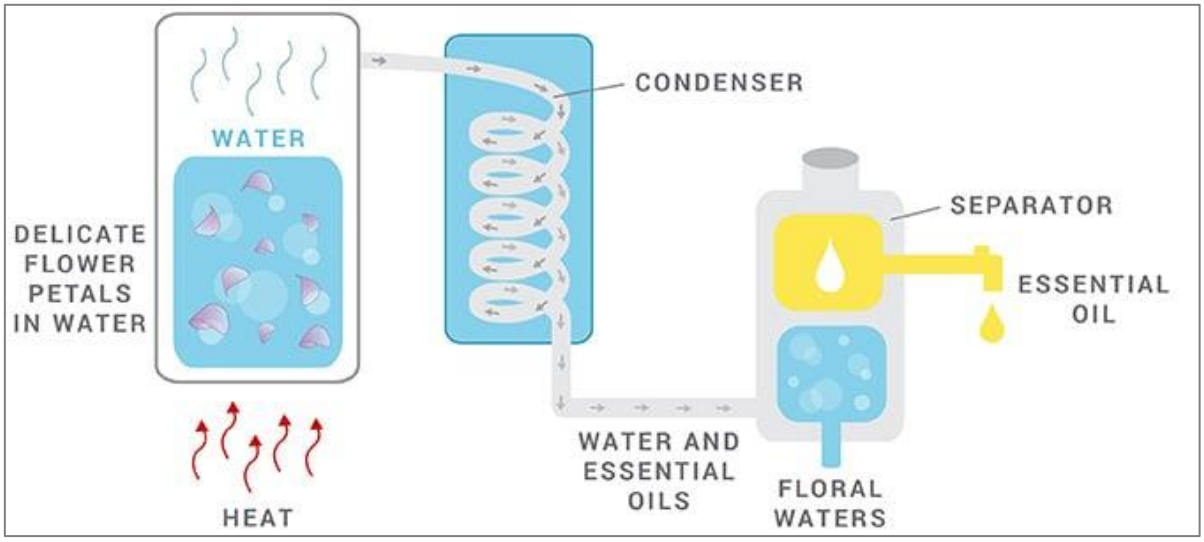

Figure 01: *Schematic representation of essential oil extraction via hydrodistillation (New Directions Aromatics, 2024).*

This efficient process entails submerging botanical matter in water inside an extraction flask, applying heat to the combination, converting the resulting vapors into liquid form, and isolating the essential oils from the aqueous phase using tools like a separation funnel or Clevenger equipment. It is highly efficient in extracting oils from powders such as groundwood and spice powders and challenging materials like nuts, wood, and roots (Hanif et al., 2019). The residual water, possibly with a pleasant scent, is commonly referred to as hydrolate, hydrosol, herbal water, essential water, flower water, or herbal distillate (New Directions Aromatics, 2024).

Steam distillation

Steam distillation, a commonly employed extraction method in the industry, is highly effective and versatile for large-scale manufacturing (*Figure 02)*.

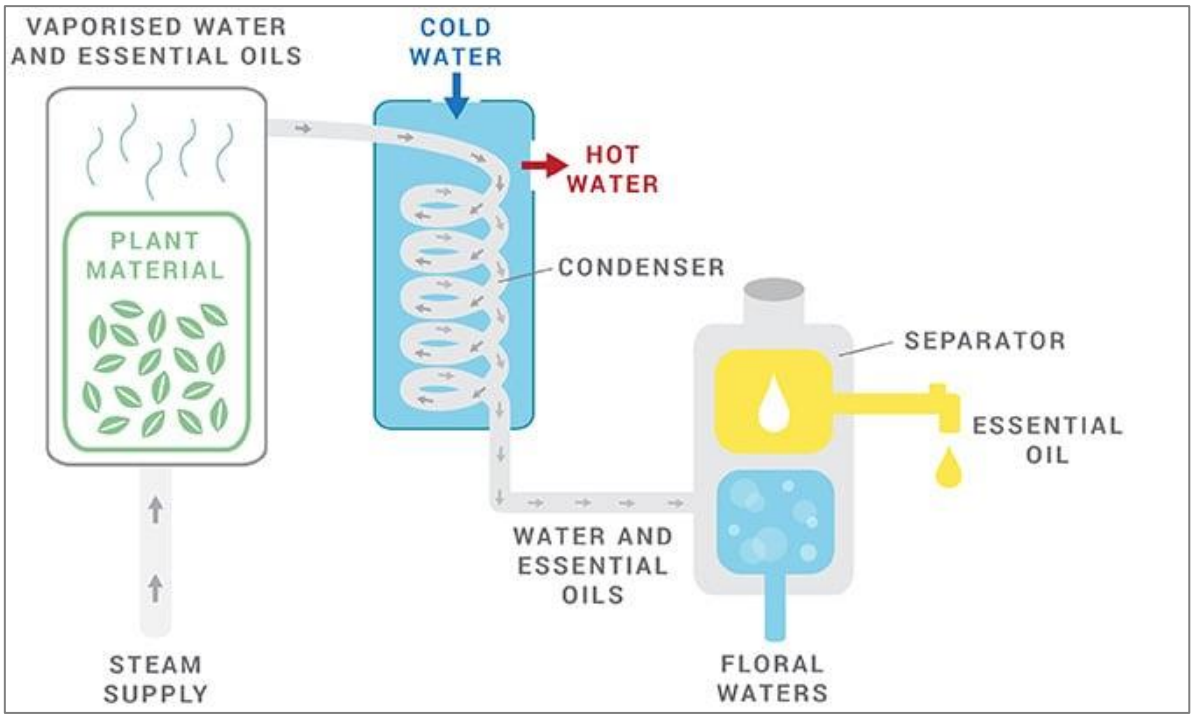

Figure 02: *Schematic representation of essential oil extraction via steam distillation (New Directions Aromatics, 2024).*

This procedure entails the transmission of steam through a fragrant botanical substance enclosed in a perforated biomass container. The steam's thermal energy enables the plant to release volatile chemicals that are transported to the condenser section. The condenser facilitates the separation of essential oils from the mixture of steam and condensed water (Gavahian et al., 2020; Hanif et al., 2019).

Solvent extraction

Solvent extraction is a technique to obtain essential oils from plant materials, particularly those sensitive to heat, such as blossoms (*Figure 03).*

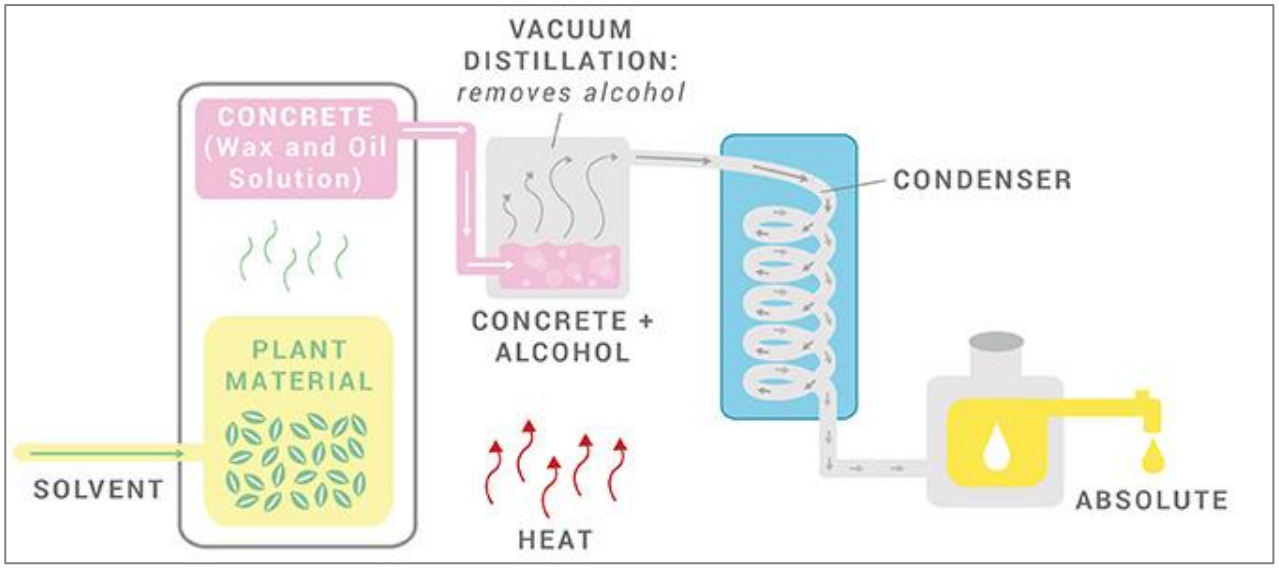

Figure 03: *Schematic representation of essential oil extraction via solvent (New Directions Aromatics, 2024).*

This method involves submerging the plant material in a solvent solution, commonly using hydrocarbons like hexane, ethanol, petroleum ether, or methanol to dissolve the plant components. The resulting solution is then filtered and concentrated via distillation, with the option to introduce pure alcohol to separate the oil (Hanif et al., 2019; Stratakos & Koidis, 2016). While this approach is economical and efficient due to the use of hot solvents, it does leave behind solvent residues that could trigger allergies and affect the immune system. However, when alcohol is used as a solvent, it is of food-grade quality and considered safe for ingestion. The perfume industry commonly uses this method (Stratakos & Koidis, 2016).

Cold pressing

Cold pressing, often referred to as expression, is the most ancient technique for extracting essential oils (*Figure 04)*. It is predominantly used for citrus fruits like lemon, orange, bergamot, and grapefruit (Hanif et al., 2019; Stratakos & Koidis, 2016).

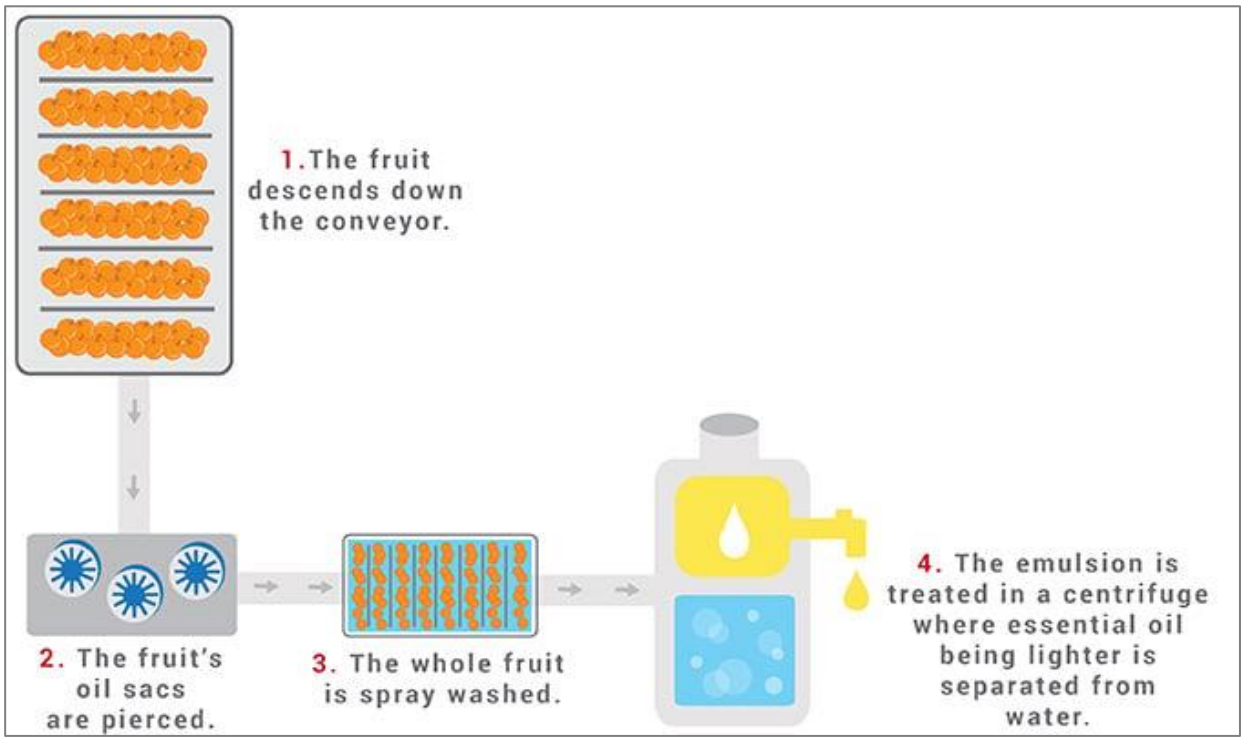

Figure 04: *Schematic representation of essential oil extraction via cold pressing (New Directions Aromatics, 2024).*

This method involves physically rupturing the vital oil glands present in the peel and cuticles of the fruit to release the oil. The process includes extracting the rinds from the fruit, cutting them into small pieces, and applying pressure to create a diluted mixture. This mixture is then centrifugated to separate the essential oil. The use of mechanical means is crucial in preventing the decomposition of aldehydes in citrus peels, making cold pressing a preferred method. Essential oils obtained through cold pressing typically have a shorter shelf life compared to those derived from alternative procedures (Hanif et al., 2019; Stratakos & Koidis, 2016).

Enfleurage

Enfleurage is an ancient and intricate technique used to extract essential oils, primarily from flowers such as jasmine (*Figure 05).*

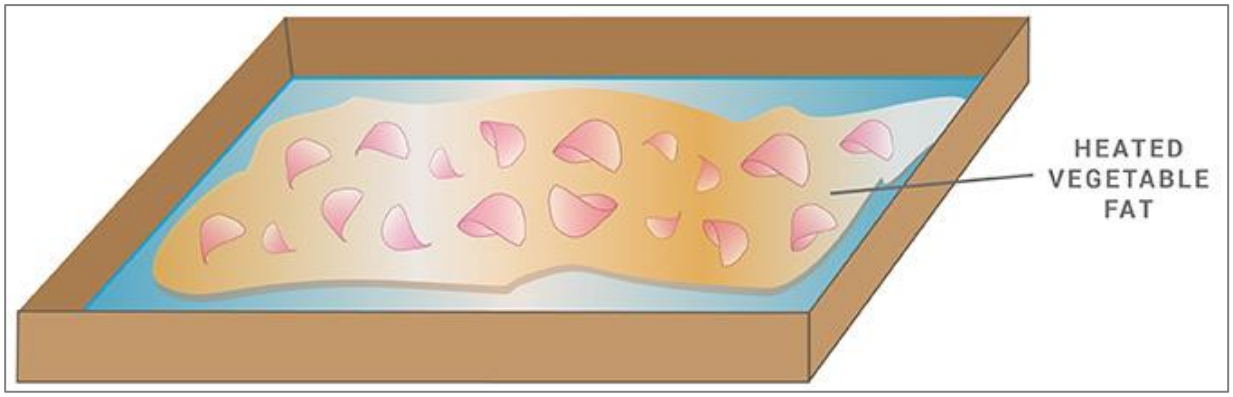

Figure 05: *Schematic representation of essential oil extraction via enfleurage (New Directions Aromatics, 2024).*

The process involves applying pure, odorless cold or hot fat to the petals, which absorb the oils emitted by the flowers. The fat is continually replaced with fresh flowers until it becomes saturated with essential oils. The fat-saturated fragrance is called "enfleurage pomade.". The saturated fat is then treated with alcohol to extract the oils, and the pure essential oil is obtained by evaporating the alcohol (Hanif et al., 2019; Stratakos & Koidis, 2016). Despite its effectiveness, Enfleurage is now considered time-consuming, expensive, and largely outdated. It has no significant applications in today's food sector (Stratakos & Koidis, 2016).

Maceration

Maceration is a method employed to extract medicinal qualities from plant material by immersing it in vegetable oil, which is subsequently heated and filtered (*Figure 06).*

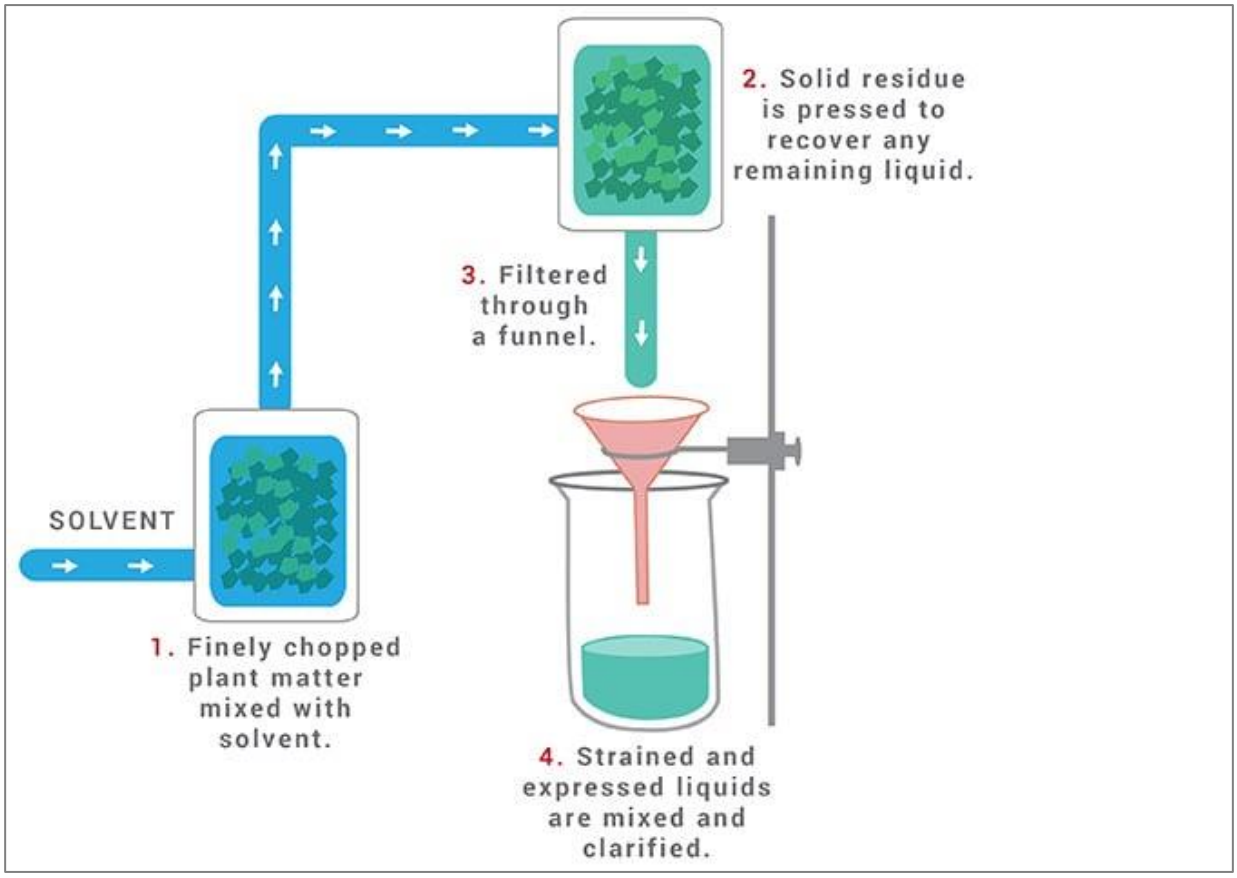

Figure 06: *Schematic representation of essential oil extraction via maceration (New Directions Aromatics, 2024).*

This procedure yields a substance called “infused oil” rather than a pure “essential oil” (Hanif et al., 2019). Carrier oils are essential in the process of maceration as they act as solvents, effectively collecting plant components that are heavier and bigger and cannot be removed through distillation. This technique retains more of the plant's essential elements, producing more abundant oil in therapeutic chemicals. In order to inhibit the development of rancidity and the growth of microorganisms, it is advisable to ensure that the plant material is thoroughly dehydrated. It is recommended to use either 5% Vitamin E or Wheatgerm oil, which has a high concentration of Vitamin E (New Directions Aromatics, 2024).

1.4.2. **Advanced extraction methods**

Supercritical CO_2 extraction

CO_2 extraction is an advanced method used in modern extraction technologies, employing carbon dioxide (CO_2) as a solvent to extract essential oils from plant materials (*Figure 07)*, (Hanif et al., 2019). In this process, CO_2 is utilized under high pressure and specific temperatures to optimize the extraction of volatile compounds. Initially, CO_2 is chilled to temperatures between 35 and 55 °F and then pressurized to 1000 psi as it is pumped through the plant material. Under these conditions, CO_2 is in a liquid state, which acts as a solvent to dissolve essential oils and other compounds such as pigments and resins from the plant material. In supercritical CO_2 extraction (SCO_2), CO_2 is heated to approximately 87 °F and pressurized to 8000 psi, transitioning it into a supercritical state, where it behaves like a dense fog or vapor. In this state, CO_2 effectively penetrates the plant material, extracting essential oils. Upon reducing the pressure, CO_2 reverts to its gaseous state, leaving behind the concentrated essential oils (Hanif et al., 2019; New Directions Aromatics, 2024).

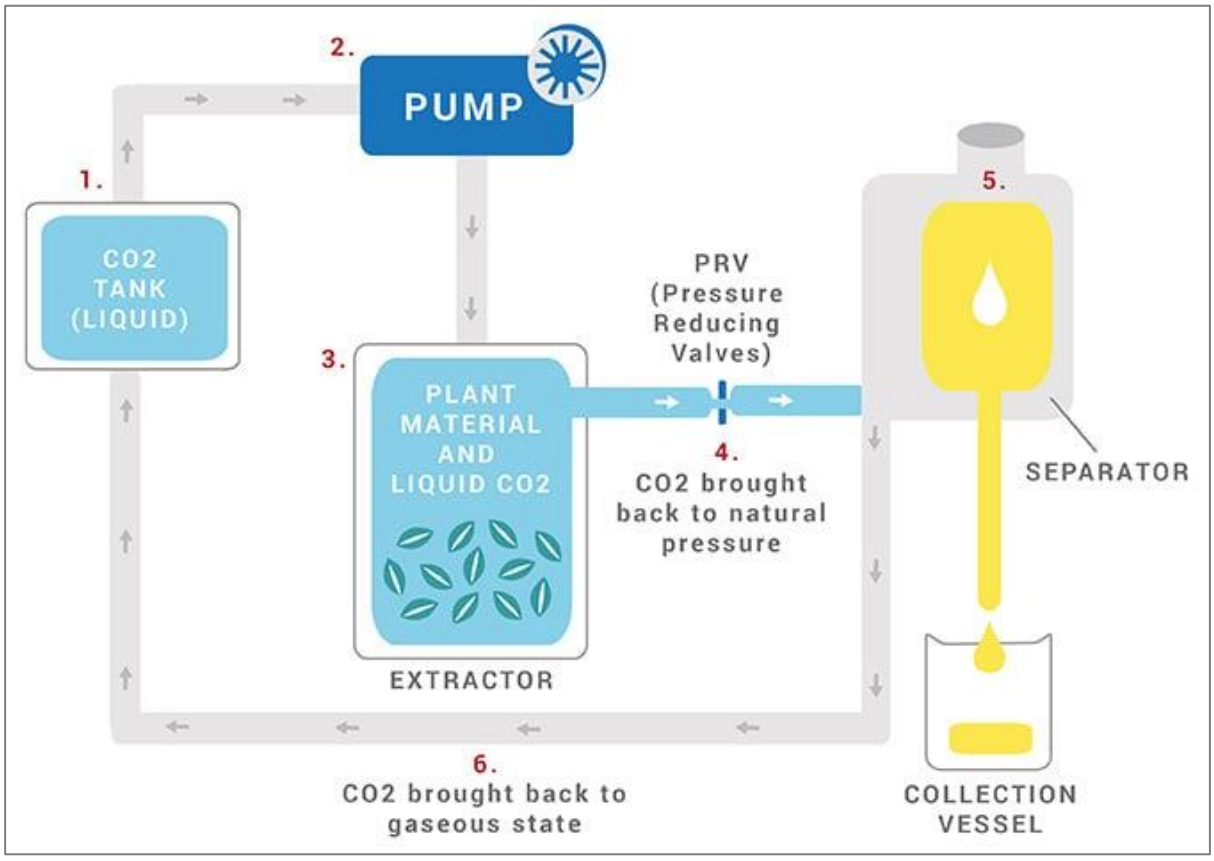

Figure 07: *Schematic representation of essential oil extraction via supercritical CO_2 (New Directions Aromatics, 2024).*

The use of CO_2 in its supercritical state enhances the purity of the essential oils obtained, as it closely preserves the essence of the original plant material (Hanif et al., 2019). Additionally, CO_2 is colorless, odorless, and easily removable, posing minimal environmental and health risks due to its complete and harmless nature (New Directions Aromatics, 2024). This extraction method does not introduce potentially harmful solvents, making it a safe and environmentally friendly option for producing essential oils.

Microwave-assisted extraction

Microwave-assisted extraction (MAE) is a modern method that uses microwave radiation to improve the extraction of substances from plant materials. This technique utilizes a distinct heating mechanism that operates on the principle of friction (*Figure 08).*

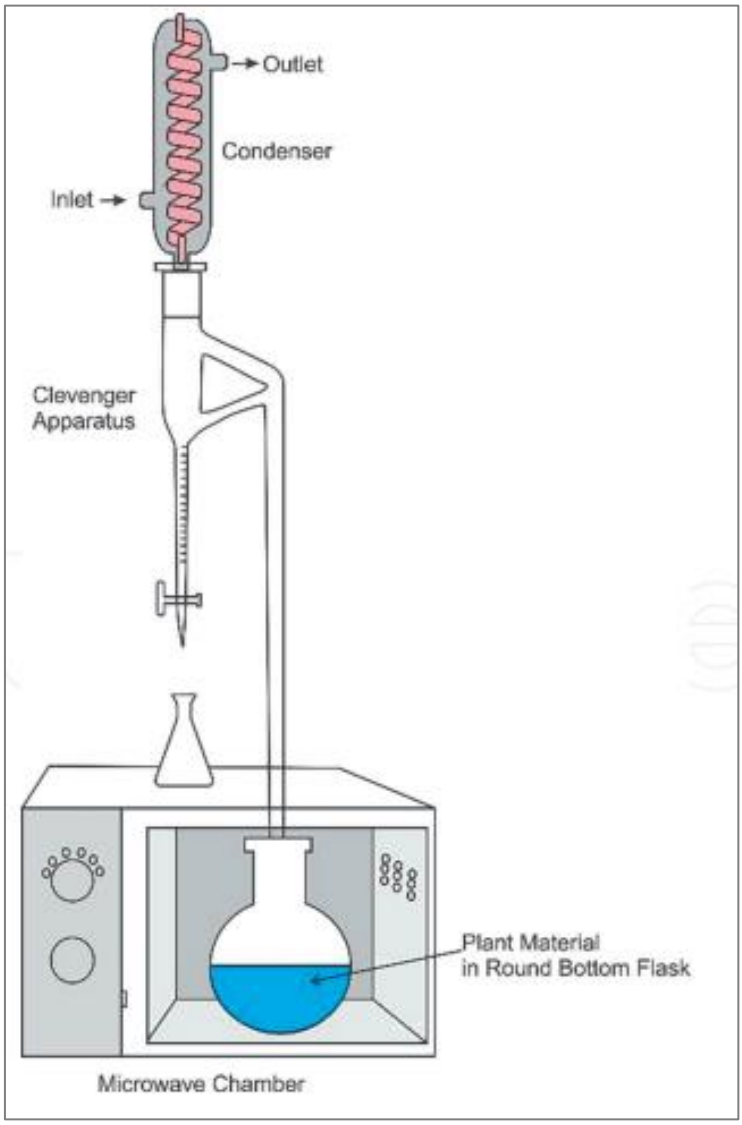

Figure 08: *Schematic representation of essential oil extraction via microwave-assisted extraction (Mahawer et al., 2022).*

In this process, microwaves produce heat by exploiting the dielectric characteristics of the extracted compounds. MAE stands out due to its capacity to achieve superior and targeted heating compared to conventional extraction techniques, resulting in increased extraction yields and decreased extraction durations. The technique generally entails exposing plant materials to microwaves, with or without the use of organic solvents or water, in a regulated manner. This technology provides numerous benefits compared to traditional methods, such as considerably reduced extraction times, frequently completing the operation in minutes instead of hours, and enhanced cost-efficiency. Nevertheless, MAE often necessitates a more significant number of organic solvents, which can affect its level of environmental sustainability. However, the process is well regarded for its simplicity, effectiveness, and enhanced selectivity in extracting valuable chemicals (Mahawer et al., 2022; Stratakos & Koidis, 2016).

Turbo distillation extraction

The turbo extraction, also known as turbo distillation, is a specialized method developed to extract essential oils efficiently from tough and fibrous plant materials, including roots, seeds, and bark (*Figure 09).*

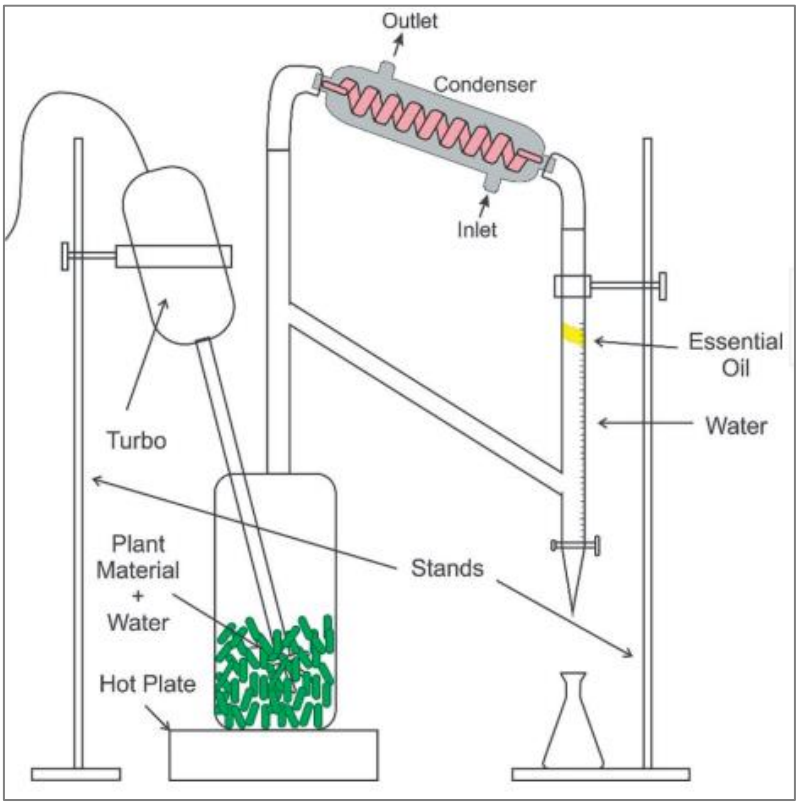

Figure 09: *Schematic representation of essential oil extraction via turbo distillation (Mahawer et al., 2022).*

This procedure involves immersing the plant material in water and introducing steam into the mixture. The key feature of turbo extraction is the continuous agitation of the mixture with a stainless-steel stirrer, which significantly enhances the extraction process. The method operates within a closed-loop system, ensuring consistent circulation of water through the plant material. Turbo extraction offers substantial advantages over traditional water distillation, including significantly reduced distillation durations, lower energy consumption, and minimal degradation of volatile components. It is particularly effective in extracting essential oils from challenging-to-extract compounds like spices and wood (Hanif et al., 2019; Mahawer et al., 2022).

Ultrasound-assisted extraction

Ultrasound-assisted extraction UAE is an innovative method that utilizes ultrasonic waves to improve the effectiveness and rapidity of extracting chemicals from plant materials (*Figure 10).*

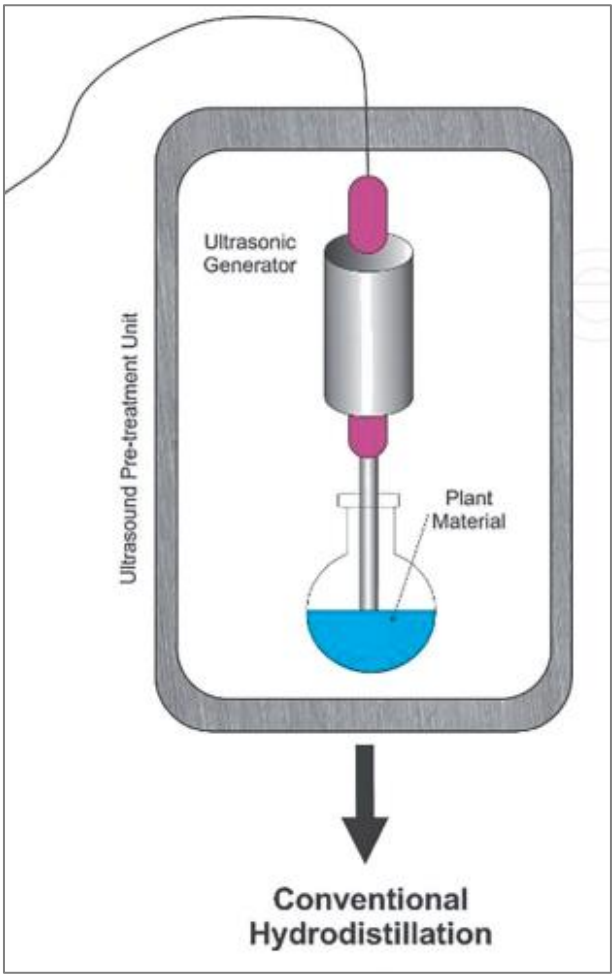

Figure 10: *Schematic representation of essential oil extraction via ultrasound-assisted extraction (Mahawer et al., 2022).*

This approach employs cavitation phenomena, in which ultrasonication generates tiny bubbles that collapse forcefully. The rupture of these bubbles generates significant mechanical forces that disturb cell membranes, thereby enhancing the mass transfer rate and release of essential oils or other extractable substances. The UAE provides various benefits, including shorter extraction durations, low influence on the quality and taste of the extracted substances, and the possibility of using less or no organic solvents. This is because the UAE is effective with solvents generally acknowledged as safe. The efficacy of UAE is impacted by factors such as the frequency and intensity of the ultrasound, duration of incubation, and temperature. Although the initial investment for ultrasound equipment can be expensive, the UAE is known for its capacity to deliver greater productivity in a shorter period compared to traditional methods. This makes ultrasound a versatile choice for a wide range of conventional and contemporary extraction procedures (Mahawer et al., 2022; Stratakos & Koidis, 2016).

1.5.How does the Choice of Extraction Method Affect the Quality of Essential Oils?

The choice of extraction method is essential as it can affect the preservation of sensitive compounds, prevent undesirable reactions, influence yield and composition, and ensure compatibility with specific plant materials. Different methods can alter the chemical composition and aroma, ultimately impacting the quality of the essential oils.

Preservation of sensitive compounds: Methods like steam distillation and cold-press extraction involve lower temperatures, preserving heat-sensitive compounds and enhancing the overall quality and aroma of the oil (Stratakos & Koidis, 2016).

Prevention of hydrolyzation and oxidation: Certain extraction techniques can minimize hydrolyzation or oxidation, which can otherwise degrade the quality of essential oils by preserving their integrity (Stratakos & Koidis, 2016).

Impact on yield and composition: The extraction method can affect essential oils' yield and chemical composition. For example, hydrodistillation and steam distillation may lead to variations in chemical composition and antibacterial activity, influencing overall quality (Zhang et al., 2018).

Suitability for specific plant materials: Certain plant materials require specific extraction methods. Cold-press extraction is ideal for citrus fruit peels, while steam distillation is widely used for various plants. Choosing the appropriate method ensures the extraction of high-quality oils.

Modern extraction techniques: Techniques such as supercritical fluid extraction offer benefits like reduced organic solvent use and higher selectivity, contributing to the quality of the extracted oils (Souiy, 2024).

Table 01 provides a comparative overview of these methods, highlighting the advantages and disadvantages of each extraction technique.

Table 01: *Advantages and disadvantages of essential oil extraction techniques.*

Extraction Technique	Advantages	Disadvantages	References
Hydrodistillation	Simple and traditional method Low cost	Long extraction times Possible degradation of heat-sensitive compounds Requires careful temperature control	(Hanif et al., 2019)
Steam Distillation	Widely used Effective for a variety of plant material Low cost	Long extraction times Potential for thermal degradation of volatile components	(Hanif et al., 2019)
Solvent Extraction	Suitable for delicate and heat-sensitive materials High yield Suitable for low-yield plants	Use of potentially toxic solvents Residual solvent contamination	(Stratakos & Koidis, 2016)
Cold-Press Extraction	No heat used, preserving oil integrity Environmentally friendly	Limited to citrus peels Lower yield compared to other methods	(Stratakos & Koidis, 2016)
Enfleurage	Preserves the fragrance of delicate flowers Traditional, gentle method	Very time-consuming Labor-intensive Low yield	(Stratakos & Koidis, 2016).

		Expensive, requires specialized equipment	
Maceration	Simple and inexpensive Suitable for heat-sensitive materials	Long extraction times Lower yield Potential for solvent contamination	(Hanif et al., 2019)
CO_2 Extraction	High yield No solvent residues Effective for a wide range of materials Preserves aroma	High cost of equipment Requires technical expertise	(Hanif et al., 2019)
Turbo Distillation	Reduces extraction time Lower energy consumption Minimizes degradation of volatiles	Specialized equipment required Limited to coarse and hard plant materials	(Mahawer et al., 2022)
Microwave-Assisted Extraction	Fast extraction times High selectivity Effective under normal conditions	Requires more organic solvents Potential environmental impact	(Mahawer et al., 2022)
Ultrasound-Assisted Extraction	Short extraction times High yield Reduced use of organic solvents Minimal thermal damage	High capital cost Potential equipment deterioration	(Mahawer et al., 2022)

2. CHEMICAL COMPOSITION OF ESSENTIAL OILS

2.1. Analytical Techniques for Essential Oil Characterization

Unravelling the chemical composition of essential oils is a challenging endeavor. Several constituents comprising a particular essential oil exist in small amounts, making them difficult to detect. Certain entities exhibit such a high degree of similarity that distinguishing them with absolute certainty becomes challenging, while others present a level of complexity that makes their identification problematic. Commonly employed techniques for analyzing the composition of essential oils include gas chromatography (GC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy. Chromatographic techniques are employed to partition essential oils into their distinct constituents to facilitate their identification using specialized methods. In recent years, the sensitivity of analytical methods used for organic compounds has significantly increased. This advancement is of paramount importance, as it has reached a level where it is now possible to identify even tiny amounts of substances, including pollutants such as pesticides.

2.1.1. Gas Chromatography (GC)

Gas Chromatography (GC) is a fundamental analytical technique for separating and analyzing volatile compounds within a mixture, making it especially valuable for essential oil characterization. These oils, composed of various volatile organic compounds, can be efficiently resolved and profiled using this method.

The sample is vaporized and introduced into a chromatographic column, where it is carried by an inert gas (mobile phase). The column's interior is coated with a stationary phase that interacts with the sample compounds based on their volatility and polarity. These interactions lead to the separation of the components as they traverse the column. The compounds elute at different times, known as retention times, and are visualized as peaks on a chromatogram (Karasek & Clement, 1988).

2.1.2. Mass Spectrometry (MS)

Mass Spectrometry (MS) is a highly sensitive technique used to determine the mass-to-charge ratio of ions, providing critical insights into the molecular weight and structure of compounds.

The analyte is ionized, often through electron impact or chemical ionization, generating charged particles. These ions are accelerated into a mass analyzer, where they are separated according to their mass-to-charge ratios. A detector quantifies the ions, creating a mass spectrum that illustrates the relative abundance of ions across varying mass-to-charge values (Karasek & Clement, 1988).

2.1.3. Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS combines the separation efficiency of GC with the identification power of MS, offering a robust platform for analyzing complex mixtures like essential oils.

The essential oil sample is first fractionated by GC into its individual components. Each separated compound is then introduced into the MS for ionization, structural analysis, and quantification. The resulting data, represented as mass spectra, provide detailed qualitative and quantitative information, enabling precise profiling of essential oil constituents (Karasek & Clement, 1988).

2.1.4. Flame Ionization Detection (FID)

Flame Ionization Detection (FID) is an effective detection method in gas chromatography, particularly for quantifying organic compounds.

As the GC-separated compounds enter a hydrogen-air flame, they undergo ionization. The resulting ions produce an electrical current proportional to the amount of carbon in the sample, which is recorded as a signal. FID offers high sensitivity for detecting carbon-based compounds and delivers reliable quantitative data (Holm, 1999).

2.1.5. Gas Chromatography-Flame Ionization Detection (GC-FID)

The GC-FID technique integrates the separation capabilities of GC with the sensitivity of FID, making it a widely used method for analyzing volatile organic compounds in essential oils.

The sample is vaporized, separated in the GC column, and analyzed by FID, which detects ionized carbon atoms resulting from combustion in a hydrogen flame. This method is recognized for its accuracy in quantifying and identifying essential oil components (Barber, 1958).

2.1.6. Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy is an advanced technique for elucidating molecular structures by exploiting the magnetic properties of atomic nuclei.

The sample is subjected to a strong magnetic field and irradiated with radiofrequency waves, causing nuclei to resonate at frequencies specific to their chemical environment. The resulting spectra provide detailed information about the molecular framework, functional groups, and connectivity. NMR enables direct analysis of essential oils without prior separation, making it invaluable for quality control and structural characterization (AbouZid, 2016).

2.1.7. Infrared (IR) Spectroscopy for essential oils

Infrared (IR) Spectroscopy, especially Fourier Transform Infrared (FT-IR) Spectroscopy, is a pivotal analytical tool for assessing the molecular structure and functional groups in essential oils.

The technique measures the absorption of infrared light by the sample, generating spectra that reflect molecular vibrations. These spectra identify functional groups such as carbonyl (C=O), hydroxyl (O-H), and alkyl (C-H) groups. FT-IR is particularly advantageous for rapid quality control and molecular fingerprinting in the fragrance and flavor industries (Agatonovic-Kustrin et al., 2020).

2.2.Essential Oil Constituents

The composition of essential oils is complex, comprising more than 300 distinct components. These substances are commonly characterized by their organic nature, volatility, and low molecular weight, typically below 300. Under ambient settings, the vapor pressure of these substances enables them to exist in a partially vaporized state at room temperature. Volatile components encompass a diverse range of chemical classes, such as alcohols, ethers, oxides, aldehydes, ketones, esters, amines, amides, phenols, and heterocycles, with terpenes being the predominant category.

Alcohols, aldehydes, and ketones play a pivotal role in shaping the aromatic profiles of essential oils. They contribute to the creation of fruity notes, such as those derived from (E)-nerolidol, floral notes like linalool, citrus notes associated with limonene, and herbal notes from β-selinene. The majority of constituents found in essential oils belong to the vast terpene family, encompassing a multitude of chemicals that have been chemically identified within these oils.

Functionalized alcohols such as geraniol and β-bisabolol, ketones like menthone and p-vetivone, aldehydes like citronellal and sinensal, esters like α-tepinyl acetate and cedryl acetate, and phenols like thymol are among the compounds that fall into this category.

Furthermore, essential oils contain non-terpenic chemicals synthesized through the phenylpropanoid pathway. These molecules include eugenol, cinnamaldehyde, and safrole.

According to biogenetical analysis, terpenoids and phenylpropanoids have dissimilar primary metabolic precursors and are produced via separate biochemical pathways. Terpenoids are generated through the physiological processes of mevalonate and mevalonate-independent (deoxyglucose phosphate), whereas phenylpropanoids are made via the shikimate pathway. Numerous scholarly investigations have examined the biosynthesis routes of terpenoids and phenylpropanoids, encompassing analysis of the enzymes and mechanisms implicated and the genes responsible for encoding these enzymes (Dhifi et al., 2016).

2.3.**Factors Influencing Chemical Composition**

A wide range of elements influence plant chemical composition, which can be split into two categories. The contents of essential oils are determined by endogenous factors such as the plant's anatomical and physiological features, as well as the biosynthetic pathways involved in the production of volatile chemicals. Exogenous factors influencing the chemical composition of essential oils include environmental conditions (such as climate, altitude, humidity, and soil quality), harvest timing, and vegetative cycle stage. Furthermore, the extraction procedures used, such as steam distillation, solvent extraction, and cold pressing, might have an impact on these oils' chemical profiles (Barra, 2009).

2.3.1. **Plant species and varieties**

Various plant species and varieties display unique chemical profiles as a result of genetic differences. The observed differences may influence the biosynthesis of secondary metabolites, essential for plant defense and adaptation. Research indicates that genetic diversity within populations can result in variations in chemical composition, especially in response to environmental factors such as climate and soil conditions (Pacheco-Hernández et al., 2021). Certain cultivars may exhibit increased levels of phytochemicals, influenced by their genetic composition and breeding methodologies (Tiwari & Cummins, 2013).

2.3.2. Geographic and Environmental factors

Geographic and environmental factors significantly influence the chemical composition of plants. Soil type, moisture levels, elevation, and proximity to water bodies significantly influence nutrient availability and overall plant health. Variations in soil nutrients such as nitrogen, phosphorus, and potassium have been correlated with the richness and distribution of plant species, subsequently influencing their chemical profiles (Pant et al., 2021; Thammanu et al., 2021). Climatic factors, including temperature and precipitation patterns, influence the distribution and abundance of plant species, thereby affecting their chemical diversity (Gimenez et al., 2020).

2.3.3. Harvesting techniques

The methods utilized in harvesting can substantially influence the chemical composition of plant materials. The timing of harvest and the techniques employed can affect the concentrations of phytochemicals and other metabolites in plants. Research demonstrates that inadequate harvesting methods can result in quality degradation, whereas optimal practices can improve the retention of beneficial compounds (Pietrzak & Nowak, 2021). Seasonal variations influence metabolite levels, with certain compounds peaking at specific times of the year, thereby requiring precise harvest timing to optimize chemical quality (Gimenez et al., 2020).

2.3.4. Storage conditions

Post-harvest storage conditions are essential for preserving the chemical integrity of plant materials. Temperature, humidity, and light exposure are factors that can cause the degradation of sensitive compounds. Improper storage can lead to the degradation of antioxidants and other phytochemicals, consequently reducing the nutritional and therapeutic value of the plants (Pietrzak & Nowak, 2021). Controlled storage conditions are crucial for maintaining the chemical composition of harvested plant products.

3. BIOLOGICAL POTENTIALS

3.1. **Antioxidant Potential**

Free radicals and reactive oxygen species (ROS) cause the oxidation of biomolecules, leading to molecular alterations and chronic disorders such are inflammation, cancers, cardiovascular diseases, brain dysfunction, arteriosclerosis, Alzheimer's disease, Parkinson's disease, diabetes, asthma, immune-related issues, and age-related conditions (S. Khan et al., 2023; Mahawer et al., 2022).

Essential oils (EOs) are recognized as valuable sources of natural antioxidants, supported by various experimental models (S. Khan et al., 2023; Sharmeen et al., 2021). They exhibit significant antioxidant activity, often confirmed by physicochemical methods (Mahawer et al., 2022). This activity is largely due to the composition of EOs, particularly phenolic compounds and other secondary metabolites with conjugated double bonds, which show substantial antioxidant properties (Hanif et al., 2019).

Monoterpene phenols, such as thymol and carvacrol, are notable for their redox properties and their role in neutralizing free radicals and decomposing peroxides (S. Khan et al., 2023; J. Sharifi-Rad et al., 2019; Sharmeen et al., 2021). The phenolic structure of these compounds underpins their antioxidant potential (Burt, 2004; Hanif et al., 2019).

Additionally, the antioxidant activity of EOs is bolstered by other compounds such as alcohols, ketones, aldehydes, ethers, and monoterpenes, including linalool, geranial/neral, 1,8-cineole, isomenthone, menthone, citronellal, α-terpinolene, α-terpinene, and β-terpinene (Hanif et al., 2019). These diverse compounds collectively enhance the overall antioxidant capacity of essential oils, making them effective agents in combating oxidative stress and related diseases.

3.2. **Anti-inflammatory Potential**

Inflammation is an innate defensive reaction triggered by infection or tissue damage to fight against intruders such as microbes or non-self-cells and eliminate injured or deceased host cells. The outcome of this response includes oxidative burst, the release of cytokines, heightened permeability of endothelial cells, and the infiltration of blood leukocytes into the interstitium (Hanif et al., 2019). Inflammatory illnesses are commonly identified by symptoms such as pain, redness, and swelling, which can result in a decline in essential bodily processes. For many years, essential oils (EOs) have been utilized to relieve pain and inflammation, often

demonstrating superior efficacy compared to numerous prescription analgesics (Elshafie & Camele, 2017; Koh et al., 2002).

Inflammation enhances the metabolic process of arachidonic acid and the functioning of other enzymes, such as nitric oxide synthases, oxygenases, and peroxidases (Hanif et al., 2019). EOs are believed to exert their anti-inflammatory effects by competing with arachidonic acid for integration into cell membranes. This competition leads to the creation of prostaglandins and eicosanoids that have undergone modest modifications. These transformed substances cause a reduced level of inflammation because they trigger less COX-2 production (Elshafie & Camele, 2017).

Essential oils (EOs) are used as anti-inflammatory medicines for treating inflammatory conditions like arthritis, allergies, and rheumatism (Hanif et al., 2019; Maruyama et al., 2005). The active anti-inflammatory components in essential oils limit the release of histamine or lower the generation of inflammation mediators. 1,8-cineole, which is found in many essential oils (EOs), has been shown to suppress leukotrienes (LTB4) and prostaglandin (PGE2) (Hanif et al., 2019; Yoon et al., 2000).

The anti-inflammatory effects of essential oils (EOs) are not only attributed to their antioxidant capabilities but also to their interactions with signaling cascades, such as regulatory transcription factors and cytokines, and the modulation of pro-inflammatory gene expression (Hanif et al., 2019). Moreover, the utilization of essential oils (EOs) for treating inflammation is advantageous because they have fewer adverse effects than numerous synthetic and conventional medications (Elshafie & Camele, 2017).

3.3.**Anti-cancer Potential**

Typically, cancer chemotherapies employ potent medicines that are toxic to cells in order to specifically target rapidly dividing cell populations. However, due to their lack of selectivity, these treatments also cause significant harm to healthy cells, leading to severe side effects. Essential oils derived from natural sources and their components play a crucial role in preventing and treating cancer (Mahawer et al., 2022).

Essential oils (EOs) have significant anticancer effects through diverse routes. A critical role of EOs is their ability to act as antioxidants, enabling them to disrupt mitochondrial functioning in human cells. This interference decreases metabolic activities such as heightened cellular metabolism, excessive mitochondrial synthesis, and persistent oxidative stress, alleviating

conditions that contribute to developing malignant tumors. Significantly, terpenoids and polyphenols found in plant oils can strongly trigger apoptosis or necrosis, which effectively inhibits the proliferation of tumor cells, providing reassurance about the potential of these oils in cancer treatment (M. Sharma et al., 2022).

Essential oils (EOs) possess chemo-preventive characteristics due to their antioxidant, antiproliferative, and antimutagenic activities. Additionally, they enhance detoxification and exhibit synergistic activity through their constituents. The generation of reactive oxygen species (ROS) is directly associated with oxidation and inflammation, which can result in cancer development. Oxidative stress has the potential to harm the DNA found in mitochondria, leading to an increase in the occurrence of mutations and facilitating the development of cancerous transformations. In addition, reactive oxygen species (ROS) also initiate signaling pathways that control cellular proliferation, angiogenesis, and metastasis, promoting tumor growth. EO components combine with ROS to create reactive phenoxy radicals, which counteract more ROS and inhibit further oxidative harm. In addition, EOs stimulate the production of antioxidant enzymes, including catalase, superoxide dismutase, glutathione peroxidase, and glutathione. This process enhances the antioxidant activity within cells, considerably decreasing tumor size by lowering oxidative stress and blocking cancer progression pathways (Mahawer et al., 2022 and references therein).

3.4.**Anti-diabetic Potential**

Hyperglycemia is a medical disease linked to diabetes, which occurs when the body cannot create or properly use insulin to control normal glucose levels in the blood. Controlling postprandial hyperglycemia in type 2 diabetes care requires the inhibition of α-glucosidase and α-amylase, as these enzymes play a significant role in the digestion of carbohydrates. α-amylase hydrolyzes complex carbohydrates into disaccharides, whereas α-glucosidase hydrolyzes starch and disaccharides into glucose or monosaccharides. By suppressing these enzymes, breaking down carbs is slowed, decreasing glucose absorption into the bloodstream. Essential oils can attach themselves to the active sites of α-amylase and α-glucosidase, functioning as inhibitors and creating enzyme-inhibitor complexes. This process ultimately hinders the activities of these enzymes (Mahawer et al., 2022 and references therein).

3.5.**Anti-cholinesterase Potential**

The potential of essential oils (EOs) to inhibit the activity of anticholinesterase enzymes has attracted interest due to their potential therapeutic use in neurodegenerative diseases such as

Alzheimer's disease (AD). AD is characterized by oxidative stress, the accumulation of amyloid plaques, neurofibrillary tangles, memory loss, and cognitive decline. The primary cause of the anticholinesterase activity of EOs is their chemical contents, specifically terpenes and phenolic compounds. These chemicals can hinder the activity of cholinesterase enzymes by either directly attaching to the enzyme's active site or by creating complexes with the enzyme that inhibit its function. This inhibition extends the duration of the effect of acetylcholine at cholinergic synapses, which could help reduce the cholinergic deficiencies linked to Alzheimer's disease and provide a natural method for treating the symptoms of the condition (Lima et al., 2024).

3.6.**Anti-viral Potential**

Essential oils (EOs) are receiving much attention due to their potent antiviral capabilities in combating viruses such as influenza and coronaviruses. The oils contain an intricate combination of volatile phytochemicals, such as monoterpenes, sesquiterpenes, and phenylpropanoids, contributing to their wide-ranging antiviral effectiveness. Recent research has also investigated the potential of essential oils in combating SARS-CoV-2, which causes COVID-19 (Asif et al., 2020). These studies have emphasized the ability of EOs to regulate immune responses and limit viral replication. EOs may exert antiviral effects by destroying viruses' protective outer layer, rendering them inactive. Additionally, they might hinder viral reproduction by interfering with viruses entering host cells or interrupting the assembly and release of new viral particles (Mustafa et al., 2023; Reichling, 2022).

3.7.**Anti-bacterial Potential**

Essential oils (EOs) demonstrate significant antibacterial action due to three primary factors: their hydrophobic nature, chemical composition, and the specific microbes they target (Fisher & Phillips, 2008; Holley & Patel, 2005). Hydrophobic EOs can enter cell walls and cytoplasmic membranes and cause structural disturbances that increase membrane permeability. This leads to the loss of ions, decreased membrane potential, failure of proton pumps, and depletion of ATP, ultimately leading to the death of bacterial cells (Dorman & Deans, 2000; Hanif et al., 2019; Mahawer et al., 2022). *Figure 11* presents the recognized antibacterial mechanisms of essential oils (Mahawer et al., 2022).

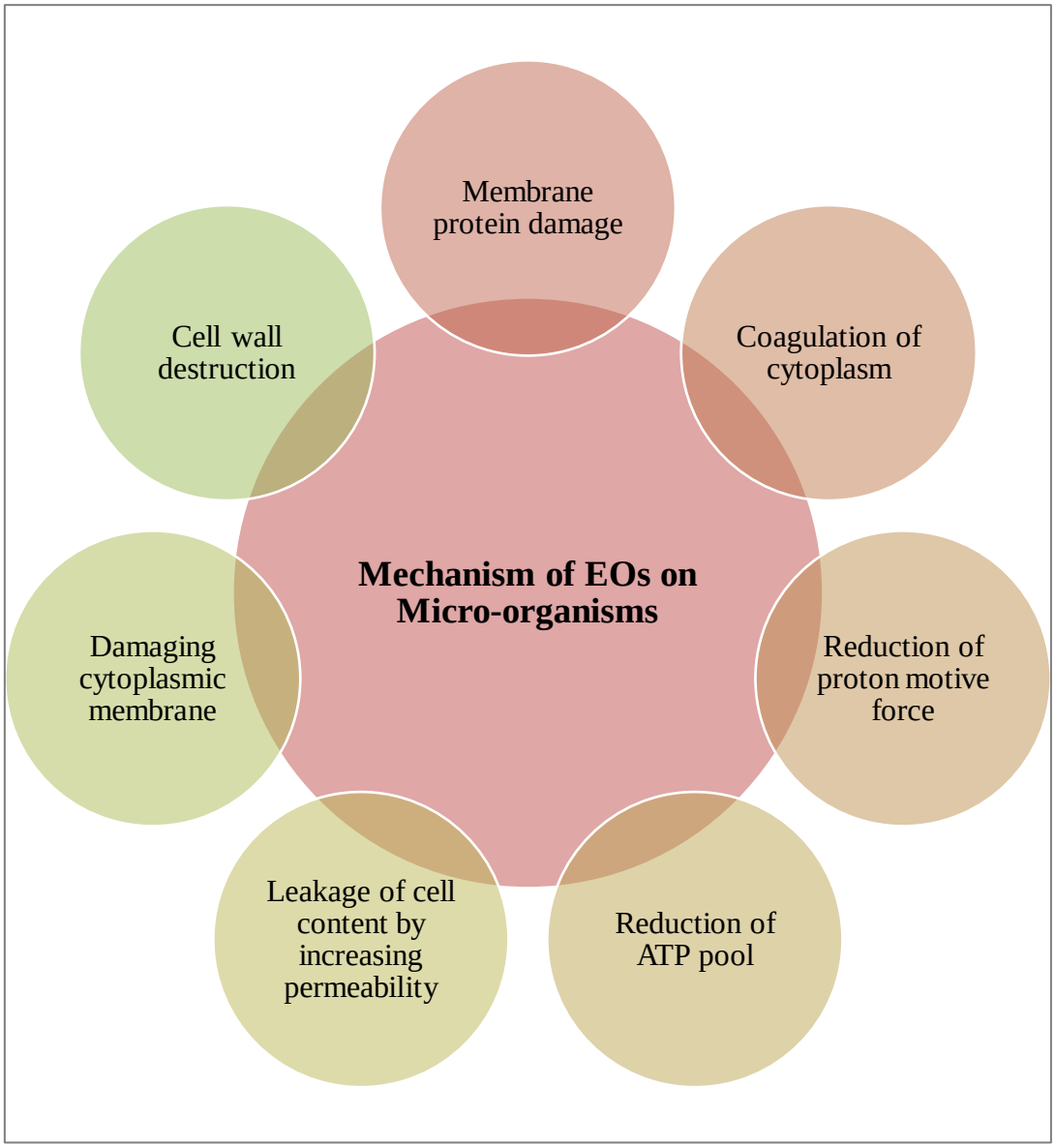

Figure 11: *Mechanisms of essential oil action on micro-organisms (Mahawer et al., 2022).*

Phenolic chemicals, such as eugenol, thymol, and carvacrol, are key players in the antibacterial properties of essential oils. These substances have the unique ability to cause cell contents to clump together and disrupt the integrity of cytoplasmic membranes, electron flow, proton driving force, and active transport mechanisms. Additionally, terpenoids in essential oils interact with specific enzymes, further enhancing their antibacterial activity (Hanif et al., 2019; Mahawer et al., 2022).

Furthermore, essential oils play a significant role in coagulating cytoplasmic lipids and proteins, thereby causing additional damage to the cells. EOs are particularly effective against gram-positive bacteria due to the inherent disparities in the composition of their cell walls. Gram-positive bacteria have a readily accessible peptidoglycan coating that can be easily

penetrated by essential oils. In contrast, gram-negative bacteria have an outer membrane consisting of a double layer of phospholipids connected by lipopolysaccharides, which prevents the penetration of EO and provides them with increased resistance (Hanif et al., 2019; Mahawer et al., 2022).

3.8. Antifungal Potential

Essential oils (EOs) possess high levels of phenylpropanoids, such as eugenol, and monocyclic sesquiterpene alcohols, including α-bisabolol, which can inhibit dermatophytes and spores. Essential oils derived from several species of *Melaleuca* demonstrate antifungal properties against *Aspergillus niger*. In addition, essential oils derived from orange, Lemongrass, and grapefruit can suppress the growth of *Aspergillus niger* and has fungicidal properties against *Aspergillus flavus*, *Penicillium verrucosum*, and *Penicillium chrysogenum*. Essential oils that contain a high amount of terpenoids are highly successful in combating both drug-sensitive and drug-resistant harmful yeasts, particularly *Candida albicans*. The antifungal actions of EOs involve several mechanisms, including interrupting the cell cycle, blocking membrane ergosterol and signalling pathways, and permeabilizing the mitochondrial membrane, all of which ultimately lead to apoptosis and cell death (Nazzaro et al., 2017).

3.9. Allelopathic Potential

The allelopathic capacity of essential oils (EOs) stems from their abundant presence of secondary metabolites, including terpenoids, which have a crucial function in the protective systems of plants. Allelopathy, as defined by the International Allelopathy Society (IAS), refers to the phenomenon in which secondary metabolites, known as allelochemicals, influence the growth and development of other organisms in agricultural and biological systems. Essential oils possess allelopathic effects by altering the physiological processes of target plants due to their bioactive terpenoids. This interruption can manifest in multiple ways, including reduced germination percentage, altered mean germination time, and decreased seedling vigor. The precise impacts of essential oils rely on their chemistry and the vulnerability of the target species, making them an effective tool for controlling plant interactions in agricultural environments (Hanif et al., 2019; Mirmostafaee et al., 2020; Zheljazkov et al., 2021).

3.10. Repellent and Insecticidal Potential

Essential oils are a rich source of chemically diverse substances that enable them to repel insects and act as insecticides (Hanif et al., 2019). These chemicals employ a variety of methods

to deter and eliminate insects. In their insecticidal role, some essential oils interact with the neuromodulator octopamine, while others disrupt GABA-gated chloride channels, revealing a fascinating neurotoxic mode of action. The exploration of essential oils as a pioneering approach for developing essential oil-based insecticides is driven by their potential for revolutionizing pest management (Amaral et al., 2017).

The application of essential oils in plant protection is extensive, encompassing their use as insecticides, herbicides, nematicides, and fungicides (Mahawer et al., 2022). Essential oils have demonstrated detrimental effects on both grain storage facility pests and flying insects. Eucalyptus and Gaultheria oils, derived from plants in the *Myrtaceae* and *Ericaceae* families, have been found to possess potent toxicity, leading to the effective and dependable elimination of insects, according to Mateeva and Karov (1983, as cited in Hanif et al., 2019). Essential oils can enter the bodies of insects through ingestion, inhalation, or skin absorption. Furthermore, EOs exhibit fumigant toxicity (Regnault-Roger & Hamraoui, 1995).

Specific examples highlight the effectiveness of essential oils against various insect species. *Eucalyptus saligna* oil has been shown to kill *Anopheles funestus*, *Pediculus capitis*, *Periplaneta orientalis* , and *Cimex lectularius* within 2–30 minutes (Hanif et al., 2019).

4. SYNERGISM BETWEEN THE COMPONENTS OF ESSENTIAL OILS

Essential oils consist of complex combinations of many molecules, and their biological qualities may arise from the collaborative interaction between these molecules rather than the individual actions of the primary components alone. The majority of the literature concentrates on the primary components found in specific essential oils, including terpineol, eugenol, thymol, carvacrol, carvone, geraniol, linalool, citronellol, nerol, safrole, eucalyptol, limonene, and cinnamaldehyde. These components are commonly examined using gas chromatography. The main components of essential oils often mirror the biophysical and biological characteristics of the oils from which they are extracted. The effects of these components vary depending on their concentration, whether examined separately or within the oil as a whole.

The concept of synergistic interactions among the several compounds in an essential oil, as opposed to the effects of just one or two primary components, is a field of active research. The influence of additional subordinate molecules on the activity of the primary constituents is still being determined. Moreover, many constituents likely play a role in determining the scent, thickness, consistency, hue, ability to penetrate cells, and affinity for cell walls and membranes of the oil. Oil distribution within cells is significant because it dictates the radical reactions that depend on their cellular compartmentation. Hence, from a biological standpoint, analyzing the complete composition of oil provides more comprehensive insights than investigating only specific constituents, as synergy holds greater importance (Bakkali et al., 2008 and references within).

5. ESSENTIAL OIL'S QUALITY

Ensuring the quality and purity of essential oils is important to facilitate their efficient utilization, and various techniques exist for evaluating these characteristics. The sensory evaluation process is an essential preliminary stage, wherein oils of superior quality should have a consistent, strong, and appealing fragrance devoid of undesirable undertones or chemical odors. Furthermore, experienced evaluators frequently analyze the color and viscosity of the oil, as nuanced variations might indicate its purity level. Physical examinations contribute to the assessment of quality. Determining the oil's refractive index, density, and optical rotation is essential in identifying potential contamination when the obtained findings differ from the expected ranges.

Gas chromatography and mass spectrometry (GC/MS) analysis is widely regarded as the most dependable approach for evaluating the chemical composition of essential oils. This analytical technique offers a comprehensive understanding of the components present in the oil and aids in identifying any unforeseen substances or minor contaminants. The quality of essential oils is substantially influenced by sourcing practices. Oils categorized as organic, wildcrafted, or certified organic generally exhibit superior quality, attributed to meticulous cultivation and harvesting techniques. While simple tests provide initial insights, laboratory-based GC/MS analysis is the ultimate method for evaluating an essential oil's purity, ensuring that users utilize oils of the highest quality (Bhagat, 2024).

6. ECONOMIC ASPECTS OF ESSENTIAL OILS

6.1. **Production and Trade of Essential Oils**

Obtaining accurate estimates of the worldwide production and trade of essential oils is difficult since there is a need for precise data on domestic production and exports for oils that are produced in large quantities. These oils are often categorized under more general product codes, making tracking harder. Hence, global production figures should be approached with caution, as they often fail to account for domestic consumption and are based on data from a restricted set of countries. Indeed, numerous countries from every continent significantly contribute to the production of essential oils. The global output in 2017 was anticipated to be more than 150,000 tones, valued at almost 6 billion USD. This marked a substantial increase from the production of 45,000 tons in 1990. Based on multiple economic evaluations, it was projected that the growth would persist until the 2020s, resulting in an estimated yearly production of 370,000 tons, with a value exceeding 10 billion USD (2018-dollar value) (Barbieri & Borsotto, 2018 and references therein).

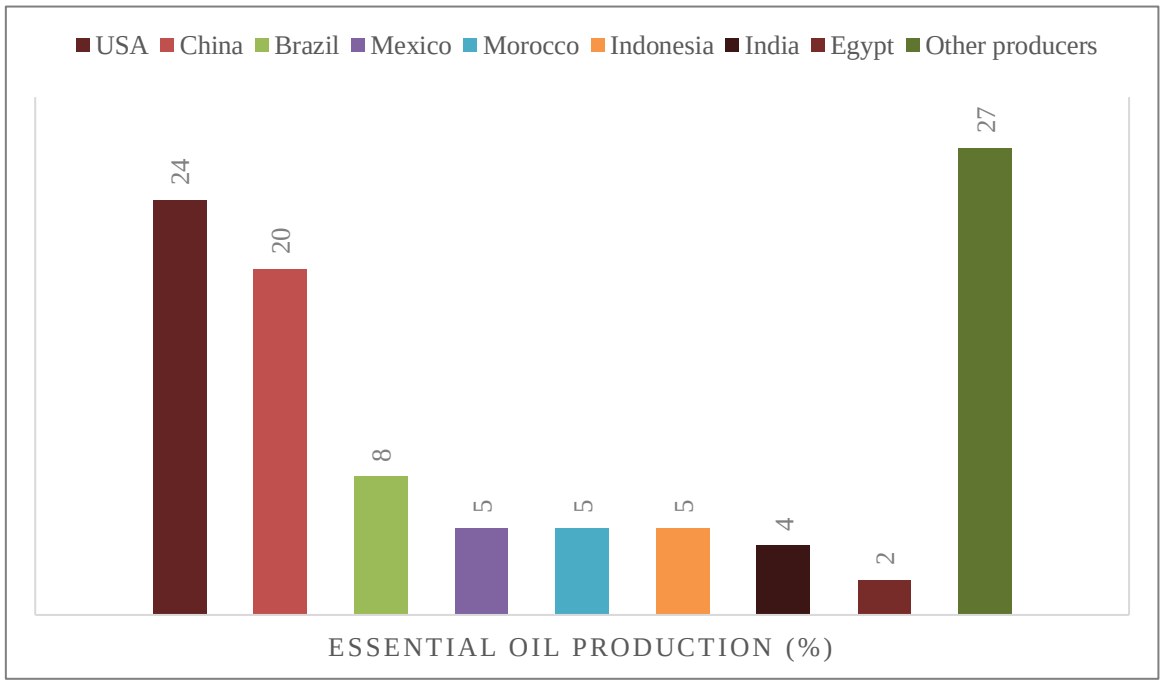

Figure 12: *Largest producers of essential oils (Devi et al., 2015).*

Out of the estimated 3,000 recognized essential oils, around 150 have commercial significance and are traded globally. As presented in *Figure 12* (Devi et al., 2015), The United States is the largest producer, accounting for 24% of the total output. China follows closely after with a 20% share. Other significant producers include Brazil (8%), Mexico (5%), Morocco

(5%), Indonesia (5%), India (4%), and Egypt (2%). Developing countries contribute to over 65% of the global production. Western Europe dominates the fragrance and cosmetic sector, accounting for over 31% of the global market. North America and the Asia Pacific region follow it (C. Barbieri & Borsotto, 2018; Devi et al., 2015).

The United States of America is the top country in terms of imports, accounting for a 14% share. It is also a significant contributor to global exports, representing 17% of the total. France, India, the UK, and Brazil are other prominent exporters. Globally, the production value of the 18 most significant essential oils accounts for around 75% of the total. Although oils such as rose, jasmine, and vetiver are traded in lower quantities, they substantially impact the market due to their high value (C. Barbieri & Borsotto, 2018; Devi et al., 2015).

Although aromatherapy is a relatively new and expanding market, its application has long been recognized in the food, flavoring, and scent industries. The soft drink industry is the primary consumer of essential oils, particularly those derived from Citrus oil. Every 'cola' is made using lemon or lime essential oils. Additional notable users include producers of alcoholic beverages, sweeteners, dairy products, confections, and desserts, as well as fast food and processed food manufacturers, all within the food industry. Essential oils are also utilized in various other sectors, including pharmaceuticals, cosmetics, vitamins, herbs, and feeds (C. Barbieri & Borsotto, 2018).

6.2.**Demand for Essential Oils**

The primary sources of demand for essential oils are four markets: food and beverage (35%), fragrances, cosmetics, and aromatherapy (29%), household (16%), and pharmaceutical (15%). The food and beverage industry dominates the market due to the acknowledged health advantages of essential oils as natural components that capture their origin's essence. Widely utilized essential oils comprise orange, lemon, and lime oils. Orange oil is particularly favored in the culinary industry for its distinct citrus taste and invigorating aroma. The increasing public awareness of the health advantages of essential oils has led to a rise in the demand for food and beverage items that incorporate these oils. However, it's the growing consumer awareness regarding health and the role of essential oils in natural and organic hygiene products that is driving the worldwide essential oils market. Moreover, the increasing need for natural flavors and fragrances in cosmetics, perfumes, and relaxation products is anticipated to drive the need for essential oils. Primary flavor and fragrance makers purchase essential oils, and their sales rise reflects significant market changes. From 2012 to 2016, there was a remarkable 7% increase

in global sales of flavor and fragrance makers, reaching €25 billion. The rising demand for natural cosmetics and flavorings mainly fueled this growth, indicating a positive shift in the market (Barbieri & Borsotto, 2018 and references therein).

6.3. Consumption of Essential Oils

Food and beverage makers, compelled by consumer health awareness, are substituting dangerous additives with natural alternatives, such as essential oils. The significant increase in essential oil output is mainly driven by rapid industrialization and growing disposable incomes in developing nations such as China, India, Vietnam, and Thailand. The USDA reports that the primary purchasers of essential oils are the United States (40%), Western Europe (30%), and Japan (7%). The sales of essential oils are intricately linked to consumer education, as a more profound comprehension results in increased demand and sales expansion. The aromatherapy market, which encompasses both professional and personal usage, is a prominent illustration of this phenomenon. In the United States, many skilled aromatherapists produce and promote their own products, resulting in market fragmentation characterized by multiple small-scale players. Food flavoring businesses are progressively creating natural flavorings, utilizing essential oils as crucial components. Despite the challenges in attaining consistent natural flavorings due to the variability in the composition of essential oils, their potentials in the food and beverage industry are inspiring and drives the industry forward (Barbieri & Borsotto, 2018 and references therein).

6.4. Regulations on Essential Oils

Gaining accurate data regarding the production, use, and regulatory frameworks of essential oils in Algeria might take a lot of work due to the limited progress of this industry in the country. More comprehensive resources are needed due to the limited availability of local data and the early stage of sector development. The most relevant Algerian legal reference appears in the *Journal Officiel de la République Algérienne*, No. 30, dated 16 May 2012, which addresses essential oils indirectly under the section regulating food additives.

Given these limitations, this study turned to the European Union's legal framework to establish a more structured reference and facilitate meaningful comparisons. The EU provides a well-developed and rigorous set of regulations concerning essential oils, encompassing production standards, quality assurance, and safety requirements. These regulations are distributed across several legal instruments depending on the intended application of the

essential oil—whether as food flavorings, cosmetic ingredients, or additives in animal feed (Barbieri & Borsotto, 2018). By examining this comprehensive legislative model, Algeria may gain insights to support the development of appropriate national regulations that foster industry growth while ensuring consumer safety.

Regulation (EC) No 1334/2008: the primary objective is to safeguard human health and facilitate the unrestricted circulation of food items within the European Union by regulating flavorings used in food. Flavorings are compounds added to food to alter its taste or aroma. Before these flavorings may be used, a risk assessment is required. Flavoring essential oils must adhere to safety regulations and avoid deceptive practices towards consumers. Stringent regulation often only applies to natural flavorings if they present potential health hazards. The legislation mandates that only sanctioned essential oils and flavorings, which pose no threat to consumer well-being, are allowed in food products.

Regulation (EC) No 1223/2009: governs the utilization of essential oils in cosmetic products. This regulation aims to maintain a balance between market innovation and consumer safety. This document outlines the obligations of the responsible person, who can be a human or a formal business, to ensure that cosmetic products meet safety regulations, have accurate labelling, and can be traced back to their source. Cosmetics must only use safe essential oils that are appropriately labelled to ensure customers are aware of any potential hazards. This rule also prohibits using animals to test cosmetic items and substances while encouraging the adoption of alternative testing methods. The labelling rules necessitate the specification of the essential oils employed, guaranteeing that consumers are adequately informed about the constituents present in their cosmetic products.

Regulations (EC) No 1831/2003 and *No 429/2008:* govern the use of additives in animal nutrition. These regulations define additives as compounds added to feed or water to accomplish specified effects. These restrictions ban the utilization of antibiotics as feed additives and mandate that only authorized additives, such as essential oils, are allowed to be advertised and utilized. Essential oils used as supplements in animal feed must adhere to safety and effectiveness criteria, guaranteeing that they do not present a hazard to animal well-being or the food supply. The laws provide comprehensive guidelines for novel additives' application, evaluation, and authorization procedures, guaranteeing that they adhere to stringent safety standards.

To summarize, the European Union has a comprehensive regulatory framework that regulates essential oils and addresses the overall safety of these oils and their specific uses in food, cosmetics, and animal nutrition. Its purpose is to safeguard health and encourage advancements in this field. To guarantee the safety of essential oils for human and animal use and to provide customers and users with accurate information regarding any potential risks, these oils must adhere to the specified laws and are appropriately labelled.

7. PRACTICAL APPLICATIONS OF ESSENTIAL OILS

This chapter aims to provide practical insights into the applications of essential oils, exploring their utilization in different settings and shedding light on their role in promoting well-being. As we navigate through the multifaceted landscape of essential oils, we will uncover the profound impact these extracts can have on our daily lives and the industries that harness their potential for a wide array of purposes.

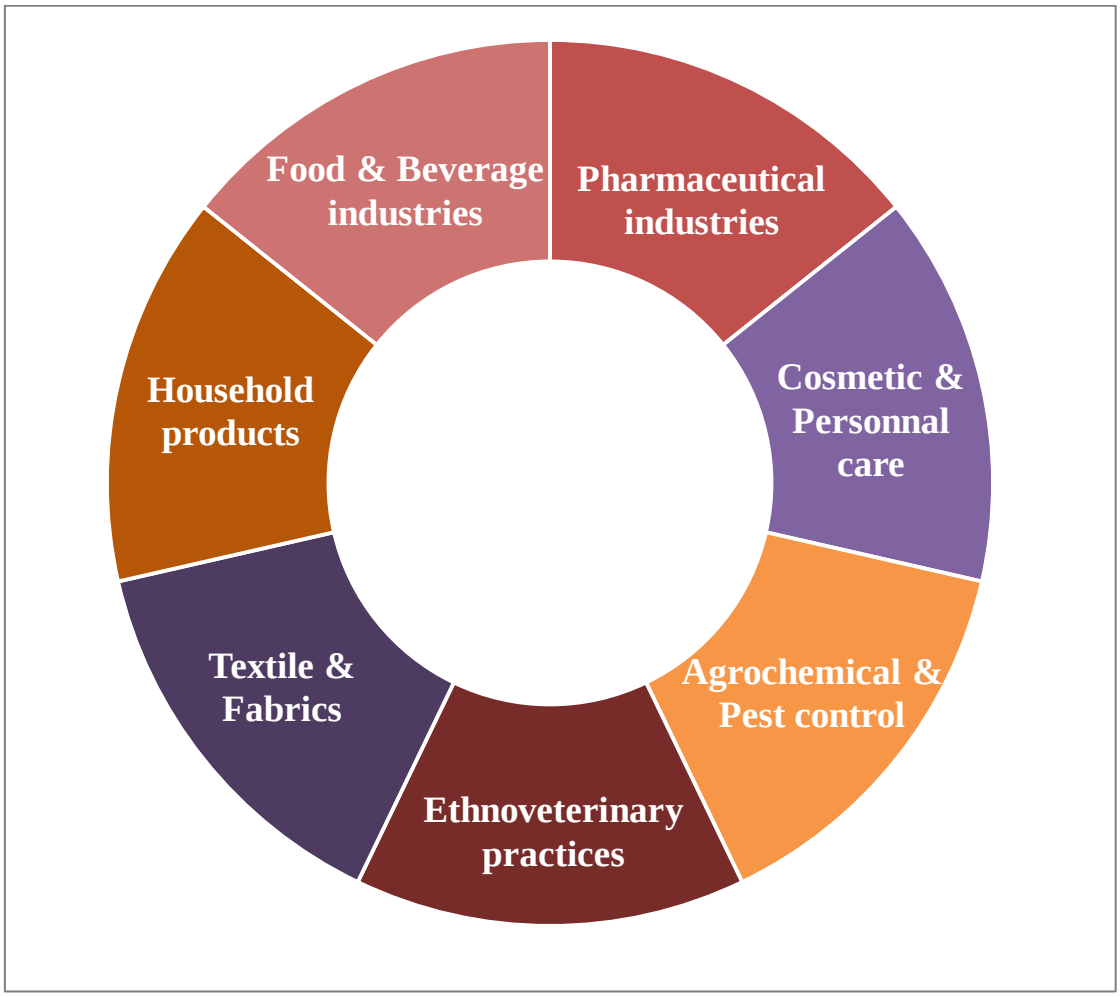

Figure13: *Fields of use for essential oils.*

7.1.**Food and Beverages**

The use of essential oils (EOs) in the food industry is both extensive and evolving, offering numerous benefits while posing challenges that demand careful consideration. Derived from aromatic herbs and spices, EOs have been widely incorporated into food products primarily for their ability to enhance sensory qualities such as flavor and aroma. Their concentrated nature makes them powerful flavoring agents, with applications in baked goods, beverages, confectionery, snacks, and savory dishes. For example, the EO of sweet basil is extensively used to impart its distinctive aroma and flavor to dishes like salads, pizzas, and soups (Fernández-López & Viuda-Martos, 2018). Similarly, the leaf and berry oils of allspice (*Pimenta dioica*) serve as flavoring agents in meat products and confectioneries, though regulatory limits such as a maximum of 0.025% for berry oil are strictly observed to ensure consumer safety. Other examples include oregano oil, which enhances the sensory properties of minced beef while remaining nearly undetectable post-cooking, and Alpinia galanga oil, widely used in traditional cuisines across Southeast Asia (Mariod, 2016 and references therein).

Beyond their role as flavoring agents, EOs have gained attention for their functional properties, particularly in food preservation. With strong antimicrobial and antioxidant activities, EOs have demonstrated the ability to delay spoilage, extend shelf life, and enhance food safety. Carvacrol, for instance, produces a “warmly pungent” aroma on fish and has been found to delay spoilage in fresh kiwi and honeydew melon without compromising sensory qualities. These properties make EOs attractive natural alternatives to synthetic preservatives, addressing growing consumer demand for clean-label and health-conscious products. However, effective application requires an in-depth understanding of their properties, including the minimum inhibitory concentrations required for microbial inhibition, the range of target microorganisms, and their interactions with complex food matrices. Encapsulation and nanotechnology are emerging as innovative solutions to overcome challenges related to EO stability, volatility, and controlled release, further expanding their utility in modern food systems (Ríos, 2016).

Despite these advantages, the use of EOs is not without risks, necessitating rigorous safety and regulatory considerations. As highly concentrated substances, EOs can cause adverse reactions such as toxicity or overpowering flavors when used inappropriately. For example, excessive use can lead to unbalanced dishes where EO flavors dominate rather than complement other ingredients. Furthermore, the bioactive compounds in EOs may provoke skin irritation or

allergic reactions if mishandled. To mitigate these risks, regulatory bodies like the U.S. Food and Drug Administration (FDA) and the World Health Organization (WHO) enforce stringent safety standards. These include evaluating toxicological profiles, establishing maximum permissible levels, and requiring compliance with labeling and quality standards to prevent foodborne illnesses and ensure consumer safety. Additionally, food manufacturers must adopt robust safety processes throughout the production chain to align with these regulations and avoid costly recalls.

The dual role of EOs as flavoring agents and preservatives highlights their versatility, yet their integration into food systems must balance efficacy with safety. Emerging trends, such as using EOs as antimicrobial agents for food packaging and crop protection, underscore their potential to revolutionize the food industry while addressing global concerns about food quality and safety. However, further research is needed to fully elucidate the mode of action of many EO components and optimize their application in diverse food matrices. By advancing knowledge in this area, the food industry can leverage the full potential of EOs, offering natural and effective solutions to meet evolving consumer demands and regulatory requirements.

7.1.1. Innovative uses of essential oils in the food sector

Essential oils are increasingly in demand across industries such as perfumery, aromatherapy, cosmetics, pharmaceuticals, and food. Research focuses on their biological properties, biosynthesis, chemical analysis, and industrial applications, particularly their antimicrobial properties and uses as flavors, fragrances, and active compounds in pharmaceuticals. There is a notable trend toward sustainable biotechnological production methods, highlighting their expanding applications and innovative production processes (K. H. C. Başer & Buchbauer, 2010).

Micro and Nano encapsulation

Micro and nanoencapsulation are advanced techniques used to enhance the stability, bioavailability, and controlled release of essential oils (EOs) in food applications. These methods involve enclosing active compounds within a protective matrix, allowing for better integration into food systems.

Microencapsulation typically refers to the encapsulation of materials ranging from 1 to 5000 micrometers, while nanoencapsulation involves particles smaller than 1 micrometer. Various

techniques such as spray drying, coacervation, and solvent evaporation are employed to create these encapsulated systems (Yammine et al., 2024).

The encapsulation process protects EOs from environmental factors like oxidation, evaporation, and degradation. This is particularly important for EOs that are sensitive to heat and light. Additionally, encapsulated EOs can provide a controlled release mechanism, ensuring that their antimicrobial and antioxidant properties are activated at the right time during food processing or storage (S. Sharma et al., 2022; Yammine et al., 2024).

Encapsulated EOs can be integrated into food products or packaging materials, enhancing their shelf life and safety. For instance, nanoliposomes have been used to encapsulate hydrophobic compounds such as garlic oil, demonstrating significant antimicrobial effects when incorporated into chitosan films (S. Sharma et al., 2022).

Active packaging

Active packaging is an innovative approach that integrates active substances into packaging materials to extend the shelf life of food products while maintaining their quality.

Active packaging systems can release antimicrobial agents or antioxidants directly into the food environment. This helps in creating a barrier against microbial growth and oxidative reactions. Essential oils serve as natural active agents that can be incorporated into various packaging matrices (Alonso et al., 2024; Carpena et al., 2021). Common active agents include essential oils, organic acids, enzymes, and biopolymers. These agents can be designed to interact with the packaged food, either by scavenging oxygen or by releasing antimicrobial compounds that inhibit spoilage organisms (Carpena et al., 2021; S. Sharma et al., 2022).

The use of natural essential oils in active packaging aligns with consumer preferences for clean-label products. Unlike synthetic preservatives, EOs are perceived as safe and beneficial for health. Moreover, active packaging can reduce food waste by prolonging freshness without compromising flavor or safety (Carpena et al., 2021; S. Sharma et al., 2022).

Edible coatings

Edible coatings represent a sustainable alternative to traditional packaging methods by providing a thin layer on food products that offers protection while being consumable.

Edible coatings can effectively limit moisture loss, reduce gas exchange, and provide a barrier against microbial contamination. Incorporating EOs into these coatings enhances their antimicrobial properties and antioxidant activity, which helps preserve food quality during storage (Antonino et al., 2024; Das et al., 2021; Ju et al., 2019). Common materials for edible coatings include polysaccharides (like alginate and chitosan), proteins (such as whey protein), and lipids. The incorporation of EOs into these matrices not only improves their functional properties but also allows for the gradual release of bioactive compounds over time (Antonino et al., 2024; Carpena et al., 2021; Ju et al., 2019).

Studies have shown that edible coatings enriched with EOs can significantly inhibit microbial growth and lipid oxidation in various food products such as fruits, meats, and dairy items. For example, coatings containing cinnamon EO have demonstrated effectiveness in extending the shelf life of chilled pork by reducing microbial counts and delaying oxidative changes (Alonso et al., 2024; Ju et al., 2019).

The innovative applications of micro and nanoencapsulation, active packaging, and edible coatings utilizing essential oils highlight the potential for enhancing food preservation methods in a natural and sustainable manner. These technologies not only address consumer demands for healthier options but also contribute to reducing food waste by extending shelf life while maintaining sensory qualities. As research continues to evolve in this field, the integration of essential oils into food systems is likely to expand further, offering new opportunities for improving food safety and quality.

Table 02: *Application of essential oils in different food products (Saeed et al., 2022 and references within)*

Product	Essential oil	Target microorganism	Modern technology	Examples of food products
Meat and meat products	Thyme, oregano, eugenol, clove and coriander essential oils, Winter savory essential oil	*Listeria monocytogenes*	Modified atmosphere packaging	Fresh chicken, breast meat, pork preservation
Seafoods	Oregano, thymol, grapefruit seed and lemon essential oil	*Photobacterium phosphoreum Mesophilic bacteria*	Modified atmosphere packaging	Cod fillets, salmon
Fruits and vegetables	Lemon grass, ginger oregano, lemongrass and vanillin essential oils	*Salmonella spp., Listeria spp., Salmonella enteritidis, Escherichia coli O157:H7, Bacillus cereus*	Edible food coatings	Pear and apple juice, Pieldesapo melon, Fuji apples, eggplant salad
Dairy products	Orange thyme, clove and cinnamon essential oil	*Pseudomonas spp., Escherichia coli, Salmonella senftenberg, Staphylococcus aureus, Enterococcus hirae, Staphylococcus aureus, Bacillus licheniformis, Salmonella enteritidis*	Nanoencapsulation	Skimmed milk, low fat milk, full cream milk, low fat cheese
Cereal-based products	Citrus peel, oregano, thyme, basil, cinnamon, sage, rosemary, anise and black cumin essential oil	*Bacillus cereus*, Fungi	Active packaging	Bread, fresh amaranth-based pasta, rice-based food products, gluten-free sliced bread, bakery products

7.2.**Pharmaceuticals**

Essential oils (EOs) have garnered considerable interest in the pharmaceutical sector owing to their varied therapeutic potentials and pharmacological activities. Oils derived from aromatic plants are acknowledged for their antimicrobial, anticancer, anti-inflammatory, and antiviral properties, positioning them as significant candidates for drug formulations and diverse therapeutic applications (Aziz et al., 2018; Cimino et al., 2021). Essential oils like eucalyptus (*E. globulus*), peppermint (*Mentha piperita*), anise (*P. anisum*), sage (*Salvia officinalis*), clove (*S. aromaticum*), and tea tree (*M. alternifolia*) have established medicinal applications. Eucalyptus oil functions as an expectorant for cough and bronchitis, peppermint oil acts as a decongestant for the respiratory tract, and anise oil serves as a carminative. Clove oil exhibits antiseptic and analgesic properties commonly utilized in dentistry, whereas tea tree oil is acknowledged for its antiacne efficacy against Gram-positive bacteria, establishing its importance in dermatological therapies (K. H. C. Başer & Buchbauer, 2010). Furthermore, essential oils improve the sensory characteristics of pharmaceutical formulations, enhancing taste and aroma to promote patient adherence.

The chemical complexity of essential oils, consisting of various active constituents, supports their diverse pharmacological activities and wide-ranging applicability. Recent research has focused on their role as skin permeation enhancers, offering innovative approaches for transdermal drug delivery (Aziz et al., 2018). The advancement of encapsulation technologies, including lipid-based delivery systems, seeks to overcome challenges related to essential oils, such as hydrophobicity, instability, and high volatility, thus enhancing their bioavailability and therapeutic efficacy (Cimino et al., 2021). These systems facilitate controlled release, thereby enhancing the sustained activity of essential oils and broadening their application in long-term treatments. Nanotechnology presents opportunities through the development of particulate delivery systems that enhance the targeting and efficacy of essential oils, addressing challenges related to their incorporation into chemotherapeutic protocols (Adeyemi et al., 2023).

Despite these advancements, the integration of essential oils into pharmaceuticals poses challenges. Their significant volatility, limited solubility in water, and possible toxicity necessitate meticulous formulation to guarantee safety and effectiveness. Novel delivery systems, such as nano-encapsulation and lipid carriers, are essential for overcoming these limitations. These methods stabilize the oils and facilitate precise targeting and extended activity, essential for therapeutic efficacy. Furthermore, advancements in research have

enhanced the characterization and standardization of EO-based products, facilitating their use in novel pharmaceutical matrices and combination therapies.

The integration of essential oils into the pharmaceutical industry signifies a promising but intricate frontier. Their natural origin and significant pharmacological activities establish them as essential elements in the advancement of contemporary therapeutics. Despite ongoing challenges associated with their physicochemical properties and formulation, advancements in encapsulation and nanotechnology offer effective solutions, enabling the realization of their full potential for medicinal applications. Ongoing research and innovation suggest that essential oils will assume a more significant role in meeting global healthcare demands.

7.3.**Fragrances, Cosmetics and Personal care**

Essential oils are integral to the cosmetics and personal care sector, contributing to improvements in skincare, haircare, fragrance enhancement, and therapeutic uses. Their diverse properties, such as antioxidant, antibacterial, and anti-inflammatory effects, make them essential in formulations designed to enhance skin and hair health (Guzmán & Lucia, 2021). Geranium and lavender essential oils are commonly used in skincare products to improve skin elasticity, postpone visible signs of aging, and diminish inflammation, providing a natural alternative to synthetic compounds. Essential oils, including tea tree oil and peppermint oil, are recognized in haircare for their effectiveness in treating scalp conditions like dandruff and irritation, attributed to their strong antibacterial and antifungal properties (Guzmán & Lucia, 2021). These oils are frequently utilized topically, diluted with carrier oils, or incorporated into compresses, sprays, baths, and massages, demonstrating their versatility in diverse applications (Kiruthika & Vishali, 2023).

In addition to their functional advantages, essential oils play a crucial role in augmenting the sensory attractiveness of beauty products. Natural fragrance compounds serve to conceal unpleasant odors and enhance the appeal of cosmetics, thereby significantly affecting consumer perception and comfort (Sharmeen et al., 2021). Essential oil-derived fragrances, including those from lavender, thyme, and citrus plants, offer appealing aromas while also enhancing emotional and psychological well-being. Functional or wellness fragrances are designed to enhance mood, alleviate stress, and encourage relaxation, integrating scientific and sensory advantages in beauty products (Guzmán & Lucia, 2021). This dual functionality corresponds with the growing consumer preference for products that provide both aesthetic and therapeutic benefits.

The economic significance of essential oils in this industry is considerable. The global production of essential oils, especially from aromatic plants such as salvia, lavender, and thyme, has increased markedly to satisfy the rising demand for high-quality and innovative perfumes. The superior quality of essential oil-based products is contingent upon high-quality production technology and the meticulous selection of raw materials (Ríos, 2016). Nonetheless, it is important to acknowledge that not all consumers can tolerate fragrances, regardless of whether they are natural or synthetic. Fragrance-free formulations serve as a suitable alternative for these individuals, promoting inclusivity in product offerings.

The diverse applications of essential oils in cosmetics and personal care products underscore their significance as functional and sensory enhancers. Their role in skincare, haircare, and wellness, along with their economic importance and adaptability, highlights their persistent significance in the beauty sector. However, the necessity for safety measures and attention to individual preferences is crucial for their sustainable and effective application.

7.4.**Aromatherapy**

Aromatherapy, defined as the use of aromatic plant extracts and essential oils for therapeutic purposes, encompasses a broad range of practices and effects (Concise Oxford Dictionary, 1995, as cited by Lis-Balchin, 2010). This discipline has evolved into three distinct branches: aromatherapy, aromatology, and aromachology. Aromachology focuses on the psychological effects of scents, such as inducing relaxation, happiness, or a sense of achievement, through their direct impact on the brain. Aromatherapy, in its traditional sense, addresses both physical conditions, such as menstrual or digestive disorders, and psychological conditions, like chronic depression, by leveraging the therapeutic properties of essential oils through inhalation or skin absorption. Aromatology extends the use of essential oils to internal applications under medical supervision, emphasizing their biochemical effects (Lis-Balchin, 2010).

The psychological and therapeutic effects of aromatherapy are attributed to the diverse properties of essential oils, which include anti-inflammatory, antibacterial, sedative, and immunomodulatory activities (Żukowska & Durczyńska, 2024). Essential oils such as lavender, eucalyptus, and peppermint are widely used for their ability to promote relaxation, improve sleep quality, and alleviate stress and anxiety (Fung et al., 2021). The mechanism of these psychological effects lies in the connection between the olfactory system and the limbic system, which governs emotions and memory. Inhalation of essential oils stimulates the limbic system, eliciting responses that can lower blood pressure, reduce respiratory rates during panic attacks,

and elevate mood. For instance, lavender oil has been extensively studied for its calming effects, while peppermint oil is noted for its capacity to relieve tension headaches and enhance cognitive clarity (Ali et al., 2015; Fung et al., 2021).

Aromatherapy practices include diffusion, topical application, and adding essential oils to bathwater. However, the safety of these practices is paramount. Essential oils should be pure, properly diluted, and used according to guidelines to prevent adverse reactions (Kiruthika & Vishali, 2023). Despite promising preclinical and clinical findings regarding the effects of essential oils on the central nervous system, further research is needed to establish their full pharmacological potential and validate their therapeutic claims (Lizarraga-Valderrama, 2021).

In summary, aromatherapy represents a multifaceted approach to enhancing both psychological well-being and physiological health, offering a natural and holistic avenue for managing stress, improving mood, and supporting physical healing.

7.5.**Agrochemicals and Pest control**

Essential oils are increasingly acknowledged in agricultural practices, particularly as environmentally sustainable alternatives to synthetic pesticides. Natural products derived from plants present multiple benefits, such as low toxicity to mammals and high biodegradability, rendering them appropriate for integrated pest management and organic farming systems (Garrido-Miranda et al., 2022; Park & Tak, 2016). Research indicates that essential oils are effective in managing arthropod pests in agricultural settings. Essential oils such as neem oil and peppermint oil are utilized in pest control, providing a natural alternative that reduces detrimental environmental effects compared to traditional chemical pesticides (Park & Tak, 2016). Their biocontrol potential encompasses the management of plant diseases, weeds, and pests, thereby enhancing sustainable horticultural practices, particularly in organic farming (Cagáň et al., 2022; Chang et al., 2022).

The increasing focus on sustainable farming practices is evident in the initiatives of the European Commission and the German government's proposal to substantially decrease the use of synthetic and hazardous pesticides. This movement has initiated the exploration and development of biopesticides, such as microbial pesticides and phytopesticides, which show potential as effective pest control solutions while reducing the adverse environmental effects linked to conventional chemical pesticides (Ayilara et al., 2023).

Essential oils are increasingly recognized for their role in managing yard and garden pests, offering a non-toxic and effective approach to pest control, thereby supporting the movement towards more sustainable agricultural practices (Ayilara et al., 2023). Bio-solarization is being incorporated into pest management strategies, thereby minimizing toxic exposure and enhancing soil health.

Essential oils provide a viable alternative to synthetic pesticides, contributing to sustainable agricultural practices through their biodegradable and effective properties. The transition to natural pest management solutions is a significant component of the global initiative to decrease pesticide usage and promote a more environmentally sustainable agricultural practice (Ayilara et al., 2023; Garrido-Miranda et al., 2022).

7.6.**Veterinary Medicine**

Essential oils are gaining increasing recognition for their applications in veterinary practices, offering significant benefits for animal health and welfare. These natural compounds, derived from aromatic plants, possess a range of bioactive properties that can be leveraged to support livestock management and companion animal care. Scientific studies have highlighted their antioxidant and antimicrobial effects, which make them effective against bacterial and fungal infections in animals, enhancing both health outcomes and food quality in livestock production (Ebani & Mancianti, 2020; Nehme et al., 2021).

In addition to physical health benefits, essential oils are increasingly used in animal aromatherapy to address emotional and psychological conditions. For companion animals such as dogs, cats, and horses, essential oils are employed to alleviate stress, anxiety, and other behavioral issues. This holistic approach aligns with a growing interest in natural and alternative therapies for animals, promoting a gentle and integrative method of care that supports emotional and physical well-being. For instance, lavender oil is known for its calming properties, while peppermint and eucalyptus oils are often used to address respiratory conditions (Nehme et al., 2021).

The expanding use of essential oils in veterinary care necessitates a focus on safety and efficacy. Various resources, including books and guidelines, have been developed to aid pet owners and veterinary professionals in their application. These materials emphasize the importance of informed use and provide insights into safe administration practices, reflecting the rising demand for naturopathic approaches to animal healthcare (Ebani & Mancianti, 2020;

Nehme et al., 2021). The key considerations for the safe use of essential oils in veterinary practices are:

Dosage and Administration: Essential oils must be administered in appropriate doses and through safe routes, such as inhalation, topical application, or controlled ingestion. The choice of method should be tailored to the species and specific health condition being addressed.

Quality and Purity: High-quality, therapeutic-grade essential oils from reputable suppliers are crucial. Oils should be tested for contaminants and adulterants to ensure safety and efficacy.

Potential Adverse Effects: Essential oils may cause side effects such as skin irritation, respiratory distress, or gastrointestinal upset if improperly used. Careful selection and testing are essential to minimize harm, particularly for animals with sensitive systems.

Medication Interactions: Some essential oils may interact with conventional medications, potentially altering their effects or causing adverse reactions. Monitoring and consultation with a veterinary professional are recommended when using essential oils alongside other treatments.

Species-Specific Sensitivities: Animals vary significantly in their tolerance to essential oils. For example, cats lack certain liver enzymes to process specific compounds found in some essential oils, making them more susceptible to toxicity. Tailoring oil selection to the species is essential to prevent harm.

Essential oils present a valuable, natural option for enhancing animal health and well-being. Their applications range from addressing infections and improving food quality in livestock to supporting the emotional health of companion animals. However, their use requires careful consideration of factors such as dosage, quality, species-specific sensitivities, and potential interactions with medications. As the integration of essential oils into veterinary care continues to grow, consulting with veterinary professionals remains essential to ensure their safe and effective application. Further research and education will help refine their use, enabling their broader acceptance in veterinary practices.

7.7.**Household Products**

The use of essential oils as natural cleaning products is increasingly being embraced as an effective and environmentally friendly alternative to synthetic cleaning agents. These oils are

well-known for their potent cleaning properties, including antimicrobial, antifungal, and antiviral actions, which make them highly effective in disinfecting and sanitizing various surfaces.

Essential oils are derived from natural sources, offering a non-toxic, eco-friendly solution that poses less risk to both human health and the environment compared to commercial cleaning products that are often laden with harmful chemicals. Additionally, when used in small amounts, high-quality essential oils can deliver significant cleaning benefits, making them a cost-effective solution over time.

One of the main advantages of using essential oils for cleaning is their versatility. These oils can be incorporated into DIY cleaning recipes, such as all-purpose cleaners, toilet bowl cleaners, and linen sprays. Commonly paired with other natural ingredients like lemon juice, baking soda, and vinegar, essential oils help create personalized, customized cleaning solutions tailored to specific needs, eliminating the need for multiple specialized products (doTERRA, 2024; Hess, 2018). This customizability is not only more sustainable but also reduces the reliance on a range of chemical-laden products, further decreasing environmental impact.

Moreover, essential oils contribute to a greener approach by reducing the release of synthetic chemicals into the ecosystem. As they are biodegradable and derived from renewable natural resources, essential oils present a sustainable alternative to the petrochemical-based substances commonly found in conventional cleaning agents. The reduction of synthetic chemicals in household cleaning products, therefore, directly benefits environmental health by minimizing the pollution of water systems and the atmosphere.

Despite these numerous benefits, it is essential to acknowledge that essential oils must be used with care, as improper use can lead to toxicity, allergic reactions, or sensitivities, especially when used in excessive quantities or mixed with certain medications. Following safe usage guidelines is crucial to avoid adverse effects, ensuring that essential oils remain a beneficial and safe option for cleaning. In summary, essential oils offer a highly effective, customizable, and eco-friendly solution for cleaning that can replace synthetic chemicals, providing both health benefits and environmental protection when used appropriately.

7.8.**Textiles and Fabrics**

The incorporation of essential oils into textiles is an emerging field that merges functionality with sensory appeal, offering innovative solutions for hygiene, aesthetics, and even therapeutic

applications. This growing area of research emphasizes the versatility of essential oils, which are valued for their significant antibacterial properties and ability to impart a natural, long-lasting fragrance to fabrics. Studies have shown that essential oils like lavender, thyme, and clove are effective against bacteria such as *Staphylococcus aureus*, inhibiting their growth when integrated into textiles (El-Molla & El-Ghorab, 2022). This antibacterial property not only promotes hygiene by reducing microbial load but also extends the lifespan of textiles by preventing bacterial-induced degradation. These features are particularly advantageous in healthcare settings, sportswear, and everyday clothing, where hygiene and durability are critical.

In addition to antibacterial effects, essential oils enhance the sensory properties of textiles by providing a natural fragrance. Techniques such as embedding essential oils into β-cyclodextrin inclusion complexes have been proven to stabilize and prolong the scent on fabric surfaces (Farouk et al., 2022). Commonly used oils like lavender, lemon, rosemary, and salvia create an enduring fragrance that enhances the user experience. Such fragrance-enhanced textiles can also contribute to emotional well-being, aligning with the principles of aromatherapy by promoting relaxation and comfort during use.

The use of essential oils in textiles resonates with the rising consumer demand for sustainable and multifunctional products. Aromatherapy textiles, which incorporate essential oils, offer a unique combination of antimicrobial protection, fragrance, and therapeutic benefits (Mehta & MacGillivray, 2022). These products cater to eco-conscious consumers by providing an environmentally friendly alternative to synthetic chemicals, appealing to those seeking sustainable and health-conscious living solutions.

Beyond general clothing, essential oils are being explored for broader applications in the textile industry. For example, they are incorporated into finishing processes to impart both antibacterial properties and enduring fragrances to fabric substrates. Popular choices include lavender, lemon, rosemary, and salvia, known for their soothing aromas and antimicrobial activity. Additionally, essential oils are increasingly found in laundry products, offering a natural way to scent clothing with oils such as bergamot, peppermint, tea tree, eucalyptus, and grapefruit (Farouk et al., 2022; N. Singh et al., 2017).

This integration of essential oils into textiles represents a promising innovation, providing solutions that address both functional and sensory demands. By combining antibacterial

efficacy, therapeutic potential, and long-lasting fragrance, these textiles meet the dual goals of effectiveness and sustainability. As consumer interest in environmentally responsible and health-promoting products continues to grow, the use of essential oils in textiles is poised to play a transformative role in the future of the industry.

8. TOXICOLOGY AND SAFETY OF USE OF ESSENTIAL OILS

Essential oils, derived from plant materials through various extraction methods such as steam distillation and cold pressing, are widely used in aromatherapy, cosmetics, and food products for their aromatic and therapeutic properties. Although often promoted as "natural" and safer alternatives to synthetic chemicals, essential oils carry a range of potential risks and adverse reactions that need to be carefully considered. The growing popularity of essential oils has led to an increase in their use, but this has also raised concerns about their toxicity and safety profiles, particularly when misused or in individuals with certain sensitivities.

The adverse effects of essential oils have been well-documented in clinical and experimental studies. One of the most common side effects of essential oils is allergic contact dermatitis. This reaction is often observed when essential oils come into contact with the skin, and can result in redness, swelling, and itching. It has been reported that allergic reactions can be triggered by certain compounds in essential oils, including terpenes and phenols, which are known to be highly reactive. In some cases, these reactions may even escalate to systemic issues, such as headaches, dizziness, and nausea (Lis-Balchin, 2010). The severity of these reactions varies based on the oil, its chemical composition, and the individual's susceptibility (Pathoulas & Senna, 2024). Additionally, while essential oils are often considered safe when used appropriately, their overuse or improper application can lead to more serious consequences, such as respiratory distress or toxicity in internal organs.

In the United States, approximately 6 million people suffer from skin allergies to fragrances, a condition that has a significant impact on their quality of life (Lis-Balchin, 2010). Symptoms of fragrance allergies include dizziness, fatigue, and difficulty concentrating, in addition to respiratory issues such as shortness of breath. Fragrance materials, including essential oils, are readily absorbed through the skin and respiratory system, and once absorbed, they can cause systemic effects. The inhalation of fragrance compounds, in particular, is known to trigger a range of respiratory problems, from mild irritation to severe asthma attacks, particularly in sensitive individuals (Lis-Balchin, 2010). The American Lung Association recognizes perfumes and fragrances as triggers for asthma, and many fragrance materials are known to be respiratory irritants, although a few may act as sensitizers. Unfortunately, the vast majority of these materials have not been thoroughly evaluated for their effects on lung function, highlighting a significant gap in research.

Asthma, which affects a growing number of individuals worldwide, is a condition that is exacerbated by fragrances in some cases. Studies have shown that fragrances can make airways more susceptible to injury and allergens, and they may even trigger asthma in certain individuals. Moreover, some individuals may experience asthma-like symptoms even in the absence of bronchial obstruction, further complicating the issue. The use of fragrances in aerosol form can exacerbate these symptoms, causing respiratory issues that can range from mild irritation to acute asthma attacks (Carpena et al., 2021).

The difficulty in identifying the specific chemicals responsible for adverse reactions is another challenge. In many cases, the exact components of fragrances, including essential oils, are not disclosed due to trade secrets within the perfume and cosmetic industries. Furthermore, the diverse range of synthetic chemicals used in fragrances makes it challenging to pinpoint the exact cause of allergic reactions, as many of these materials may act synergistically or modify each other's effects when combined (Lysdal & Johansen, 2009).

8.1.**Mechanisms of Toxicity**

Essential oils, like other chemicals, exert their effects through complex mechanisms of action that can vary depending on the specific oil and the route of exposure. Inhalation is a common route of exposure, particularly in aromatherapy and other therapeutic applications. Upon inhalation, essential oils pass through the respiratory system and can reach the bloodstream, where they may exert systemic effects. For instance, some essential oils, such as cedarwood, pine, and juniper, can be toxic at high doses or when ingested, potentially causing acute kidney or liver failure (Lysdal & Johansen, 2009). While many essential oils are considered safe when used in moderation, their toxic potential increases with excessive exposure or improper usage.

The ability of essential oils to interact with various biological systems is also a key factor in their toxicity. For example, oils high in phenolic compounds, such as rosemary and sage, have been shown to exhibit pro-oxidant activity. This can lead to DNA damage and lipid peroxidation, processes that are associated with increased risk of cancer and other chronic diseases (Dosoky & Setzer, 2021). Ingestion of essential oils can also result in digestive system disturbances, such as nausea, vomiting, and diarrhea, particularly when consumed in excessive amounts.

8.2.**Special Populations and Precautions**

Certain populations may be more vulnerable to the toxic effects of essential oils. Babies, young children, and the elderly are particularly at risk, as their bodies may be less capable of detoxifying the compounds present in essential oils. Pregnant and breastfeeding women should exercise extra caution, as some oils may have teratogenic effects or affect milk production. As a result, strict adherence to guidelines on maximum doses and routes of administration is crucial for ensuring safety.

It is also important to consider the potential for essential oils to interact with other medications, including both conventional and herbal remedies. These interactions, although not fully understood, could modify the effects of essential oils or cause unintended side effects. For example, certain essential oils may interfere with the metabolism of drugs, potentially leading to either enhanced or diminished drug effects. This highlights the need for further toxicological research into the interactions between essential oils and medications, particularly for individuals who are undergoing treatment for chronic conditions.

The use of essential oils in beauty products is governed by safety and regulatory standards to ensure consumer protection. In the United States, the Food and Drug Administration (FDA) regulates essential oils used in foodstuffs, supplements, and cosmetics. Similarly, in the European Union, the European Directorate for the Quality of Medicines & HealthCare (EDQM) provides guidance on the quality and associated risks of essential oils in cosmetic products.

Although often perceived as safer alternatives to synthetic chemicals, essential oils are not without risks. Documented adverse reactions range from mild skin irritation to severe respiratory distress and organ toxicity in clinical and experimental contexts. With the growing use of essential oils across various products, there is a pressing need to better understand their potential toxicity and to implement precautions for safe use. While many essential oils are generally recognized as safe (GRAS) when used appropriately, gaps in knowledge remain—particularly concerning their interactions with other substances and their effects on vulnerable populations. Until further research addresses these gaps, users should adhere to established safety guidelines and seek professional advice, especially for therapeutic applications.

9. LIMITATIONS AND CHALLENGES IN THE USE OF ESSENTIAL OILS

The use of essential oils (EOs) across various fields such as food, medicine, cosmetics, and agriculture presents significant opportunities but also notable limitations and challenges. This overview elaborates on these aspects based on current research and applications.

9.1.**Limitations**

9.1.1. **Volatility and Stability**

High Volatility: EOs are characterized by their volatility, which can lead to a substantial loss of efficacy during storage and processing. This volatility makes it challenging to maintain consistent concentrations in formulations (Cimino et al., 2021; I. R. Singh & Pulikkal, 2022).

Sensitivity to Environmental Factors: Essential oils are sensitive to light, temperature, and oxygen, making them prone to degradation. Exposure to these elements can result in the loss of active compounds and overall effectiveness (Cimino et al., 2021; Ren et al., 2022).

9.1.2. **Low water solubility**

Hydrophobic Nature: EOs are inherently hydrophobic, which limits their application in aqueous systems unless appropriate carriers or emulsifiers are used. This characteristic poses challenges in formulating EOs for use in beverages or water-based products (Cimino et al., 2021; I. R. Singh & Pulikkal, 2022).

9.1.3. **Dosage and Safety concerns**

Potent Bioactivities: The strong bioactive properties of EOs can lead to cytotoxicity or allergic reactions if not used at safe concentrations. Determining the appropriate dosage for effectiveness while minimizing adverse effects remains a complex issue (Cimino et al., 2021; Żukowska & Durczyńska, 2024).

Regulatory Compliance: The lack of standardized guidelines for safe usage complicates the formulation process, particularly in consumer products where safety is paramount (I. R. Singh & Pulikkal, 2022).

9.1.4. Standardization issues

Variability in composition: The chemical composition of EOs can vary significantly due to environmental factors, seasonal changes, and geographical origins. This variability can hinder reproducibility and consistency in product formulations (I. R. Singh & Pulikkal, 2022).

9.1.5. High cost of extraction

Expensive extraction methods: Traditional extraction methods such as steam distillation and cold pressing are often costly and time-consuming. Additionally, limited availability of raw materials can further escalate costs (I. R. Singh & Pulikkal, 2022).

9.1.6. Regulatory barriers

Strict Regulations: Many countries impose stringent regulations that require comprehensive safety and efficacy testing before EOs can be marketed in food, cosmetics, or pharmaceutical products. This regulatory landscape can slow down innovation and market entry (I. R. Singh & Pulikkal, 2022).

9.2.Challenges

9.2.1. Lack of comprehensive research

Limited Clinical Trials: There is a scarcity of clinical trials and *in vivo* studies on the therapeutic applications of EOs, particularly in medical fields. This lack of data restricts their broader acceptance and utilization in healthcare settings (I. R. Singh & Pulikkal, 2022).

9.2.2. Resistance mechanisms in microbes

Microbial Resistance: The prolonged use of EOs as antimicrobial agents may lead to the development of resistance mechanisms in microbes. This is particularly concerning when EOs are used frequently or at sub-lethal concentrations (Cimino et al., 2021; Ren et al., 2022).

9.2.3. Delivery systems

Technical hurdles: Developing effective delivery systems that allow for controlled release and targeted action of EOs remains a significant challenge. Innovations in this area are crucial for maximizing the benefits of EOs while minimizing their limitations (Cimino et al., 2021; I. R. Singh & Pulikkal, 2022).

9.2.4. **Environmental impact**

Sustainability concerns: Overharvesting aromatic plants for EO production poses risks to biodiversity and can lead to ecological imbalances. Sustainable sourcing practices are essential to mitigate these impacts (I. R. Singh & Pulikkal, 2022).

9.2.5. **Consumer perception and education**

Public skepticism: There is often skepticism among consumers regarding the safety and efficacy of EOs compared to synthetic alternatives. Educating consumers about the benefits and proper use of EOs is vital for market acceptance (I. R. Singh & Pulikkal, 2022).

10. BOTANICAL AND PHYTOCHEMICAL PROFILE OF THE SELECTED PLANTS

10.1. **Lemongrass (*Cymbopogon citratus*)**

10.1.1. **Botanical description**

Taxonomy and Classification

The genus *Cymbopogon* comprises approximately 55 species. *Cymbopogon citratus* (DC. ex *Nees*) Stapf., commonly referred to as lemongrass, is one of the most recognized species within the genus. It is also known by several botanical synonyms, including *Andropogon citratus DC.* ex *Nees, Andropogon ceriferus Hack., Andropogon nardus subsp. ceriferus* L. *(Hack.) Hack., Andropogon roxburghii Nees ex Steud*., and *Cymbopogon nardus subvar. citratus* (L.) *Rendle (DC. ex Nees) Roberty* (Lawal et al., 2017).

Cymbopogon derives its name from the Greek terms "kymbe" (meaning boat) and "pogon" (meaning beard), which describe the distinct arrangement of its flower spikes (Shah et al., 2011). Widely recognized across different regions, *C. citratus* is known by various common names, reflecting its global significance. In English, it is referred to as West Indian lemongrass or lemongrass. In Spanish, it is called hierba limón or zacate de limón, while in French, it is known as citronelle or verveine des Indes. In China it's called xiang mao (CABI, 2022). In Arabic it is known as Laymūniyya (ليمونية) . The classification of *C. citratus* is as follows:

Kingdom	Plantae
Phylum	Angiosperms
Class	Monocots
Order	Poales
Family	Poaceae
Genus	*Cymbopogon*
Species	*Cymbopogon citratus*

Morphology

C. citratus leaves are strap-like, measuring between 0.5–1 inch (1.3–2.5 cm) in width and approximately 3 feet (0.9 m) in length. They possess gracefully drooping tips. These evergreen leaves exhibit a bluish-green coloration and emit a citrus aroma when crushed.

Figure 14: *C. citratus mature plant (POWO, 2025).*

The leaves exhibit a simple structure with an entire margin and a linear shape. They possess parallel venation, contributing to their uniform appearance. Most of the leaves emerge directly from the soil, typically without a stem. These leaves are fragrant and maintain their presence throughout the year, highlighting their persistence. With a blade length ranging from 18 to 36 inches and a green coloration, the leaves also display a showy appearance during the autumn season. Common lemongrass plants encountered in cultivation are usually cultivars and are unlikely to produce flowers. Flowering panicles are rarely observed. The inflorescences are 30–60 cm in length and have a nodding habit. The partial inflorescences are organized as paired racemes of spikelets that are subtended by spathes (Shah et al., 2011).

Figure 15: *Leaves of C. citratus (Shutes, 2025).*

Geographical distribution and Habitat

Cymbopogon citratus is indigenous to South and Southeast Asia, with India and Sri Lanka serving as its primary centers of origin. Over time, it has been extensively cultivated in tropical and subtropical regions worldwide. In Asia (India, Indonesia, Malaysia, Thailand, and the Philippines), in Africa (Nigeria, Madagascar, and Kenya) and in the Americas (Brazil, Mexico, and throughout the Caribbean). The species demonstrates remarkable ecological adaptability, thriving in diverse environments, including open grasslands, plantations, and household gardens (Lawal et al., 2017).

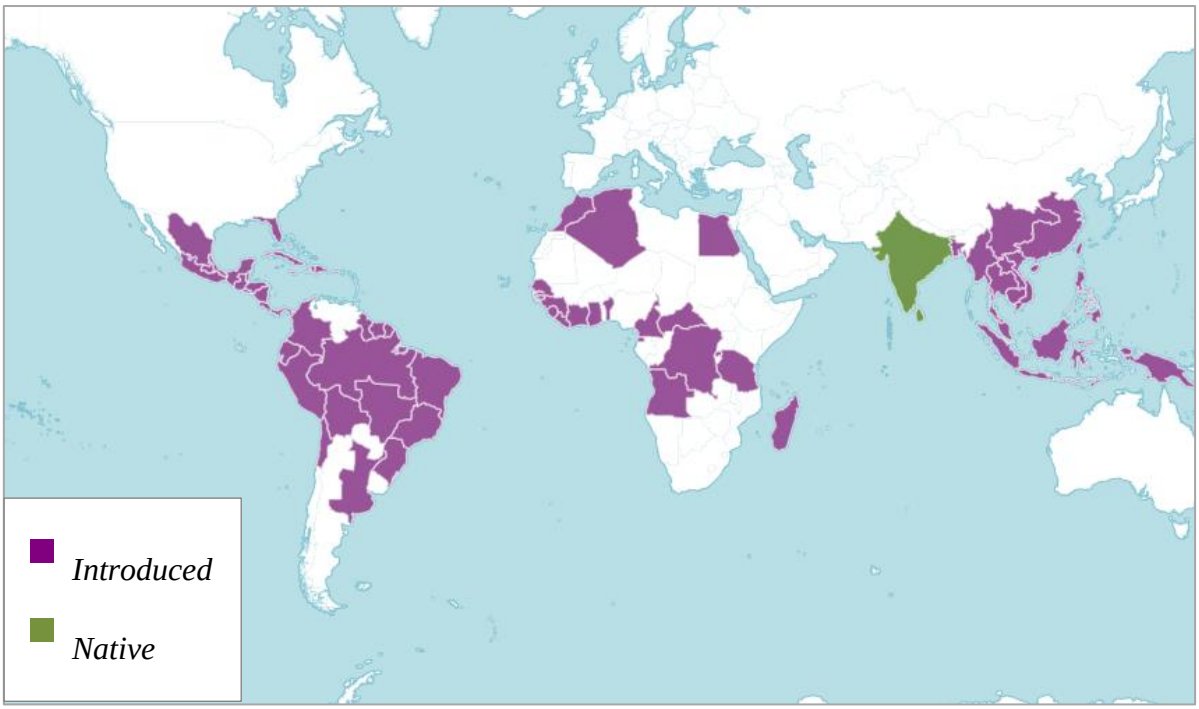

Figure 16: *Native and introduced regions of C. citratus (POWO, 2025).*

Ecological Adaptation and Growth Conditions

Cymbopogon citratus demonstrates exceptional ecological adaptability, enabling its cultivation in diverse environments. It thrives predominantly in tropical and subtropical climates, where temperatures range between 20°C and 30°C. The plant favors well-drained sandy loam soils with a pH range of 5.0 to 8.4 and can endure nutrient-poor soils, although it exhibits enhanced growth when supplemented with organic amendments. With moderate water requirements, it flourishes in regions receiving 750–2,500 mm of annual rainfall and shows considerable drought tolerance once fully established. Optimal growth and essential oil production are achieved under full sunlight conditions. Propagation is primarily conducted through vegetative methods, such as root division or stem cuttings, as seed propagation remains uncommon due to the plant's infrequent flowering. This resilience and adaptability make *Cymbopogon citratus* a versatile species suitable for a range of climatic and soil conditions (Skaria et al., 2012).

10.1.2. **Ethnobotanical and Traditional uses**

Lemongrass (*Cymbopogon citratus*) has a long-standing historical and cultural significance, particularly in traditional medicine and culinary practices. Native to South and Southeast Asia, it played an essential role in Ayurvedic and Traditional Chinese Medicine (TCM), where it was valued for its therapeutic properties in treating fevers, digestive ailments, and infections.

Historical records highlight its use in religious rituals, incense, and perfumery due to its refreshing citrus aroma, while its essential oil was traded along ancient spice routes, symbolizing wealth and utility in early economies. In countries such as India and Indonesia, lemongrass continues to represent purification and health in traditional ceremonies (S. Mukherjee et al., 2024).

Ethnobotanical applications of *Cymbopogon citratus* encompass a broad spectrum of uses, particularly for digestive health, where lemongrass infusions address indigestion, bloating, and gastric ulcers while stimulating appetite. It is also recognized as a natural antipyretic, known as "fever grass" in the Caribbean, where it is consumed to reduce fevers and alleviate respiratory infections due to its antimicrobial properties. Additionally, lemongrass leaves are applied topically as poultices to prevent infections and treat fungal conditions, while its essential oil is widely used as an insect repellent and antifungal agent. Lemongrass is also associated with mental health benefits, with infusions commonly used to relieve anxiety and insomnia due to their calming effects (Avoseh et al., 2015).

Beyond medicinal applications, it serves as a versatile culinary ingredient in Asian cuisines, adding a citrusy flavor to soups, teas, and curries. Furthermore, lemongrass infusions are employed as natural deodorants and disinfectants in households. Its widespread traditional use extends across regions, with Ayurvedic detoxification therapies in India, postpartum herbal wraps in Indonesia and Malaysia, herbal baths and hypertension remedies in Africa, and febrile treatments in the Caribbean and South America. Such extensive use across cultures underscores the plant's global relevance and its continued importance in both modern and traditional practices (Burt, 2004; Majewska et al., 2019; S. Mukherjee et al., 2024).

10.1.3. **Key phytochemicals**

The essential oil extracted from *Cymbopogon citratus* has garnered significant attention due to its rich phytochemical composition, predominantly comprising volatile compounds such as terpenoids, which are responsible for its distinct aroma and therapeutic properties. Among its key constituents, Citral, a mixture of Geranial and Neral, represents approximately 70–80% of the oil. This compound imparts the characteristic lemon fragrance and demonstrates potent antimicrobial, antifungal, and antioxidant activities, making it valuable in pharmaceuticals, food preservation, and perfumery (Shah et al., 2011).

Another notable compound is β-myrcene, accounting for 5–12% of the oil, which contributes to its anti-inflammatory and analgesic effects. Similarly, limonene, present in concentrations ranging from 1–4%, exhibits strong antioxidant and anticancer properties. Geraniol, comprising 2–4% of the oil, is recognized for its antifungal and sedative effects, while caryophyllene, although present in minor quantities, functions as a cannabinoid receptor agonist, providing anti-inflammatory benefits. In addition to these major components, the oil contains trace levels of α-pinene, linalool, and citronellal, which work synergistically to enhance the plant's biological activities (Shah et al., 2011).

10.1.4. **Biological and Medicinal properties**

The essential oil of *Cymbopogon citratus*, primarily composed of Citral, demonstrates broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as various fungi. Its mechanism of action involves disrupting microbial cell membranes, leading to cell death, making it an effective natural preservative. The oil also exhibits significant antioxidant activity, due to its phenolic compounds, flavonoids, and terpenoids, which scavenge free radicals and help mitigate oxidative stress linked to aging and chronic diseases (Boukhatem et al., 2014; Salaria et al., 2020; Viktorová et al., 2020).

Additionally, *Cymbopogon citratus* shows anti-inflammatory properties by inhibiting pro-inflammatory cytokines and enzymes involved in inflammation, suggesting its potential in managing conditions like arthritis and inflammatory bowel diseases. It also offers cytoprotective effects, protecting liver and colon cells from oxidative stress and toxins. Beyond these activities, the plant demonstrates anticancer potential by inducing apoptosis in cancer cells and antidiabetic properties by inhibiting enzymes that regulate blood sugar. Furthermore, it exhibits cardioprotective effects by improving lipid metabolism and preventing LDL cholesterol oxidation, as well as neuroprotective properties, potentially benefiting conditions like Alzheimer's disease (Kobenan et al., 2021; Madi et al., 2020; Onyedikachi et al., 2021; Salaria et al., 2020).

10.1.5. **Safety profile**

Cymbopogon citratus is generally recognized as safe (GRAS) by the U.S. FDA for use as a flavoring and medicinal agent. However, caution is advised for individuals with hypersensitivity or those using high doses of essential oil directly without dilution (FDA, 1985).

10.1.6. **Commercial and Industrial applications**

Cymbopogon citratus has established itself as a versatile resource with extensive applications across the food, cosmetic, agricultural, and emerging technology sectors. In the food industry, lemongrass is prized for its high Citral content, making it a widely used flavoring agent in teas, soups, curries, and desserts, particularly in Southeast Asian, African, and Latin American cuisines. Its essential oil is also incorporated into beverages, confectioneries, and baked goods, providing a natural citrus flavor. Beyond its culinary uses, lemongrass serves as a natural preservative due to its potent antimicrobial properties, which inhibit microbial growth in perishable foods such as meat, fish, and dairy. Effective against common foodborne pathogens like *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella spp*., it extends shelf life and enhances food safety. Additionally, lemongrass is utilized as a functional food ingredient in health drinks and fortified foods, leveraging its antioxidant, anti-inflammatory, and antimicrobial properties to promote wellness (Faheem et al., 2022; Majewska et al., 2019).

In the cosmetic and fragrance industries, lemongrass essential oil is a sought-after ingredient in skincare products, including creams, toners, and masks, valued for its astringent properties that tighten skin and reduce oiliness, as well as its antibacterial effects that combat acne and infections. Its soothing and hydrating characteristics make it a popular choice in natural formulations. The oil's refreshing citrus scent is also widely used in perfumes, aromatic oils, and aromatherapy products, often blended with complementary oils such as lavender and eucalyptus to alleviate stress, anxiety, and fatigue. Moreover, its antifungal properties make it a valuable component in shampoos and conditioners, promoting scalp health, reducing dandruff, and encouraging hair growth (De Andrade et al., 2023; Tran et al., 2021).

In agriculture, lemongrass essential oil serves as an eco-friendly pesticide, offering insecticidal activity against pests like mosquitoes, ticks, ants, and aphids through compounds such as Citral and Geraniol, which disrupt insect nervous systems and feeding behaviors. It also plays a role in post-harvest protection, where its vapors help prevent fungal contamination in stored crops, thereby extending the shelf life of grains, fruits, and vegetables. Furthermore, lemongrass extracts act as plant growth enhancers, stimulating seed germination and improving plant vigor when used as biofertilizers, thus promoting sustainable agricultural practices (Aćimović et al., 2020).

Emerging applications highlight lemongrass’s growing relevance in modern industries. It is being explored for biodegradable antimicrobial packaging materials designed to inhibit

microbial growth on food surfaces, contributing to eco-friendly food preservation. In veterinary medicine, lemongrass is added to animal feed to boost immunity and control infections, as well as to formulations for managing ticks and ectoparasites in livestock and poultry. Additionally, its biomass shows promise as a renewable energy source, particularly for bioethanol and biodiesel production (Valková et al., 2022).

Economically, *Cymbopogon citratus* continues to contribute significantly to global markets, especially in countries such as India, Brazil, Indonesia, and Africa, where its cultivation and essential oil production drive agricultural and industrial growth. The rising global demand for lemongrass essential oil underscores its importance as a sustainable and multifunctional resource across industries (Mwithiga et al., 2024).

10.1.7. **Sustainability and Conservation status**

Cymbopogon citratus (Lemongrass) is not classified as endangered by the IUCN and is widely cultivated in tropical and subtropical regions for its culinary and medicinal uses (IUCN, 2016). As a domesticated species, it faces minimal pressure on wild populations, although localized threats from unsustainable practices and habitat conversion remain concerns. Sustainable farming techniques, including intercropping, organic methods, rainwater harvesting, and crop rotation, promote soil health and biodiversity. Low-input agriculture and repurposing biomass reduce environmental impact, while genetic conservation through seed banking enhances resilience (R. Verma, 2024).

Although largely cultivated, excessive harvesting in wild habitats may cause biodiversity loss, soil degradation, and ecosystem imbalances. Key approaches include promoting small-scale farming, establishing sustainable harvesting guidelines, and investing in research for pest-resistant varieties. Policies encouraging sustainable practices and farmer education further support conservation efforts (R. Verma, 2024).

10.2. **Peruvian pepper tree (*Schinus molle*)**

10.2.1. **Botanical description**

Taxonomy and Classification

Schinus molle belongs to the family Anacardiaceae, which includes economically and ecologically important species such as cashews (*Anacardium occidentale*) and poison ivy (*Toxicodendron radicans*). Classified as a distinct species, *S. molle* has retained its original botanical name since its first description by Linnaeus in 1753. Synonyms for the species are rare, with *Schinus areira* occasionally cited as an alternative name, a botanical variety, or even a cultivar. Additionally, three other varieties—*S. molle var. argentifolius*, *S. molle var. hassleri*, and *S. molle var. rusbyi*—have been reported. However, their taxonomic status remains uncertain (CABI, 2019).

The genus name *Schinus* is derived from the Greek word shinos, referring to the mastic tree (*Pistacia lentiscus*), a related species that shares morphological similarities. The specific epithet *molle* is attributed to the Quechua word mulli, which denotes the tree in its native Peruvian context (CABI, 2019).

The species is widely recognized under different common names across various languages. In English, it is called the Peruvian pepper tree, Brazilian pepper tree, and false pepper tree. Spanish speakers refer to it as aguaribai or false pimiento, while it is known as faux poivrier or poivrier d'Amérique in French and aroeira-do-matto in Portuguese (CABI, 2019). In Arabic it is known as Al-Felfel Az-Zaïf (الفلفل الزائف). The classification of *S. molle* is as follows:

Kingdom	Plantae
Phylum	Angiosperms
Class	Eudicots
Order	Sapindales
Family	Anacardiaceae
Genus	*Schinus*
Species	*Schinus molle*

Morphology

Schinus molle is a deciduous tree or shrub that can grow up to 10 meters in height. The species exhibits several distinctive morphological characteristics.

Figure 17: *Schinus molle mature tree (CABI, 2019).*

The leaves are pinnately compound, ranging from 20 to 35 cm in length, with 11 to 41 leaflets. These leaflets are lanceolate, finely serrated, and arranged alternately along the leaf stalk. When crushed, the leaves release a characteristic aromatic scent. The flowers of *Schinus molle* are small, typically pink to white in color, and are arranged in panicles. Blooming from spring to early summer, the flowers possess five petals and are primarily pollinated by insects. The tree produces small, round, red fruits that resemble berries and contain a single seed. These fruits are of significant value in culinary and medicinal contexts, often utilized for their aromatic and therapeutic properties (Garzoli et al., 2019; Machado et al., 2019).

Figure 18: *S. molle fruits and foliage (CABI, 2019).*

Geographical Distribution and Habitat

Schinus molle is native to the subtropical regions of South America, primarily in the Andean foothills and the dry valleys of Peru, Chile, and Argentina. It has since spread to tropical and subtropical regions worldwide, including Africa, the Mediterranean, and the southwestern United States. The plant thrives in a variety of environments, from arid, rocky soils to more fertile, irrigated areas (Razzak et al., 2023).

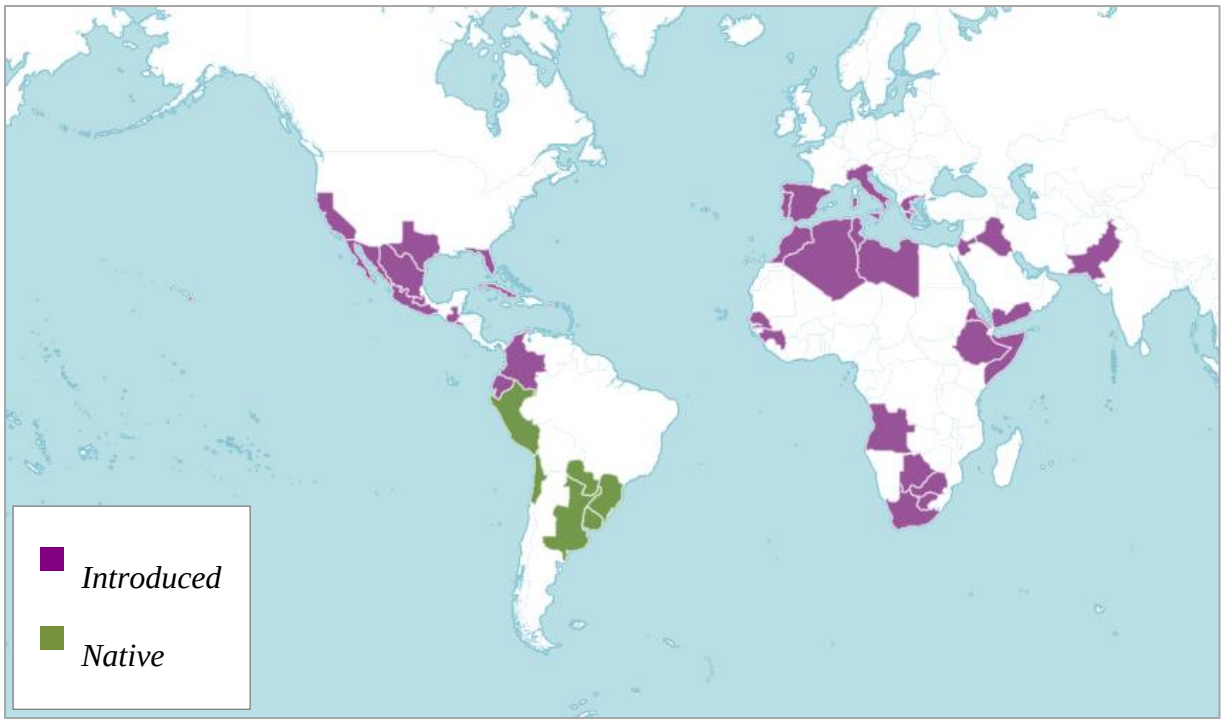

Figure 19: *Native and introduced regions of S. molle (POWO, 2025b).*

Ecological Adaptation and Growth Conditions

Schinus molle is highly adaptable, tolerant of dry conditions, and resistant to environmental stress. It can grow in sandy, clayey, or rocky soils and is resistant to both drought and low temperatures. This plant is known for its ability to fix nitrogen, making it useful for improving soil quality in degraded areas. It grows best in full sunlight and requires moderate to low water levels, making it ideal for xerophytic habitats (Orwa et al., 2009).

10.2.2. **Ethnobotanical and Traditional Uses**

Schinus molle, known for its medicinal and spiritual significance, has been an integral part of indigenous cultures in South America for thousands of years. Particularly in Peru and Chile, the plant is highly regarded not only for its healing properties but also for its role in cultural and spiritual rituals. The leaves, bark, and fruits of *Schinus molle* are traditionally used in folk medicine, where they are believed to possess protective and purifying qualities. Indigenous peoples have long associated the plant with rituals aimed at safeguarding health, ensuring well-being, and providing spiritual protection (Guala et al., 2016; Valdez, 2019).

In traditional medicine, *Schinus molle* has been used to address a variety of health conditions. Its fruits and leaves are commonly employed as remedies for gastrointestinal issues, such as dysentery and diarrhea. The leaves are also applied topically to alleviate pain from headaches,

muscular aches, and other body pains. The bark of *Schinus molle* is often used in decoctions to treat inflammatory conditions like arthritis and rheumatism, showcasing the plant's broad anti-inflammatory potential. Additionally, the plant's antimicrobial properties make it valuable for wound healing, where its extracts are used to treat infections and prevent further complications (Taylor, 2005).

The indigenous knowledge surrounding *Schinus molle* is rich and complex, with this plant being used in combination with other traditional remedies. In the Andean region, the resin of *Schinus molle* is used to make incense, which is burned during purification rituals, believed to cleanse both individuals and their surroundings. This practice underscores the spiritual value of the plant, which is considered a means of enhancing well-being and maintaining balance. *Schinus molle* is also featured in local culinary traditions, where its aromatic fruits are used as a spice to flavor dishes, highlighting its versatility beyond medicinal uses. Through its many applications, *Schinus molle* continues to play a crucial role in the daily lives of indigenous communities (Guala et al., 2016; Ravindran, 2017).

10.2.3. **Key Phytochemicals**

Schinus molle essential oil is composed of several bioactive phytochemicals, with key components including limonene, α-pinene, and α-phellandrene. Limonene, a monoterpene, is known for its strong citrus aroma and is widely studied for its antimicrobial, antioxidant, and anti-inflammatory properties. It is effective against a range of pathogens, including Gram-positive and Gram-negative bacteria. α-Pinene, another prominent monoterpene, contributes to the oil's distinctive pine-like fragrance and exhibits significant anti-inflammatory, antimicrobial, and bronchodilator effects. It also plays a role in enhancing cognitive function and promoting respiratory health. α-Phellandrene, a lesser-known monoterpene, adds to the oil's citrusy scent and enhances its antimicrobial and antioxidant activities. This compound has been shown to possess antibacterial and antifungal properties, particularly against pathogenic microorganisms such as *Candida* species. Collectively, these key phytochemicals contribute to the essential oil's broad-spectrum bioactivity, making *Schinus molle* oil valuable in industries ranging from food preservation to cosmetics and pharmaceuticals (Kouachi et al., 2024).

10.2.4. **Biological and Medicinal Properties**

Schinus molle has demonstrated significant antimicrobial properties against a wide range of pathogens, including both bacteria and fungi. Its essential oil, containing key compounds such

as limonene and α-pinene, has been found to effectively inhibit the growth of Gram-positive and Gram-negative bacteria, as well as fungal species like *Candida albicans*. This antimicrobial activity positions *Schinus molle* as a promising natural alternative to synthetic antimicrobial agents. Additionally, the plant exhibits strong antioxidant properties, attributed to its rich content of flavonoids and phenolic compounds, which help neutralize harmful free radicals and protect cells from oxidative stress and damage (Bouhenna et al., 2021; Martins et al., 2014; Rouibi et al., 2010).

Beyond its antimicrobial and antioxidant effects, *Schinus molle* is recognized for its potent anti-inflammatory actions. Extracts and essential oils from the plant have been shown to inhibit pro-inflammatory mediators, including TNF-α and IL-6, which play a crucial role in inflammatory responses. Furthermore, *Schinus molle* has cytoprotective effects, promoting the survival of healthy cells under conditions of stress. The plant also exhibits analgesic properties, effectively reducing pain associated with conditions such as arthritis and musculoskeletal disorders. These diverse bioactivities highlight the therapeutic potential of *Schinus molle* in treating various health conditions (N. Aboalhaija et al., 2021; N. H. Aboalhaija et al., 2019; Feriani et al., 2020, 2021).

10.2.5. **Safety Profile**

Schinus molle is generally recognized as GRAS (Generally Recognized as Safe) by the FDA, (1985). Although toxicological studies are limited, available research indicates the plant is safe at appropriate doses, with low acute toxicity observed in animal models. However, caution is advised for pregnant or lactating women due to insufficient safety data for these populations.

10.2.6. **Commercial and Industrial Applications**

Schinus molle, commonly known as the Peruvian pepper tree, has a wide range of commercial and industrial applications due to its beneficial properties. One of the key areas of use is the extraction of its essential oil, which is highly valued in the fragrance and cosmetics industries. The oil is known for its pleasant, spicy aroma and is incorporated into perfumes, soaps, lotions, and skincare products. Its antimicrobial and antioxidant properties further enhance its appeal, offering not only a pleasing scent but also skin-protecting benefits. In addition to its essential oil, *Schinus molle*'s extracts are utilized in herbal supplements, teas, and topical applications, thanks to their anti-inflammatory, analgesic, and healing properties (Ravindran, 2017).

In the culinary world, *Schinus molle* is utilized as a spice in several South American cultures. The tree's peppery fruits are dried and ground into a seasoning, which is used to flavor meats, sauces, and traditional dishes. In some regions, it is also employed to flavor fermented beverages, further demonstrating its versatility in food preparation (Razzak et al., 2023).

Schinus molle's value extends beyond its medicinal and culinary uses into the field of agriculture. The plant is frequently incorporated into agroforestry systems, where it plays a vital role in soil restoration. As a nitrogen-fixing species, it is used to improve soil fertility in degraded lands. Additionally, its ability to act as a natural windbreak and its aesthetic appeal make it a popular choice in landscaping and urban greening projects, contributing to environmental sustainability (Ravindran, 2017).

10.2.7. **Sustainability and Conservation Status**

Schinus molle, commonly known as the Peruvian pepper tree, is not classified as endangered or threatened by the International Union for Conservation of Nature (IUCN, 2016). It is widely cultivated across various regions, including its native South America, the Mediterranean, Africa, and the southwestern United States. While it is not at significant risk of extinction, it can become invasive in non-native areas, where it may disrupt local ecosystems by outcompeting native species.

Sustainable cultivation practices of *Schinus molle* focus on maintaining ecological balance while maximizing the plant’s benefits. These include agroforestry and soil restoration through its nitrogen-fixing properties, low water consumption once established, and organic farming practices that avoid synthetic fertilizers and pesticides. Additionally, controlled harvesting in plantations can prevent overexploitation of wild populations. In non-native regions, management strategies are essential to prevent *Schinus molle* from becoming invasive, and maintaining biodiversity in surrounding ecosystems is crucial (Orwa et al., 2009).

Despite its resilience, overharvesting of *Schinus molle*, especially for its fruits and bark in the essential oil industry, could lead to resource depletion, habitat degradation, and reduced genetic diversity. To mitigate these impacts, conservation strategies include expanding cultivation in controlled environments, establishing sustainable harvesting regulations, and promoting public awareness about sustainable practices. These efforts ensure that *Schinus molle* can remain a valuable resource without compromising biodiversity or environmental health.

10.3. **Felty germander (*Teucrium polium*)**

10.3.1. **Botanical Description**

Taxonomy and Classification

The genus *Teucrium* is classified under the Lamiaceae family, it represents the second-largest genus within the subfamily, exhibiting a subcosmopolitan distribution and comprising 434 recognized taxa (Navarro, 2020). According to international botanical classifications, the genus has been divided into six sections encompassing forty-nine species. Notable examples include *Teucrium arduini* L., which is assigned to the *Stachyobotrys* section; *Teucrium chamaedrys* L., representing the *Chamaedrys* section; *Teucrium scordium* L., categorized within the *Scorodonia* section; and *Teucrium polium* L. and *Teucrium montanum* L., which belong to the *Polium* section. While most members of this genus are perennial herbs, subshrubs, or shrubs, *Teucrium botrys* L. is an exception, being an annual herbaceous species (Jaradat, 2015).

Teucrium polium L. is commonly referred to by various vernacular names, reflecting its widespread recognition. In Arabic, it is known as Jaäda (الجعدة), while in French, it is called pouliot de montagne, and germandée tomenteuse. English designations include golden germander, felty germander, germander, mountain germander, and cat thyme. In Italian, it is termed camendrio di montagna, polio, polio primo, and timo bianco, whereas in German, it is identified as poleigamander and berggamander (Jaradat, 2015). The classification of *T. polium* is as follows:

Kingdom	Plantae
Phylum	Angiosperms
Class	Dicotyledons
Order	Lamiales
Family	Lamiaceae
Genus	*Teucrium*
Species	*Teucrium polium*

Morphology

Figure 20: *T. polium mature plant (Dönmez et al., 2024).*

The leaves of *Teucrium polium* are small, with an ovate to lanceolate shape, and are densely covered with silvery-gray trichomes that impart a woolly texture. The leaf margins are either entire or slightly serrated, and the leaves emit an aromatic fragrance when crushed. The flowers are small, tubular, and exhibit a pale-yellow to white coloration, typically arranged in terminal spikes or dense clusters. These flowers are characterized by a two-lipped corolla, which is a distinctive feature of the Lamiaceae family. The stems are erect or ascending, woody at the base and herbaceous toward the apex. They are covered with soft hairs, which contribute to the plant's resistance to drought. *Teucrium polium* has a deep, fibrous root system, which is essential for water absorption, particularly in arid environments (Navarro, 2020; Özcan, 2020).

Figure 21: *T. polium flowers (Dönmez et al., 2024).*

Geographical Distribution and Habitat

The Mediterranean region and its surrounding floristic areas serve as the primary center of diversity for the genus *Teucrium*, with approximately 90% of the species found globally occurring in this region. *Teucrium polium* is widely distributed across the Mediterranean Basin, North Africa, the Middle East, and parts of Europe. It is commonly found in dry, rocky, and semi-arid environments, thriving particularly in sandy or calcareous soils. This species grows in open fields, along roadsides, and in low scrublands, at altitudes ranging from sea level up to 2000 meters (Navarro, 2020).

Figure 22: *Native and introduced regions of T. polium (POWO, 2025c)*

Ecological Adaptation and Growth Conditions

Teucrium polium exhibits notable drought resistance, having adapted to arid and semi-arid climates through the presence of trichome-covered leaves that minimize water loss. The species prefers well-drained soils, particularly sandy to calcareous types, but is also capable of tolerating nutrient-poor conditions. *Teucrium polium* thrives in full sunlight, although it can also survive in partial shade. Additionally, it demonstrates a high degree of temperature tolerance, withstanding a broad temperature range that spans from mild winters to hot summers (Dönmez et al., 2024; Pavlova et al., 2020).

10.3.2. **Ethnobotanical and Traditional Uses**

Teucrium polium has a rich history of use in traditional medicine, particularly in the Mediterranean and North African regions, where it has been valued since antiquity. Ancient cultures, including those of Greece, Egypt, and Rome, utilized the plant for its medicinal properties, with notable figures such as Dioscorides and Theophrastus documenting its effectiveness in treating gastrointestinal disorders and as an antidote for poisons. The genus name "*Teucrium*" is believed to honor Teucer, the legendary founder of Salamis, renowned for his knowledge of medicinal plants. The common name "golden germander" likely refers to its yellowish flowers and its historical use in early herbal remedies (Jarić et al., 2020; Meguellati et al., 2019).

In traditional medicine, *Teucrium polium* has been widely used for a range of ailments. It has been employed as a remedy for digestive issues, such as indigestion, bloating, and gastrointestinal cramps, acting as a carminative and antispasmodic to alleviate stomach discomfort. The plant has also been used to treat inflammatory conditions, including arthritis, skin irritations, and wounds, and is valued for its potential antioxidant and antimicrobial properties, often prepared as teas or tinctures. In addition, *Teucrium polium* has been included in detoxifying formulas for its hepatoprotective effects and used in folk medicine as a mild expectorant for respiratory health issues like coughs and colds. The plant also holds anthelmintic properties, serving as a remedy for intestinal worms in some cultures (Jarić et al., 2020; Senouci et al., 2019).

Beyond its medicinal applications, *Teucrium polium* has culinary uses, especially in North African cuisine, where it is added as a spice to impart a mild bitterness to stews, salads, and meat dishes, often in combination with other herbs to enhance flavor (Jarić et al., 2020)

Indigenous communities in the Mediterranean region have long relied on traditional knowledge passed down through generations, using *Teucrium polium* as a local remedy for common ailments. This knowledge remains deeply embedded in the cultural practices of rural populations, with the plant being either harvested from the wild or cultivated for personal and community use. Local herbalists or healers play a vital role in preserving these traditions, preparing infusions, poultices, or oils derived from the plant for therapeutic purposes. The indigenous understanding of the plant's medicinal properties has formed the basis for much of the current research into its bioactive compounds, highlighting the importance of preserving and validating traditional herbal practices (Meguellati et al., 2019).

10.3.3. **Key Phytochemicals**

The essential oil of *Teucrium polium* is highly valued for its potent biological activities, which are primarily attributed to its diverse phytochemical composition. Among the key compounds identified in the oil are α-Fenchene, a monoterpene known for its antimicrobial and antifungal properties, and α-Citral, which contributes a citrus-like aroma and exhibits both anti-inflammatory and antimicrobial effects. β-Citral, often found alongside α-Citral, enhances the oil's bioactivity, particularly in terms of its antimicrobial action. Additionally, Germacrene D, a sesquiterpene, offers potential antifungal and anti-inflammatory benefits, while Limonene, a terpene, is recognized for its antioxidant, anti-inflammatory, and anticancer properties. Collectively, these compounds play a central role in the plant's reputation for its antimicrobial,

anti-inflammatory, and antioxidant effects, which are integral to its traditional medicinal uses (Grafakou et al., 2020).

10.3.4. **Biological and Medicinal Properties**

Research has established that *Teucrium polium* exhibits notable antimicrobial activity, demonstrating effectiveness against a wide range of pathogenic microorganisms, including both bacteria and fungi. Studies have emphasized its broad-spectrum antimicrobial action against both Gram-positive and Gram-negative bacteria. Additionally, the plant's antioxidant properties are attributed to its high phenolic content, which plays a key role in scavenging free radicals and mitigating oxidative stress, a contributing factor in many chronic diseases (Belmekki et al., 2013; Hechachna et al., 2023; Saleh et al., 2020a).

Beyond its antimicrobial and antioxidant properties, *Teucrium polium* also displays significant anti-inflammatory, cytoprotective, and potential anticancer activities. The plant has been shown to inhibit the production of pro-inflammatory cytokines, which could make it beneficial for managing inflammatory conditions such as arthritis. Furthermore, certain extracts of *Teucrium polium* have been reported to offer cytoprotective benefits, protecting cells from damage caused by oxidative stress or toxic agents. Preliminary studies also suggest that the plant may possess anticancer potential, particularly in inhibiting cell proliferation in various cancer cell lines. Additionally, *Teucrium polium* is sometimes used in traditional medicine for its analgesic properties, providing relief for conditions such as headaches and musculoskeletal pain (Benchikha et al., 2022; Bendjabeur et al., 2018; J. Sharifi-Rad et al., 2019).

10.3.5. **Safety Profile**

Teucrium polium is generally regarded as safe for short-term use in moderate doses, its potential toxicity at higher concentrations, particularly with prolonged use or in sensitive individuals, warrants careful consideration. More extensive clinical studies and human trials are needed to fully understand its pharmacokinetics and long-term safety profile (Bahramikia & Yazdanparast, 2012).

10.3.6. **Commercial and Industrial Applications**

Teucrium polium has diverse commercial and industrial applications, particularly in the food, cosmetic, fragrance, and agricultural sectors. In the food industry, its aromatic compounds, especially terpenes like Limonene and Citral, impart a citrusy fragrance, making it a valuable

natural flavoring agent for a variety of products, including beverages, sauces, and confectionery. Additionally, the plant's antimicrobial properties offer promising potential as a natural preservative, aiding in food safety and extending shelf life, particularly in organic and natural food products (Estrada-Castillón et al., 2018; Maccioni et al., 2007).

In the cosmetic and fragrance industries, *Teucrium polium* essential oils are prized for their bioactive properties, such as antimicrobial and antioxidant effects, which make them beneficial in skincare products. These oils are incorporated into creams, lotions, and ointments for their anti-inflammatory and protective qualities, helping to maintain skin health and prevent premature aging. Furthermore, the plant is used in perfumes and body sprays for its subtle floral and citrus-like fragrance, which blends well with other notes (Bahramikia et al., 2022; Hamdy A, 2023).

In agriculture, *Teucrium polium* shows promise as a natural pesticide and growth enhancer. The plant's essential oils possess insecticidal properties, effective against pests like aphids and ants, making it a suitable alternative for organic farming and integrated pest management systems. Additionally, some studies suggest that *Teucrium polium* may improve plant growth, especially in drought-prone areas (Ebadollahi & Taghinezhad, 2020; Ravan et al., 2019).

10.3.7. **Sustainability and Conservation Status**

Teucrium polium is not currently classified as endangered or threatened, with a wide distribution across the Mediterranean, Middle Eastern, and North African regions (IUCN, 2016). However, like many wild plant species, it faces significant pressures from habitat destruction, overharvesting, and climate change. While it is cultivated in certain areas for medicinal purposes, wild populations may be at risk in some regions due to unsustainable harvesting practices.

Sustainable cultivation of Teucrium polium can be achieved through proper management practices. Incorporating the plant into agroforestry systems, where it is grown alongside other crops, can enhance biodiversity and reduce the pressure on wild populations. Additionally, its ability to thrive in poor soils makes it an excellent candidate for low-input farming systems, which minimize the need for fertilizers and water, further promoting sustainability (Dönmez et al., 2024).

Overharvesting of wild populations presents a potential threat to the plant's survival, particularly if harvested unsustainably. To mitigate this risk, conservation strategies should include regulating harvesting by setting limits on the amount of wild Teucrium polium collected each year. Promoting the cultivation of the plant for both commercial and medicinal use can also reduce the pressure on wild populations. Furthermore, restoration projects aimed at rehabilitating degraded habitats where the plant once thrived are essential for ensuring its long-term survival.

METHODOLOGY

1. RESEARCH DESIGN

1.1. **Research Approach**

A quantitative research approach was adopted in this study to objectively analyze and measure the biological and chemical properties of essential oils extracted from three Algerian medicinal plants. The focus was on obtaining numerical data through analytical techniques and *in vitro* assays, allowing for precise, statistically validated results regarding antioxidant, anti-enzymatic, anti-inflammatory, and antimicrobial activities. This quantitative approach was chosen to ensure reliable, reproducible data, essential for establishing the bioactivity of these essential oils.

1.2. **Study Design**

The study employed an experimental research design. After collecting and drying the plants, essential oils were extracted using a Clevenger-type apparatus through hydrodistillation. Following extraction, essential oils underwent GC-MS and GC-FID analysis to establish their chemical composition. For the biological assays, a series of *in vitro* tests (antioxidant, anti-enzymatic, anti-inflammatory, antimicrobial, and toxicity tests) were performed to assess each oil's efficacy and safety. Statistical analysis via ANOVA was conducted to analyze the data and validate the findings.

2. MATERIALS AND METHODS

2.1.Sample Preparation

2.1.1. Collection of Plant Sample

The plants examined in this research were sourced from three provinces in Algeria (Fig.23) in October 2022.

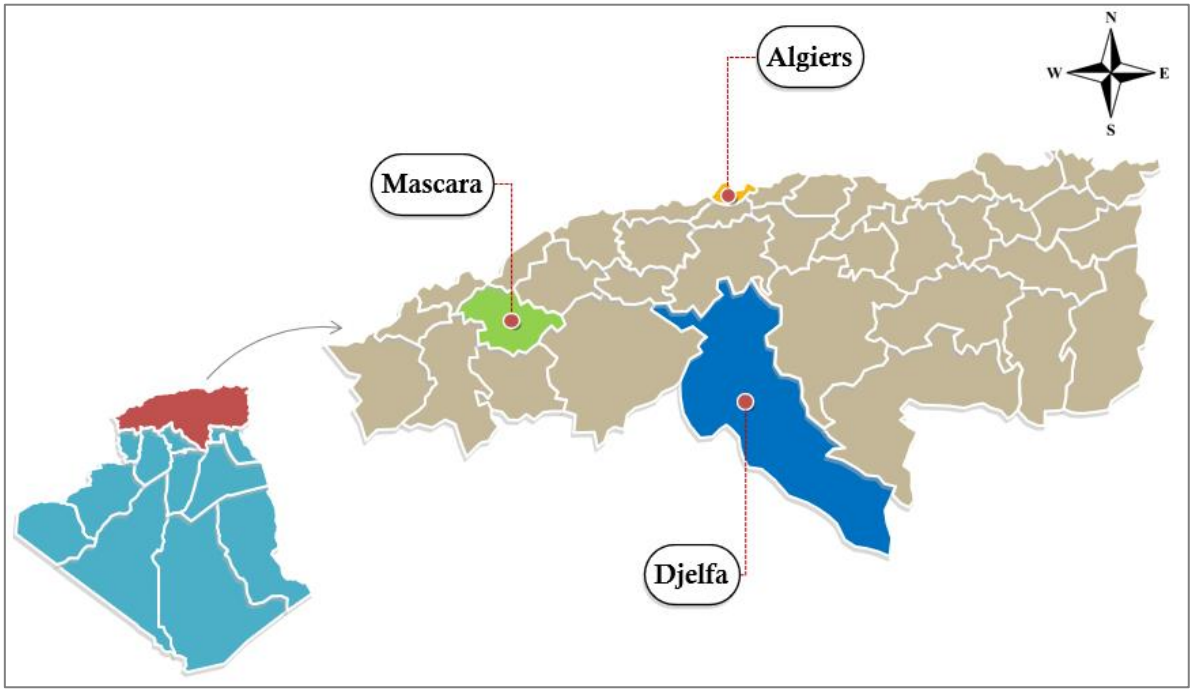

Figure 23: *Geographic map representing the different harvest stations: Algiers, Djelfa and Mascara.*

Cymbopogon citratus was acquired from a plant nursery in Birtouta, Algiers, whereas *Teucrium polium* was obtained from a local herbalist who collected it from the Senalba forest in Djelfa. The leaves of *Schinus molle* were gathered from the trees located in the dormitory garden of Mascara University *(Table 06).*

Table 03: *Cymbopogon citratus, Schinus molle and Teucrium polium harvest locations.*

Plant species	Province	Harvest station	Location
Cymbopogon citratus	Algiers	Plant nursery in Birtouta	36°38'25.8"N 2°59'02.6"E
Schinus molle	Mascara	Mascara University's dormitory garden	35°24'15.5"N 0°07'11.2"E
Teucrium polium	Djelfa	Senalba forest	34°38'47.3"N 3°10'50.9"E

2.1.2. Preparation of Plant Sample

The plant species were identified and authenticated by Prof. Benarba Bachir from Mascara University, Algeria. The voucher specimens have been deposited at the research laboratory of Mascara University. The leaves were carefully cleaned and dried in the shade for ten days at ambient temperature (25 ± 2 °C). Immediately prior to the extraction process, the aerial parts of the three plants were carefully cut into small pieces to facilitate the hydrodistillation procedure.

2.2.Essential Oil Isolation

A Clevenger-type apparatus was employed for the extraction of essential oils via hydro-distillation. After a 4-hour extraction, the essential oils and hydrolate were separated through decantation, as described by Öztürk et al., (2009). Subsequently, the essential oil was dehydrated with anhydrous sodium sulfate (Na_2SO_4) and stored in sterile amber glass vials at 4°C until further analysis. The essential oil yield was determined using the following formula:

$$\text{Yield of essential oil (\%)} = \frac{\text{Essential oil obtained (g)}}{\text{Raw materials used (g)}} \times 100$$

2.3.Phytochemical Analysis

2.3.1. GC-FID and GC/MS Analysis

The composition of the essential oil was analyzed using both GC-FID and GC-MS techniques, following the protocol outlined by Alfred Ngenge et al., (2021). For the gas chromatography (GC) analysis, a nonpolar fused silica capillary column (Rxi-5MS, Restek)

was employed, with dimensions of 30 meters in length, 0.25 millimeters in internal diameter, and a 0.25-micron film thickness. A Flame Ionization Detector (FID) was used for detection. The injector temperature was set at 250°C, while the detector was maintained at 270°C. Helium (He) was used as the carrier gas, flowing at 1.4 mL/min, with an injection volume of 0.2 µL and a split ratio of 20:1. The oven was initially set at 60°C for 5 minutes, then increased to 240°C at a rate of 4°C per minute, and maintained at 240°C for an additional 10 minutes. The percentage composition of the essential oil was determined using the GC10 GC computer program. Qualitative analysis of the components was also performed by the peak superimposition method, using reference standards where applicable. All analyses were conducted in triplicate, and the data were averaged to obtain mean values.

For the GC-MS analysis, a nonpolar fused silica capillary column (Rxi-5MS, Restek) was coupled with an ion trap mass spectrometer. The column dimensions were the same as in the GC-FID analysis (30 meters in length, 0.25 millimeters in internal diameter, and 0.25-micron film thickness), and helium was again used as the carrier gas at a flow rate of 1.4 mL/min. The oven temperature program was identical to that used for GC-FID analysis. The injector and transfer line temperatures were set at 220°C and 290°C, respectively, while the ion source was kept at 200°C. A 0.2 µL sample was injected using a 1:20 split ratio. Electron ionization (EI) was performed at 70 eV, and the mass spectrum was recorded in the range of 28–650 mass-to-charge (m/z), with a scan duration of 0.5 seconds and an inter-scan latency of 0.1 seconds.

Component identification was based on GC retention indices, which were calculated using a series of C_7-C_{30} alkanes (Supelco), and validated through computer matching with the Wiley, NIST14, and TRLIB libraries. Additionally, the fragmentation patterns of the mass spectra were compared with literature data (Adams, 2007). Quantification of the essential oil constituents was carried out using internal normalization. Co-injection with authentic compounds was employed to enhance the accuracy of identification when possible.

2.4. **Biological Activity Assays**

2.4.1. **Antioxidant Activities**

β-Carotene/linoleic acid assay

The assessment of lipid peroxidation inhibitory activity followed the β-carotene/linoleic acid test system outlined by Çayan et al., (2019). A mixture containing 0.5 mg β-carotene in 1 mL of chloroform, 25 µL linoleic acid, and a 200 mg Tween 40 emulsifier was prepared. After

evaporating the chloroform under vacuum, 100 mL of distilled water saturated with oxygen was added by vigorous shaking. A 160 µL solution of β-carotene–linoleic acid was combined with 40 µL samples at different concentrations. Zero-time absorbance was measured at 470 nm using a 96-well microplate reader immediately after adding the emulsion to each tube. The emulsion's absorbance was measured again at the same wavelength after incubating the plate for 2 hours at 50ºC. Absorbance measurements continued until the color of β-carotene disappeared. BHA and α-tocopherol served as antioxidant standards for activity comparison. The same procedure was repeated with used antioxidant standards and a blank. The bleaching rate (R) of β-carotene was calculated according to Eq. (1).

$$R = \frac{\ln \frac{a}{b}}{t} \qquad (1)$$

Where: ln = natural log, a = absorbance at time zero, and b = absorbance at time t (120 min).

The calculation of antioxidant activity was expressed as a percentage of inhibition in comparison to the control, utilizing Eq. (2).

$$\text{Antioxidant activity } (\%) = \frac{\text{R control} - \text{R sample}}{\text{R control}} \times 100 \qquad (2)$$

DPPH free radical-scavenging assay

The spectrophotometric determination of free radical scavenging activity was conducted following the method given by Çayan et al., (2019). DPPH, in its radical form, exhibits absorption at 517 nm, which decreases upon reduction by an antioxidant or a radical species. In brief, 40 µL of sample solutions at various concentrations were combined with 160 µL of 0.4 mM methanol solution of DPPH. After a period of thirty minutes, the absorbance was measured at 517 nm using a 96-well microplate reader. BHA and α-tocopherol served as antioxidant standards in the DPPH free radical scavenging assay. The inhibition activity (I), representing the capability of scavenging, was calculated using the Eq. (3).

$$\text{Inhibition } (\%) = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \qquad (3)$$

ABTS $^{\bullet+}$ radical decolorization assay

The determination of ABTS $^{\bullet+}$ scavenging activity through spectrophotometric analysis was carried out in accordance with the method outlined by Öztürk et al., (2011). The ABTS $^{\bullet+}$ radical cation was generated by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, and the resulting solution was stored in the dark at room temperature for 12 hours. Although the oxidation of ABTS began immediately, the absorbance did not reach its maximum and stable state until more than 6 hours had passed. The radical cation remained stable in this form for over 2 days when stored in the dark at room temperature. Prior to use, the ABTS $^{\bullet+}$ solution was diluted to achieve an absorbance of 0.708 ± 0.025 at 734 nm with ethanol. Subsequently, 160 µL of the ABTS $^{\bullet+}$ solution was added to 40 µL of sample solution in ethanol at various concentrations. After 10 minutes, the absorbance was measured at 734 nm using a 96-well microplate reader. The percentage inhibitions were calculated for each concentration relative to a blank absorbance (ethanol). BHT, α-tocopherol, and quercetin served as antioxidant standards for the purpose of comparing activity levels. The scavenging capability of ABTS $^{\bullet+}$ was determined using the Eq. (3).

Cupric-reducing antioxidant capacity

The Cupric-Reducing Antioxidant Capacity (CUPRAC) test was conducted using the methodology previously described by Apak et al., (2004). In summary, each well of a 96-well microplate received 50 µL of Cu (II) (10 mM), 50 µL of neocuproine (7.5 mM), and 60 µL of NH4 Ac buffer (1 M, pH 7.0) solutions. Subsequently, 40 µL of the extract at various concentrations was added to the initial mixture, resulting in a final volume of 200 µL. After 1 hour, the absorbance was measured at 450 nm against a reagent blank using a 96-well microplate reader. The obtained results were compared with those of BHA and α-tocopherol, which were used as antioxidant standards.

Metal-chelating activity

The essential oils' chelating activity on Fe^{2+} was assessed following Djebili et al., (2022) method with slight adjustments. An 80 µL solution of the samples (dissolved in ethanol at various concentrations) was mixed with 40 µL of 0.2 mM $FeCl_2$. Subsequently, 80 µL of 0.5 mM ferrene was added. The mixture was vigorously shaken and left at room temperature for 10 minutes. Afterward, the absorbance was measured at 593 nm. EDTA served as a positive control for comparing the activity. The metal chelating activity was calculated using the Eq. (3).

2.4.2. Enzyme Inhibitory Activities

Anticholinesterase Activity

Anticholinesterase activity was assessed by measuring the inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using a spectrophotometric method, as described by Ellman et al., (1961) and Taş-Küçükaydın et al., (2023) with some modifications. AChE was sourced from electric eels and BChE from horse serum, with acetylthiocholine iodide and butyrylthiocholine chloride serving as substrates. The cholinesterase activity was quantified using 5,5′-Dithio-bis(2-nitrobenzoic) acid (DTNB). Incubation was performed at 25°C for 15 minutes, involving the reaction mixture consisted of 130 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution at various concentrations in ethanol, and 20 µL of AChE or BChE enzyme solution in buffer. Following the addition of 20 µL of 0.5 mM DTNB, the reaction was initiated by adding 20 µL of 0.71 mM acetylthiocholine iodide or 0.2 mM butyrylthiocholine chloride. The degradation of these substrates was monitored by measuring absorbance at 412 nm using a 96-well microplate reader. The yellow 5-thio-2-nitrobenzoate anion, formed due to the reaction between DTNB and thiocholine produced during enzymatic degradation, allowed for measurement of enzyme activity. The results are given as percentage inhibition (at a concentration of 200 µg/mL) and IC_{50} values (50% inhibition concentration).

Anti-urease Activity

Anti-urease activity was determined by quantifying ammonia production using the indophenol method, as outlined by Weatherburn, (1967) and Tamfu et al., (2020). A reaction mixture was prepared with 100 mM sodium phosphate buffer (pH 8.2), 25 µL of urease enzyme solution (obtained from Jack bean), and 50 µL of 100 mM urea. The mixture was incubated at 30°C for 15 minutes, after which 10 µL of sample solution was added. Following incubation, 45 µL of 1% (w/v) phenol reagent and 70 µL of 0.005% (w/v) alkali reagent were added to each well. After an additional 50 minutes of incubation, absorbance was measured at 630 nm using a microplate reader. Thiourea was used as the standard compound, and the results were expressed as the IC_{50} value, representing the concentration of the sample that inhibited 50% of the urease activity.

Anti-tyrosinase Activity

Tyrosinase inhibition was evaluated using the spectrophotometric method described by Masuda et al., (2005). The assay utilized mushroom-derived tyrosinase with L-DOPA as the substrate. A reaction mixture containing 150 µL of 100 mM sodium phosphate buffer (pH 6.8), 10 µL of sample solution, and 20 µL of tyrosinase enzyme solution was incubated at 37°C for 10 minutes. Subsequently, 20 µL of L-DOPA were added to the mixture. After 10 minutes of incubation at 37°C, absorbance was measured at 475 nm using a 96-well microplate reader. The results were expressed as the percentage reduction in enzyme activity at a concentration of 200 µg/mL and IC_{50} values.

Antidiabetic Activity - α-Amylase and α-Glucosidase Inhibition Assay

The α-amylase and α-glucosidase inhibitory activities were assessed using the methods described by Küçükaydın et al., (2021). For the α-amylase inhibitory assay, the starch-iodine method was employed. A phosphate buffer solution containing 0.1 units/mL of α-amylase (from swine pancreas) at pH 6.9 was prepared. To the buffer, 50 µL of α-amylase solution and 25 µL of sample solution were added in a 96-well microplate. After incubation at 37°C for 10 minutes, 50 µL of starch solution (0.05%) was added, and the mixture was further incubated for 10 minutes. The reaction was stopped by adding 25 µL of 0.1 M hydrochloric acid and 100 µL of Lugol's solution. Absorbance was measured at 565 nm using a 96-well microplate reader.

For the α-glucosidase inhibitory assay, 50 µL of α-glucosidase (from *Saccharomyces cerevisiae*) at 0.1 units/mL in phosphate buffer (0.01 M, pH 6.0), 25 µL of PNPG (4-N-nitrophenyl-α-D-glucopyranoside) in phosphate buffer (0.01 M, pH 6.9), and 50 µL of phosphate buffer (0.01 M, pH 6.9), were mixed in a 96-well microplate. After adding 10 µL of sample solution, the mixture was incubated at 37°C for 20 minutes. The reaction was terminated by adding 90 µL of sodium carbonate solution (0.1 M), and absorbance was measured at 400 nm using a 96-well microplate reader. Acarbose was used as the standard for both assays.

2.4.3. **Anti-Inflammatory Activities**

HRBC Membrane Stabilization Assay - Heat-Induced Hemolysis

The anti-inflammatory potential was evaluated by assessing heat-induced hemolysis of Human Red Blood Cell (HRBC) membranes, following the procedure described by Sunmathi et al., (2016), with minor modifications. Fresh blood was collected from healthy volunteers who had abstained from anti-inflammatory or contraceptive medications for two weeks prior to

sample collection. The blood was mixed with an equal volume of sterilized Alsevers solution, composed of 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water. The blood solution was then centrifuged at approximately 840.19 g for 10 minutes. The resulting cell pellet was separated and washed three times with an Isosaline solution (0.85%, pH 7.2). The packed blood volume was adjusted to a 10% (v/v) suspension using Isosaline. A total of 1 mL of the HRBC suspension was added to 1 mL of the essential oil sample at different concentrations. After incubating the tubes in a water bath at 56°C for 30 minutes, the samples were allowed to cool to room temperature and then centrifuged at approximately 583.93 g for 5 minutes. The absorbance of the supernatant was measured at 560 nm using a spectrophotometer. Diclofenac sodium was used as a positive control. The membrane stabilizing activity was calculated using the formula:

$$\text{Hemolysis inhibition (\%)} = ((\text{Absorbance of control} - \text{Absorbance of sample})/ \text{Absorbance of control}) \times 100$$

Egg Albumin Denaturation Inhibition Assay

The Egg Albumin Denaturation Assay was performed *in vitro* following the methodology of Bhutia, (2020). A 0.2 mL sample of egg albumin, extracted from fresh hen eggs and dissolved in a 0.5% (w/v) aqueous solution, was mixed with 2 mL of essential oil and 2.8 mL of phosphate-buffered saline (PBS) at pH 6.4. The mixture was incubated in a water bath at 37°C for 15 minutes, followed by heating at 70°C for 5 minutes. After cooling, the absorbance was measured at 660 nm. Distilled water of the same volume was used as the standard. Diclofenac sodium served as the reference compound. The inhibition of albumin denaturation was expressed as a percentage.

$$\text{Inhibition of Egg Albumin Denaturation (\%)} = ((\text{Absorbance of control} - \text{Absorbance of sample})/ \text{Absorbance of control}) \times 100$$

2.4.4. **Antimicrobial Activities**

Bacterial and Fungal Strains

A set of bacterial and fungal strains was carefully chosen for various assays in this study. The tested microorganisms were obtained from the *Microbiology Research Laboratory at Muğla Sıtkı Koçman University, Muğla, Türkiye*. Bacterial strains included *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. Additionally, fungal strains *Candida albicans* ATCC 10239 and *Candida tropicalis* ATCC 13803 were selected for *in vitro* antimicrobial and anti-biofilm activities. In the context of quorum sensing inhibition and violacein inhibition assays, biosensor strains *Chromobacterium violaceum* CV026 and CV12472, respectively, were employed. Furthermore, *Pseudomonas aeroginosa* PA01 was utilized for swarming and swimming motility inhibition assays. The above-mentioned bacterial strains were cultured in Nutrient Broth (NB, Difco), while *Candida albicans* and *Candida tropicalis* were cultivated in Sabouraud dextrose broth (SDB, Difco). Throughout the study, microbial cultures were maintained on their respective agar slants at 4 °C and utilized as stock cultures.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) refers to the lowest concentration of a sample where there is no observable growth of microorganisms. The microtiter broth dilution method, as outlined by (Popova et al., 2021; Tamfu, Ceylan, Fru, et al., 2020), was utilized in this investigation to determine the minimum inhibitory concentration (MIC). The test medium employed was Mueller-Hinton Broth (MHB), with the bacterial density being standardized to 5×10^5 colony-forming units (CFU) per mL. Bacterial suspensions (100 µL) were introduced into the wells of 96-well microtiter plates alongside samples containing different final concentrations, spanning from 0.03125 µL/mL to 2 µL/mL. Afterwards, the microplates were placed in an incubator at 37 °C for 24 hours. After incubation, the absorbance was measured at 550 nm (Tamfu, Ceylan, Fru, et al., 2020). The negative controls consisted only of Mueller-Hinton broth (MHB), whereas the positive controls consisted of MHB supplemented with the bacterial inoculum.

Biofilm Inhibition Assays

To evaluate the impact of the essential oil at concentrations of 1, 1/2, 1/4, 1/8, and 1/16 MIC on the biofilm-forming ability of selected bacterial and fungal strains, a microplate biofilm

assay was employed following the procedures outlined by Merritt et al., (2005) and Tamfu et al., (2020). In brief, 1% of overnight cultures of isolates was added to 200 µL of fresh Tryptose-Soy Broth (TSB) supplemented with 0.25% glucose. The cultures were then cultivated in the presence and absence of the essential oil without agitation for 48 hours at 37 ºC. Wells containing TSB with cells served as the control.

After incubation, the wells were washed with water to remove non-adherent bacteria. The remaining bacteria were stained with 0.1% crystal violet solution for 10 minutes at room temperature. Subsequently, the wells were washed again to remove the crystal violet solution. For plates containing Gram-negative bacteria and yeast, 200 µL of 95% ethanol was added, while wells containing Gram-positive bacteria received 33% glacial acetic acid. Biofilm stains were solubilized at room temperature. After shaking and pipetting the wells, 125 µL of the solution from each well was transferred to a sterile tube, and the volume was adjusted to 1 mL with distilled water. Finally, the optical density (OD) of each well was measured at 550 nm (Merritt et al., 2005; Tamfu, Ceylan, Fru, et al., 2020). The percentage of inhibition of the tested essential oils was calculated using the formula:

$$\text{Biofilm inhibition (\%)} = ((OD550\ \text{Control} - OD550\ \text{Sample})/OD550\ \text{Control}) \times 100$$

Violacein Inhibition Assay

To evaluate its potential for quorum sensing inhibition (QSI), the essential oils were analyzed qualitatively using *Chromobacterium violaceum* CV12472 Alfred Ngenge et al., (2021). A 10 µL culture of *C. violaceum* that had been grown overnight and adjusted to an optical density (OD) of 0.4 at 600 nm was placed into sterile microtiter plates containing 200 µL of Luria-Bertani (LB) broth and incubated in either the presence or absence of sub-MIC concentrations of essential oils. The positive control consisted of LB broth containing *C. violaceum* CV12472. The incubation process was carried out at a temperature of 30°C for 24 hours, during which a decrease in the formation of violacein pigment was noticed. The measurement of absorbance was conducted at 585 nm. The experiment was conducted three times, and the percentage of violacein inhibition was determined using the following formula:

$$\text{Violacein inhibition (\%)} = ((OD585\ \text{Control} - OD585\ \text{Sample})/OD585\ \text{Control}) \times 100$$

Methodology

Quorum-Sensing Inhibition (QSI) Assay

Quorum sensing inhibition (QSI) was evaluated using the previously documented method Alfred Ngenge et al., (2021). Preparation involved combining 1.3 g of agar, 2.0 g of Tryptone, 1.0 g of sodium chloride, and 200 mL of deionized water to create a 5 mL solution of warm molten Soft Top Agar. Subsequently, 100 µL of a previously cultured CV026 solution was combined with 20 µL of a 100 µg/mL C6HSL solution, which served as an external source of AHL (acyl homoserine lactone). The mixture was homocombined and promptly put onto the surface of a hardened Luria Bertani Agar (LBA) plate as a top layer. After the overlay solidified, wells with a diameter of 5 mm were created on each plate. The essential oil, sterilized using a filter, was added to each well in a concentration below the minimum inhibitory concentration (sub-MIC), using a volume of 50 µL. The QSI was observed as a pale or off-white circular area encircling the well, in contrast to the dense purple growth of the activated CV026 bacteria. The experiment was replicated thrice. The widths of the QSI zones on the assay plates were assessed following a three-day incubation period at 30 °C.

Swimming and Swarming Motility Inhibition Assay

The swimming and swarming motility studies were performed according to the techniques outlined by Tamfu et al., (2020). To conduct swarming experiments, a 5 µL sample of an overnight culture of *P. aeruginosa* (PA01), obtained from the *Microbiology Research Laboratory at Muğla Sıtkı Koçman University, Muğla, Türkiye*, which had an optical density of 0.4 at 600 nm, was introduced at the center of swarming plates. The plates were produced by combining 1% peptone, 0.5% NaCl, 0.5% agar, and 0.5% filter-sterilized D-glucose in a medium. Different concentrations of essential oils (50, 75 and 100 µg/mL) were added to the plates.

The *P. aeruginosa* (PA01) strain was inoculated at the center of swimming agar medium plates for the swimming motility assay. The swimming agar medium comprised 1% Tryptone, 0.5% NaCl, 0.3% agar, and 0.5% filter-sterilized D-glucose. Different concentrations of essential oils (50, 75 and 100 µg/mL) were introduced into the medium. The plates were encased in Saran Wrap to avoid dehydration. Subsequently, the samples were placed in an incubator set at 30 °C and positioned upright for 16 hours. Following incubation, the movement of bacterial cells through swimming and swarming was observed and documented by measuring the areas of swim and swarm. Control plates without the essential oil were also prepared.

2.4.5. **Cytotoxic Activity**

Cell Culture

The human non-tumorigenic CCD18-Co cell line was cultured in RPMI (Roswell Park Memorial Institute) growth medium supplemented with 10% fetal bovine serum (FBS). The cells were maintained in a CO_2 incubator at 37°C under conditions of 5% CO_2 and 95% humidity. Upon reaching 80% confluence, the cells were washed with phosphate-buffered saline (PBS). Trypsin–EDTA (1×) was used to passage the cells. The CCD18-Co cell line was provided by Dr. Aydın Demiray from Pamukkale University, Faculty of Medicine, Department of Medical Genetics, and the Assessment Centre for Genetic Patients.

MTT Assay

Cytotoxicity was assessed using the MTT assay, as described by Plumb, (1999) and González-Sarrías et al., (2022), on the CCD18-Co human colon cell line. Cells were seeded into 96-well plates at a density of 1000 cells per well. After 24 hours of cultivation, the cells were treated with verbascoside and essential oils at four different concentrations, ranging from 200 µg/mL to 25 µg/mL, and incubated for 24, 48, or 72 hours. The experiments were conducted independently in triplicate, with a control group that received no treatment. Following incubation, 10 µL of MTT reagent (Applichem), at a concentration of 5 mg/mL in PBS, was added to each well. After a 4-hour incubation, the medium was aspirated, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan blue crystals formed within the cells. The absorbance of each well was measured at 540 nm using a microplate reader. The IC_{50} value was determined based on the time curve (24, 48, and 72 hours) that produced the highest percentage of cytotoxicity. IC_{50} values were calculated using the AAT Bioquest IC_{50} calculator and were cross-validated with the IC_{50} values derived from dose–response data, where extract concentrations were used as the independent variable (x) and cytotoxicity percentages as the dependent variable (y), following the formula $IC_{50} = y = a * x + b$.

2.5.**Statistical Analysis**

The statistical analysis was conducted using Microsoft Excel® with GraphPad® Prism8 software. The experiments were performed in triplicate. Statistical significance of the differences was assessed using analysis of variance (ANOVA), and the results were deemed significant at a p-value of less than 0.05.

RESULTS & DISCUSSION

1. PHYTOCHEMICAL ANALYSIS

1.1. Extraction Yield of Essential Oils

The results of hydrodistillation for the three plants are presented in *Table 07*. *Figure 24* illustrates the color and appearance of these essential oils. *Cymbopogon citratus* exhibited the highest extraction yield, reaching 1.99%, the essential oil was characterized by a bright yellow color and a pronounced citrus scent. This was followed by *Schinus molle*, which yielded 0.69% of yellow oil with spicy notes. *Teucrium polium* yielded the lowest amount of light yellow, earthy-toned oil (0.18%).

Table 04: *Extraction yield and organoleptic profile of Cymbopogon citratus, Schinus molle, and Teucrium polium essential oils.*

Essential oil sample	Extraction yield (%)	Sensory characteristics
CCEO	1.99	Light yellow oil with strong, fresh, lemony notes
SMEO	0.69	Pale yellow oil to colorless with spicy, woody notes
TPEO	0.18	Light yellow oil with herbaceous, earthy notes.

CCEO: *Cymbopogon citratus* essential oil. SMEO: *Schinus molle* essential oil. TPEO: *Teucrium polium* essential oil.

Cymbopogon species are widely recognized for their notable essential oil yields. Remarkably, our *Cymbopogon citratus* essential oil (CCEO) yield (1.99%) surpassed those reported from the Blida region, southwest of Algiers, where yields of 0.8 ± 0.1% and 0.6% were documented by Benoudjit et al., (2022) and Boukhatem et al., (2014), respectively. However, our yield was slightly lower than the 2.12% obtained via hydrodistillation from *Cymbopogon* species cultivated in Egypt (Mohamed Hanaa et al., 2012) and the 2.65% reported in India (Dutta et al., 2014). Hydrodistillation of fresh Lemongrass yielded 0.37% in Brazil (Almeida et al., 2018) and 1.10% in Benin (Degnon et al., 2017).

These variations in *C. citratus* essential oil yield can be attributed to several factors that influence both the quality and quantity of the essential oils. Environmental conditions, such as climate and soil composition, play a critical role. Additionally, geographical origin, geobotanical conditions, farming practices, plant maturity stage, photoperiod, harvest timing,

genetic differences, extraction methods, and drying techniques prior to distillation significantly contribute to yield variability (Benoudjit et al., 2022; Dutta et al., 2014; Majewska et al., 2019). Furthermore, the specific plant organs used for extraction strongly impact both the yield and chemical composition of the essential oil (Mubarak et al., 2015; Olayemi, 2017).

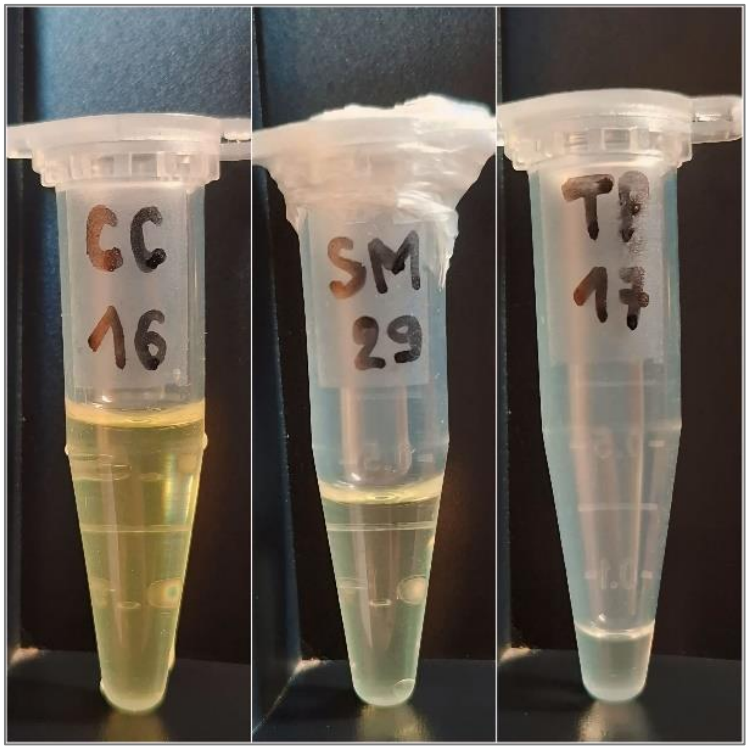

Figure 24: *Cymbopogon citratus, Schinus molle, and Teucrium polium essential oils.*

The hydrodistillation of *Schinus molle* leaves produced a pale-yellow essential oil with a mildly pungent aroma, yielding 0.7% (w/w). This value aligns with the range reported in the literature (0.1–3.0%, as per Abderrahim et al., 2018). Comparable results were obtained by Machado et al., (2019), who documented a yield of 0.78% (v/w) from air-dried *S. molle* leaves, resulting in a pale-yellow, viscous oil with a faintly pungent fragrance. Similarly, Hamdan et al., (2016) investigated the hydrodistillation of fresh *S. molle* plant parts from Egypt. They reported yields of 0.7% for fruits, 1% for leaves, 0.7% for stems, and 0.8% for flowers (v/w), all producing pale-yellow oils with a slightly pungent, pepper-like aroma. These findings collectively confirm the variability in yield and aroma across plant parts and studies.

The yield of essential oil from *Teucrium* species is typically moderate. The yield of our *T. polium* essential oil (TPEO) aligns with values documented in Algerian literature, which span from 0.1% to 1.7% (Ahmad et al., 2007; Belmekki et al., 2013; Bendif et al., 2018; Bendjabeur

et al., 2018; Gherib et al., 2022; Hammoudi et al., 2013; Hechachna et al., 2023; Lograda et al., 2014, p. 2012013; Maizi et al., 2019).

Previous studies indicate that the essential oil yield from the aerial parts of *Teucrium polium* L. differs based on the plant's growth stage, particularly between the vegetative and flowering phases. Furthermore, winter leaves of *T. polium* displays distinct morphological and anatomical traits not found in summer leaves, which may enhance essential oil production in winter. This adaptation likely serves as a defensive response to chilling stress (Lianopoulou et al., 2014; Maizi et al., 2019).

1.2.Chemical Composition of Essential Oils

The essential oils of *Cymbopogon citratus*, *Schinus molle*, and *Teucrium polium* were evaluated using gas chromatography with flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS), which detected 39, 72, and 73 components, respectively. These components comprised 100.0% of the essential oil composition. A detailed analysis of the essential oils' composition is provided in *Table 15 (Appendix 02).*

Oxygenated monoterpenes are the predominant components in *Cymbopogon citratus* essential oil (CCEO), accounting for 90.15% of its total composition. Monoterpene constituted 6.26%, sesquiterpene hydrocarbons accounted for 2.41%, and oxygenated sesquiterpenes were present in trace amounts at 0.90%. Among the major compounds identified in CCEO, α-Citral (43.36%) and β-Citral (36.16%) were the most abundant, followed by β-Pinene (5.03%) and Trans-geraniol (3.29%).

Numerous studies have examined the chemical composition of *Cymbopogon citratus* essential oil, emphasizing its characteristic richness in oxygenated monoterpenes, particularly Citral isomers. These compounds, predominantly Geranial (Citral A) and Neral (Citral B), are well-documented as the main constituents of CCEO, and their abundance is a critical marker of essential oil quality (Majewska et al., 2019).

The chemical composition of *Cymbopogon citratus* essential oil shows significant variation depending on geographical origin, extraction methods, and other environmental factors. In Algeria, Boukhatem et al., (2014) reported Geranial (42.2%), Neral (31.5%), and β-Myrcene (7.5%) as the major components of CCEO. In contrast, Benoudjit et al., (2022)'s findings highlighted Isogeranial (41.77%), Neral (43.75%), β-Pinene (5.77%), Geranial (3.78%), and Isoneral (1.90%) as the predominant compounds.

In Africa, studies also revealed notable variability. Mohamed Ali, (2017) analyzed Sudanese CCEO and identified Citral (34.8%), Neral (30.72%), β-Myrcene (11.28%), Geraniol (5.54%), 1,3,4-trimethyl-3-cyclohexene-1-carboxaldehyde (2.20%), and Citronellol (1.34%) as the main components. Similarly, Mohamed Hanaa et al., (2012) conducted a study in Egypt to evaluate the chemical composition of CCEO obtained through different drying techniques. Geranial (31.53%, 39.86%, and 37.24%), Neral (30.08%, 34.52%, and 31.28%), and Myrcene (16.61%, 14.49%, and 15.42%) were the primary compounds in oils extracted from lemongrass leaves dried in the sun, shade, and oven, respectively. These results highlight the impact of post-harvest processing on essential oil composition.

In Asia, presumed to be the native region of *C. citratus*, variations in chemical profiles were also evident. A study from India evaluated the composition of CCEO under different drying methods and consistently found Citral (Neral and Geranial) and β-Myrcene as the major compounds across fresh, oven-dried, shade-dried, and sun-dried samples, although the percentages of major and minor compounds varied (Dutta et al., 2014). In Malaysia, Tajidin, (2012) studied CCEO at different plant ages, identifying β-Myrcene, 3-Undecyne, Neral, Geranial, Nerol, Geranyl acetate, and Juniper camphor as the main components. However, their concentrations fluctuated with plant maturity, with some compounds increasing, decreasing, or even disappearing entirely.

In South America, CCEO also exhibited distinctive chemical profiles. Cortes-Torres et al., (2023) analyzed fresh leaves from Mexico and identified β-Myrcene (10.7%), Z-Geranial (32.1%), and E-Geranial (32.9%) as the major components. In Brazil, Almeida et al., (2018) reported Neral (36.37%), Geranial (53.20%), and Geraniol (2.66%) as the primary constituents. Similarly, Pino et al., (2018) identified Neral and Geranial as the dominant volatile compounds in CCEO from Ecuador, reaffirming the global recognition of *Cymbopogon* species for their Citral-rich profile.

The high Citral content in CCEO, observed in our sample, aligns with Barbosa et al., (2008) criterion for high-quality lemongrass oil, which necessitates a minimum of 75% Citral content. This underscores the significance of Citral concentration as a determinant of the essential oil's commercial and therapeutic value. However, it is noteworthy that the chemical composition of CCEO is influenced by numerous factors, including the plant's developmental stage, harvest timing, and environmental conditions (R. K. Verma et al., 2015).

Although harvesting methods exert minimal influence on essential oil yield, they can affect the Citral content. Factors such as fertilizer application and soil microbiota, particularly rhizosphere fungi, have been reported to enhance Citral concentrations in lemongrass (Shaikh, et al., 2019). Additionally, the proportion of young to older leaves at harvest plays a pivotal role, as younger leaves contribute to higher Citral levels, thereby improving essential oil quality (Tajidin, 2012).

Geographical origin also profoundly affects the chemical composition of CCEO. Essential oils from *C. citratus* cultivated in Brazil, Asia, and Africa exhibit variations in their major chemotypes. Notably, Myrcene is a significant component in African CCEO samples (Majewska et al., 2019). However, our Algerian CCEO sample lacked Myrcene, suggesting a distinctive chemical profile influenced by the unique environmental and geobotanical conditions of its growth region.

Besides Citral and Myrcene, other notable compounds such as Geraniol, Citronellal, and Limonene are frequently detected in *C. citratus* essential oil, often in concentrations exceeding 1% in certain samples (Majewska et al., 2019). These secondary constituents, although present in smaller amounts compared to Citral, contribute significantly to the essential oil's aromatic profile and biological properties.

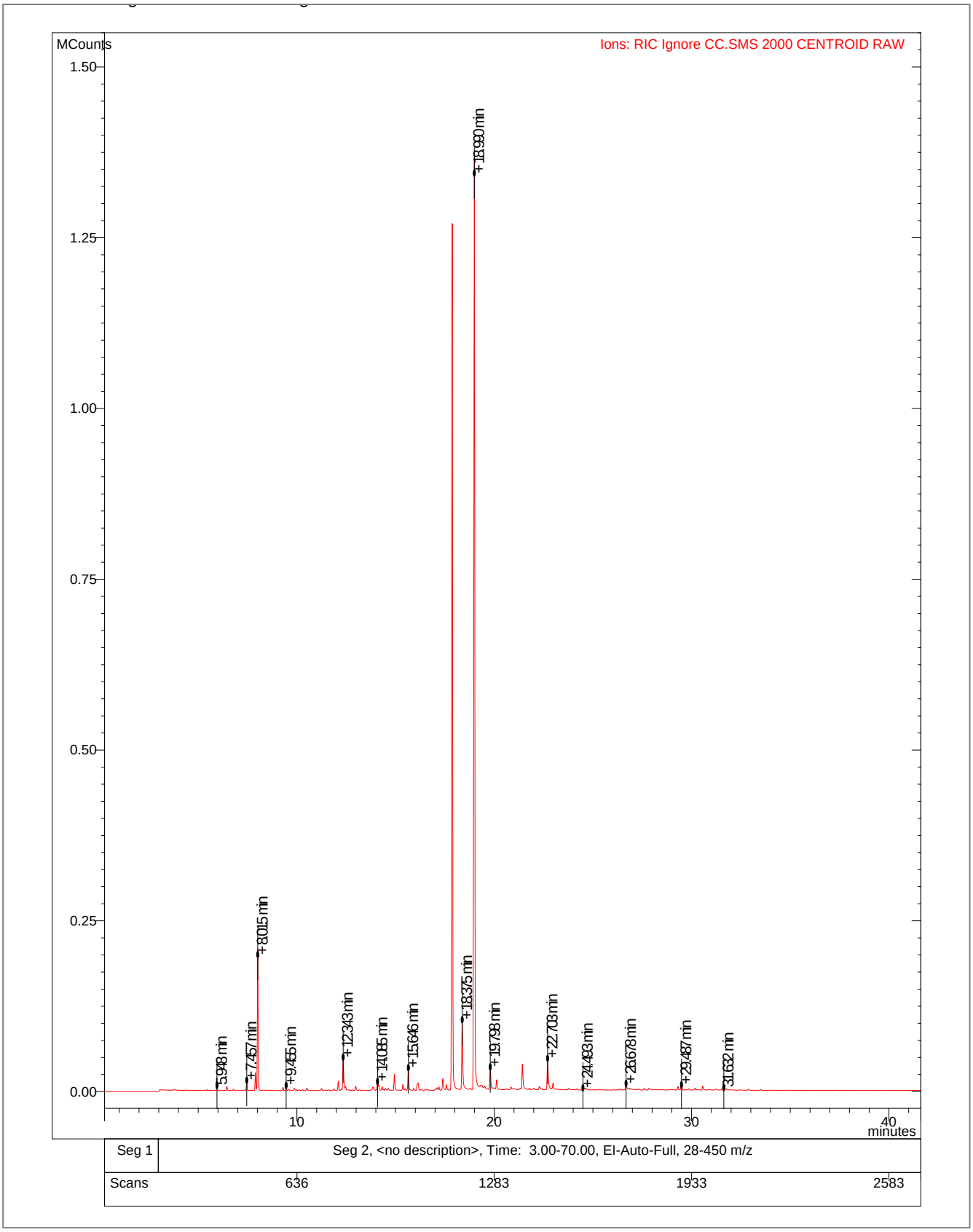

Figure 25: *GC-MS chromatogram of Cymbopogon citratus essential oil.*

Results & Discussion: Phytochemical Analysis

The chemical composition of *Schinus molle* essential oil (SMEO) revealed the presence of 72 components that collectively accounted for 100.0% of the oil's composition. Monoterpenes emerged as the dominant group, constituting 43.02% of the total, while oxygenated monoterpenes contributed 4.96%. Sesquiterpene hydrocarbons were significant as well, comprising 24.47%, alongside a notable proportion of oxygenated sesquiterpenes at 27.37%. The main constituents identified were α-Phellandrene (12.70%), Limonene (11.90%), Germacrene D (10.15%), and α-Cedrene oxide (5.52%).

Comparable analyses of *S. molle* essential oils from the Mascara region of Algeria indicated a distinct composition, with Shyobunone (10.14%), 1-Phellandrene (9.63%), α-Cadinol (7.46%), δ-Cadinene (7.45%), and Germacrene D (7.09%) as the most prevalent compounds (Lalia et al., 2023). Similarly, Martins et al., (2014) investigated the essential oils from the leaves and fruits of *S. molle* collected in southeastern Portugal. They reported α-Phellandrene (25.9%), Limonene (11.7%), β-Myrcene (11.1%), β-Phellandrene (10.5%), and Elemol (9.0%) as the principal components in leaf-derived oils, while the fruit essential oils were predominantly composed of β-Myrcene (51.3%), Limonene (14.1%), α-Phellandrene (14.0%), and β-Phellandrene (11.0%).

Across various studies, α- and β-Phellandrene consistently appear as significant constituents of *S. molle* essential oils, often ranking among the major components (A. Aboalhaija et al., 2019; Bachheti et al., 2018; Belhamel et al., 2008; Bendaoud et al., 2010; Eryigit et al., 2017; Guerra-Boone et al., 2013; Hayouni et al., 2008; Martins et al., 2014; Zahed et al., 2011). However, discrepancies in the concentration of these compounds have been documented. For instance, essential oils derived from *S. molle* leaves and berries in two regions of Kabylie, Algeria, exhibited low levels of α-Phellandrene (0.4–3.0%) and β-Phellandrene (0.5–1.6%). In contrast, essential oils from Algiers recorded only 1.10% α-Phellandrene and no β-Phellandrene (Bouhenna et al., 2021). Similarly, Kouachi et al., (2024) and Vicenço et al., (2020) noted the complete absence of Phellandrene in their respective studies of *S. molle* essential oils from Algeria and Brazil.

The variation in the chemical composition of essential oils is influenced by numerous factors, including seasonal changes, phenological stages, geographical conditions, ecological factors, genetic diversity, and extraction methodologies (Eryigit et al., 2017; Garzoli et al., 2019). Notably, drying processes can significantly alter the composition of essential oils. Silva et al., (2023) observed that drying *S. molle* leaves enhanced the concentration of major

constituents compared to fresh leaves. Similarly, Singh, (2020) highlighted the critical role of drying duration and conditions in determining the yield and volatile component content of essential oils.

Climatic and environmental conditions, such as altitude and temperature, also play pivotal roles in shaping the chemical profile of essential oils. Vokou et al., (1993) emphasized that variations in these factors could lead to significant differences in the yield and composition of essential oils. Consequently, the chemical diversity observed in *S. molle* essential oils underscores the importance of contextual factors and methodological considerations in essential oil research.

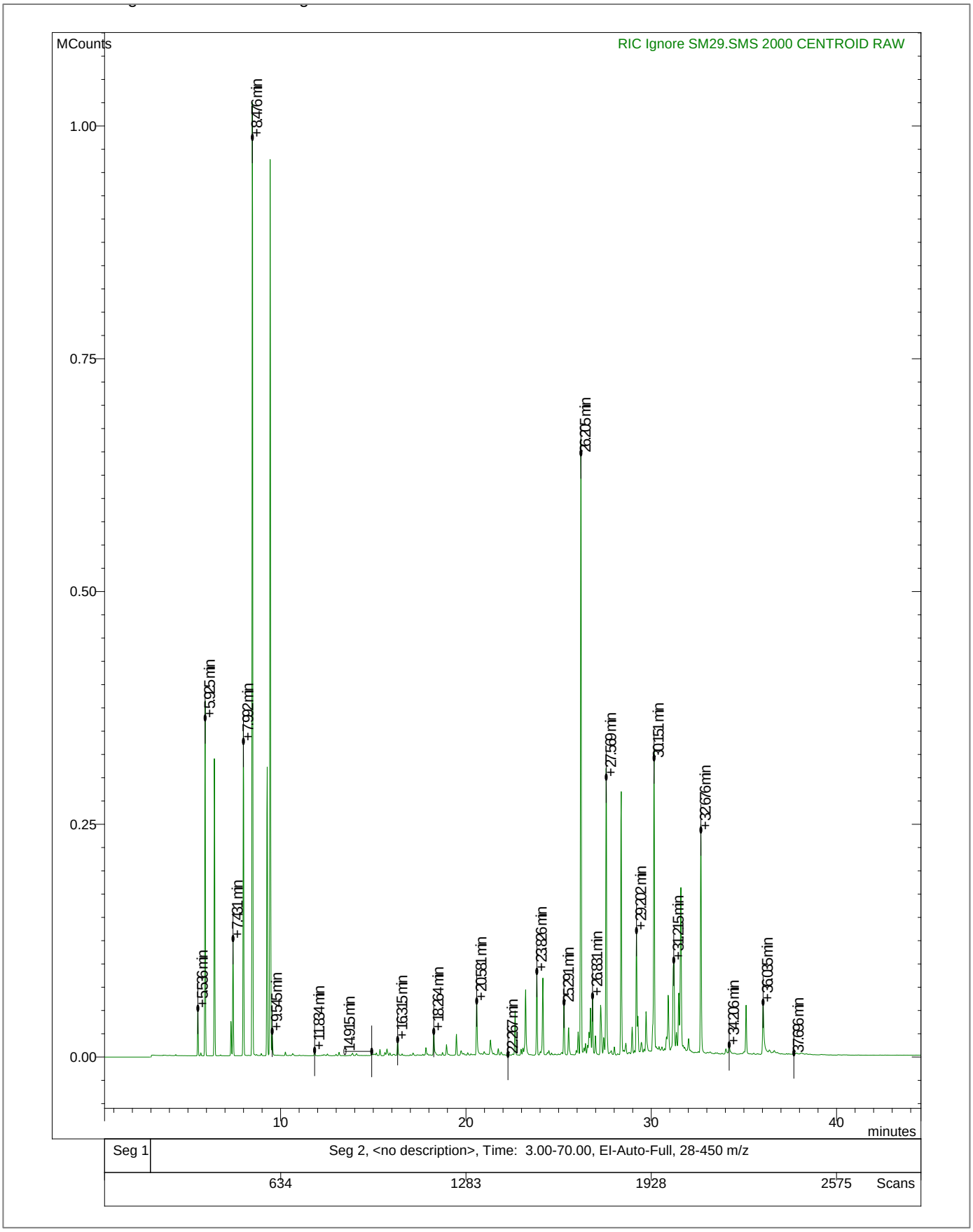

Figure 26: *GC-MS chromatogram of Schinus molle essential oil.*

Results & Discussion: Phytochemical Analysis

The essential oil of *Teucrium polium* comprised oxygenated monoterpenes and monoterpenes as the primary constituents, with respective values of 38.81% and 35.34%. Oxygenated sesquiterpenes constituted 13.19%, while sesquiterpene hydrocarbons accounted for 11.76%. A sequence of significant chemicals with comparably analogous quantities was noted. The predominant compound was α-Fenchene (16.45%), succeeded by α-Citral (8.37%), β-Citral (6.85%), Germacrene D (6.25%), and Limonene (6.21%).

Prior research has documented significant variations in the essential oil composition of *Teucrium polium*, with such differences also observed across various regions and provinces of Algeria. Research conducted in the central region of Northern Algeria, specifically in M'sila province, has revealed that the essential oil of *Teucrium polium* (TPEO) comprises β-Pinene (33%), Germacrene D (17%), Myrcene (10%), Limonene (8%), Bicyclogermacrene (3%), trans-β-Guaiene (1.7%), Spathulenol (1.6%), and β-Bourbonene (1.3%) as its principal components (Chabane et al., 2021). Conversely, TPEO from the Bousaada district identifies t-Cadinol (18.3%), Germacrene D (15.3%), and β-Pinene (10.5%) as the principal constituents, accompanied by Carvacrol (5.5%), Bicyclogermacrene (5.5%), and α-Pinene (4.1%) (Kerbouche et al., 2015).

In Eastern Algeria, essential oils of *T. polium* from Setif province comprised α-Pinene (14.1-18.0%), β-Pinene (15.3-18.1%), Germacrene D (3.8-19%), Myrcene (8.2-10.4%), and Limonene (5.3-8.7%) (Lograda et al., 2014). In Ain Melilla-Aures, the predominant chemicals discovered were α-Cadinol (46.8%), 3-β-hydroxy-α-muurolene (22.5%), α-Pinene (9.5%), and β-Pinene (8.3%) (Ahmad et al., 2007).

In Western Algeria, essential oils derived from *T. polium* in Mascara province were identified to comprise significant compounds including Limonene (29.87%), Spathulenol (17.24%), Camphor (8.20%), Pinocarvone (7.76%), tau-Cadinol (5.41%), Pinene oxide (4.78%), and α-Terpineol (4.6%) during the plant's vegetative phase. During the flowering stage, the primary chemicals were Limonene (26.39%), Spathulenol (13.29%), 1-Adamantanemethylamine (9.80%), Pinocarvone (5.60%), β-Myrcene (4.02%), tau-Cadinol (3.67%), and α-Phellandrene (3.45%) (Maizi et al., 2019). Belmekki et al., (2013) identified Germacrene D (25.81%), Bicyclogermacrene (13%), β-Pinene (11.69%), and Carvacrol (8.93%) as the principal constituents of *T. polium* essential oils in the Tlemcen province. Djabou et al., (2012) identified β-Pinene (16.6%), Germacrene D (14.8%), α-Pinene (7.2%),

Spathulenol (6.4%), Limonene (5.6%), Bicyclogermacrene (5.5%), and Myrcene (2.9%) as the predominant ingredients.

In the Amour Range of the Saharan Atlas in Southern Algeria, essential oils derived from *Teucrium polium* in Laghouat province exhibited seasonal compositional change. In autumn, the principal components were β-Pinene (23.97%), γ-Muurolene (17.77%), Carvacrol (11.59%), Bicyclogermacrene (7.21%), α-Pinene (8.13%), and Limonene (6.38%). During winter, the predominant chemicals were Camphor (22.09%), Eucalyptol (13.70%), α-Pinene (10.90%), β-Pinene (7.47%), Camphene (7.76%), Borneol (8.24%), and α-Terpineol (6.53%) (Hechachna et al., 2023). Furthermore, TPEO from Tamanrasset province in the Central Hoggar region was identified to comprise dl-Limonene (11.18%), δ-Cadinene (10.02%), and Trans-β-caryophyllene (9.15%) as its principal constituents (Hammoudi et al., 2013).

Research on two subspecies of *Teucrium polium* in Morocco demonstrated varied essential oil compositions. *T. polium subsp. aurum* was predominantly comprised of caryophyllene (19.13%), γ-Muurolene (13.02%), τ-Cadinol (11.01%), α-Gurjunene (9.2%), Rosifoliol (8.79%), and 3-Carene (7.04%). Simultaneously, *T. polium subsp. polium* comprised 3-Carene (16.49%), γ-Muurolene (14.03%), α-Pinene (9.94%), α-Phellandrene (6.93%), and Caryophyllene (7.51%) as its principal components (El Atki et al., 2020). In Turkey, the principal constituents of TPEO comprised β-Caryophyllene (8.8%), t-Cadinol (6.2%), (E)-Nerolidol (5%), α-Cadinol (5.4%), and α-Pinene (4.7%) (Gizm, 2022). In Iran, the essential oil of *T. polium* predominantly comprised α-Pinene (40.52–54.05%), β-Pinene (17.36–23.3%), and Limonene (10.10–15.19%) (Reaisi et al., 2019).

The content of our *Teucrium polium* essential oil is consistent with previously documented characteristics in the literature. Nonetheless, it is significant for its remarkably high concentration of α-Fenchene, a constituent that seems to be little documented in current research. The only study, we found, that supports our findings is by Al-Otaibi & AlMotwaa, (2022), who documented a similar α-Fenchene content of 20% in *T. polium* samples from Riyadh, Saudi Arabia.

A study by Reaisi et al.,(2019) shown that the composition of *Teucrium* species is markedly affected by a complex interplay of elements, including genetic and physiological characteristics such as ecotype, chemotype, and phenological stage. Environmental and edaphic elements, including elevation, soil nutrients, precipitation, and climate, significantly influence the

phytochemical composition. An elevation increase was observed to augment specific elements of *T. orientale* and *T. polium*, including β-Pinene and Limonene, while diminishing others, such as α-Pinene and Sabinene. Reaisi et al., (2019) research demonstrates that management practices, such as drying and extraction techniques, are crucial in determining the quality and quantity of essential oils. This study emphasizes the interaction between ecological factors and plant-specific traits in shaping essential oil profiles, corroborating comparable findings among diverse medicinal plant species (Reaisi et al., 2019 and its associated references).

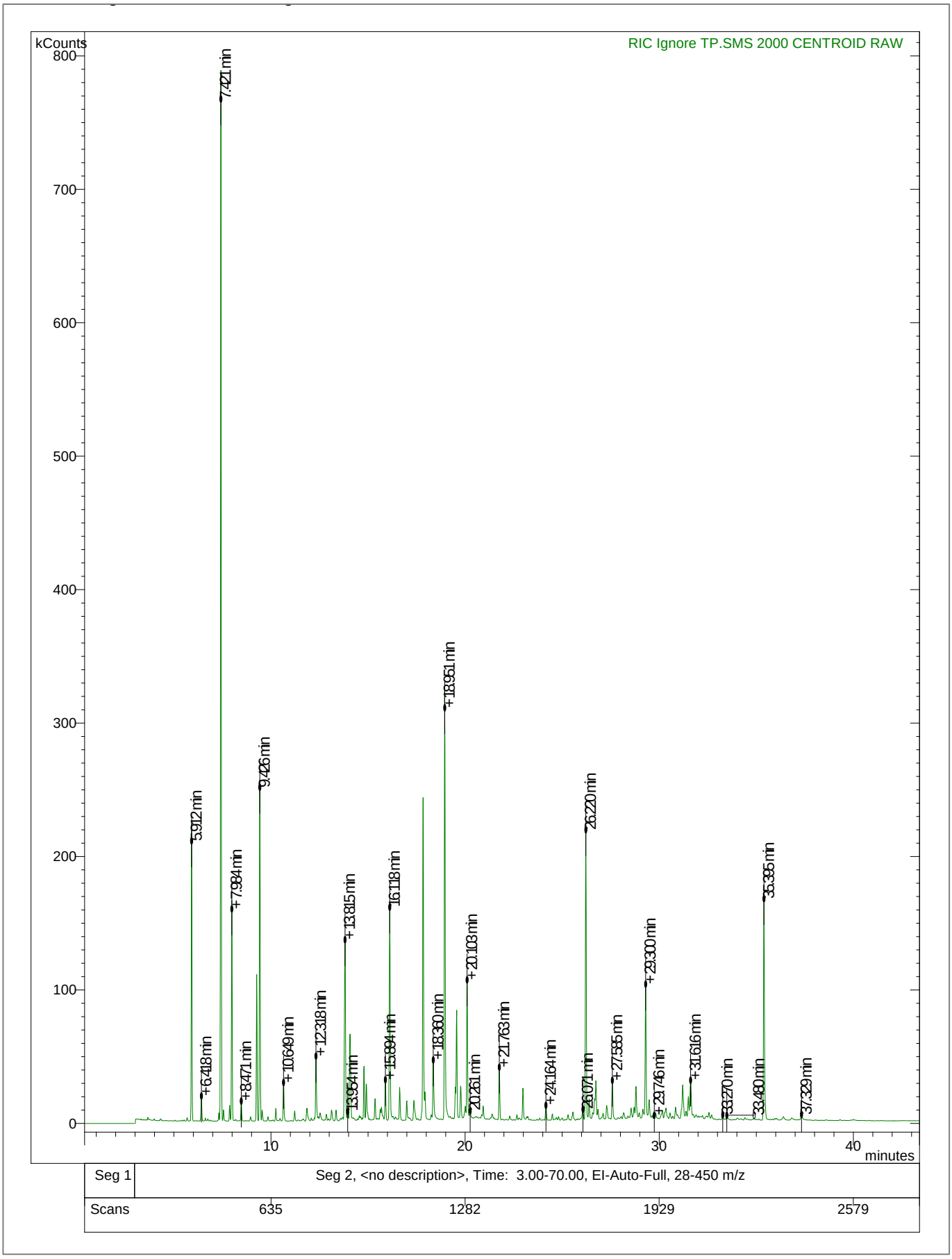

Figure 27: *GC-MS chromatogram of Teucrium polium essential oil*

2. BIOLOGICAL POTENTIALS

2.1. **Antioxidant Capacity**

The evaluation of antioxidant activity is designed to measure the ability of substances to combat the detrimental effects of oxidative stress. The β-Carotene-linoleic acid assay specifically examines the capacity to inhibit lipid oxidation, while the DPPH, ABTS+, CUPRAC, and metal chelating assays assess the potential to neutralize free radicals, reduce metal ions, and exhibit diverse antioxidant properties. Collectively, these assays provide a comprehensive assessment of the essential oil samples' antioxidative potential, which is crucial for determining their effectiveness in protecting against oxidative damage.

Table 05 and *Figure 28* illustrates the antioxidant activity of the three essential oil samples, which varied from moderate to low levels across different assays. The CCEO sample demonstrated the highest antioxidant activity overall, outperforming TPEO and SMEO. In the metal chelating assay, CCEO exhibited the most significant activity at a concentration of 200 µg/mL, with an inhibition percentage of 45.17 ± 0.82%. Additionally, it showed a substantial activity in the β-Carotene-linoleic acid assay (35.80 ± 0.68%), ABTS+ radical scavenging (23.17 ± 0.21%), and DPPH free radical scavenging (14.67 ± 0.94%). TPEO, in contrast, exhibited an inhibition percentage of 31.02 ± 0.55% in the metal chelating assay and had lower activity in the β-Carotene-linoleic acid (25.17 ± 0.51%), ABTS+ radical (20.88 ± 0.33%), and DPPH free radical (10.52 ± 0.56%) assays. SMEO displayed the lowest antioxidant activity among the samples, with inhibition percentages of 22.65 ± 0.76% in the metal chelating assay, 18.90 ± 0.48% in the β-Carotene-linoleic acid assay, 15.34 ± 0.23% in the ABTS+ radical assay, and 11.93 ± 0.84% in the DPPH free radical assay. In the CUPRAC assay, TPEO yielded the lowest absorbance value at 200 µg/mL (0.17 ± 0.03), followed by SMEO (0.22 ± 0.01), and CCEO, which had the highest absorbance value (0.39 ± 0.04). Notably, the IC_{50} and $A_{0.50}$ values for all three essential oils across all assays exceeded 200 µg/mL, indicating that their antioxidant activities are relatively low compared to the standards α-Tocopherol, BHA, and EDTA.

Table 05: *Antioxidant activity of Cymbopogon citratus, Schinus molle and Teucrium polium essential oils by β-Carotene-linoleic acid, DPPH•, ABTS•+, CUPRAC and metal chelating assays*[a].

Samples/ Standards	β-Carotene-linoleic acid assay		DPPH• assay		ABTS•+ assay		CUPRAC assay		Metal chelating assay	
	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	$A_{0.50}$ (µg/mL)	Absorbance (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)
CCEO	>200	35.80±0.68	>200	14.67±0.94	>200	23.17±0.21	>200	0.39±0.04	>200	45.17±0.82
SMEO	>200	18.90±0.48	>200	11.93±0.84	>200	15.34±0.23	>200	0.22±0.01	>200	22.65±0.76
TPEO	>200	25.17±0.51	>200	10.52±0.56	>200	20.88±0.33	>200	0.17±0.03	>200	31.02±0.55
α-Tocopherol	2.10±0.05	95.73±0.44	38.20±0.50	84.25±0.36	34.75±0.55	83.63±0.34	60.45±0.30	70.12±0.15	NT	NT
BHA	1.50±0.03	96.20±0.28	19.50±0.30	87.90±0.44	12.70±0.10	88.93±0.21	25.40±0.42	85.67±0.36	NT	NT
EDTA	NT	NT	NT	NT	NT	NT	NT	NT	5.50±0.25	94.40±0.35

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).
CCEO: *Cymbopogon citratus* essential oil. SMEO: *Schinus molle* essential oil. TPEO: *Teucrium polium* essential oil.
NT: not tested.

The antioxidant activity of *Cymbopogon citratus* essential oil (CCEO) has been extensively studied, demonstrating its potential as a natural antioxidant source. Our findings align with the literature, highlighting significant antioxidant properties through various mechanisms, including radical scavenging, metal chelation, and lipid peroxidation inhibition.

Several studies emphasize the strong radical scavenging activity of CCEO, particularly in DPPH and ABTS assays. Salaria et al., (2020) reported that CCEO from Indian leaves exhibited dose-dependent antioxidant activity, with an IC_{50} value of 91.0 ± 9.25 µg/mL for DPPH and slightly higher values for ABTS and FRAP assays. Although these values are less potent than standard ascorbic acid, they reflect a considerable radical scavenging capacity. Our results further support this, particularly in DPPH assays, underscoring the effective hydrogen-donating ability of CCEO.

Similarly, Kumar et al., (2017) and Rhimi et al., (2022) demonstrated remarkable DPPH and ABTS radical scavenging activities in lemongrass essential oil. This activity is likely attributed to the presence of Citral, a key active compound in CCEO. However, as Widelska et al., (2018) revealed, the antioxidant activity of lemongrass essential oil cannot be solely attributed to its major components (e.g., Neral and Geranial). Instead, the synergistic effect of all constituents appears to play a critical role. This synergism might explain why our CCEO sample displayed superior antioxidant activity compared to individual compound analyses.

The metal-chelating potential of CCEO is another notable antioxidant mechanism. Olaiya et al., (2016) highlighted the high level of metal-chelating activity in CCEO, which aligns with our observations. Metal chelation is critical for mitigating oxidative stress as it prevents the catalytic activity of transition metals like Fe^{2+} and Cu^{2+} in promoting free radical formation. Our findings confirm that CCEO's highest antioxidant potential was observed in the metal-chelation assay, emphasizing its capacity to act as a preventative antioxidant.

Lawrence et al., (2015) demonstrated that CCEO effectively scavenged radicals in DPPH and nitric oxide (NO) assays, as well as in the β-Carotene bleaching method, comparing favorably with synthetic antioxidants such as BHT and gallic acid. This corroborates the hypothesis that CCEO could serve as a natural alternative to synthetic antioxidants, addressing the growing consumer demand for safer and more sustainable food preservation strategies.

The holistic antioxidant potential of CCEO appears to derive from the interplay of its diverse bioactive components. As Widelska et al., (2018) showed, isolated compounds such as Neral, Geranial, and Citronellal did not exhibit the same level of activity when tested individually, reinforcing the importance of the oil's complex matrix. This synergistic effect likely amplifies the overall antioxidant capacity of CCEO, which was evident in our study as well.

Our findings, corroborated by previous studies, reaffirm the strong antioxidant potential of CCEO. Its natural and synergistic properties position it as a viable alternative to synthetic antioxidants, paving the way for its incorporation into health-related applications.

The antioxidant activity of *Schinus molle* essential oil (SMEO) was assessed using multiple assays, revealing the lowest inhibitory percentages in comparison to *C. citratus* and *T. polium* essential oils. These findings align with prior research that consistently highlights the limited antioxidant capacity of essential oils derived from *S. molle* leaves. For instance, Bouhenna et al., (2021) compared different *S. molle* extracts, including methanol, chloroform, ethyl acetate, butanol, and essential oils, across several assays such as DPPH, ABTS, Galvinoxyl, β-carotene bleaching, ferrous chelation, reducing power, and CUPRAC. Among these, essential oils consistently displayed the lowest antioxidant potential, with IC_{50} values ranging from >200 µg/mL to 400 µg/mL and A_{50} values between >50 µg/mL and 200 µg/mL. Conversely, the ethyl acetate extract exhibited the highest antioxidant potential.

Similarly, Abderrahim et al., (2018) reported that essential oils from *S. molle* leaves and berries had IC_{50} values of 6898.6 ± 219.1 µg/mL to 8643.4 ± 364.9 µg/mL against DPPH and 741.0 ± 4.1 µg/mL to 5048.0 ± 31.5 µg/mL against ABTS, reinforcing the limited antioxidant capacity of these oils. Belhoussaine et al., (2022) observed moderate to low activity in *Schinus* species from Morocco, where *S. molle* leaves and fruits exhibited radical inhibition percentages of 17.99% and 53.30%, respectively, compared to 49.31% and 77.82% for *S. terebinthifolius*. Notably, fruits generally exhibited higher antioxidant activity than leaves, reflecting similar trends across different studies.

The chemical composition of essential oils plays a crucial role in determining their antioxidant potential. Prior studies, such as those by Kelen & Tepe, (2008) and Harkat-Madouri et al., (2015), have suggested that a high concentration of monoterpenes coupled with a limited presence of oxygenated terpenes typically corresponds to reduced antioxidant activity. Eryigit et al., (2017) further noted that essential oils with α-Phellandrene as a predominant monoterpene

often exhibit lower antioxidant activity. This observation aligns with the composition of the SMEO sample analyzed in the present study, which showed higher antioxidant activity than previously reported samples, likely due to its elevated α-phellandrene content.

Overall, while *S. molle* essential oil demonstrates measurable antioxidant properties, its efficacy remains relatively low compared to synthetic standards and other plant-derived extracts. Variability in antioxidant activity across studies underscores the influence of chemical composition, extraction methods, and environmental factors on the bioactive potential of essential oils. Further investigations focusing on optimizing extraction methods and exploring synergistic effects with other natural antioxidants could enhance the utility of *S. molle* essential oils in functional applications.

The antioxidant properties of *Teucrium polium* essential oil (TPEO) exhibit significant variability, as seen by comparisons between our sample and those documented by Saleh et al., (2020), Al-Otaibi & AlMotwaa, (2022), Mahmoudi, & Nosratpour, (2013) and Maizi et al., (2019). Our TPEO sample exhibited a DPPH scavenging activity of 10.52±0.56% at a concentration of 200 µg/mL. Conversely, Saleh et al., (2020) indicated that a significantly greater concentration of 800 µg/mL was required for their TPEO sample to achieve, yet not surpass, 20% scavenging activity, implying that our sample may exhibit a more potent antioxidant impact at reduced concentrations. Simultaneously, Al-Otaibi & AlMotwaa, (2022)'s sample had superior efficacy, attaining 50% inhibition at merely 61.38 µg/mL. Subsequent evaluations of additional antioxidant assays, including ABTS, CUPRAC, and β-Carotene bleaching, revealed elevated IC_{50} values for our sample compared to those documented by Bendjabeur et al., (2018). The elevated IC_{50} value of our TPEO sample indicates a comparatively diminished antioxidant capacity, as IC_{50} values are negatively related to antioxidant efficacy.

The variability in antioxidant activity among studies is largely linked to the chemical composition of TPEO. Maizi et al., (2019) observed that oils derived from vegetative aerial portions in winter exhibited superior antioxidant activity (IC_{50} = 3.90 mg/mL) compared to those extracted during the flowering phase (IC_{50} = 16.14 mg/mL). Seasonal variations in key components, including oxygenated monoterpenes, monoterpene hydrocarbons, and sesquiterpenes, significantly influence antioxidant effectiveness. These variances are believed to stem from both developmental phases and inherent chemical disparities in the essential oils,

which affect the total antioxidant network and capabilities for reducing oxidative stress (De Cássia Da Silveira E Sá et al., 2013; Noacco et al., 2018).

Alongside developmental stage effects, environmental factors, including climate, geographical location, and genetic variability, also impact TPEO's chemical composition and antioxidant capability. Assaeed et al., (2020) and Abd-ElGawad et al., (2019) posited that variations in environmental conditions and genetic factors could substantially modify the composition of TPEO derived from aerial parts throughout the flowering phase, resulting in divergent antioxidant responses. Moreover, Saleh et al., (2020) recognized that variations in plant drying and oil extraction techniques serve as further factors contributing to these inconsistencies since these processes might affect the stability and concentration of antioxidant components in TPEO.

Determining the specific molecules that confer antioxidant capabilities to TPEO is difficult due to its complex composition. Oxygenated monoterpenes, sesquiterpenes, and monoterpene hydrocarbons are significant constituents that enhance its free radical scavenging properties. Identifying particular molecules responsible for this activity is challenging due to the efficacy being affected by both the presence and the relative quantities of various constituents. The diversity in antioxidant efficacy found among studies may stem from changes in the quantities of these chemicals influenced by various environmental variables (Bendjabeur et al., 2018).

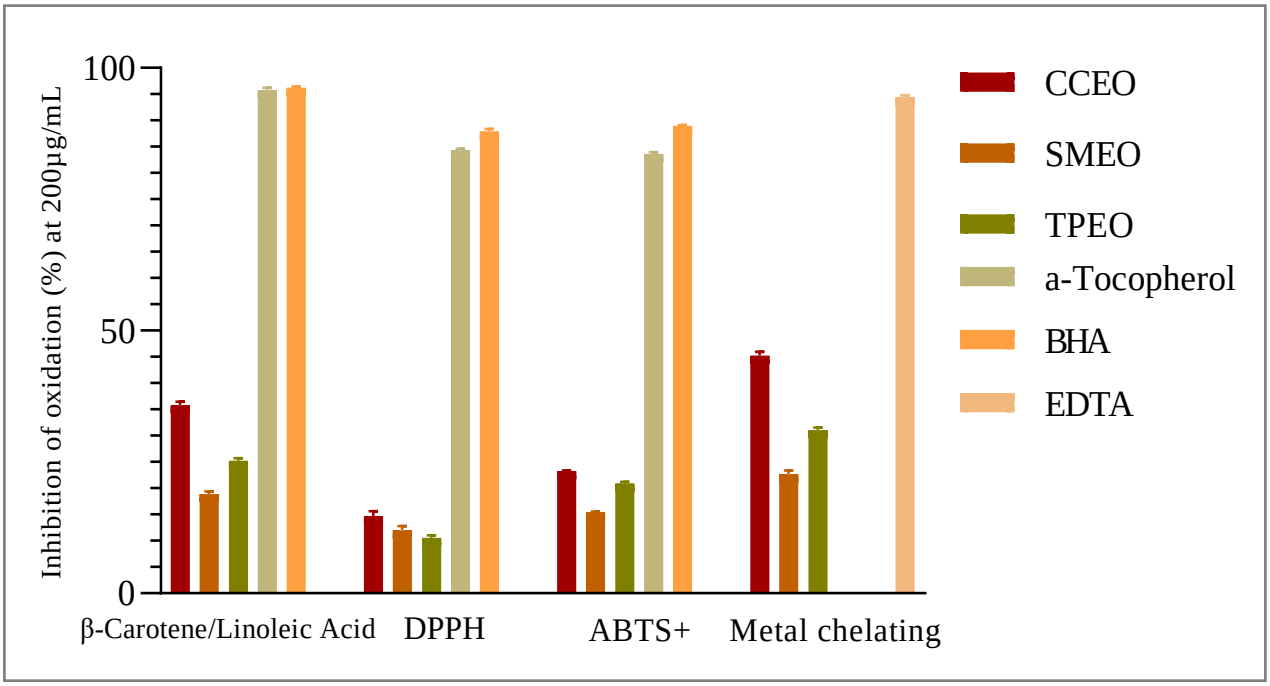

Figure 28: *Antioxidant potential of CCEO, SMEO, TPEO and their corresponding standards.*

2.2.Enzyme Inhibitory Capacity

2.2.1. Anti-cholinesterase activity

Inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) is considered an effective approach for addressing neurological disorders such as Alzheimer's disease, senile dementia, ataxia, and myasthenia gravis (S. Kumar, 2015). The exploration of diverse phytochemicals and potential plant species as cholinesterase inhibitors underscores the significant contribution of natural sources to medicinal advancements (P. K. Mukherjee et al., 2007).

Table 06 and *Figure 29* illustrates that all three essential oil samples displayed a moderate yet efficient inhibition against butyrylcholinesterase (BChE) at 200 µg/mL. Among the tested essential oils, CCEO exhibited the most potent inhibitory activity, with an inhibition percentage of 53.27 ± 0.90%. TPEO and SMEO exhibited comparable levels of inhibition, with percentages of 38.97 ± 0.25% and 35.71 ± 0.32%, respectively. On the other hand, the inhibition of acetylcholinesterase (AChE) was slightly less pronounced. CCEO demonstrated an inhibition value of 41.62 ± 0.77%, TPEO displayed 30.57 ± 0.93% inhibition, while SMEO revealed the lowest inhibition at 20.88 ± 0.45%. The standard Galantamine exhibited much greater inhibitory activities, with values of 89.25 ± 0.48% against AChE and 79.43 ± 0.60% against BChE.

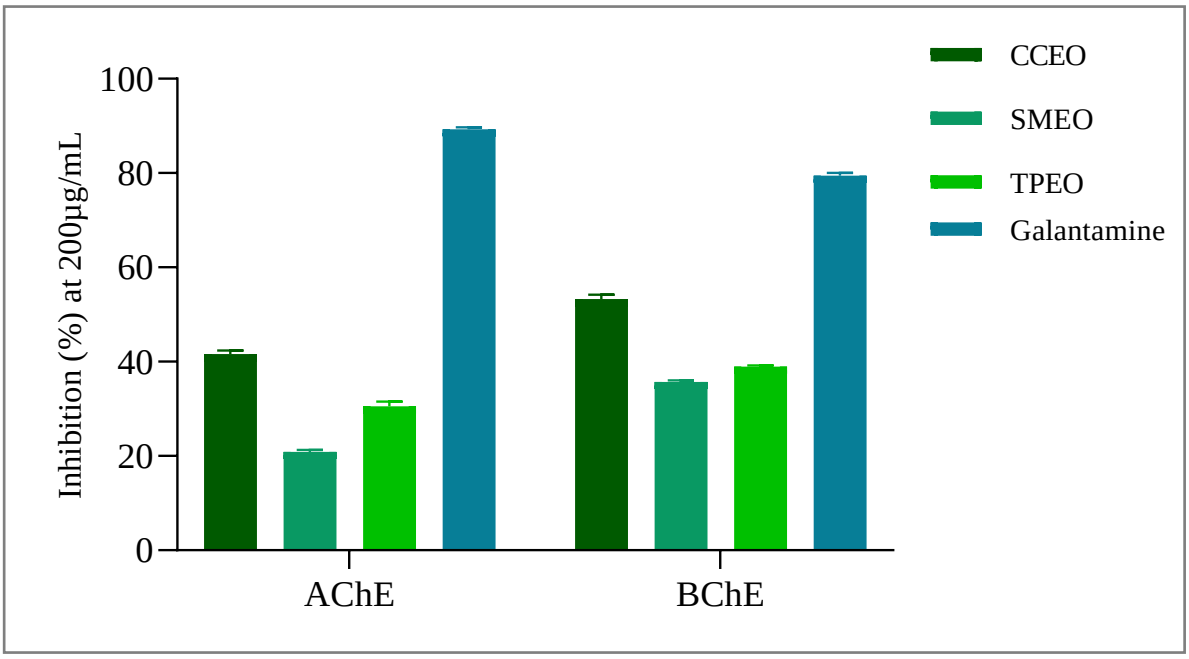

Figure 29: *Anticholinesterase potential of CCEO, SMEO, TPEO and the standard Galantamine.*

The essential oil of *C. citratus* (CCEO) demonstrates promising cholinesterase inhibitory activity, highlighting its potential for therapeutic applications, particularly in neurodegenerative diseases like Alzheimer's disease. This activity is influenced by its chemical composition, seasonal variations, and potential synergistic interactions among its components.

Seasonal changes significantly impact the cholinesterase inhibitory activity of CCEO. Madi et al., (2020) reported that the winter oil sample exhibited the highest acetylcholinesterase (AChE) inhibition (IC_{50}= 2.86 ± 0.17 mg/mL), despite having lower Citral content than samples from other seasons. Interestingly, isolated Citral demonstrated potent AChE inhibition (IC_{50} =0.21 ± 0.01 mg/mL), comparable to the standard physostigmine (IC_{50}=0.012 mg/mL). This suggests that while Citral is a key bioactive compound, the enhanced activity of winter oil could be attributed to synergistic effects with other minor constituents (Yu et al., 2011).

Cymbopogon citratus essential oil is a complex mixture of volatile constituents whose interactions significantly influence its biological activity. Owokotomo et al., (2015) highlighted that synergistic interactions among constituents could enhance AChE inhibitory effects. This is consistent with the observed high activity of winter oil, suggesting that minor constituents in the oil may amplify the activity of Citral. Such findings support the potential use of CCEO as a memory restorative agent.

CCEO exhibits activity against both AChE and butyrylcholinesterase (BChE). Kobenan et al., (2021) confirmed its inhibitory effects using a spectrophotometric method, while Chaiyana et al., (2010) demonstrated dose-dependent inhibition, with higher activity observed against BChE (59.4 ± 4.1%) compared to AChE (28.4 ± 4.4%). Furthermore, microemulsion formulations containing CCEO at 100 µL/mL inhibited both enzymes, emphasizing its potential for targeted therapeutic applications.

The inhibitory mechanism of CCEO can be attributed to its interaction with the cation-binding pocket in the active site of cholinesterase enzymes. Citral, a major component, likely binds to this hydrophobic pocket, preventing the natural substrate acetylcholine (ACh) from binding and being enzymatically cleaved (Chaiyana et al., 2010). Other hydrocarbon terpenes in the oil may also contribute to this activity, enhancing its overall inhibitory effect. This mechanism underpins the development of innovative formulations like transdermal microemulsions, enabling effective enzyme inhibition and potential therapeutic delivery.

The role of Citral in cholinesterase inhibition is well-documented. In addition to its strong binding affinity to the enzyme's active site, Citral has shown efficacy in managing mild Alzheimer's cases (Adams, 2007; Guginski et al., 2009) and improving memory and learning in animal models (Yang et al., 2009). Its contribution, alongside the synergistic effects of other oil constituents, underscores the therapeutic promise of CCEO.

The cholinesterase inhibitory potential of CCEO is a result of its complex composition, with Citral playing a pivotal role. Seasonal variations, synergistic interactions, and its dual action against AChE and BChE highlight its multifaceted activity. These findings position CCEO as a potential candidate for neuroprotective therapies, warranting further exploration into its clinical applications and formulation strategies.

The anticholinesterase activity of *S. molle* has been evaluated in several studies, revealing variability depending on the type of extract and experimental conditions. (A. Aboalhaija et al., 2019) reported that most extracts of Jordanian *S. molle* exhibited moderate to strong AChE and BChE inhibitory activities when compared to the positive control, Tacrine. Specifically, the water extract showed moderate BChE inhibitory activity but failed to inhibit AChE, whereas the chloroform extract demonstrated strong inhibition of both enzymes. Leaves' essential oil and ethyl acetate extracts exhibited moderate inhibitory activity, while fruit essential oil was inactive against BChE.

In a study by de De Souza et al., (2022), *S. molle* essential oils from Brazil demonstrated concentration-dependent enzyme inhibitory activity with an IC_{50} value of 0.047 mg/mL. Similarly, Bouhenna et al., (2021) found that methanol extracts and ethyl acetate and butanol fractions of *S. molle* were highly effective in inhibiting AChE, with IC_{50} values of 42.51 ± 1.05 µg/mL, 45.78 ± 1.59 µg/mL, and 37.35 ± 1.77 µg/mL, respectively. The chloroform fraction showed moderate AChE inhibition (IC_{50} = 148.1 ± 1.79 µg/mL). Against BChE, methanol extracts and butanol fractions exhibited the most significant activity, with IC_{50} values of 40.81 ± 1.81 µg/mL and 38.67 ± 2.03 µg/mL, respectively, followed by ethyl acetate fractions (IC_{50} = 71.85 ± 1.22 µg/mL). The essential oil extract showed weak activity against both enzymes, with IC_{50} values >200 µg/mL.

Russo et al., (2023) investigated the essential oil of *Schinus areira L.* from Argentina and found it to be more effective in inhibiting BChE (IC50 = 42.37 µg/mL) than AChE (IC_{50} = 347.3 µg/mL). Additionally, the major compound α-Phellandrene exhibited potent inhibitory

activity, with IC_{50} values of 6.04 ± 0.23 µM against AChE and 43.31 ± 0.35 µM against BChE. This aligns with earlier studies by Ustun et al., (2012), which indicated that α-Phellandrene is active against both AChE and BChE, while compounds like trans-Caryophyllene and Terpinene-4-ol strongly inhibit BChE.

The anticholinesterase potential of SMEO appears influenced by its chemical composition, particularly the presence of α-Phellandrene, which has been linked to moderate activity against both AChE and BChE. However, the overall activity of SMEO remains relatively lower than certain extracts or fractions, highlighting the need for further studies to explore its potential synergistic effects and optimize its use in therapeutic applications targeting cholinesterase enzymes.

The essential oil of *Teucrium polium* (TPEO) exhibits varying levels of inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) across several studies, revealing inconsistencies in its potency relative to other extracts or standards.

Bendjabeur et al., (2018) found that *Teucrium polium* essential oil demonstrated inhibitory activity against both AChE and BChE, although this activity was less significant compared to the standard drug, galantamine. The IC_{50} values for TPEO against AChE were reported as 89.71 ± 41 µg/mL, indicating moderate activity. In contrast, BChE inhibition exhibited lower potency, with an IC_{50} of 261.97 ± 1.70 µg/mL. Our study identified IC_{50} values exceeding 200 µg/mL for both enzymes, indicating a relatively lower inhibitory potency of the TPEO sample utilized in our research. A comparative study by Bendjabeur et al., (2018) indicated that TPEO exhibited more significant inhibitory effects than *Thymus algeriensis*, reinforcing the potential of *T. polium* in cholinesterase inhibition.

Other studies, including those by Benchikha et al., (2022), indicate that various preparations of *T. polium* may produce more potent outcomes. Benchikha et al., (2022) demonstrated *in vitro* inhibitory activity of the hydroalcoholic extract of *T. polium*, with IC_{50} values of 28.69 µg/mL for BChE and 4.93 µg/mL for AChE, exceeding the activity of galantamine, which had IC_{50} values of 34.75 µg/mL for BChE and 6.27 µg/mL for AChE. This indicates that extraction methods and the nature of the sample (oil versus hydroalcoholic extract) can affect the observed bioactivity, with specific compounds possibly enhancing or reducing the inhibitory effects.

Furthermore, research on other *Teucrium* species, including *T. montanum*, has demonstrated varying results regarding cholinesterase inhibition. Bektasevic et al., (2023) found that the

essential oil of *T. montanum* inhibited acetylcholinesterase by 51.92% and 59.32% and butyrylcholinesterase by 35.65% and 49.54% at concentrations of 1 and 2 mg/mL, respectively. Abbas et al., (2016) found that the essential oil derived from the Omanian *Teucrium mascatense* exhibited a remarkable inhibitory effect on the acetylcholinesterase enzyme, achieving over 90% inhibition at a concentration of 0.5 mg/mL. This indicates that various species within the *Teucrium* genus may demonstrate differing potencies, with the chemical composition of the oils being a significant factor in their efficacy.

Compounds such as α-Pinene, found in *T. polium* EO *(Table 15, Appendix 02)*, have demonstrated inhibitory effects on AChE and BChE (Burčul et al., 2017). The overall inhibition observed in this study may not be just due to individual compounds but rather the complex interactions among various constituents within the essential oil.

2.2.2. **Anti-diabetic activity**

Managing diabetes and its associated complications can be effectively achieved by inhibiting the enzymes α-amylase and α-glucosidase, which play a key role in starch metabolism. This approach slows down the conversion of starch into glucose, thereby aiding in the regulation of blood glucose levels (Dirir et al., 2022; Kalinovskii et al., 2023).

Table 06 and *Figure 30* displays the essential oils' antidiabetic potential. All samples exhibited a modest antidiabetic effect at 200 µg/mL, with a more significant inhibition found against α-glucosidase than α-amylase. CCEO showed the most promising results in the α-glucosidase inhibition experiment, with a percentage of 51.90 ± 0.59%. This is comparable to the standard acarbose value of 57.70 ± 0.75%. The SMEO exhibited an inhibition rate of 30.18 ± 0.48%, while the TPEO displayed the lowest inhibition rate of 29.85 ± 0.22%.

During the α-amylase inhibition test, TPEO exhibited the highest activity level with an inhibition percentage of 41.52 ± 0.32%. CCEO followed closely with a percentage of 37.43 ± 0.70%, while SMEO displayed the lowest activity at 17.65 ± 0.69%. Acarbose exhibited substantially more significant inhibition of α-amylase, with a value of 82.10 ± 0.27%.

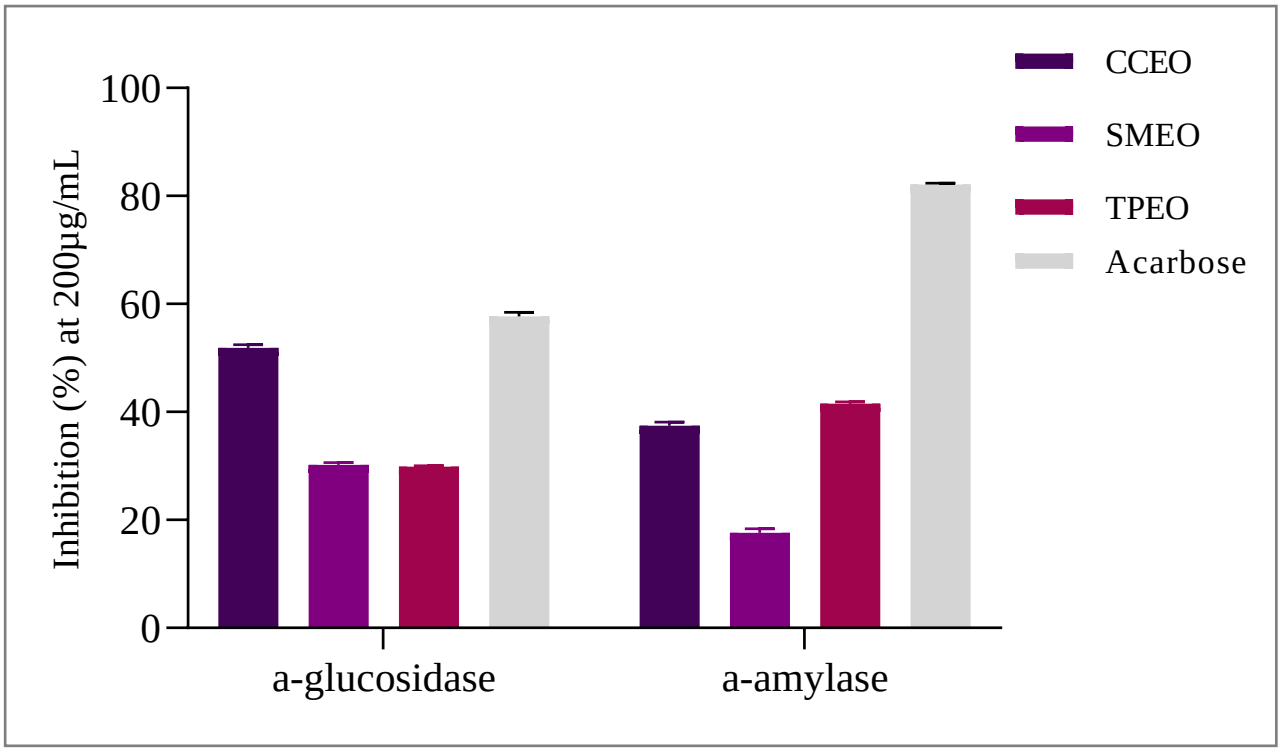

Figure 30: *Antidiabetic potential of CCEO, SMEO, TPEO and the standard Acarbose.*

Cymbopogon citratus essential oil (CCEO) demonstrates significant antidiabetic potential through various mechanisms, as evidenced by both *in vitro* and *in vivo* studies. Inhibition of key enzymes involved in glucose metabolism, such as β-glucosidase and α-amylase, highlights its potential as a natural alternative to commercial antidiabetic drugs. Mirghani et al., (2012) reported that essential oil from Lemongrass stalk showed the highest β-glucosidase inhibitory activity (89.63%) compared to leaves and commercial oil, and inhibition decreased with lower oil concentrations. Similarly, Jumepaeng et al., (2013) found that the α-amylase inhibitory activity of CCEO was ~15 times stronger than the standard drug acarbose, possibly attributed to active compounds like α-Pinene.

In vivo studies further substantiate these findings. (Bharti, 2013) observed significant improvements in glycemia, insulin levels, lipid profiles, and oxidative stress markers in diabetic rats treated with CCEO. The treatment also enhanced β-cell mass and reduced pancreatic insulitis. Garba et al., (2020) corroborated these effects in a type 2 diabetes rat model, showing improved glucose utilization, reduced insulin resistance, and amelioration of hyperlipidemia with Lemongrass tea. Additionally, the chemical composition of CCEO, including Citral, Geraniol, and Limonene, suggests a potential synergistic interaction among its constituents, contributing to its antidiabetic efficacy. The influence of harvest time on the oil's composition

further underscores the importance of optimizing production conditions to enhance its therapeutic potential.

The activity of *S. molle* against starch-metabolizing enzymes has been evaluated in various studies. Unlike our SMEO sample, which demonstrated activity against both α-amylase and α-glucosidase, Bouhenna et al., (2021) reported that *S. molle* essential oil did not exhibit any significant effect on α-glucosidase activity. However, other extracts and fractions from *S. molle* evaluated in the same study demonstrated concentration-dependent inhibition of α-glucosidase, suggesting the presence of bioactive compounds in these fractions that were absent or less active in the essential oil.

Feriani et al., (2021) explored the antidiabetic potential of methanolic extracts from fresh and mature fruits of *Schinus molle L.* and *Schinus terebinthifolius Raddi*. Their findings revealed that both species significantly inhibited α-amylase and α-glucosidase in a dose-dependent manner, outperforming the positive control, acarbose. Specifically, the scavenging activities were 84.56% and 77.49% for α-amylase, and 80.31% and 86.45% for α-glucosidase, at a concentration of 0.4 mg/mL for *S. terebinthifolius* and *S. molle*, respectively. Interestingly, *S. terebinthifolius* showed greater inhibitory capacity against α-amylase, whereas *S. molle* was more effective against α-glucosidase.

Similarly, İlgün et al., (2023) investigated the aquatic and methanolic extracts of *S. molle* leaves, raw fruits, and mature fruits against α-glucosidase and α-amylase. Their results highlighted the efficacy of the methanolic extract in inhibiting these enzymes, whereas the aquatic extract showed no effect. Furthermore, the methanolic extract was effective in reducing glucose levels in diabetic β-TC cells, demonstrating its potential as an antidiabetic agent.

The observed variability in enzyme inhibition across different studies emphasizes the importance of the extraction method and plant part in determining bioactivity. While SMEO exhibited moderate inhibitory activity, methanolic and other polar extracts from *S. molle* displayed stronger inhibition, likely due to the presence of more polar bioactive compounds not extracted into the essential oil. These findings underscore the potential of *S. molle* as a source of natural antidiabetic agents, particularly when using targeted extraction techniques.

Prior research on extracts of *T. polium* and related species indicates potential antidiabetic effects, implying that TPEO may possess bioactive properties pertinent to blood glucose regulation (Albadr et al., 2022). Numerous studies on *T. polium* extracts demonstrate the plant's

ability to inhibit enzymes associated with glucose metabolism. Benchikha et al., (2022) illustrated the anti-hyperglycemic properties of the hydroalcoholic extract of *T. polium*, which exhibited α-amylase inhibition with an IC_{50} of 111.68 µg/mL, markedly surpassing the efficacy of the standard drug acarbose (IC_{50} = 3650.93 µg/mL). This indicates that *T. polium* may serve as a natural alternative for managing hyperglycemia through enzyme inhibition. Ireng et al., (2016) demonstrated that intravenous administration of *T. polium* extract (100 mg/kg) in normoglycemic rats resulted in an acute hypoglycemic effect within 30 minutes, similar to that of insulin. This indicates that *T. polium*'s bioactivity goes beyond enzyme inhibition and may directly affect blood glucose levels through hypoglycemic action. A comparative study by Dastjerdi et al., (2014) demonstrated that both *T. polium* and *T. oliverianum* displayed significant inhibitory activity on α-amylase, indicating their potential as natural enzyme inhibitors for the management of type II diabetes. Asghari et al., (2020) noted that some *T. polium* extracts exhibited significant α-glucosidase inhibitory activity, whereas other preparations, such as a 50% methanolic extract, demonstrated only minimal α-amylase inhibition. The observed inconsistencies indicate that extract type, concentration, and application method may affect the efficacy of *T. polium* as an antidiabetic agent.

2.2.3. **Anti-urease Activity**

Urease activity presents a significant threat to human health due to its detrimental effects on cells, making it an important marker for various bacterial infections (Konieczna et al., 2012). The development of urease inhibitors from natural sources is essential to mitigate serious health risks (Tamfu, Ceylan, Fru, et al., 2020). Previous research has documented the isolation of urease inhibitors from various plants and herbs (Biglar et al., 2012; Korona-Glowniak et al., 2020; Modolo et al., 2015).

The three essential oils demonstrated moderate inhibition of urease activity, with CCEO showing the strongest effect *(Table 06, Figure 31)*. At a concentration of 200 µg/mL, CCEO achieved an inhibition rate of 58.13 ± 0.82%, making it the most effective among the essential oils tested. TPEO followed with an inhibition rate of 42.82 ± 0.73%, while SMEO exhibited a lower inhibition rate of 36.58 ± 0.47%. Despite these moderate levels, the standard reference inhibitor, thiourea, exhibited a much higher inhibition rate of 87.37 ± 0.52%, underscoring the relatively lower urease inhibition potential of these essential oils compared to standard controls.

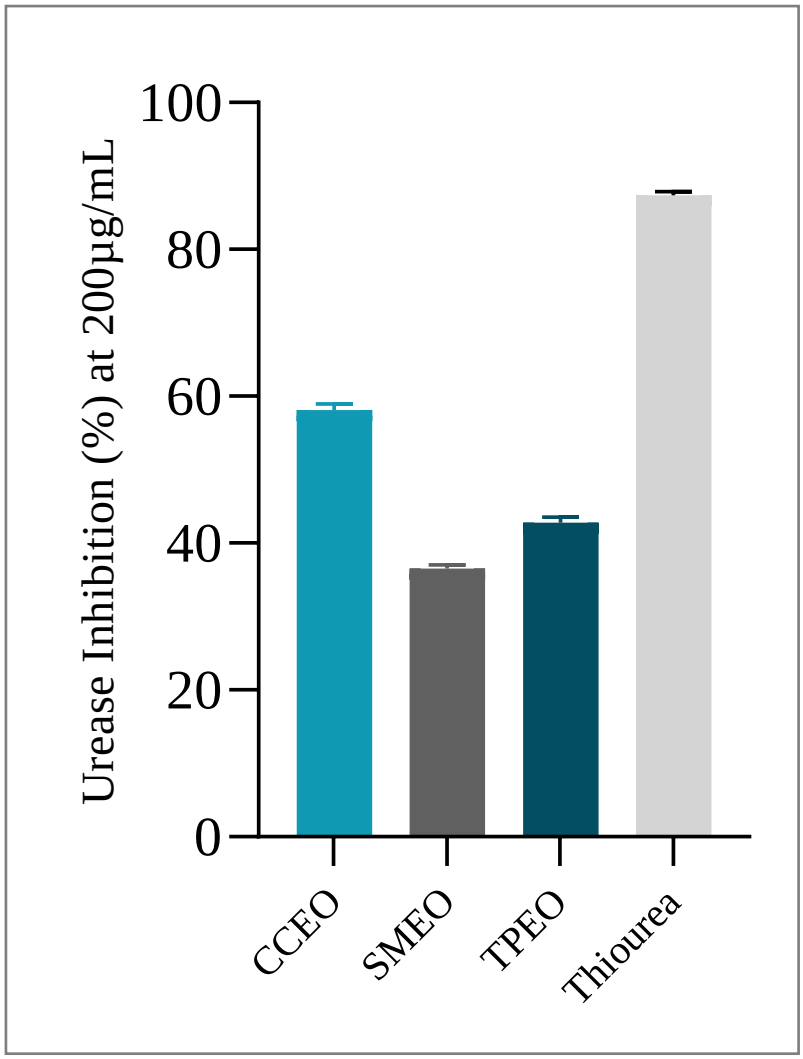

Figure 31: *Anti-urease potential of CCEO, SMEO, TPEO and the standard Thiourea.*

The urease inhibition potential of *Cymbopogon citratus* essential oil (CCEO) remains an underexplored area, with limited studies addressing its specific activity as a urease inhibitor. In the present study, our CCEO sample exhibited promising inhibition against jack bean urease, suggesting its potential to inhibit urease activity in other biological systems, including *Helicobacter pylori* urease. Nevertheless, due to structural differences between jack bean and *H. pylori* ureases, direct testing against *H. pylori* urease is necessary to validate this hypothesis and establish its clinical relevance.

Urease inhibition serves as a critical defense mechanism against *H. pylori* by preventing its adherence to the gastric mucosa. Prior studies have reported the isolation of plant-derived urease inhibitors, with mechanisms often described as noncompetitive, wherein both the inhibitor and substrate bind to the enzyme independently. For instance, Korona-Glowniak et al.,(2020) found that *Cymbopogon schoenanthus* essential oil exhibited weaker urease

inhibition (IC_{50} = 67.1 mg/L) despite demonstrating strong antimicrobial activity against *H. pylori*.

The potent urease inhibition observed in our CCEO sample can likely be attributed to its high Citral content, which constitutes 79.52% of the oil. Previous studies, such as those conducted by Lee et al., (2018), demonstrated that Citral and 4-hexen-3-one exhibit dose-dependent urease inhibition against *H. pylori* strains, including ATCC 43526 and TDR *H. pylori*. This suggests that Citral, a major component of CCEO, significantly contributes to its observed urease inhibition in jack bean assays and may exhibit similar activity against *H. pylori* urease. Further investigation is warranted to confirm these findings through specific assays targeting *H. pylori* urease and to elucidate the exact mechanism of inhibition.

The anti-urease activity of the *Schinus* genus remains underexplored. Our related article for this thesis (Kouachi et al., 2024) may be the first to analyze the anti-urease potential of *Schinus molle*. In our comparative study, SMEO exhibited moderate urease inhibitory activity at a concentration of 200 µg/mL, with an inhibition percentage of 36.6±0.5%. This activity surpassed that of *Schinus molle* essential oils obtained from other Algerian regions, which showed inhibition percentages of 34.4±0.7% from Djelfa and 28.1±0.5% from Algiers.

The potential of SMEO as a natural urease inhibitor is particularly significant, given the health risks associated with urease activity. A number of medicinal plants, including *Zygophyllum fabago* L., *Cucumis sativus* L., *Carica papaya* L., and *Annona squamosa* L., have been reported to possess urease inhibitory activity (Naz et al., 2022). This highlights the importance of identifying natural sources of urease inhibitors for therapeutic applications (Tamfu, Ceylan, Fru, et al., 2020). These plants should be further investigated to isolate pure compounds responsible for their anti-urease effects. This activity may be attributed to the synergistic effects of the active compounds, including those already identified, positioning these plants as potential candidates for developing new remedies against infections caused by urease-producing bacteria (Naz et al., 2022).

Research on the anti-urease activity of *Teucrium* species shows that different extracts have potential for urease inhibition. *Teucrium mascatense* essential oil from Oman exhibits significant urease inhibitory effects, achieving over 90% inhibition at a concentration of 0.5 mg/mL (Abbas et al., 2016). Ersoy et al., (2023) found that ethanol extracts from the aerial parts and roots of *Teucrium multicaule*, sourced from two regions in Turkey, inhibited urease activity

by 57.24% to 67.85% at a concentration of 200 mg/mL. However, the anti-urease activity of *Teucrium* species remains insufficiently studied, much like their antityrosinase potential (Ersoy et al., 2023). In contrast, other *Teucrium* species show minimal urease inhibition. Ahmad et al., (2007) reported that crude extracts and fractions of *Teucrium royleanum* exhibited no activity against urease. Studies, including Kafarski & Talma, (2018), highlight the complexity of anti-urease mechanisms and emphasize the importance of identifying plant-based urease inhibitors for their therapeutic potential.

2.2.4. **Anti-tyrosinase Activity**

Tyrosinase is a critical oxidase enzyme involved in melanin synthesis, playing a key role in skin pigmentation. Its inhibition has therapeutic potential in managing hyperpigmentation disorders and protecting against skin malignancies like cutaneous melanoma. Essential oils, with their diverse chemical compositions, have shown promise as natural tyrosinase inhibitors. In addition to medical applications, tyrosinase inhibition is important in the food industry to prevent enzymatic browning, which affects the color, taste, and nutritional quality of fruits and vegetables by reducing the bioavailability of essential amino acids. Compounds such as arbutin, kojic acid, and hydroquinones are well-known tyrosinase inhibitors widely used in cosmetics for skin whitening and in food preservation strategies (Boghrati et al., 2016; Hassan et al., 2023).

Regarding tyrosinase inhibition *(Table 06, Figure 32)*, SMEO displayed the highest inhibitory activity among the essential oils, achieving an inhibition rate of 35.80 ± 0.41% at 200 µg/mL. CCEO demonstrated a moderate inhibition rate of 29.85 ± 0.53%, while TPEO had a bit lower tyrosinase inhibition at 25.98 ± 0.60%. In comparison, the standard inhibitor, kojic acid, was significantly more effective, showing an inhibition rate of 83.54 ± 0.56%.

The anti-tyrosinase potential of *Cymbopogon citratus* essential oil (CCEO) has been evaluated in various studies, with findings indicating moderate activity that may be attributed to its chemical composition. In a comparative study of 19 medicinal plants, CCEO demonstrated an IC_{50} value of 132.16 ± 2.544 µg/mL, which, while less potent than the standard kojic acid (IC_{50} = 2.28 ± 0.054 µg/mL), surpassed the activity of other essential oils in the study and showed better results than our CCEO sample (IC_{50} > 200 µg/mL) (Aumeeruddy-Elalfi et al., 2016). Geranic acid isomers, key components of the ethyl acetate-soluble extract of Lemongrass, exhibited strong tyrosinase inhibition with IC_{50} values of 0.14 and 2.3 mM, suggesting their significant role in the observed activity (Masuda et al., 2005). Among six edible

plants tested for tyrosinase inhibition, the essential oil from fresh *C. citratus* leaves displayed the strongest activity with an IC_{50} of 0.5 mg/mL, highlighting its potential as a natural inhibitor (Saeio et al., 2011). Previous studies have correlated tyrosinase inhibitory activity with the abundance of Citral, comprising Geranial and Neral, which were the most abundant compounds in the current CCEO sample according to GC-MS analysis *(Table 15, Appendix 02).* These findings suggest that the anti-tyrosinase activity of CCEO may largely depend on these major constituents, underscoring the need for further exploration of their synergistic effects and optimization to enhance its efficacy (Matsuura et al., 2006; Saeio et al., 2011).

Similar to the lack of research on the anti-urease activity, there is limited literature on the anti-tyrosinase effects of *Schinus molle*. Our study (Kouachi et al., 2024) may be the first to investigate this aspect of *Schinus molle* essential oil. The SMEO collected from the Mascara region exhibited moderate tyrosinase inhibitory activity, with an inhibition percentage of 35.8±0.4% at a concentration of 200 µg/mL. This result was slightly lower than the tyrosinase inhibition observed in *S. molle* essential oils from Algiers (36.7±0.9%) but higher than that from Djelfa (31.5±0.6%). These results indicate that geographic location and the chemical composition of essential oils influence their anti-tyrosinase inhibitory potential.

A comparative study by (Cheraif et al., 2020) on six essential oils from Algeria suggested that the presence of compounds such as α- and β-Pinene, Limonene, p-Cymene, and Germacrene D could contribute significantly to the anti-tyrosinase potential of these plants. Tyrosinase inhibition is considered beneficial for managing skin pigmentation disorders and has been associated with the protective effects of essential oils against skin malignancies (Hassan et al., 2023).

Comparative findings indicate that various species within the *Teucrium* genus, along with different extracts from *T. polium*, demonstrate significant anti-tyrosinase effects. A study in Iran identified two phenylpropanoid glycosides—Verbascoside and Poliumoside—and two flavonoids—Jaranol and Isorhoifolin—extracted from the methanolic extract of *T. polium var. gnaphalodes*, demonstrating significant antioxidant and anti-tyrosinase activities. Jaranol has been identified for its potential use as a tyrosinase inhibitor, similar to other established tyrosinase inhibitors utilized in the cosmetics and food sectors (Boghrati et al., 2016). This indicates that the constituents of *T. polium* possess properties that may be advantageous in addressing conditions related to excessive melanin production or oxidative stress, where the demand for tyrosinase inhibitors is significant. The ethanolic extract of *Teucrium multicaule*

aerial parts and roots from two regions in Turkey demonstrated comparable and significant tyrosinase inhibitory activity of 47.03±0.72% to 66.17±0.92% (at 200 µg/mL) (Ersoy et al., 2023). A recent study on *Teucrium chamaedrys* L. cell suspension extract demonstrated its capacity to inhibit tyrosinase activity *in vitro* and to reduce melanin levels in B16-F10 melanoma cells (Pruccoli et al., 2024). This finding supports the hypothesis that *Teucrium* species may be utilized in cosmetic formulations for skin-whitening or as natural tyrosinase inhibitors, thereby expanding the therapeutic options of plant-derived tyrosinase inhibitors.

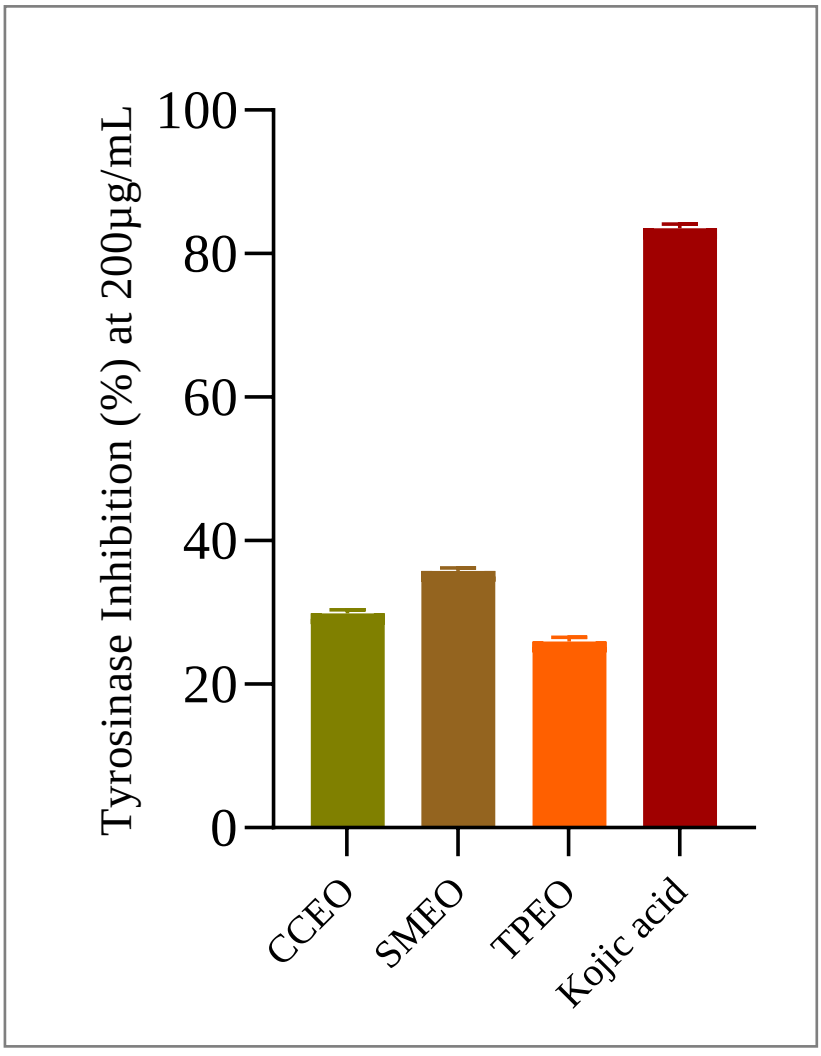

Figure 32: *Anti-tyrosinase potential of CCEO, SMEO, TPEO and the standard Kojic acid.*

Table 06: Antidiabetic, cholinesterase, urease and tyrosinase inhibitory activities of Cymbopogon citratus, Schinus molle and Teucrium polium essential oils [a]

	Cholinesterase inhibitory activity				**Anti-diabetic activity**							
	AChE		BChE		α-glucosidase		α-amylase		**Urease inhibitory**		**Tyrosinase inhibitory**	
Samples/ Standards	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)
CCEO	>200	41.62±0.77	168.1±0.95	53.27±0.90	>200	51.90±0.59	>200	37.43±0.70	123.7±1.18	58.13±0.82	>200	29.85±0.53
SMEO	>200	20.88±0.45	>200	35.71±0.32	>200	30.18±0.48	>200	17.65±0.69	>200	36.58±0.47	>200	35.80±0.41
TPEO	>200	30.57±0.93	>200	38.97±0.25	>200	29.85±0.22	>200	41.52±0.32	>200	42.82±0.73	>200	25.98±0.60
Galantamine	5.50±0.20	89.25±0.48	42.20±0.35	79.43±0.60	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*
Acarbose	*NT*	*NT*	*NT*	*NT*	128.5±0.62	57.70±0.75	32.50±0.45	82.10±0.27	*NT*	*NT*	*NT*	*NT*
Thiourea	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	8.20±0.36	87.37±0.52	*NT*	*NT*
Kojic acid	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	23.50±0.44	83.54±0.56

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$). NT: not tested.
CCEO: *Cymbopogon citratus* essential oil. SMEO: *Schinus molle* essential oil. TPEO: *Teucrium polium* essential oil.

2.3.**Anti-Inflammatory Capacity**

The anti-inflammatory efficacy of *Cymbopogon citratus*, *Schinus molle*, and *Teucrium polium* essential oils was evaluated through the Human Red Blood Cell (HRBC) Membrane Stabilization assay and the Egg Albumin Denaturation assay. The data in *Table 07* and *Figure 33* indicate that all three oils exhibited significant anti-inflammatory effects relative to diclofenac sodium.

The membrane of human erythrocytes has a composition similar to that of the lysosomal membrane, making the HRBC membrane stabilization experiment a suitable approach for assessing the anti-inflammatory properties of plant extracts. The red cell membrane stabilization activity of the three essential oil samples was quite comparable *(Table 07, Figure 33)*. CCEO and TPEO exhibited the highest denaturation inhibition at a concentration of 200 µg/mL, with a percentage of 55.13±0.48% and 52.13±0.11%, respectively. The SMEO showed a denaturation inhibition of 48.64±0.47%. At the same concentration, diclofenac sodium exhibited an inhibition rate of 75.31±0.34%.

Table 07: *HRBC membrane stabilization and Egg Albumin Denaturation inhibition of Cymbopogon citratus, Schinus molle, and Teucrium polium essential oils*[a]

	HRBC Membrane	**Egg Albumin**
Samples/ Standard	Inhibition (%) of denaturation (at 200 µg/mL)	Inhibition (%) of denaturation (at 200 µg/mL)
CCEO	55.13 ±0.48	51.05 ± 0.45
SMEO	48.64 ± 0.47	35.19± 0.31
TPEO	52.13 ±0.11	42.83 ±0.57
Diclofenac sodium	75.31 ± 0.34	64.28 ±0.39

[a] Values represent the means ± SD of three parallel sample measurements ($p < 0.05$).
CCEO: *Cymbopogon citratus* essential oil. SMEO: *Schinus molle* essential oil. TPEO: *Teucrium polium* essential oil.

The Egg Albumin Denaturation assay, on the other hand, is valuable for measuring the inhibition of protein denaturation, a process closely associated with inflammatory responses.

Our analysis found that CCEO inhibited 51.05±0.45% of Egg Albumin denaturation at 200 µg/mL. TPEO showed a little lower activity of 42.83±0.5%, while SMEO exhibited the lowest inhibition at 35.19±0.31%. However, the inhibition result obtained from Diclofenac sodium, which showed a 64.28±0.39% inhibition rate, was more significant than the inhibition rate of our plants' essential oils *(Table 07, Figure 33)*.

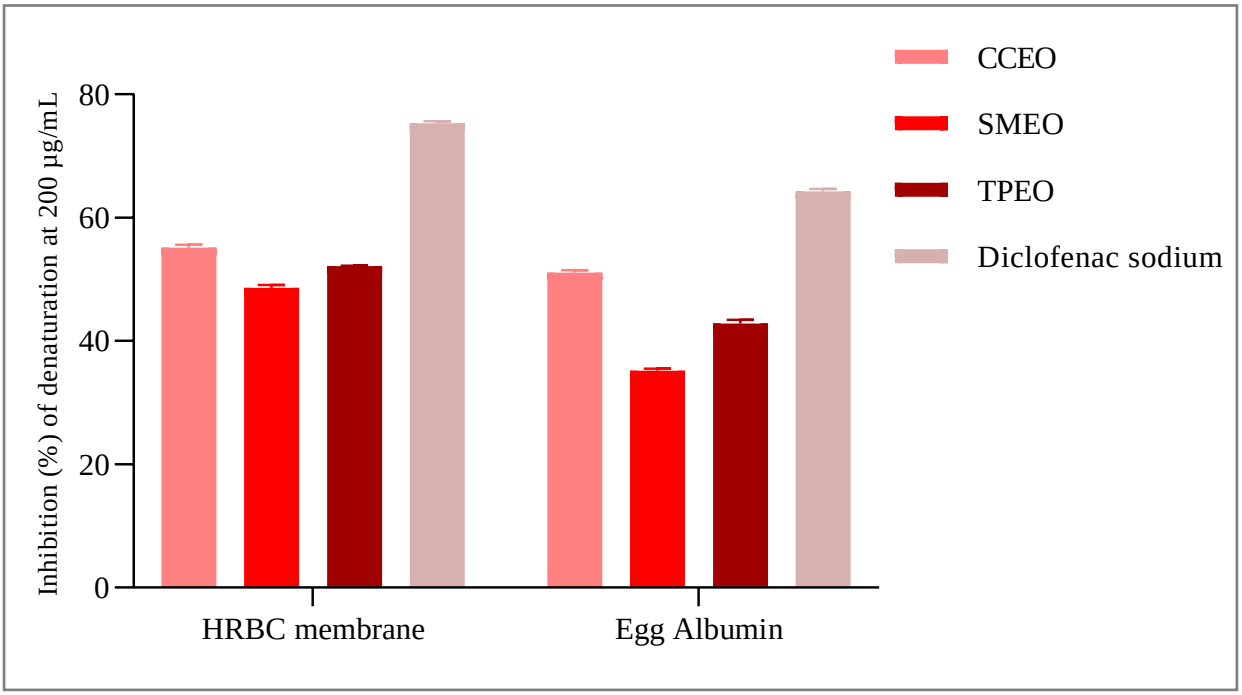

Figure 33: *Anti-inflammatory potential of CCEO, SMEO, TPEO and the standard Diclofenac sodium.*

Numerous studies have evaluated the anti-inflammatory activity of *Cymbopogon citratus* essential oils (CCEO) using both *in vitro* and *in vivo* models. Salaria et al., (2020) investigated the *in vitro* anti-inflammatory activity of CCEO by assessing egg albumin denaturation at concentrations ranging from 50 to 400 µg/mL. The results demonstrated a concentration-dependent inhibition of protein denaturation, with CCEO exhibiting superior activity (IC_{50} = 397.11 ± 1.45 µg/mL) compared to the standard diclofenac (IC_{50} = 682.98 ± 7.47 µg/mL). Another *in vitro* study by Onyedikachi et al., (2021) evaluated CCEO using HRBC membrane stabilization assays, including heat-induced hemolysis and hypotonic solution tests. The results indicated a dose-dependent anti-inflammatory response, with inhibition rates of 42.43 ± 4.27% and 39.08 ± 0.24% at 200 µg/mL, respectively. Notably, the inhibition values obtained in these studies were lower than those observed with our CCEO sample at equivalent concentrations. Talabi et al., (2018) also examined protein denaturation inhibition, comparing aqueous and hexane fractions of *C. citratus* leaves. The hexane fraction exhibited stronger anti-denaturation

activity than the aqueous fraction, suggesting that the anti-inflammatory properties of *C. citratus* may be attributed to non-polar bioactive compounds. Furthermore, the hexane fraction demonstrated superior stabilization of human erythrocytes against hypotonicity-induced hemolysis, reinforcing its potential as a membrane stabilizer.

In vivo studies further support the anti-inflammatory potential of CCEO. Boukhatem et al., (2014) evaluated the effect of orally administered Lemongrass essential oil at doses of 10–200 mg/kg using a carrageenan-induced paw edema model. Results showed significant anti-inflammatory activity, with CCEO achieving stronger inhibition of edema than the standard diclofenac at 240 minutes post-administration. In another *in vivo* assay, topical pre-treatment with CCEO resulted in dose-dependent reductions in croton oil-induced ear edema. CCEO demonstrated superior anti-inflammatory activity compared to diclofenac topical gel, suggesting its potential efficacy in managing inflammation (Boukhatem et al., 2014).

The precise mechanism underlying the anti-inflammatory effects of CCEO remains to be fully elucidated. However, it is hypothesized that various bioactive compounds, including Citral, Geranial, Neral, and Carvone, may contribute to modulating inflammatory pathways. These compounds have been associated with the inhibition of pro-inflammatory mediators and cytokines, such as IL-1β, IL-6, and TNF-α, which are involved in inflammatory responses (Boukhatem et al., 2014).

Studies have highlighted the anti-inflammatory properties of various parts and extracts of the *S. molle* plant. For instance, Feriani et al.,(2020) evaluated the erythrocyte protective effects of crude polysaccharides extracted from *Schinus terebinthifolius Raddi* and *S. molle* fruits. Their findings indicated that the anti-hemolysis inhibitory capacity of these polysaccharides was inferior to that of ascorbic acid. Additionally, administering these extracts to rats prior to carrageenan injection significantly reduced paw edema. In another study, Feriani et al., (2021) also reported that methanolic extracts of *S. terebinthifolius* and *S. molle* effectively decreased carrageenan-induced edema in a dose-dependent manner. The study further revealed that malondialdehyde (MDA) levels, a marker of lipid peroxidation, were significantly elevated ($p < 0.01$) in the paw and liver tissues of rats exposed to carrageenan. Pre-treatment with *S. terebinthifolius* and *S. molle*, however, significantly reduced ($p < 0.05$) these MDA levels, suggesting their potent antioxidative and anti-inflammatory effects.

Previous investigations have identified bioactive compounds in *S. molle* essential oils, such as Limonene (d'Alessio et al., 2013), α-Phellandrene (Siqueira et al., 2016), and Camphene (Quintans-Júnior et al., 2013), which are believed to contribute to their anti-inflammatory properties. The presence of these compounds likely accounts for the activity observed in the SMEO sample *(Table 15, Appendix 02).*

The anti-inflammatory potential of *Teucrium* species has been assessed in various studies using different types of extracts. M. Sharifi-Rad et al., (2022) assessed the anti-inflammatory properties of methanolic extracts from the aerial parts and roots of *T. polium* during three phenological phases: vegetative, flowering and seeding. The study indicated that the different extracts had significant membrane-stabilizing activities in human red blood cells, suggesting anti-inflammatory potential. The extract collected at the flowering stage exhibited the highest activity, with a stabilizing effect of 68.5% at 200 µg/mL., which surpassed the activity observed in our TPEO sample (52.13 ± 0.11%) at the same concentration. Benchikha et al., (2022) conducted an *in vitro* evaluation of the anti-inflammatory potential of *Teucrium polium* hydroalcoholic extract using a BSA denaturation assay. The results showed that at a concentration of 2 mg/mL, the extract exhibited notable anti-inflammatory activity, achieving 97.53% inhibition of protein denaturation. This effect was comparable to that of the standard drug diclofenac sodium, which produced 100% inhibition at the same concentration.

Numerous research has confirmed the anti-inflammatory properties of different *Teucrium* species using *in vivo* methods. Menichini et al., (2009) examined the *in vivo* anti-inflammatory properties of essential oils derived from *Teucrium flavum*, *Teucrium montbretia ssp. heliotropiifolium*, *Teucrium polium ssp. capitatum*, and *Teucrium brevifolium* at a concentration of 200 µg/mL. These investigations demonstrated a decrease in paw edema in animal models, akin to the suppression observed with anti-inflammatory medications. Subsequent *in vivo* investigations by Rahmouni et al., (2017) demonstrated that pre-treatment with an aqueous extract of *Teucrium polium* significantly mitigated inflammation and the rise in paw volume induced by carrageenan injection, with a notable reduction evident one hour post-treatment. Benchikha et al., (2022) documented analogous anti-inflammatory properties, indicating that oral treatment of *T. polium* aerial component extracts markedly diminished carrageenan-induced paw edema in mice ($p < 0.001$), exhibiting efficacy comparable to diclofenac sodium at a dosage of 10 mg/kg. The anti-inflammatory characteristics of *T. polium* may be ascribed to its bioactive components, such as α- Pinene, β-Pinene and Limonene, which are acknowledged

for their anti-inflammatory actions (d'Alessio et al., 2013; Salehi et al., 2019). Furthermore, Passos et al., (2007) linked the significant anti-inflammatory properties of essential oil of *Cordia verbenacea* to key chemicals like Caryophyllene and Carvacrol, both recognized for their anti-inflammatory effectiveness. Collectively, these investigations underscore the considerable anti-inflammatory potential of *Teucrium* species, mostly attributable to their varied bioactive compounds.

2.4.**Antimicrobial Capacity**

The efficacy of the three essential oil samples against the tested bacteria and fungi was supported by previous studies using the disc diffusion method as a preliminary step, which served as a foundation for conducting our research (El Atki et al., 2020; Mehreteab et al., 2023; Ujilestari et al., 2019; Valková et al., 2022).

2.4.1. **Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of a substance, such as an essential oil or antibiotic, required to inhibit visible microbial growth. Effectiveness of MIC values for essential oils can also depend on the type of pathogen; Gram positive bacteria typically exhibit lower MIC values than Gram negative bacteria, which are generally more resistant due to their cell wall structure. According to studies conducted by (Burt, 2004) and (Cosentino et al., 1999):

- Low MIC (High Antimicrobial Activity): MIC values ≤ 1 µL/mL (or µg/mL for other substances) are considered to indicate strong or potent antimicrobial activity.

- Moderate MIC: MIC values between approximately 1 to 10 µL/mL suggest moderate or intermediate antimicrobial activity.

- High MIC (Low Antimicrobial Activity): MIC values ≥ 10 µL/mL usually indicate lower antimicrobial efficacy or weak activity.

Table 08 presents the Minimum Inhibitory Concentration (MIC) values for our *Cymbopogon citratus, Schinus molle* and *Teucrium polium* essential oils against six microorganisms: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans,* and *Candida tropicalis*.

For antibacterial activity, CCEO demonstrated the most potent effects with a MIC value of 0.25 µL/mL against *B. subtilis*, 0.5 µL/mL against *E. coli*, and 2 µL/mL against both *S. aureus* and *P. aeruginosa*. This indicates that CCEO has the most potent antibacterial activity among the tested essential oil samples. SMEO showed MIC values of 1 µL/mL against *B. subtilis* and *E. coli*, 2 µL/mL against *S. aureus*, and > 2 µL/mL against *P. aeruginosa*, suggesting moderate antibacterial activity. TPEO exhibited higher MIC values of 2 µL/mL against *B. subtilis* and *S. aureus* and > 2 µL/mL against *E. coli* and *P. aeruginosa*, indicating lower antibacterial potency.

For antifungal activity, CCEO showed strong effects with a MIC of 0.5 µL/mL against both *C. albicans* and *C. tropicalis*. SMEO exhibited MIC values of 1 µL/mL against both *C. albicans* and *C. tropicalis*, indicating moderate antifungal activity. TPEO had MIC values greater than 2 µL/mL against both *C. albicans* and *C. tropicalis*, suggesting the lowest antifungal activity among the tested essential oil samples.

Overall, the essential oils exhibit both antibacterial and antifungal properties, with CCEO showing the highest potency, followed by SMEO and then TPEO.

Table 08: *MIC values and Biofilm inhibition percentages of C. citratus, S. molle and T. polium essential oils.*

Micro-organisms Tested	*S. aureus* ATCC 25923	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*P.aeruginosa* ATCC 27853	*C. albicans* ATCC 10239	*C. tropicalis* ATCC 13803
MIC (µl/ml)						
CCEO	2	0.25	0.5	2	0.5	0.5
SMEO	2	1	1	>2	1	1
TPEO	2	2	>2	>2	>2	>2
Biofilm percentage inhibition (%)						
CCEO						
MIC	47.88±0.39	70.11±1.44	58.10±2.02	45.39±0.65	55.56±3.07	49.83±0.43
MIC/2	34.75±2.21	57.73±0.74	47.21±1.34	31.37±1.23	41.50±2.98	36.03±0.14
MIC /4	18.64±1.79	29.55±2.22	24.22±1.53	16.61±0.63	21.66±0.91	19.53±0.22
MIC /8	9.53±0.39	11.78±0.61	11.97±0.97	NI	10.77±0.43	9.76±0.11
SMEO						
MIC	35.38±1.72	48.08±1.62	54.83±2.63	-	42.87±1.38	40.74±0.19
MIC/2	28.60±2.31	33.90±0.39	37.69±0.27	-	36.25±1.09	29.97±0.30
MIC /4	14.19±1.43	17.34±1.51	19.45±0.24	-	16.50±0.21	13.80±0.14
MIC /8	NI	10.25±0.62	9.52±1.26	-	NI	7.74±0.17
TPEO						
MIC	23.09±1.30	25.28±2.02	-	-	-	-
MIC/2	14.41±2.28	10.16±0.95	-	-	-	-
MIC /4	9.32±0.42	NI	-	-	-	-
MIC /8	NI	NI	-	-	-	-

CCEO: *Cymbopogon citratus* essential oil. SMEO: *Schinus molle* essential oil. TPEO: *Teucrium polium* essential oil. NI: No inhibition

Cymbopogon citratus is widely acknowledged for its notable antimicrobial properties against a diverse range of bacterial and fungal strains. Previous research has emphasized its efficacy in this context. For instance, Boukhatem et al., (2014) investigated *C. citratus* essential oils sourced from Algeria and reported significant antimicrobial activity against Gram-positive bacteria, along with pronounced antifungal effects against *Candida albicans* and *C. parapsilosis*. The minimum inhibitory concentrations (MIC) varied between 0.019 and 1.25 mg/mL for Gram-positive bacteria and yeasts, identifying *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida albicans* as the most susceptible strains. Furthermore, the diameter of the inhibition zone was found to increase proportionally with higher essential oil concentrations.

In contrast to our findings, Viktorová et al., (2020) observed that the antifungal activity of *C. citratus* essential oil surpassed its antibacterial activity. The oil demonstrated the highest efficacy against *Candida albidus*, with IC_{50} values ranging from 180 to 570 µL/L. Among the tested organisms, the lowest activity was observed against Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Salmonella enterica* (Viktorová et al., 2020). This observation aligns with our results, where *P. aeruginosa* exhibited the greatest resistance to our CCEO sample.

When compared to Lemongrass essential oil, Citral—the principal component of CCEO—demonstrated a considerably lower IC_{50}, indicating no specific antimicrobial selectivity. Unlike the whole essential oil, Citral effectively inhibited the growth of both bacteria and yeasts within a similar concentration range (Viktorová et al., 2020). Additionally, β-Pinene, a compound identified in CCEO, has been reported to exhibit antifungal activity. Other constituents, including Limonene, α-Pinene, and Carvacrol, have also displayed potent antibacterial and antifungal properties. The antimicrobial mechanism of Carvacrol has been associated with ergosterol depletion, cytoplasmic leakage, and membrane disruption. Moreover, α-Pinene has shown higher antibacterial efficacy against Gram-positive strains compared to Gram-negative bacteria, as reported by Hechachna et al., (2023).

The antimicrobial potential of *Schinus molle* essential oils (SMEO) has been extensively documented by previous studies. Deveci et al., (2010) investigated the antimicrobial activity of *S. molle* essential oils and hexanic extracts derived from leaves and fruits collected in Turkey. The findings highlighted that the extracts exhibited superior antimicrobial activity compared to the essential oils. Notably, the essential oil obtained from unripe fruits showed no antimicrobial

activity, whereas the oil extracted from ripe fruits demonstrated effectiveness against *E. coli* O157:H7 and *E. coli*.

The essential oil derived from leaves exhibited more pronounced antibacterial activity, particularly against *E. coli* O157:H7, *B. cereus*, and *S. aureus*, including methicillin-resistant *S. aureus* (MRSA). However, despite its higher antimicrobial activity, the leaf extract failed to show activity against *E. coli* and *S. typhimurium*, while displaying only weak activity against *E. faecalis* and *C. albicans* (Deveci et al., 2010).

With regard to antifungal activity, Martins et al., (2014) reported that both leaf and fruit EOs were effective against *Aspergillus* spp. and *Fusarium oxysporum*. However, the leaf EO was the only sample effective against *Rhizopus* spp. In terms of comparative efficacy, leaf EO demonstrated stronger antibacterial activity than fruit EO, particularly against Gram-positive strains, although both exhibited weaker activity against Gram-negative bacteria. Additionally, fruit EO showed higher antifungal activity against *Aspergillus japonicus* and *Aspergillus niger*, while leaf EO demonstrated greater effectiveness against *Rhizopus stolonifer*, *Aspergillus japonicus*, and *Rhizopus oryzae*.

Further supporting these observations, Rouibi et al., (2010) reported that the leaf EO of *S. molle* exhibited strong antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida albicans*.

The variations observed in the antimicrobial activity of *S. molle* EOs may be attributed to differences in the chemical composition and concentrations of active components in the oils, as well as variations in the susceptibility of target microorganisms. Given the complexity and variability of EO compositions, establishing a direct correlation between specific components and antimicrobial efficacy remains challenging.

The antimicrobial potential of *Teucrium polium* essential oil (TPEO) has been well-documented, with studies demonstrating its broad-spectrum activity, especially against gram-positive bacteria and some gram-negative strains. Belmekki et al., (2013) found that *Staphylococcus aureus* and *Escherichia coli* were notably sensitive to TPEO, as evidenced by the highest inhibition zone (16 mm) and a moderate minimum inhibitory concentration (MIC) of 3 µl/ml, suggesting a strong inhibitory effect against these organisms. In contrast, *Pseudomonas aeruginosa* demonstrated resistance to TPEO, reflecting the selective efficacy of this essential oil against different bacterial types.

Geographical differences in the antimicrobial activity of TPEO have also been observed. For example, Lograda et al., (2014) analyzed TPEO from two regions within the Setif Province and reported distinct variations in effectiveness. The TPEO sourced from Beni Aziz, Setif exhibited high antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and the yeast *Saccharomyces cerevisiae*, with lower activity against *Bacillus cereus*. Conversely, the TPEO from Boutaleb, Setif was particularly effective against *Bacillus cereus* and displayed moderate action against *Escherichia coli* but no detectable activity against *Staphylococcus aureus* or *Saccharomyces cerevisiae*. This regional variability suggests that the local environmental conditions may influence the chemical composition of the essential oil, thereby affecting its antimicrobial properties.

Seasonal variation further impacts the effectiveness of TPEO. Hechachna et al., (2023) observed that TPEO collected in winter was more potent than that collected in autumn, with MIC values of 5 µl/ml and 10 µl/ml, respectively, against *Bacillus subtilis, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The seasonal fluctuation in MIC values likely corresponds to changes in the concentration of bioactive compounds, which may vary in response to environmental conditions throughout the year.

The antimicrobial activity of TPEO is largely attributed to its composition, which includes moderate levels of oxygenated compounds such as Carvacrol, Thymol, Terpinen-4-ol, and α-Terpineol—compounds known for their broad-spectrum antifungal effects (Belmekki et al., 2013). The presence of Germacrene D, a terpene with recognized antimicrobial potential (Ngassapa et al., 2003), also contributes to TPEO's efficacy. Essential oils containing terpenes are widely reported to possess antimicrobial activity, as noted by Dorman & Deans, (2000). Additionally, minor components in the oil may contribute to its overall activity through synergistic interactions with the main active compounds (Marino et al., 2001).

2.4.2. **Biofilm inhibition potential**

Table 08 displays the antibiofilm efficacy of CCEO, SMEO, and TPEO, expressed as the percentage of biofilm inhibition at various concentrations (MIC, MIC/2, MIC/4, MIC/8).

Among the tested essential oils, CCEO showed the most significant inhibition of biofilm formation for *S. aureus* at the minimum inhibitory concentration (MIC), with a rate of 47.88 ± 0.39%. SMEO followed with a rate of 35.38 ± 1.72%, while TPEO had the lowest rate at 23.09 ± 1.30%. With decreasing concentration (MIC/2, MIC/4), all extracts exhibited a notable

decline in their ability to inhibit biofilm formation. At a concentration of MIC/8, CCEO exhibited its lowest activity, with an inhibition percentage of 9.53 ± 0.39%. In contrast, SMEO and TPEO showed no activity at this concentration.

When testing *B. subtilis*, CCEO demonstrated the most significant inhibition of biofilm formation at MIC, with a rate of 70.11 ± 1.44%. SMEO followed with a 48.08 ± 1.62% rate, and TPEO had the lowest rate at 25.28 ± 2.02%. The decline in biofilm inhibition was particularly notable when using lower concentrations, and TPEO showed no activity at MIC/4 and MIC/8, highlighting the concentration-dependent nature of the oils' efficacy.

For *E. coli*, CCEO and SMEO demonstrated comparable and significant biofilm inhibition at MIC, with rates of 58.10 ± 2.02% and 54.83 ± 1.62%, respectively. The level of inhibition decreased as the concentrations decreased, and at MIC/8, CCEO and SMEO showed the least amount of action with 11.97% ± 0.97 et 9.52 ± 1.2, respectively. While TPEO sample exhibited no inhibitory effect on *E. coli*.

Only CCEO showed biofilm inhibition against *P. aeruginosa*, compared to SMEO and TPEO, which have shown no efficacy. The inhibition percentages were 45.39 ± 20.65% at MIC, 31.37 ± 1.23% at MIC/2, and 16.61 ± 0.63% at MIC/4. However, no activity was observed at MIC/8.

For *C. albicans*, CCEO exhibited a significant biofilm inhibition rate of 55.56 ± 3.07% at the MIC, while SMEO showed a slightly lower inhibition rate of 42.87 ± 1.38%. With a decrease in concentration, both CCEO and SMEO exhibited a notable decline in their ability to inhibit biofilm formation. The most minor activity was recorded at a concentration of MIC/4 for SMEO, with a value of 16.50 ± 0.24% and at MIC/8, with a value of 10.77 ± 0.43% for CCEO. TPEO sample has shown no efficacy against *C. albicans*.

Regarding *C. tropicalis*, CCEO exhibited the most significant biofilm inhibition at MIC, with a rate of 49.83 ± 0.43%. SMEO followed closely with a biofilm inhibition rate of 40.47 ± 0.19%. As the concentration declined, the ability to inhibit biofilm formation reduced considerably, reaching its lowest level at MIC/8 with 9.76 ± 0.11% for CCEO and 7.74 ± 0.17% for SMEO. While TPEO sample has shown no efficacy against *C. tropicalis*.

To summarize, the essential oils displayed variable levels of antibiofilm action. CCEO generally exhibited the most potent inhibition against all tested microorganisms and at various concentrations, followed by SMEO and TPEO. Notably, TPEO showed no antifungal activity.

Biofilm formation is a critical factor in both medical and industrial contexts, with anti-biofilm compounds acting at two primary stages—Adhesion of planktonic cells to surfaces and Disruption of mature biofilms. Previous studies have reported that 0.125% Lemongrass essential oil effectively inhibited biofilm formation of both methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) strains *in vitro* (Adukwu et al., 2012). Similarly, Viktorová et al., (2020) demonstrated that both Citral and lemongrass essential oil inhibited the adhesion of Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria in a dose-dependent manner. Notably, in contrast to direct antimicrobial activity, a greater inhibitory effect was observed on *P. aeruginosa* adhesion compared to *S. aureus*.

In terms of mature biofilm disruption, Viktorová et al., (2020) reported that Citral exhibited concentration-dependent activity, significantly disrupting bacterial biofilms, particularly those formed by Gram-negative bacteria (*P. aeruginosa*), whereas Lemongrass essential oil was not as effective. Additionally, Citral demonstrated activity against both phases of biofilm formation—adhering and matured cells.

A study by Khosakueng et al., (2024) further confirmed the anti-biofilm potential of lemongrass essential oils. It demonstrated reduced biofilm formation in all three tested bacterial strains, including *K. pneumoniae* and *P. aeruginosa*, across all tested sub-MIC concentrations, supporting previous findings of its broad-spectrum antibiofilm activity.

Complementary findings by Scotti et al., (2021) highlighted the potent antibiofilm activity of *Cymbopogon martini*, *Cymbopogon citratus*, and *Cymbopogon flexuosus* essential oils against all tested *E. coli* strains. Investigating the mechanism of action, Gao et al., (2020) analyzed the impact of Lemongrass essential oil and Citral on the architecture of *C. albicans*/*S. aureus* dual-species biofilms. Using confocal laser scanning microscopy (CLSM), the study revealed significant alterations in biofilm matrix components, including nucleic acids, proteins, and carbohydrates. Treatments with Lemongrass essential oil and Citral resulted in a more dispersed biofilm structure, with Lemongrass essential oil demonstrating marginally greater efficacy than Citral in reducing matrix density.

Regarding fungal biofilm inhibition, Khan & Ahmad, (2012) tested the potential of *C. citratus* essential oil against 23 drug-resistant clinical isolates and 4 reference strains of *Candida spp.* (C. *albicans, C. tropicalis, C. glabrata*, and *C. krusei*). The data demonstrated the highest cidal activity from *Cymbopogon citratus* in a concentration-dependent manner, whereas antifungal drugs were less effective. These observations suggest that the essential oils target cell membranes in both planktonic and sessile cells of *C. albicans*, overcoming the resistance mechanisms associated with biofilm formation. The potent anti-biofilm activity of *Cymbopogon citratus* against *C. albicans* implies its effectiveness against adaptive resistance mechanisms exhibited by *Candida* biofilms.

Several studies have highlighted the antibiofilm potential and antimicrobial properties of *Schinus* species. Cutro et al., (2023) investigated the antibiofilm activity and mechanism of action of *Schinus areira* essential oil against *Staphylococcus aureus*. The findings demonstrated a significant, dose-dependent reduction in biofilm formation across all tested concentrations, with a maximum effect observed at 0.5× MIC, leading to approximately 75% biofilm inhibition. This effect was comparable to gentamicin at the same concentration. The assays further revealed the essential oil's capacity to both inhibit biofilm formation and eradicate preformed biofilms. Mechanistic insights suggested multiple bacterial targets, including disruptions at the membrane level and interference with DNA, emphasizing the essential oil's ability to combat both planktonic and biofilm-associated cells, thus reinforcing its potential for antimicrobial applications.

İlgün et al., (2023) examined the antimicrobial activity of methanolic and aqueous extracts from the leaves, ripe, and unripe fruits of *S. molle*. While these extracts exhibited moderate bactericidal effects against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, no biofilm inhibition was observed, indicating that their antimicrobial activity may be limited to planktonic cells rather than biofilm structures.

In a related study, Barbieri et al., (2014) reported notable antiadhesion activity of the aqueous alcohol extract of *Schinus terebinthifolius* against biofilms formed by *Streptococcus mutans* UA159 and *Candida albicans* ATCC10231. These findings suggest the potential of *S. terebinthifolius* extracts in disrupting microbial adhesion, a critical step in biofilm development.

Similarly, Romero et al., (2016) assessed the antibiofilm activity of *Schinus fasciculatus* along with other medicinal plants commonly used in Argentina against *Bacillus* strains and

clinical isolates of coagulase-negative *Staphylococcus*. The results indicated that *S. fasciculatus* extract was the most effective antimicrobial agent against *Staphylococcus sp.* Mcr1 and *Bacillus sp.* Mcn4. However, it displayed low biofilm inhibition activity against *Staphylococcus sp.* Mcr1 and no significant biofilm inhibition against *Bacillus sp.* Mcn4, suggesting a stronger action against planktonic cells than biofilm-associated forms.

Regarding the antibiofilm potential of *Teucrium polium*, studies on methanolic, ethanolic, and aqueous extracts from various *Lamiaceae* plants, including *Teucrium chamaedrys* and *Teucrium montanum*, have demonstrated significant biofilm inhibition against *Pseudomonas aeruginosa* PAO1. Notably, the ethanolic extract of *T. chamaedrys* displayed considerable biofilm degradation, reducing pre-existing biofilm by approximately 42.26% at a concentration of 625 µg/mL, highlighting the promising antibiofilm applications of *Teucrium* species (Oalđe Pavlović et al., 2024). In contrast, our TPEO showed no biofilm inhibition against *P. aeruginosa*, potentially due to the differences in composition between extracts and essential oils.

Additionally, sesquiterpenes isolated from *Teucrium polium* extracts have been shown to inhibit *Staphylococcus aureus* biofilm formation (Elmasri et al., 2014), suggesting that TPEO could potentially target biofilm-forming bacterial strains like *S. aureus*. This aligns with other findings on the antibiofilm activity of TPEO against *S. aureus*, though our results for *E. coli* were negative. In contrast, essential oils from *Lippia origanoides*, *Thymus vulgaris*, *Origanum majorana*, and *Elettaria cardamomum* have demonstrated substantial anti-biofilm efficacy against *E. coli*. Specifically, *Lippia origanoides* achieved over 70% biofilm inhibition and reduced *E. coli* motility by 55%, while *Thymus vulgaris* reduced motility by 47%, likely by interacting with the lipid bilayer's hydrophobic core and disrupting membrane stability (Martínez et al., 2021). Similarly, *Origanum majorana* essential oil effectively inhibited biofilms of both *E. coli* and *S. aureus*, possibly due to synergistic interactions among its components rather than individual monoterpenes alone (Ghazal et al., 2022). Additionally, *E. cardamomum* essential oil prevented *E. coli* biofilm formation with inhibition rates ranging from 64.29–85.59% across various concentrations (Abdullah et al., 2021). These findings point to the potential of these essential oils as alternative natural agents for managing *E. coli* biofilms.

Similarly, *Bacillus subtilis* biofilms were effectively inhibited by *Citrus aurantium* and *Coriandrum sativum* essential oils, mirroring the results obtained with TPEO (Kačániová et al., 2020).

In terms of antifungal biofilm activity, several *Lamiaceae* essential oils—including those from *Ocimum basilicum*, *Lavandula angustifolia, Melissa officinalis*, *Mentha piperita*, *Salvia officinalis,* and *Mentha spicata*—have been investigated against *Candida albicans* biofilms. Most of these oils showed no antifungal biofilm inhibition, except *Melissa officinalis*, likely due to its geraniol and citronellol content, which have recognized antifungal properties (Serra et al., 2018). This is consistent with our TPEO results, which showed no activity against *C. albicans* or *C. tropicalis* biofilm formation. Conversely, oils such as clove and thyme are known for their anti-biofilm efficacy, particularly against *Candida* spp., and can effectively prevent surface colonization (Rajkowska et al., 2019). Studies have also documented the antifungal potential of *Salvia officinalis* and *Thymus vulgaris* essential oils in reducing *C. albicans* biofilm adhesion, making them viable candidates for antifungal applications like denture cleansers. Notably, essential oils from *Thymus vulgaris* and *Carum copticum* have shown potent anti-biofilm activities against various *Candida* species, often outperforming fluconazole even at sub-MIC concentrations (Rajkowska et al., 2019).

2.4.3. Quorum sensing, Violacein production, and Bacterial motility Inhibition Capacity

The zone of inhibition, measured in millimeters, is an indicator of the antimicrobial efficacy of a substance, reflecting the size of the clear area surrounding a disc, well, or strip on an agar plate where microbial growth is suppressed. Similar to MIC values, there is no universally fixed standard for interpreting zones of inhibition, as this can vary depending on the microorganism, the compound, and experimental conditions. According to (Barry, 1991), zones greater than 15 mm typically indicate effective inhibition for many plant extracts and essential oils. While the Clinical and Laboratory Standards Institute (CLSI) guidelines provide specific values for various types of antimicrobials, they are not directly applicable to essential oils. However, they can serve as useful comparative references. For example, zones ≥ 18 mm for antibiotics or highly active compounds are generally considered effective in clinical settings. Essential oils often have variable inhibition zones based on their diffusion abilities, volatility, and chemical composition, which is why some studies focus on MIC values alongside or instead of inhibition zones. Based on general observations, the following guidelines are often used for interpreting zones of inhibition:

- Zones ≥ 15–20 mm are typically considered to demonstrate strong antimicrobial activity.
- Zones between 10–15 mm indicate moderate antimicrobial activity.

- Zones ≤ 10 mm generally suggest weak or marginal antimicrobial activity.

Table 09 present the Minimum Inhibitory Concentration (MIC) values and the corresponding violacein inhibition percentages against *C. violaceum* CV12472 at MIC and sub-MIC concentrations. CCEO and TPEO demonstrated the ability to inhibit violacein production in *C. violaceum* CV12472, with MIC values of 1 µL/mL. In contrast, SMEO had a higher MIC value of 2 µL/mL. The visual appearance of violacein inhibition results is shown in *Figure 34*.

At MIC, CCEO and TPEO demonstrated total inhibition (100 ± 0.0%) of violacein production, whereas SMEO inhibited it by 76.23 ± 1.00%. The inhibitory effects at MIC/2 were as follows: 92.94 ± 1.0% for CCEO, 41.93 ± 0.5% for SMEO, and 30.84 ± 1.3% for TPEO. The inhibition values at MIC/4 were 49.28 ± 0.65% for CCEO, 24.31 ± 0.24% for SMEO, and 15.59 ± 1.70% for TPEO. At MIC/8, CCEO showed a 26.90 ± 0.4% inhibition, and SMEO demonstrated a 10.48 ± 0.17% inhibition. However, TPEO did not show any violacein inhibition action at this dosage. Ultimately, CCEO was the only sample that achieved a MIC/16, exhibiting a violacein inhibition of 12.06 ± 0.1%.

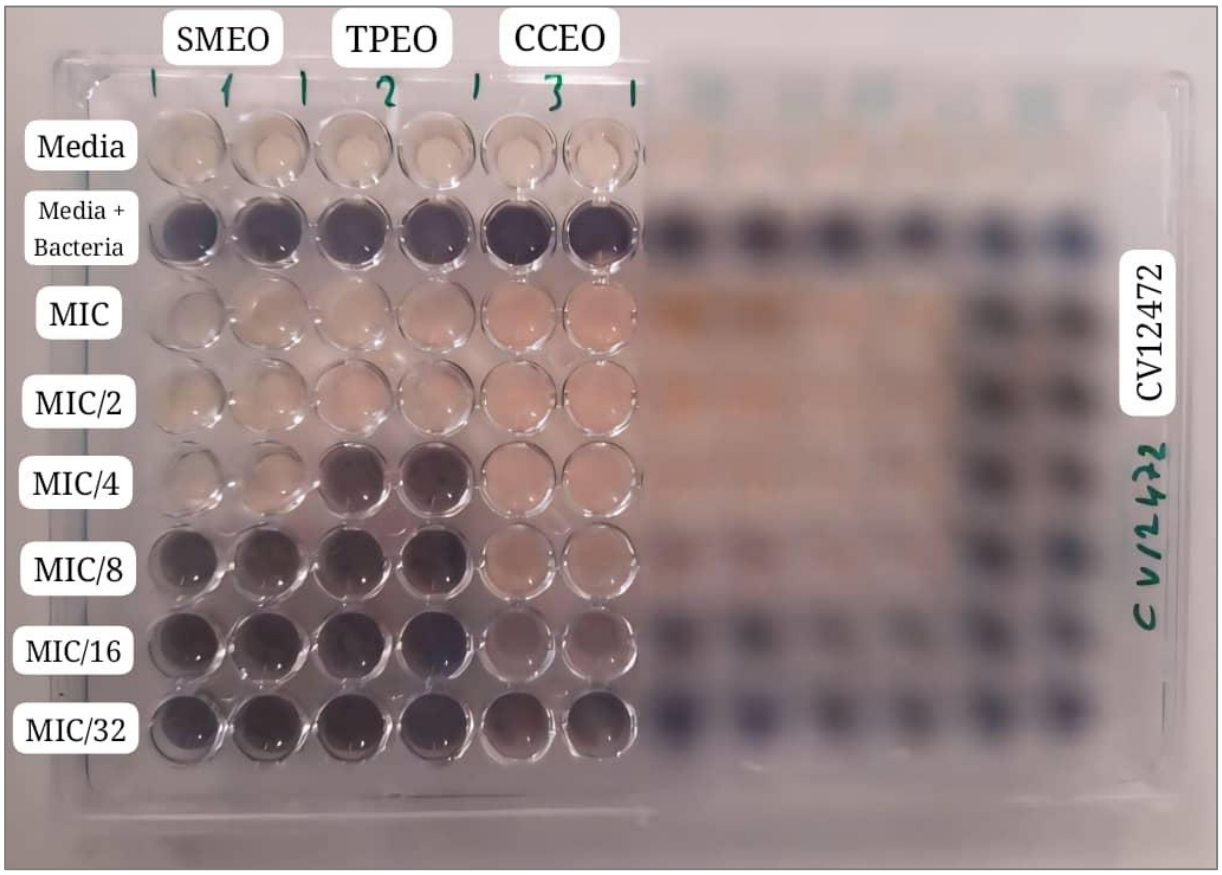

Figure 34: *Violacein inhibition potential of Cymbopogon citratus, Schinus molle and Teucrium polium essential oils.*

Table 09: *MIC values, Violacein inhibition percentage and Quorum sensing inhibition zones of Cymbopogon citratus, Schinus molle and Teucrium polium essential oils.*

	CCEO		**SMEO**		**TPEO**	
	CV12472	CV026	CV12472	CV026	CV12472	CV026
MIC (µl/mL)	1	0.5	2	0.5	1	2
	Violacein Inhibition (%)	Anti-QS zone (mm ±SD)	Violacein Inhibition (%)	Anti-QS zone (mm ±SD)	Violacein Inhibition (%)	Anti-QS zone (mm ±SD)
MIC	100±0.00	24.0±1.00	76.23 ± 1.00	21.0 ± 0.5	100±0.00	7±0.50
MIC/2	92.94±1.00	18.5±0.4	41.93 ± 0.5	15.0 ± 0.00	30.84±1.30	-
MIC/4	49.28±0.65	12.0±0.0	24.31 ± 0.24	10.0 ± 0.3	15.59±1.70	-
MIC/8	26.90±0.42	8.0±0.0	10.48 ± 0.17	7.5 ± 0.5	-	-
MIC/16	12.06±0.10	-	-	-	-	-

CCEO: *Cymbopogon citratus* essential oil. SMEO: *Schinus molle* essential oil. TPEO: *Teucrium polium* essential oil.

The quorum sensing (QS) inhibition zones for CCEO, SMEO, and TPEO against *C. violaceum* CV026 were assessed in the presence of AHL at both the minimum inhibitory concentration (MIC) and sub-MIC concentrations. The findings are displayed in *Figure 35* and *Table 09*. The minimum inhibitory concentration (MIC) values against *C. violaceum* CV026 were 0.5 µL/mL for CCEO and SMEO and 2 µL/mL for TPEO.

At the minimum inhibitory concentration (MIC), all samples showed moderate quorum sensing inhibition (QSI) levels, with inhibition zones measuring 24.0 ± 1.0 mm for CCEO, 21.0 ± 0.5 mm for SMEO, and 7.0 ± 0.5 mm for TPEO. At the minimum inhibitory concentration (MIC/2), the CCEO exhibited inhibitory effects of 18.5 ± 0.4 mm, while the SMEO showed inhibitory effects of 15.0 ± 0.0 mm. The inhibition zones at MIC/4 were 12.0 ± 0.0 mm for

CCEO and 10.0 ± 0.3 mm for SMEO. At the MIC/ 8, CCEO displayed an inhibition zone measuring 8.0 ± 0.0 mm, while SMEO showed an inhibition zone measuring 7.5 ± 0.5 mm. On the other hand, the TPEO did not reveal any quorum sensing inhibition (QSI) activity from MIC/2.

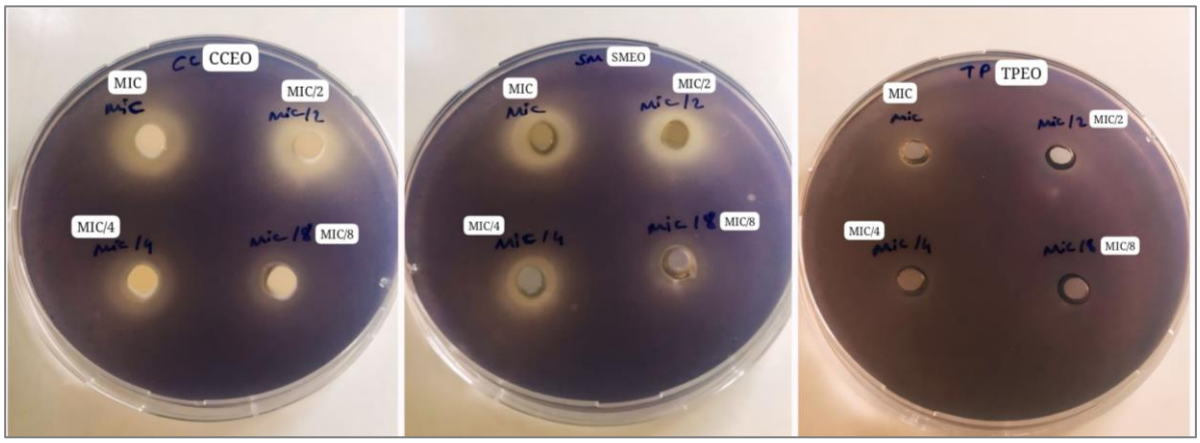

Figure 35: *Quorum sensing plates showing inhibition zones of Cymbopogon citratus, Schinus molle and Teucrium polium essential oils.*

The inhibition of swimming and swarming motility results are shown in *Figures 36*, *37* and *Table 10*. CCEO emerged as the most effective sample for inhibiting swarming motility, with inhibition percentages of 77.78 ± 0.52% at 100 µL/mL, 60.00 ± 0.16% at 75 µL/mL, and 42.00 ± 2.06% at 50 µL/mL. SMEO followed in effectiveness, showing inhibition percentages of 62.22 ± 0.65% at 100 µL/mL, 40.00 ± 0.00% at 75 µL/mL, and 28.89 ± 0.30% at 50 µL/mL. TPEO demonstrated the lowest inhibition, with percentages of 53.10 ± 0.58% at 100 µL/mL, 29.64 ± 1.44% at 75 µL/mL, and 15.57 ± 0.33% at 50 µL/mL.

Table 10: *Swarming and Swimming motility inhibition activities of CCEO, SMEO and TPEO.*

	Swarming motility (% Inhibition)			**Swimming motility (% Inhibition)**		
Concentrations (µg/mL)	CCEO	SMEO	TPEO	CCEO	SMEO	TPEO
100 µg/ml	77.78±0.52	62.22 ± 0.65	53.10±0.58	78.57±1.29	64.29 ± 0.97	57.14±1.96
75 µg/ml	60.00±0.16	40.00 ± 0.00	29.64±1.44	50.00±0.00	42.86 ± 1.44	35.71±1.08
50 µg/ml	42.00±2.06	28.89 ± 0.30	15.57±0.33	42.86±0.24	28.57 ± 1.75	14.29±0.28

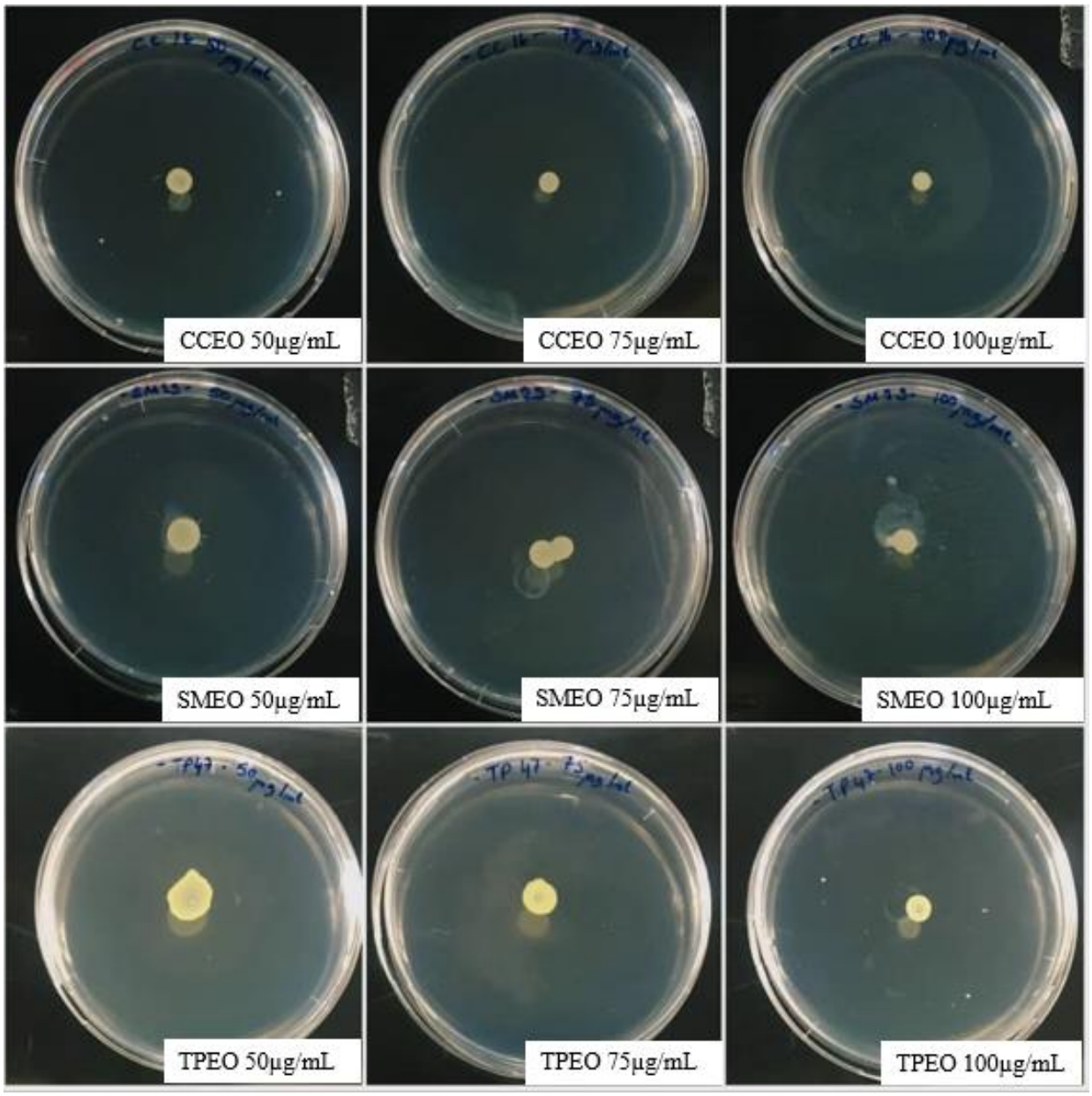

Figure 36: *Anti-swarming activity of Cymbopogon citratus, Schinus molle and Teucrium polium essential oils.*

For the inhibition of swimming motility (*Figure 31*), CCEO was also the most effective, exhibiting inhibition percentages of 78.57 ± 1.29% at 100 µL/mL, 50.00 ± 0.00% at 75 µL/mL, and 42.86 ± 0.24% at 50 µL/mL. SMEO showed inhibition percentages of 64.29 ± 0.97% at 100 µL/mL, 42.86 ± 1.44% at 75 µL/mL, and 28.57 ± 1.75% at 50 µL/mL. TPEO had the lowest inhibition, with percentages of 57.14 ± 1.96% at 100 µL/mL, 35.71 ± 1.08% at 75 µL/mL, and 14.29 ± 0.28% at 50 µL/mL.

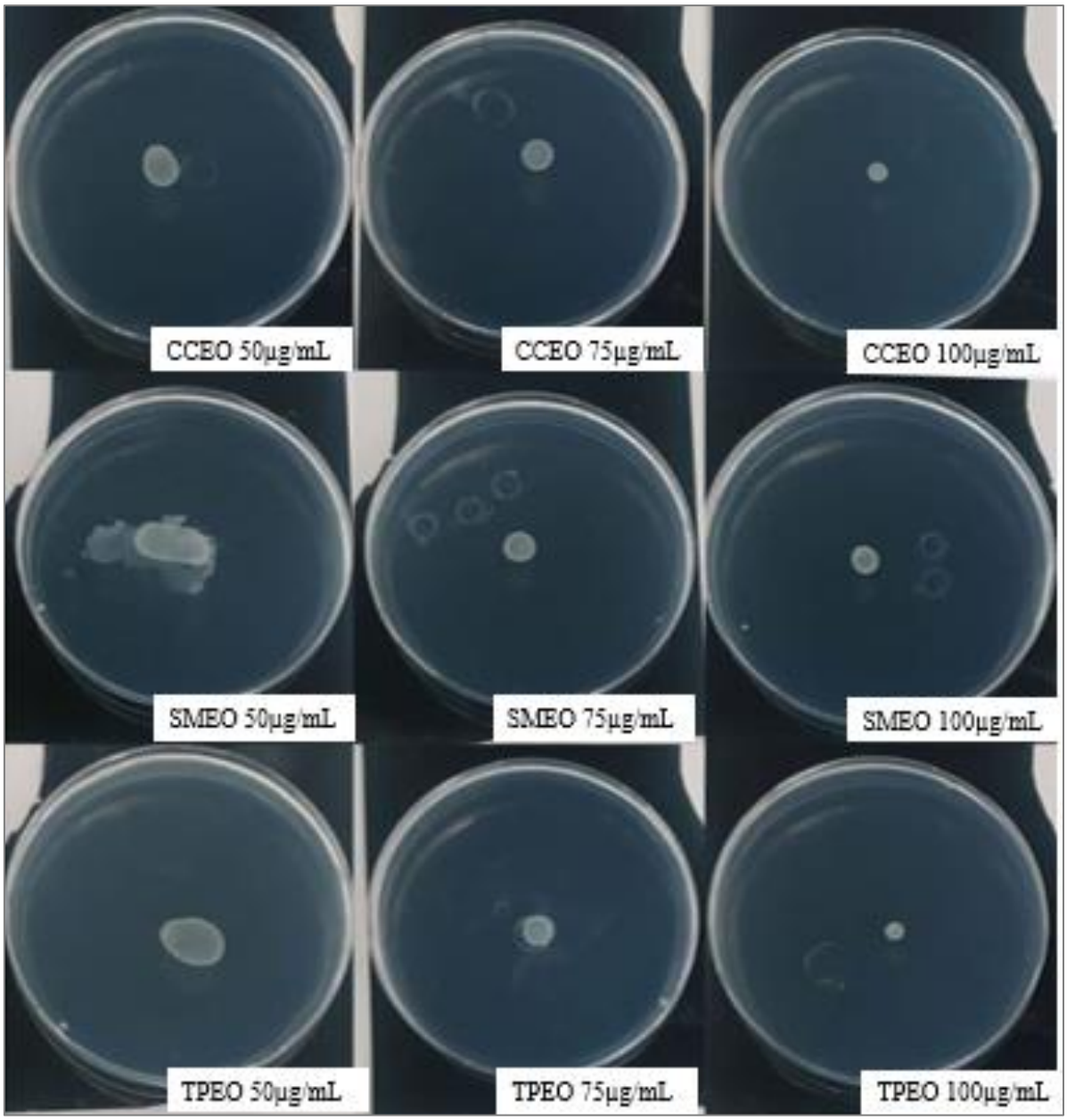

Figure 37: *Anti-swimming activity of Cymbopogon citratus, Schinus molle and Teucrium polium essential oils.*

Swimming, swarming, and violacein inhibition are frequently employed as indications of quorum sensing (QS) activity, particularly in bacterial pathogens. McClean et al., (1997) developed a biosensor system utilizing *Chromobacterium violaceum* as a model organism to identify quorum sensing inhibition via violacein production. The regulation of violacein production in *C. violaceum* by quorum sensing implies that the blockage of QS pathways results in diminished violacein synthesis, hence serving as a dependable marker for QS inhibition. Choo et al., (2006) investigated violacein production as a marker for quorum sensing in *C.*

violaceum, illustrating how QS inhibitors impede violacein synthesis, so offering a measurable method to evaluate QS activity.

Rasko & Sperandio, (2010) examined the interplay between swarming and swimming motility and quorum sensing (QS), highlighting how QS governs motility, which is crucial for bacterial pathogenicity and colonization. Quorum sensing systems regulate flagellar mobility in several bacteria, hence affecting swarming and swimming behaviors. Daniels et al., (2004) investigated the impact of quorum sensing (QS) on surface motility, including swarming and swimming, in *Pseudomonas aeruginosa*, elucidating the mechanisms via which QS governs different motility forms to promote biofilm formation and pathogenicity. Waters & Bassler, (2005) conducted a thorough study on quorum sensing in bacteria, elucidating its regulation of motility, biofilm formation, and several virulence factors.

Quorum sensing (QS) plays a pivotal role in bacterial communication, regulating various cellular processes, including virulence, motility, and biofilm formation. The inhibitory effects of *C. citratus* essential oil (CCEO) and its major component, Citral, on QS mechanisms have been extensively studied, highlighting their potential in combating microbial infections.

Studies have demonstrated the ability of CCEO to inhibit bacterial quorum sensing without directly affecting bacterial growth. Viktorová et al., (2020) evaluated the impact of CCEO and Citral using the sensor system of *V. campbellii*. Both compounds disrupted communication systems rather than cell proliferation, with Citral exhibiting slightly stronger inhibitory effects than the essential oil. Similarly, Gao et al., (2020) found that lemongrass EO and Citral effectively eradicated dual-species biofilms of *C. albicans* and *S. aureus* by hindering interactions and breaking down biofilm matrices. The treatment suppressed genes responsible for quorum sensing, cell adhesion, and virulence factors, demonstrating CCEO's broad-spectrum efficacy against biofilm-associated pathogens.

Furthermore, Naik & Premanath, (2024) highlighted the anti-QS activity of *C. citratus*, showing reductions in capsular polysaccharide, exopolysaccharide, and siderophore production. These findings suggest that CCEO can be utilized to develop alternative intervention strategies for infections caused by multidrug-resistant (MDR) strains of *K. pneumoniae*.

Khosakueng et al., (2024) evaluated the anti-QS potential of CCEO against *C. violaceum* by measuring violacein production, a QS-regulated pigment. Lemongrass EO exhibited a dose-

dependent reduction in violacein synthesis, achieving significant inhibition at sub-MIC levels. At MIC/2 and MIC/4, violacein production was reduced by 91.4% and 44.1%, respectively, without affecting bacterial viability. These findings underscore the ability of CCEO to selectively target QS-regulated pathways while preserving bacterial survival, which minimizes the likelihood of resistance development.

Motility inhibition is another critical factor in reducing bacterial virulence. Khosakueng et al., (2024) investigated the effect of CCEO on swarming motility in *P. aeruginosa*. Surprisingly, lemongrass EO increased swarming motility at different concentrations, reaching almost three times higher than the control. Non-motile strains (*K. pneumoniae* and *S. epidermidis*) showed no response to treatment, highlighting the specific interaction of CCEO with motile bacteria.

Contrary to expectations, the observed increase in motility might be linked to the disruption of QS-regulated mechanisms, potentially affecting the coordination required for biofilm formation. This phenomenon warrants further investigation to elucidate the exact pathways involved and to determine whether increased motility translates to reduced biofilm stability or facilitates dispersal, thereby making bacteria more susceptible to treatment.

The combined findings suggest that CCEO and Citral exhibit multifaceted antimicrobial actions, including quorum sensing inhibition, biofilm disruption, and modulation of bacterial motility. Their ability to suppress virulence factors and degrade biofilm matrices provides a strong foundation for their use as therapeutic agents, particularly against polymicrobial and MDR infections. Furthermore, their low cost and safety profile make them promising candidates for pharmaceutical and industrial applications.

Future studies should focus on elucidating the molecular mechanisms underlying these effects and optimizing formulations to enhance efficacy. Fractionation approaches to isolate bioactive compounds responsible for QS inhibition may further improve therapeutic outcomes (Viktorová et al., 2020).

The antimicrobial properties of extracts and essential oils from *Schinus* species have been thoroughly studied and have shown significant effectiveness against various microorganisms (Belhamel et al., 2008). However, there is limited research on their effects on bacterial communication systems, specifically quorum sensing (QS) activity and violacein inhibition. Pellegrini et al., (2014) documented a consistent inverse correlation between violacein pigment

production and *C. violaceum* counts when subjected to varying concentrations of essential oils. A significant decrease of 50% in violacein production was reported when *Schinus molle* essential oils were used at a concentration of 0.005% (v/v). The experiment revealed the strong inherent ability of pure *Schinus molle* oils to inhibit quorum sensing. Similarly, our SMEO demonstrated substantial suppression of QS at lower doses. Tamfu et al., (2020) highlighted that only substances hindering violacein formation without impacting bacterial growth could be considered authentic quorum-sensing inhibitors. This attribute was noted in our SMEO. In contrast to these findings, specific studies have shown that ethanolic extracts were inefficient in reducing violacein synthesis by *C. violaceum*. Zaki, (2013) propose that the presence of chemicals with anti-quorum sensing activity may be limited to the volatile fraction. In addition, a study conducted by Adonizio et al., in 2006 found that both ethanolic and aqueous extracts of *Schinus molle* showed no noticeable effect in inhibiting quorum sensing. The specific method via which essential oils affect the Quorum Sensing system has yet to be fully understood, mainly due to the complex combination of active chemicals found in them. However, current proposals imply that essential oils may exert their influence by limiting the generation of AHL or slowing the communication system between cells Cáceres et al., (2020).

The earliest bacterial adhesion and movement stages rely heavily on swimming and swarming motilities, which are vital in creating biofilms. Although the significance of these movements is recognized, more focused research needs to examine the effects of *Schinus molle* essential oils on swimming and swarming motilities. These motilities enable the synchronized motion and collective perception of *Pseudomonas aeruginosa*, hence aiding in the creation of biofilms. Significantly, our SMEO had captivating effects in this aspect. The chemicals found in *Schinus molle* essential oils may have a crucial function in removing the pathogenicity of this opportunistic pathogen, which is known for producing serious human infections (Tamfu, Ceylan, Fru, et al., 2020), by limiting its motilities. In addition, *Schinus* essential oils have been recognized as substances that prevent the production of biofilms. In their study, Cutro et al., (2023) found that *Schinus areira* can suppress the formation of *Staphylococcus aureus* biofilms and break down existing biofilms.

The analysis of quorum sensing (QS) inhibition and motility effects of *Teucrium polium* extracts indicates that although specific bacterial behaviors are influenced, their effectiveness seems to be concentration-dependent and relatively restricted. Unlike our findings with *T. polium* essential oil (TPEO), which exhibited significant violacein inhibition percentages, the

methanolic extract of *T. polium* failed to inhibit violacein production in *Chromobacterium violaceum* strains CV026 and ATCC 12472 (Alreshidi et al., 2020). Nevertheless, the methanolic extract demonstrated concentration-dependent inhibition of both swarming and swimming motilities in *Pseudomonas aeruginosa*, with the maximum inhibition recorded at 100 µg/mL (35.25% for swimming and 23.66% for swarming), yet these figures remained inferior to the >50% inhibition attained by our essential oil samples in both motility assays. The data indicate that *T. polium* extracts may affect bacterial motility without directly impacting violacein production, a QS-regulated pigmentation process, as demonstrated by the minimal QS inhibition zone noted in our investigation. The results indicate a partial manipulation of the QS system, wherein specific bacterial behaviors are influenced but others remain unaffected. Moreover, the alcoholic extract of *T. polium* examined by Al-Haidari et al., (2016) shown no significant effect on QS activity.

Plants of the *Lamiaceae* family have exhibited several effects related to quorum sensing (QS). Species include *Calamintha nepeta ssp. nepeta, Lavandula stoechas, Mentha suaveolens ssp. insularis,* and *Origanum vulgare* have demonstrated the capacity to affect *Chromobacterium violaceum* via violacein inhibition, with differing MICs documented (D'Aquila et al., 2023; Poli et al., 2018). Furthermore, *Mentha longifolia* demonstrated modest quorum sensing inhibitory (QSI) activity, as reported by Al-Haidari et al., (2016). Furthermore, essential oils derived from *Ocimum basilicum, Salvia officinalis,* and *O. vulgare* have been documented to impair several motility forms, such as swimming and swarming, in *Pseudomonas* species (Pejčić et al., 2020; Rossi et al., 2018).

Additionally, studies on other QS-regulated bacterial motility functions, such as swarming and swimming in *Pseudomonas* species, further illustrate the antimicrobial potential of *Lamiaceae* plants. *O. vulgare* EO, for instance, inhibited QS-related behaviors in *Pseudomonas fluorescens*, including biofilm formation, pigment production, and motility types (Rossi et al., 2018). Similarly, *Ocimum basilicum* and *Salvia officinalis* EOs significantly reduced biofilm formation and disrupted various motility forms (Pejčić et al., 2020). These observations reinforce the notion that QS inhibition in certain *Lamiaceae* EOs may extend beyond violacein suppression to broader effects on bacterial virulence factors, potentially due to a combined effect of various terpenes and phenolic compounds.

In conclusion, while *T. polium* exhibits partial inhibitory effects on bacterial motility, further research is needed to clarify its QS-regulating capabilities. The modest violacein inhibition and

limited QS-modulating effects compared to other *Lamiaceae* species suggest a less potent profile in *T. polium*, likely influenced by its specific phytochemical composition. However, the evidence from other related plants highlights the potential for QS inhibition across the family, warranting deeper exploration into optimizing and characterizing *T. polium* extracts for antimicrobial applications

2.5.Cytotoxic Potential

The cytotoxicity assay results are presented in *Figure 38* and *Table 11*.

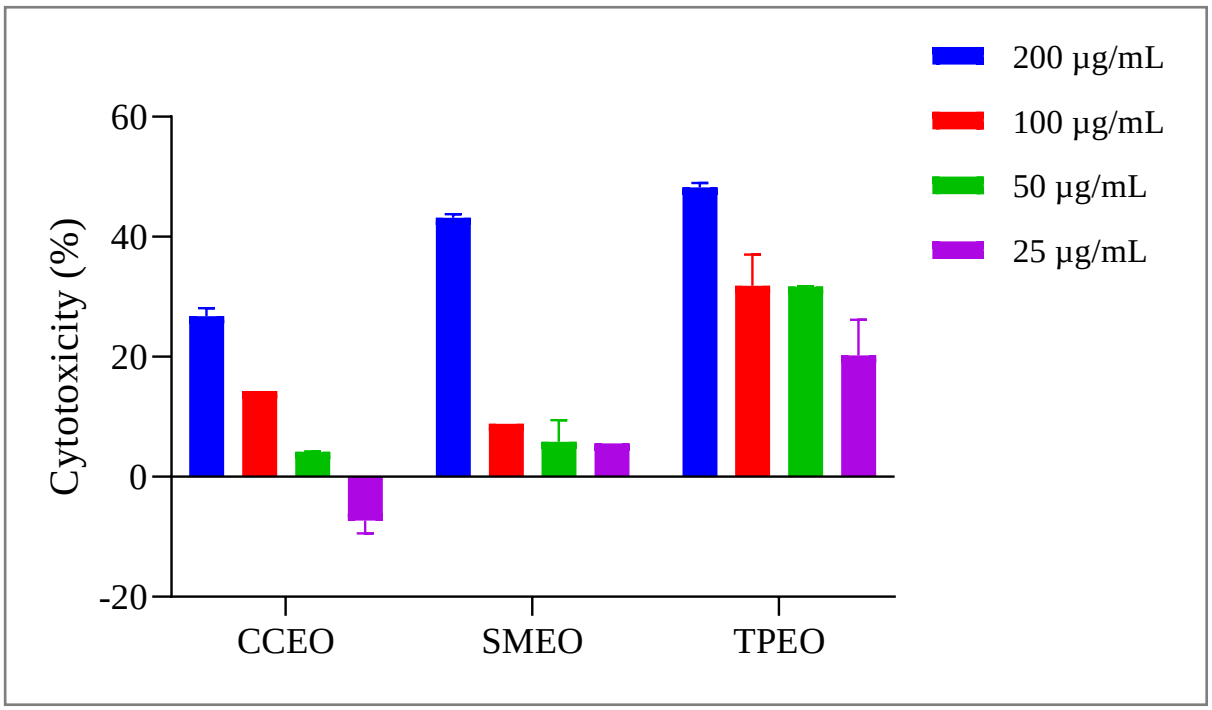

Figure 38: *Cytotoxicity percentages of CCEO, SMEO and TPEO against CCD18-Co.*

The cytotoxicity percentages varied depending on the essential oil type and concentration. CCEO and SMEO exhibited lower cytotoxicity, while TPEO showed moderate cytotoxicity; however, none of the oils reached 50% cytotoxicity at 200 µg/mL, indicating IC_{50} values greater than 200 µg/mL for all samples. These findings suggest that the essential oils extracted from *Cymbopogon citratus*, *Schinus molle*, and *Teucrium polium* growing in Algeria did not cause significant damage to healthy colon cells (CCD18-Co) at the tested concentrations (0–200 µg/mL) over a 72-hour period. This highlights the relative safety of these essential oils at these concentrations, as they do not exhibit notable toxic effects on the tested cell line.

Table 11: *IC_{50} values (± Standard Derivation) of healthy cell line (CCD18-Co) cytotoxicity assays.*

Samples	CCD18-Co IC_{50} (µg/mL)
CCEO	>200 ± 2.03
SMEO	>200 ± 1.04
TPEO	>200 ± 0.59

CCEO: *Cymbopogon citratus* essential oil. SMEO: *Schinus molle* essential oil. TPEO: *Teucrium polium* essential oil.

The assessment of the toxicity implications of essential oils represents a pivotal aspect in various domains.

Numerous research has investigated the cytotoxic characteristics of *Cymbopogon citratus* essential oil (CCEO), emphasizing its selective toxicity to cancer cells while preserving the viability of healthy cells. Bayala et al., (2018) revealed that CCEO, alongside *Cymbopogon giganteus* essential oil, exhibited notable anti-proliferative effects on multiple cancer cell lines, demonstrating efficacy comparable to the conventional anticancer drug cisplatin. Research by Trang et al., (2020) indicated that CCEO exhibits significant cytotoxic effects on various lung cancer cell lines, whereas an ethanol extract from *Cymbopogon citratus* had strong anticancer activity in lymphoma and leukemia models, efficiently inducing apoptosis (Philion et al., 2017). Conversely, research validates the safety of CCEO for healthy cells. Saeio et al., (2011) study demonstrated that the activity of CCEO on the cell viability of human peripheral blood mononuclear cells (PBMCs) was significantly non-toxic, indicating the safety of the oil samples. Experiments on mouse prostate epithelial cells (MPECs) exhibited no toxic effects, suggesting the oil's selective action on rapidly proliferating cells and its potential applicability as a therapeutic agent for cancers, including prostate cancer (Bayala et al., 2018). Furthermore, Salsabila et al., (2023) research demonstrates that CCEO, in conjunction with *Cymbopogon nardus* essential oil, is non-toxic to normal cells such as Vero and NIH-3T3, exhibiting negligible cytotoxicity and offering tissue-protective advantages by diminishing cellular

senescence and suppressing MMP-2 expression. These findings align with our results, as our CCEO also showed safety in healthy human colon cells.

The exact mechanism of action of CCEO remains inadequately explored; nevertheless, the principal constituent responsible for these effects seems to be Citral, which consists of the isomers Neral and Geranial. Ghosh, (2013) posits that the mitochondrial function of cancer cells may be affected by lemongrass and Citral, thereby explaining the heightened cytotoxicity noted in cells exposed to lemongrass essential oil and Citral. Our analysis identified Citral as the predominant chemical in CCEO, presenting an encouraging path for further research and inspiring potential therapeutic applications that could significantly impact cancer treatment.

Regarding the *Schinus molle* essential oil, Díaz et al., (2008) reported SMEO exhibited no toxicity to non-tumor mouse myoblast (C2C12) and macrophage (J774) cell lines. Likewise, prior *in vivo* investigations established the safety of *S. molle* extracts and *Schinus terebinthifolius* essential oils; assessments conducted on Wistar rats (Feriani et al., 2020) and zebrafish (Todirascu-Ciornea et al., 2019) indicated no mortality, toxic manifestations, or alterations in behavior, thereby affirming the safety of the administered doses.

Ovidi et al., (2021) performed *in vitro* tests on *Schinus molle* extracts utilizing MTT assays and found notable antiproliferative effects against two human cancer cell lines: neuroblastoma SH-SY5Y and leukemia HL60. The chemical composition of *S. molle* essential oils comprises compounds with diverse biological activities, notably Germacrene D, a sesquiterpene hydrocarbon prevalent in our SMEO sample, which may contribute to its cytotoxic effects (Casiglia et al., 2017).

The findings suggest that SMEO may exhibit selective cytotoxicity, potentially targeting cancer cells while sparing non-cancerous cell lines like CCD18-Co (N. H. Aboalhaija et al., 2019; Tayarani-Najaran et al., 2014).

Recent studies have demonstrated the selective cytotoxic properties of *Teucrium polium* extracts, suggesting a potentially beneficial therapeutic profile. No additional references regarding the application of *Teucrium polium* essential oils on healthy cells were identified for comparative analysis. A study was conducted on the aqueous extract of *T. polium*, assessing its impact on mitochondrial respiration and cell membrane integrity in healthy cell lines, specifically PC12 (rat pheochromocytoma) and HepG2 (human hepatoblastoma) cells. The findings indicated that the extract exhibited no cytotoxic effects, preserving cellular function and structure in these healthy models (Bahramikia & Yazdanparast, 2012 and references

therein). The methanolic extract of *T. polium* supports healthy peripheral blood mononuclear cells (PBMNCs) by enhancing proliferation without inducing cytotoxic responses, as demonstrated by MTT and flow cytometry analyses. In PBMNCs obtained from hepatitis C virus (HCV) patients, which are predisposed to apoptosis due to immune exhaustion, the methanolic extract of *T. polium* increased apoptosis in a dose-dependent manner (Matic et al., 2022). This selective effect is significant, as it demonstrates that *T. polium* facilitates healthy cell growth while simultaneously inducing cell death in pathological cells characterized by impaired proliferation.

The findings indicate that *T. polium* extracts may provide a dual benefit: they exhibit non-toxicity in healthy cells while demonstrating selective cytotoxicity in diseased or dysfunctional cells, thereby positioning it as a potential candidate for targeted therapeutic applications

3. SYNTHESIS OF FINDINGS

In summary, the observed variability in the biological effectiveness of the essential oils across studies can be attributed to multiple factors, including differences in plant taxonomy, geographical origin, and environmental adaptability. The three investigated plants—*Cymbopogon citratus* (Poaceae), *Teucrium polium* (Lamiaceae), and *Schinus molle* (Anacardiaceae)—belong to distinct botanical families, each characterized by unique phytochemical profiles that contribute to the variation in their bioactivities. Furthermore, the native geographic regions of these plants differ significantly, spanning North Africa, South America, and Southeast Asia, respectively. Despite these origins, all three plants demonstrated remarkable adaptability to the Algerian climate, underscoring the ecological versatility of Algerian lands to host a wide range of plant species from diverse climates.

Environmental and procedural factors, such as plant age, climatic conditions, harvest timing, and extraction methods, also play pivotal roles in influencing the yield and chemical composition of the essential oils. These compositional variations directly impact their biological activities. For instance, Citral content (a combination of neral and geranial isomers) emerged as a critical determinant of biological efficacy. Previous studies (Chaiyana et al., 2010; Cheraif et al., 2020; Matsuura et al., 2006; Saeio et al., 2011; Widelska et al., 2018) have highlighted the association of high Citral concentrations with enhanced bioactivity, alongside other key compounds such as α- and β-Pinene, Limonene, and Germacrene D. The synergy and diversity of bioactive molecules within the essential oils further amplify their therapeutic potential.

In the present study, *Cymbopogon citratus*, characterized by the highest Citral content, exhibited superior activity across most biological assays. *Teucrium polium* closely followed in performance, surpassing in α-amylase inhibition. *Schinus molle*, while less potent overall, displayed compound-specific activities; for example, its α-Phellandrene content contributed to its lower antioxidant potential (Eryigit et al., 2017). Interestingly, other studies (Russo et al., 2023; Siqueira et al., 2016) have identified α-Phellandrene as an active compound in cholinesterase inhibition and anti-inflammatory assays, highlighting the nuanced relationship between chemical composition and bioactivity.

The antimicrobial assessments revealed a more complex dynamic, strongly influenced by the microbial strains tested, the essential oil concentrations applied, and the mechanisms of action. Prior research (Houicher et al., 2016, 2018) has emphasized the importance of both the

quantity and quality of bioactive compounds in determining antifungal efficacy, as well as the susceptibility of fungal strains to variations in essential oil composition. The antimicrobial activity of terpenes and other volatile components is intricately linked to their ability to integrate into microbial cell membranes, with the lipid composition of pathogen membranes potentially modulating the oils' effectiveness (Belmekki et al., 2013; Połeć et al., 2019).

The toxicity analysis demonstrated that the essential oils from the three plant species, at the tested concentrations, did not exhibit cytotoxic effects on normal colon cells. This finding supports the safety of these oils for potential applications in areas such as aromatherapy, personal care products, and therapeutic interventions that necessitate preserving colon cell health. However, while these results are promising, further in-depth investigations are warranted to explore the oils' effects across diverse cell types, dosages, and exposure conditions.

The complexity of essential oil composition presents challenges in elucidating precise modes of action. Each constituent may exert distinct mechanisms, and minor components can play modulatory roles, potentially enhancing or altering the effects of primary bioactive molecules (Jalal et al., 2015). These findings collectively highlight the significant biological potential of the studied essential oils, with their efficacy being contingent on chemical composition, environmental influences, and the specific target microorganisms.

CONCLUSION & RECOMMENDATIONS

GENERAL CONCLUSION

This thesis investigated the multifaceted properties of essential oils extracted from *Cymbopogon citratus* (CCEO), *Schinus molle* (SMEO), and *Teucrium polium* (TPEO), collected from three distinct provinces in Algeria—Algiers, Djelfa, and Mascara—focusing on their chemical composition, biological activities, and safety profiles.

The chemical profiling of these essential oils revealed a diverse array of bioactive compounds. *Cymbopogon citratus* was notably rich in citral isomers (over 70%), a key indicator of essential oil quality. *Schinus molle* was characterized by a high concentration of α-phellandrene (12.70%), a compound commonly associated with this species. In contrast, *Teucrium polium* exhibited an unusually high level of α-fenchene (16.45%), highlighting a distinctive chemical profile. In terms of biological potentials, CCEO demonstrated the highest overall efficacy across all conducted assays, with TPEO and SMEO following in alternating order depending on the test. In the antioxidant series, all three essential oils showed their greatest activity in the metal chelating assay, with inhibition percentages ranging between 22.65±0.76% and 45.17±0.82% at 200µg/mL. In terms of anti-enzymatic properties, CCEO exhibited the strongest inhibition against AChE (41.62±0.77%) and BChE (53.27±0.90%), as well as notable activity against α-glucosidase (51.90±0.59%) and urease (58.13±0.82%). SMEO displayed the highest inhibition of tyrosinase (35.80±0.41%), whereas TPEO showed superior activity against α-amylase (41.52±0.32%). Regarding anti-inflammatory potential, all essential oils were more effective in stabilizing human red blood cell (HRBC) membranes (48.64±0.47% - 55.13±0.48%) than in preventing egg albumin protein denaturation (35.19±0.31% - 51.05±0.45%). In the antimicrobial test series, CCEO was the most potent, recording the lowest minimum inhibitory concentrations (MICs), with *Pseudomonas aeruginosa* emerging as the most resistant strain. The tested essential oils also demonstrated notable efficacy in inhibiting biofilm formation, violacein production, quorum sensing, and bacterial motility in a dose-dependent manner. Finally, cytotoxicity assays performed on healthy colon cells (CCD18-Co) confirmed the safety of all three oils at the tested concentrations, with no harmful effects observed even after 72 hours of incubation.

In conclusion, this thesis provides a comprehensive understanding of the potential and limitations of essential oils. While they hold immense promise as natural alternatives in diverse fields, further research is required to overcome challenges related to standardization, safety, and sustainability.

RECOMMENDATIONS

For Industry and Practice

Industries can use these EOs in food packaging materials to inhibit microbial growth and extend shelf life. Their natural antioxidant properties present an opportunity to replace synthetic additives in functional foods. Sustainable agricultural practices are essential to ensure a steady supply of EO-producing plants, particularly in Algeria. Partnerships with local farmers can improve yield and promote economic development. Beyond food science, EOs show promise in pharmaceuticals as antimicrobial and anti-inflammatory agents and in cosmetics for skincare and fragrance products.

For Policymakers

Establishing regulatory frameworks to standardize EO production and quality assurance is crucial. Policymakers should fund research into indigenous medicinal plants, promoting local heritage and sustainable practices. Incentives for eco-friendly extraction methods, such as supercritical CO_2 or enzymatic techniques, are necessary to minimize environmental impact.

For Researchers

Interdisciplinary collaborations are essential to address challenges like EO volatility and delivery mechanisms. Research should include bioassays targeting resistant pathogens and studies on phytochemical variability due to environmental factors. Future investigations could examine seasonal variations in EO composition and molecular mechanisms behind their bioactivities.

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APPENDICES

Appendix 01: Preparation of plant samples and essential oil extraction process.

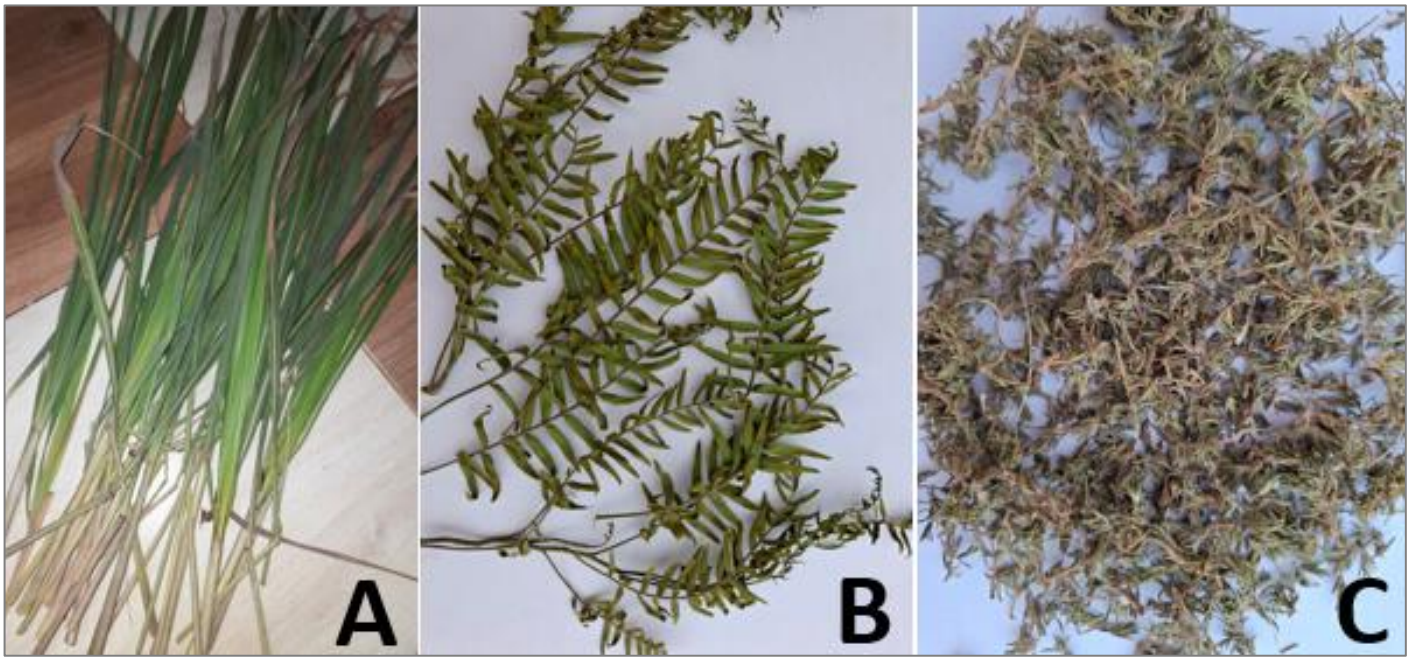

Figure 39: *Plant collection and drying process.*

A: *C. citratus* aerial parts; **B:** *S. molle* aerial parts; **C:** *T. polium* aerial parts.

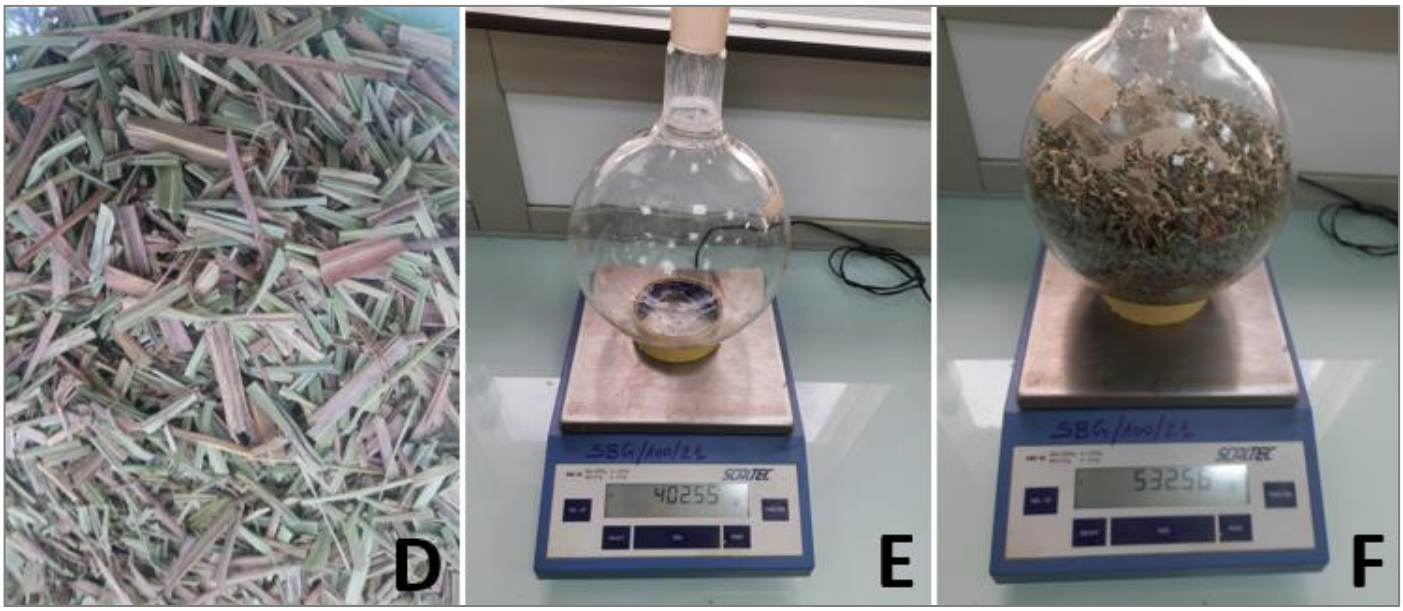

Figure 40: *Plant cutting and weight measurement for yield calculation.*

D: Cut plant material (*C. citratus);* **E**: Empty flask; **F**: Flask with plant material (*S. molle*).

Figure 41: *Essential oil extraction through hydrodistillation using a Clevenger-type apparatus*

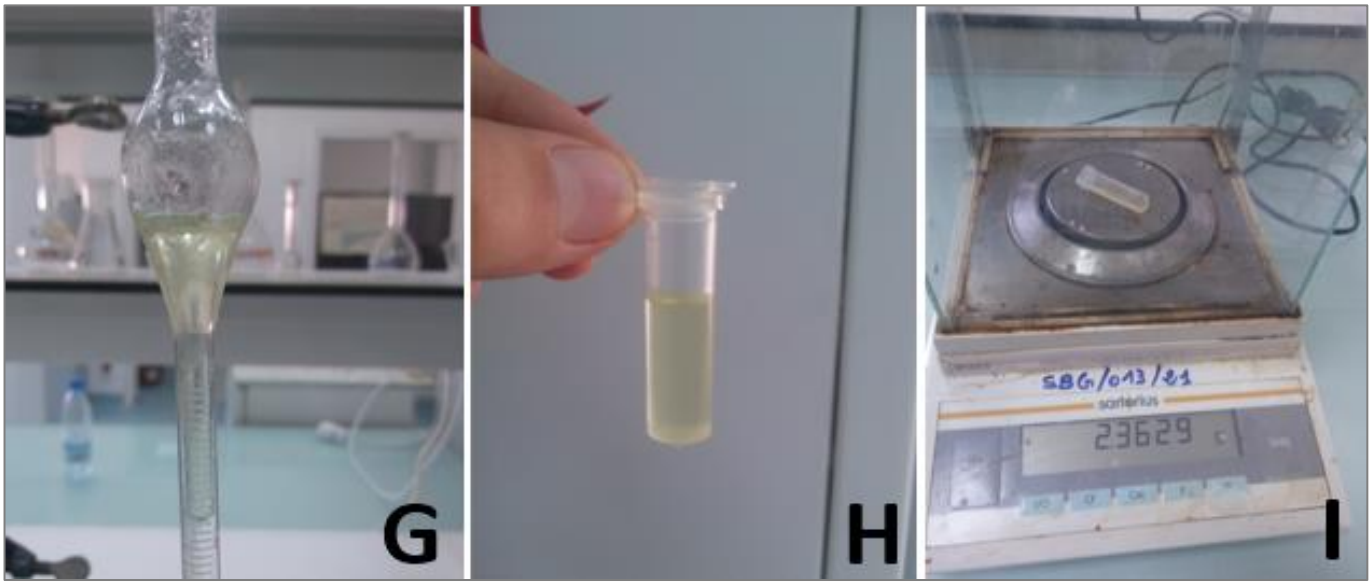

Figure 42: *Essential oil recovery and measurement for yield calculation*

G: Essential oil accumulation in Clevenger; **H**: Essential oil; **I**: Essential oil measurement

Appendix 02: Chemical Composition of Essential Oils from *Cymbopogon citratus* (CCEO), *Schinus molle* (SMEO), and *Teucrium polium* (TPEO).

Table 12: *Chemical Composition of C. citratus, S. molle and T. polium essential oils (%)*[a]

No	Compounds	RI[a]	LRI[b]	CCEO (%)	SMEO (%)	TPEO (%)	ID[c]
1	Tricyclene	921	916	-	0.61	-	MS, RI
2	α-Thujene	924	920	-	0.03	-	Co-GC, MS, RI
3	α-Pinene	928	922	0.20	4.16	4.41	Co-GC, MS, RI
4	Camphene	940	938	0.17	3.57	0.43	Co-GC, MS, RI
5	Sabinene	958	962	-	0.43	0.13	Co-GC, MS, RI
6	α-Fenchene	962	965	0.38	1.42	16.45	MS, RI
7	1-Octen-3-ol	964	966	-	-	0.20	Co-GC, MS, RI
8	6-Methyl. 5-hepten-2-one	966	970	-	-	0.26	Co-GC, MS, RI
9	β-Pinene	968	972	5.03	4.05	3.58	Co-GC, MS, RI
10	α-Phellandrene	993	998	-	12.70	0.34	Co-GC, MS, RI
11	α-Terpinene	1001	1006	-	0.03	-	Co-GC, MS, RI
12	p-Cymene	1011	1015	0.11	3.76	2.47	Co-GC, MS, RI
13	Limonene	1016	1021	0.21	11.90	6.21	MS, RI
14	1,8-Cineole	1022	1026	-	0.23	0.18	Co-GC, MS, RI
15	cis-β-Ocimene	1027	1032	0.09	-	-	MS, RI
16	trans-β-Ocimene	1034	1035	-	0.05	0.21	MS, RI
17	γ-Terpinene	1046	1047	-	-	0.77	Co-GC, MS, RI
18	cis-Linalool oxide	1057	1060	0.08	-	0.19	Co-GC, MS, RI
19	Terpineolene	1067	1073	0.07	0.08	0.34	MS, RI
20	β-Linalool	1098	1101	1.58	-	1.37	MS, RI
21	Nonanal	1102	1105	-	0.02	-	Co-GC, MS, RI
22	Octen-1-ol acetate	1112	1114	-	-	0.12	MS, RI
23	trans-p-Mentha-2,8-dienol	1120	1127	-	0.06	0.25	Co-GC, MS, RI
24	α-Campholenal	1127	1130	-	-	0.22	Co-GC, MS, RI
25	cis-p-Mentha-2,8-dien-1-ol	1137	1139	-	-	-	MS, RI
26	trans-Pinocarveol	1139	1142	0.18	-	4.50	MS, RI
27	cis-Sabinol	1141	1143	-	-	0.10	Co-GC, MS, RI
28	Camphor	1142	1146	0.39	-	2.62	MS, RI
29	β-Citronellal	1155	1157	0.09	-	-	MS, RI
30	Verbenone	1160	1161	-	-	1.05	Co-GC, MS, RI
31	Borneol	1162	1160	-	0.06	0.89	MS, RI
32	trans-2-Caren-4-ol	1163	1164	0.68	-	-	MS, RI
33	Terpinen-4-ol	1167	1170	0.25	0.09	0.42	MS, RI
34	trans-3-Caren-2-ol	1169	1172	0.98	0.05	0.19	MS, RI
35	Cryptone	1172	1175	-	0.14	0.32	MS, RI
36	α-Terpineol	1175	1179	0.09	0.05	0.80	MS, RI

37	Myrtenal	1178	1180	0.53	-	4.30	MS, RI
38	cis-Piperitol	1183	1181	-	0.03	-	MS, RI
39	Myrtenol	1185	1182	-	2.18	-	MS, RI
40	cis-Carveol	1189	1190	-	0	0.33	MS, RI
41	Bornyl formate	1199	1205	-	-	-	Co-GC, MS, RI
42	cis-Geraniol	1206	1207	0.59	-	0.59	Co-GC, MS, RI
43	trans-Carveol	1208	1209	-	-	-	MS, RI
44	Cumic aldehyde	1212	1210	-	0	-	Co-GC, MS, RI
45	β-Citral	1213	1215	36.13	0.11	6.85	Co-GC, MS, RI
46	Carvone	1220	1222	-	0.02	0.19	Co-GC, MS, RI
47	α-Limonenediepoxide	1226	1227	-	0.44	0.10	MS, RI
48	trans-Geraniol	1232	1237	3.29	-	1.43	MS, RI
49	α-Citral	1240	1243	43.36	0.15	8.37	MS, RI
50	p-Menth-1-en-9-al	1247	1252	-	-	-	MS, RI
51	Bornyl acetate	1251	1257	0.09	0.31	0.52	MS, RI
52	Thymol	1253	1255	-	-	2.05	Co-GC, MS, RI
53	Carvacrol	1257	1259	-	-	0.76	Co-GC, MS, RI
54	trans-Pinocarvyl acetate	1297	1302	-	-	0.34	Co-GC, MS, RI
55	Thymol acetate	1339	1345	0.39	0.04	-	MS, RI
56	cis-2,3-Pinanediol	1345	1348	-	0.95	-	MS, RI
57	Isopulegyl acetate	1347	1351	-	-	-	MS, RI
58	Myrtenyl acetate	1352	1356	-	0.06	0.20	MS, RI
59	Geranyl acetone	1375	1380	1.32	0.35	-	MS, RI
60	σ-Elemene	1382	1386	0.07	0.10	1.01	Co-GC, MS, RI
61	Geranyl acetate	1385	1388	0.13	0.01	-	Co-GC, MS, RI
62	α-Copaene	1387	1390	1.68	0.64	0.10	Co-GC, MS, RI
63	β-Bourbonene	1389	1393	0.36	0.10	0.63	MS, RI
64	β-Elemene	1391	1394	-	1.23	-	MS, RI
65	α-Gurjunene	1408	1410	-	1.19	-	MS, RI
66	β-Caryophllene	1414	1415	-	1.22	0.33	MS, RI
67	β-Cubebene	1420	1423	0.13	0.07	0.12	MS, RI
68	δ-Elemene	1435	1438	0.07	0.03	-	MS, RI
69	α-Caryophyllene	1447	1450	-	0.84	-	MS, RI
70	Alloaromadendrene	1456	1459	-	0.47	0.16	MS, RI
71	β-Cadinene	1462	1464	-	0.07	-	Co-GC, MS, RI
72	δ-Muurolene	1464	1466	0.03	0.36	0.25	MS, RI
73	Germacrene D	1468	1471	-	10.15	6.25	MS, RI
74	α-Selinene	1473	1475	-	0.06	0.33	MS, RI
75	Ledene	1474	1477	-	0.34	0.04	MS, RI
76	2-Tridecanone	1476	1478	0.28	-	-	MS, RI
77	δ-Gurjunene	1479	1480	-	0.70	0.93	MS, RI
78	α-Muurolene	1487	1488	-	0.86	0.23	MS, RI

79	Valencene	1490	1490	-	0.27	-	MS, RI
80	δ-Cadinene	1496	1498	-	0.98	0.35	MS, RI
81	Germacrene B	1503	1505		0.18	-	MS, RI
82	σ -Cadinene	1513	1518	0.07	4.61	0.86	MS, RI
83	cis-α-Bisabolene	1518	1522	-	-	0.17	MS, RI
84	Elemol	1523	1527	-	4.13	-	MS, RI
85	trans-Longipinocarveol	1526	1530	-	0.01	0.25	MS, RI
86	cis-Z-α-Bisabolene epoxide	1528	1536	-	-	0.25	MS, RI
87	trans-α-Nerolidol	1538	1541	-	-	0.80	MS, RI
88	Germacren-D-4-ol	1557	1562	-	1.84	-	MS, RI
89	Spathulenol	1568	1573	0.16	0.56	2.80	MS, RI
90	Caryophyllene oxide	1578	1580	0.28	0.23	0.50	MS, RI
91	Viridifloral	1580	1583	-	0.71	0.06	MS, RI
92	Aristolene epoxide	1582	1588	-	-	0.10	MS, RI
93	α-Cedrene oxide	1587	1594	0.07	5.52	0.18	MS, RI
94	Di-epi-cedrene-1-oxide	1589	1596	-	0.01	-	MS, RI
95	Ledol	1596	1600	-	3.73	0.11	MS, RI
96	Cubenol	1600	1602	0.19	0.06	0.12	MS, RI
97	Apiole	1605	1607	-	0.02	-	MS, RI
98	σ -Cadinol	1608	1612	-	0.07	0.22	MS, RI
99	δ-Eudesmol	1615	1616	-	1.09	-	MS, RI
100	tau-Cadinol	1617	1621	-	2.64	1.13	MS, RI
101	β-Eudesmol	1620	1625	-	1.01	0.48	MS, RI
102	α-Cadinol	1626	1632	0.20	3.52	1.16	MS, RI
103	Eudesm-7(11)-en-4-ol	1633	1640	-	-	0.08	MS, RI
104	Nerolidyl acetate	1690	1695	-	-	0.11	MS, RI
105	Guaiyl acetate	1712	1717	-	0.82	4.84	MS, RI
106	cis-Lanceol	1737	1742	-	1.42	-	MS, RI
			Monoterpenes	6.26	43.02	35.34	
			Oxygenated monoterpenes	90.15	4.96	38.81	
			Sesquiterpene hydrocarbons	2.41	24.47	11.76	
			Oxygenated sesquiterpenes	0.90	27.37	13.19	
			Others	0.28	0.18	0.90	
			Total identified (%)	100.00	100.00	100.00	
			Total number of components	39	72	73	

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$)
[b] (RI) Retention index: experimentally determined using homologous series of C7-C30 alkanes on Rxi-5Sil MS fused silica column
[c] (LRI) Linear retention index: taken from Adams (2007) and/or NIST14. [d] (ID) Inner diameter
Co-GC: co-injection, based on comparison with standard compounds
MS: based on comparison with WILEY, ADAMS and NIST databases

Appendix 03: Composition of Media Used in Microbiological Tests

1. **Nutrient Broth**

– Peptone: 5.0 g/L
– Beef Extract: 3.0 g/L
– Sodium Chloride (NaCl): 5.0 g/L
– pH: Adjust to 7.0 ± 0.2

2. **Sabouraud Dextrose Broth (SDB)**

– Casein peptone: 5.0 g/L
– Meat peptone: 5 g/L
– Dextrose: 20.0 g/L
– pH: Adjust to 5.6 ± 0.2

3. **Mueller-Hinton Broth (MHB)**

– Beef Infusion: 2.0 g/L
– Casein Hydrolysate: 17.5 g/L
– Starch: 1.5 g/L
– pH: Adjust to 7.3 ± 0.1

4. **Tryptic Soy Broth (TSB)**

– Tryptone: 17.0 g/L
– Soy Peptone: 3.0 g/L
– Dextrose: 2.5 g/L
– Sodium Chloride (NaCl): 5.0 g/L
– Dipotassium Phosphate (K_2HPO_4): 2.5 g/L
– pH: Adjust to 7.3 ± 0.2

5. **Luria-Bertani (LB) Broth**

– Tryptone: 10.0 g/L
– Yeast Extract: 5.0 g/L
– Sodium Chloride (NaCl): 10.0 g/L
– pH: Adjust to 7.0

6. **Luria-Bertani (LB) Agar**

- Tryptone: 10.0 g/L
- Yeast Extract: 5.0 g/L
- Sodium Chloride (NaCl): 10.0 g/L
- Agar: 15.0 g/L
- pH: Adjust to 7.0

7. **Swarming Agar Medium Plates**

- Peptone: 1%,
- NaCl: 0.5%
- Agar: 0.5%
- D-glucose: 0.5%

8. **Swimming Agar Medium Plates**

- Tryptone: 1%
- NaCl: 0.5%
- Agar: 0.3 %

9. **Molten Soft Top Agar**

For 5 mL solution of warm molten Soft Top Agar

- Agar: 1.3 g
- Tryptone: 2.0 g
- Sodium chloride: 1.0 g
- Deionized water: 200 mL

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ORIGINAL ARTICLE

Comparison of Essential Oils of *Schinus molle* L. Leaves Growing in Different Regions of Algeria in Terms of Their Chemical Compositions and Various Biological Activities

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ABSTRACT

This preliminary study investigated the chemical composition and biological properties of essential oils derived from *Schinus molle* L. leaves obtained from three specific regions in Algeria: Algiers (SMA), Djelfa (SMD), and Mascara (SMM). Gas chromatography (GC) and Gas chromatography–Mass Spectrometry (GC–MS) analyses confirmed the presence of 52 compounds (100.0%) in SMA essential oil, 50 compounds (100.0%) in SMD, and 72 compounds (100.0%) in SMM essential oils. The main components of SMA oil were camphene (31.82%), limonene (14.71%), and *p*-cymene (9.25%). In SMD and SMM oils, α-phellandrene (14.25% and 12.70%), limonene (13.02% and 11.90%), and germacrene D (10.62% and 10.15%) were the major components, respectively. The antioxidant characteristics were evaluated using five methods: β-carotene-linoleic acid, DPPH, ABTS+, CUPRAC, and Metal chelating assays. The results revealed a moderate to low anti-oxidant effect, with SMA essential oils exhibiting the highest activity. A moderate inhibitory effect against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-glucosidase, α-amylase, urease, and tyrosinase was observed, indicating anti-enzymatic activity. Nevertheless, all samples had higher IC_{50} values for both antioxidant and anti-enzymatic activities than 200 μg/mL. It was determined that regional differences in the locations where *S. molle* grows showed both qualitative and quantitative differences in essential oil components. This difference was also detected in the biological activities of the essential oils. The anti-inflammatory capacities of the three samples were assessed using Human Red Blood Cell (HRBC) membrane stabilisation and egg albumin denaturation techniques. The results showed effective anti-inflammatory activity in all three samples. The antimicrobial efficacy was evaluated using a range of tests, such as anti-quorum sensing, violacein inhibition, anti-swimming, and anti-swarming assays, demonstrating moderate activity. No toxic effects were observed at the tested dosages when assessing cytotoxicity against a healthy cell line (CCD18-Co). This thorough examination provides valuable insights into the chemical composition and bioactive properties of essential oils derived from *S. molle*. These findings indicate the possible use of these essential oils in the food, medicinal, and cosmetic industries.