CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Antimicrobial activity and chemical composition of essential oils and extracts from Indian medicinal plants

DOCTORAL THESIS

Author: Aishwarya Chaure, MSc.

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

ACKNOWLEDGEMENT-

Initially, I want to convey my appreciation to my supervisor, Prof. Ing. Ladislav Kokoška, Ph.D., for his generous guidance and invaluable advice throughout my doctoral studies. I have learned a lot from him, and I'm thankful for the chance to research in the Lab of Ethnobotany and Ethnopharmacology. I extend my gratitude to Assoc. Prof. Ing. Klára Urbanová, Ph.D., for her assistance and suggestions during all my chemical analysis sessions, and to Ing. Markéta Houdková, Ph.D., for her help with the microbiology experiments and guidance during the field trip to India. I would like to thank Prof. Mukund Lal Naik, Ph.D., and Prof. Khageshwar Singh Patel, Ph.D., for their invaluable assistance and expertise in identifying plant samples from India. I would also like to express my gratitude to Prof. Franz Bucar, Ph.D., for providing me with the opportunity to intern at his lab and deepen my understanding of the headspace technique. A special acknowledgment goes to my former colleague and best friend, Jul, whose guidance in headspace analysis and assistance with scientific writing have been indispensable. I extend my sincere gratitude to all my fellow lab mates for their unwavering support throughout this journey. Emmanuel, Genesis, Manka, and Katka, I extend my heartfelt appreciation for your presence and support.

सबसे पहले, मैं इसे अपनी दादी को समर्पित करना चाहता हूं, जिनका मुझ पर और मेरे सपनों पर अटूट विश्वास हर दिन मुझे प्रेरित करता रहता है। मुझे उनकी बहुत याद आती है। मैं अपने माता-पिता के प्रति भी आभार व्यक्त करना चाहता हूं, जिन्होंने मेरे लिए एक दृढ़ समर्थन प्रणाली बने रहने का कार्य किया, और अपनी बहन अन्मोल के प्रति, जिनके योगदान ने यह सब संभव बनाया। इसके अतिरिक्त, मैं अपने दादा और करीबी रिश्तेदारों के प्रोत्साहन और समर्थन के लिए भी सराहना व्यक्त करना चाहता हूं। अंत में, मैं प्राग में अपने दोस्तों का भी धन्यवाद करना चाहता हूं, जो यहां मेरे लिए दूसरा परिवार बन गए हैं।

This research was financially supported Czech University of Life Sciences Prague Internal Grant Agency project IGA [20223102].

CERTIFICATION

I, Aishwarya Chaure, submitted this dissertation for Ph.D. degree at the Czech University of Life Sciences Prague, Faculty of Tropical AgriSciences, declare that this dissertation is my own work unless otherwise referenced or acknowledged.

In Prague, 2024

…….

Aishwarya Chaure, MSc.

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ATCC	American Type Culture Collection
CbpA	Choline-Binding Protein A
COVID-19	Corona Virus Disease 2019
D-HS	Dynamic Headspace
DMSO	Dimethyl Sulfoxide
EMEM	Eagle's Minimum Essential Medium
EO	Essential Oil
ED	Effective dose at 50% inhibition
Esp	*Enterococcus* surface protein
FBS	Fetal Bovine Serum
FID	Flame Ionization Detector
GC	Gas chromatography
GTS	Gas Tight Syringe
Hap	*H. influenzae* autotransporter protein
Hib	*H. influenzae* type b
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HS	Headspace
IC_{50}	Half-maximal Inhibitory Concentration
IC_{80}	80% Inhibitory Concentration of Proliferation
Ig_A	Immunoglobulin A
IS	Internal standard
LRTI	Lower Respiratory Tract Infection
MHB	Muller Hinton Broth
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant *S. aureus*
MS	Mass Spectrometry
m/z	Mass-to-charge ratio
MTT	Thiazolyl blue tetrazolium bromide
NAD	Nicotinamide Adenine Dinucleotide
NMR	Nuclear Magnetic Resonance
NTHi	Non-encapsulated, non-typable *H. influenzae*
OD	Optical Density
PSaA	Pneumococcal Surface Adhesin A

PspC	Pneumococcal Surface Protein
RI	Retention Indices
RT	Retention Time
S-HS	Static Headspace
SM	Secondary Metabolites
SPME	Solid Phase Micro-Extraction
SpsA	*S. pneumoniae* secretory IgA binding protein
TI	Therapeutic Index
UNICEF	United Nations Children's Fund
UTI	Urinary Tract Infections

ABSTRACT

Despite the progress of medicine accomplished in recent decades, infectious diseases remain a major cause of human suffering in terms of both morbidity and mortality. The use of antibacterial agents such as antibiotics is essential in the treatment of patients with bacterial infections. However, multidrug resistance, drug side effects, and drug delivery challenges hinder treatment. Plant-derived products, like extracts and essential oils (EOs), offer promising alternatives due to their relative safety, cost-effectiveness, and therapeutic benefits. Among traditional medicines, Ayurvedic herbal preparations remain underexplored for their potential in combating infections. Therefore, the aim of the current study was the determination of the antimicrobial activity of ethanolic plant extracts and the evaluation of cytotoxic and antibacterial effects of EOs in liquid and vapour phases including their chemical analysis. The results indicated that the extract of *Psoralea corylifolia* L. exhibited the strongest antimicrobial effect, inhibiting the growth of *S. aureus* with minimum inhibitory concentrations (MICs) ranging from 64 to 128 μg/mL, and *C. albicans* with an MIC of 512 μg/mL. In contrast, the extracts of *Mucuna pruriens* (L.) DC. and *Cocculus hirsutus* (L.) Diels displayed weak antibacterial activity against *S. aureus*, with an MIC of 512 μg/mL. Among all the EO samples tested, *Trachyspermum ammi* (L.) Sprague EO showed the strongest antibacterial effect against *H. influenzae*, with MICs of 128 and 256 μg/mL in the liquid and vapour phases, respectively. The cytotoxicity assay revealed that *Cyperus scariosus* R.Br. EO was non-toxic with an IC-50 value greater than 258 μg/mL, whereas *Cymbopogon citratus* (DC.) Stapf and *T. ammi* were moderately toxic to normal lung fibroblasts, with IC-50 values of 19.63 and 82.04 μg/mL, respectively. Chemical analysis using gas chromatography-mass spectrometry identified α-citral, β-citral, and caryophyllene oxide as the main constituents of *C. citratus* EO. Cyperotundone, caryophyllene oxide, and cyperene were the predominant compounds of *C. scariosus* EO. In the case of *T. ammi* EO, thymol, p-cymene, and γ-terpinene were the most abundant compounds. α-citral, β-citral, and camphene were the major compounds identified in *C. citratus* EO vapours using the solid phase micro-extraction sampling technique, whereas, camphene, limonene, and α-citral were reported to be the abundant ones when analysed by gas-tight syringe technique. p-cymene, γ-terpinene, β-pinene, and thymol were identified as the predominant compounds of *T. ammi* EO vapours using the SPME and GTS sampling techniques. The results of the study indicate the considerable potential of Indian medicinal plants for the development of anti-infective preparations. Specifically, it suggests the extract of *P. corylifolia* and EO of *T. ammi* as promising agents for developing new plant-derived anti-infective preparations. However, further research focused on the isolation and characterization of the antimicrobial constituents of *P. corylifolia* extract and the *in vivo* efficacy of *T. ammi* EO will be necessary to verify their practical applicability.

Key words: antimicrobial activity; cytotoxicity; essential oils; ethanol extracts; GC/MS analysis; headspace analysis; Indian medicinal plants; vapour phase

1. INTRODUCTION

Diarrhoea and respiratory infections, including pneumonia, bronchitis, and tuberculosis, are major contributors to global mortality [1]. Moreover, urinary tract infections (UTIs) are responsible for causing significant morbidity and comorbidities, particularly in individuals with underlying conditions [2]. The burden of these diseases is especially high among children under five years old, the elderly, and immunocompromised individuals, particularly in developing countries [3,4]. In India, the incidence of lower respiratory infections and tuberculosis is 2.5 to 3.5 times higher than the global average [5]. In 2010, approximately 0.212 million deaths in India were attributed to diarrhoeal infections [6]. Fungal diseases also pose a significant burden, affecting around 57 million people in the country [7]. Major pathogens responsible for these infections include bacterial species such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, which accounted for 54.9% of total deaths worldwide in 2019 [8]. Between 2019 and 2023, fungal diseases, primarily caused by *Candida* spp., were linked to an estimated 3.8 million deaths. [9].

The common approach to treating and preventing infectious diseases involves the timely administration of antibiotics. However, irrational, and uncontrolled antibiotic use has led to the rapid emergence of multidrug-resistant pathogens [10]. Recently, nebulized antibiotics have emerged as a preferable alternative to systemic drugs for respiratory infections like cystic fibrosis and pneumonia, offering high pulmonary efficacy with minimal systemic side effects. Despite their benefits, nebulized antibiotics face challenges such as uneven distribution and deposition of aerosol particles, where larger particles accumulate in the oropharyngeal area and smaller ones in the lower airways, affecting drug delivery due to fewer active substances in smaller particles. Patient-related factors like age, physical capability, disease severity, and cognitive ability to perform inhalation techniques also impact drug delivery [11,12]. The emergence of resistant microorganisms and the challenges associated with drug delivery present major setbacks in the prevention and treatment of various infectious diseases. Hence, there is an urgent need for discovering new therapeutic antimicrobial agents [13].

Historically, plant-derived natural products have played a vital role in drug discovery, especially for infectious diseases [14]. In modern drug development, they act as a mainstay in providing novel chemical scaffolds for anti-infective drugs as well as leads that have chemically been modified and developed as antimicrobial agents. Presently, a wide range of phytochemicals, their derivatives, and complex mixtures such as extracts and essential oils (EOs) are extensively utilized in pharmaceutical, food, and cosmetic products in different forms, aiming to treat or minimize various types of infectious diseases. For example, quinine (*Cinchona* spp.), artemisinin (*Artemisia annua* L.), and its derivatives are used as antimalarial drugs whereas GeloMyrtol, a preparation based on the mixture of EOs of *Citrus limon* (L.) Osbeck, *Citrus sinensis* (L.) Osbeck, *Eucalyptus globulus* Labill. and *Myrtus communis* L., is recommended for treating chronic bronchitis [15]. In addition, EOs can be promising sources for the

development of new inhalation preparations to treat various respiratory disorders, as the physicochemical feature of being volatile at room temperature enables them to freely distribute among the lung tissues. Therefore, when compared to aerosolised antibiotics, EO vapours are easy to inhale without requiring any specific breathing technique. At the same time, this characteristic allows a uniform distribution of EOs' active substances, at a significant concentration, in the lower section of the lungs [16].

India has a rich reservoir of medicinal plants growing under different geographical and ecological conditions. In terms of biodiversity, it contains nearly 8000 plant species present on the earth, many of which have not been phytochemically and pharmacologically explored. Indian traditional system of medicine, known as Ayurveda, has played an essential role in providing health care services to human civilization. It is among the oldest medical systems in the world, using single or poly-herbal formulations in the management of various infectious diseases [17]. Several pharmaceutical companies are manufacturing and marketing different Ayurvedic formulations. For example, Bresol-NS (Himalaya, Bengaluru, India), a poly-herbal nasal spray with a combination of *Coleus aromaticus* Benth., *Eucalyptus globulus* Labill., and *Glycyrrhiza glabra* L. extracts help relieve nasal congestion due to upper respiratory tract infections. Moreover, the concept of administration of drugs through the nasal route is an integral part of Ayurvedic practices such as *dhumpana* (medicinal smoking), *nasya* (nasal administration of therapeutic oil), and *dhupanartha* (herbal fumigation) [18]. Ethnobotanical evidence supports the use of vapour-based medicines, particularly in northeast India. For example, in Manipur, the Meitei community uses the leaves of *Phlogacanthus thyrsiflorus* Nees for steam inhalation to treat pneumonia. Similarly, the stems of *Clerodendrum indicum* (L.) Kuntze and *Rotheca serrata* (L.) Steane & Mabb. are inhaled via smoke to treat acute bronchitis [19]. Among various plant species used in Indian traditional medicine for respiratory infections, *Cymbopogon citratus* (DC.) Stapf, *Trachyspermum ammi* (L.) Sprague, and certain *Cyperus* species (e.g., *Cyperus rotundus* L.) are commonly included in vapour-based remedies [20,21,22]. Additionally, plants like *Anethum graveolens* L. and *Psoralea corylifolia* L. have been documented for their efficacy in treating skin and urinary infections [23,24].

Therefore, considering the significant burden of infectious diseases and the urgent need for effective treatments, we investigated the antibacterial and antifungal activity of Indian medicinal plant extracts and EOs against a broad spectrum of pathogens responsible for causing various infections. This study presents the *in vitro* antimicrobial activity of ethanolic extracts derived from 10 plant species utilized in traditional Indian medicine using broth microdilution method. Additionally, it investigates the antibacterial properties of selected EOs extracted from Indian medicinal plants, namely *C. citratus*, *Cyperus scariosus*, and *T. ammi* against pneumonia causing pathogen in liquid and vapour phases using broth macrodilution volatilization assy. Furthermore, the study identifies the major chemical constituents responsible for the growth-inhibitory effects using gas chromatography-mass spectrometry

(GC/MS) and characterizes the chemical profile of antibacterial vapours of EO through a time series of headspace analyses employing both solid phase micro-extraction (SPME) and gas-tight syringe (GTS) sampling techniques.

2. LITERATURE REVIEW

2.1 Infectious diseases

Infectious disease or microbial infection is the ability of a microorganism to invade the host, overcome the host's immune system, survive, and multiply within the host, and produce toxins that contribute to the infection, resulting in clinical signs with either morbidity or mortality. Depending on the pathogens, infectious diseases can be classified as bacterial (e.g., tuberculosis), fungal (e.g., thrush, ringworm), parasitic (e.g., malaria, toxoplasmosis), and viral (e.g., the common cold, influenza, HIV/AIDS) infections [25]. Considering bacterial pathogenesis, these pathogens have evolved various strategies to overcome the host's defences, establish infection, and produce symptoms of the disease. The initial and crucial step is the attachment of the bacterium to the host epithelial tissue which undergoes multiplication before the removal of mucus and the shedding. To achieve this, bacteria have developed attachment mechanisms, such as pili (also known as fimbriae), which can identify and attach the bacteria to host cells. These attachment factors, commonly referred to as colonization factors, are synthesized by various bacterial pathogens and constitute a vital component of these bacteria's disease-causing strategies. Some examples of bacterial pathogens that possess pili and exhibit adherence to host tissues include *E. coli*, *Salmonella spp*., and *Streptococcus pyogenes.* Furthermore, for the invasion into the host immune system, bacterium employ numerous strategies like capsule formation (that protect them from opsonization and phagocytosis), and toxin production that can cause damage to host tissues and contribute to disease symptoms. There are two main types of toxins involved i.e., endotoxins (these are lipopolysaccharides, mainly produced by gram-negative bacteria that lead to fever, changes in blood pressure, inflammation, lethal shock, and many other toxic events). Exotoxins (include several types of protein toxins and enzymes secreted from pathogenic bacteria, mainly including cytotoxins, neurotoxins, and enterotoxins). Subsequently, certain bacteria can penetrate deeper tissues and gain access to the bloodstream, facilitating their dissemination to other regions of the body, this can result in systemic infections [26]. The general approach in the treatment of bacterial diseases is the timely administration of appropriate antibiotic therapy. However, overuse and misuse of these medications have led to the rapid emergence of antibiotic resistance. Furthermore, the risk of antibiotic-associated gut dysbiosis can result in diminished diversity of gut microbial species and altered abundance of specific taxa, thereby disrupting host immunity [27]. Concerning nebulized antibiotics, challenges arise from the absence of standardized clinical practices, hindering optimal nebulization techniques' implementation [28].

2.1.1 Epidemiology

Despite effective preventative measures and advances in the medical healthcare system, infectious disease continues to pose a severe threat to public health in low- and middle-income nations as well as high-income ones [29]. Five infectious diseases (lower respiratory infections, HIV, diarrheal diseases, tuberculosis, and malaria) were among the top ten global causes of death in the year 2018. According to the WHO, infectious diseases caused 32% of deaths worldwide, 68% of deaths in Africa, and 37% of deaths in Southeast Asia. These diseases account for 90% of the health problems worldwide and kill about 14 million people annually, 90% of whom are from the developing world [30]. Currently, bacterial, and fungal infections are major contributors to mortality and morbidity worldwide. According to the WHO in the year 2019, bacterial infections were responsible for 1.7 million deaths annually. This includes deaths from pneumonia, tuberculosis, and sepsis [31]. Whereas fungal infections are responsible for >1.5 million deaths globally per year, primarily in those with compromised immune function [32]. In Southeast Asia, candidiasis prevalence varies from 0.16 to 4.53 cases per 1000 hospital discharges and reaches 11.7 per 1000 discharges in intensive care units, a rate significantly higher compared to developed nations [33]. This disparity can be attributed to various factors such as inadequate sanitation, overcrowding, and limited healthcare access. India, with its population of 1.4 billion, carries one of the world's heaviest burdens of bacterial and fungal diseases [34].

Given major bacterial-associated infections contribute to the high instances of morbidity and mortality worldwide, the epidemiology trend of respiratory and diarrhoeal infections is focused more. Respiratory infectious diseases (e.g., bronchitis, pneumonia, and tuberculosis) are attracting the greatest attention primarily because of their serious threat to the population and public health [35]. For instance, pneumonia remains one of the most common causes of acute illness and mortality globally, responsible for more than 2.5 million deaths in 2019, and is a leading cause of death among children younger than 5 years outside the neonatal period and adults older than 65 years [36]. Considering global distribution patterns, low-income countries like sub-Saharan Africa and Southeast Asia, have documented higher fatalities. In Southeast Asia 75% of all lower respiratory tract infection (LRTI) deaths occur in children under 5 and among adults, 55% of deaths occur in those under 70 [37]. India alone contributes the highest number of deaths due to pneumonia, for about 20% of global mortality among under-five children with a current mortality rate of 33 per 1,000 live births. Annually India reports 3.6 to 4.0 million episodes of childhood pneumonia along with 0.35 to 0.37 million deaths [38]. By contrast, in high-income countries, nearly 90% of all LRTI-associated deaths occur in adults over 70. In the United States, the overall incidence in adults is around 2.5 per 1000 person-years, rising to 6.3 and 16.4 per 1000 person-years in adults aged 65–79 and 80 years or older, respectively [39].

Considering diarrhoeal diseases, it is also constituting a serious public health challenge globally, especially as the leading cause of death in children after respiratory diseases [40]. The mortality rate is

high among children aged five years and below accounting for approximately 63% of the global diarrhoea burden. In certain regions of the world like developing nations, they contribute to higher mortality rates than all other causes combined. Inadequate sanitation and limited access to clean drinking water are significant contributing factors. In Africa, Asia, and South America, diarrhoea accounts for one in eight deaths among children under five each year, and approximately 16% of child deaths in Nigeria annually [41]. As mentioned before the health burden of the disease is disproportionately high in South Asia with diarrhoea being the leading cause of death due to a communicable disease in India and accounting for 9.9 percent of the deaths in 2017 [42]. From 2016 to 2020, the prevalence of childhood diarrhoea increased from 9% to 9.2% making it the third most common responsible disease for under five mortalities in India [43].

Several major factors contribute to the emergence of infectious diseases, particularly in developing nations. One crucial aspect is the transition in demographics and behaviour, with a notable increase in vulnerable populations, especially the elderly, who are more susceptible to various bacterial diseases, even without underlying conditions. Additionally, the breakdown of public health measures has played a significant role, leading to the resurgence of infectious diseases like tuberculosis, which has seen a 15% increase in sub-Saharan African countries. Urbanization further exacerbates the issue, as overcrowding and inadequate sanitation can facilitate the transmission of diseases such as typhoid, tuberculosis, respiratory infections, and pneumonia. Moreover, the misuse of antimicrobial agents in both humans and animals accelerates the development of antibiotic resistance, exemplified by antibiotic-resistant bacterial pathogens like MRSA, responsible for approximately 171,000 invasive infections each year. Antibiotics have long been effective in treating many infectious diseases for centuries, but the emergence of resistance in microbes has posed a new challenge to researchers. Scientists are now evaluating alternatives for combating infectious diseases [44].

2.1.2 Pathogenic microorganisms

The prevalence of microbial infections leading to conditions such as diarrhoea, dysentery, UTIs, respiratory tract infections, and hospital-acquired infections remains elevated within the population of India. In the context of these diseases, the present study focused on the potential pathogens responsible for causing these infections, which include *Candida albicans*, *Enterococcus faecalis*, *E. coli*, *P. aeruginosa*, and *S. aureus*. Notably, the research placed particular emphasis on bacteria associated with pneumonia to investigate the antimicrobial effects of EO vapours. To this end, bacterial species such as *S. pneumoniae*, *S. pyogenes*, and *Haemophilus influenzae* were included in the study.

Candida albicans, a versatile yeast species, exhibits a spectrum of morphological variations. These include its yeast form, characterized by oval to round cells (2–5 μm diameter) that reproduce through budding, as well as the potential to form pseudo-hyphae—elongated, interconnected chains bridging the gap between yeast and true hyphae. True hyphae are thread-like structures with invasive properties. *C. albicans* can also produce chlamydospores in specific environments, serving as diagnostic indicators. In laboratory settings, the yeast generates germ tubes, tube-like structures emerging from cells, a distinctive feature. Moreover, *C. albicans* is recognized for its capacity to construct biofilms—complex assemblages of yeast cells and other microorganisms adhering to surfaces, contributing to enhanced resistance to antifungal agents. This morphological flexibility, known as dimorphism, responds to factors such as temperature, pH, and nutrient availability, and underpins *C. albicans* adaptability and pathogenic potential across diverse host environments [45]. It is a common commensal fungus, that colonizes in the oropharyngeal cavity, gastrointestinal and vaginal tract, and in healthy individuals' skin [46]. However, under specific circumstances, it can produce infections ranging from superficial infections of the skin (e.g., oral, or vaginal candidiasis) to life-threatening systemic infections, particularly in immune-compromised patients [47]. The burden of these infections is usually high on the developing countries, for instance around 57 million people in India, which is about 4.1% of the population, have serious fungal diseases. Out of these, about 24 million have recurrent vulvovaginal candidiasis [48]. Additionally, fungal co-infections in Coronavirus Disease 2019 (COVID-19) patients have a higher incidence of acute infections and an increased mortality rate of up to 83% despite anti-fungal treatment [49].

C. albicans is responsible for various human infections, including oral candidiasis, invasive candidiasis and vulvovaginal candidiasis. Among these, oral candidiasis is the most common fungal infection affecting the oropharynx and oesophagus of individuals with compromised immune systems. Over 90% of HIV patients develop oral candidiasis at some point during their illness. It also frequently occurs among the elderly, especially denture wearers [50]. Vulvovaginal candidiasis results from the abnormal growth of *C. albicans* and other *Candida* fungi in the female genital tract mucosa, affecting about 75% of women at least once in their lives. Women of childbearing age and HIV-infected individuals are more susceptible [51]. Invasive candidiasis is a significant opportunistic mycosis globally and a major cause of hospital-acquired infections. It leads to various infections, including bloodstream infections, heart muscle infections, UTIs, surgical wound infections, and skin abscesses related to catheter insertion [52].

The virulence factors of *C. albicans* play an active role in the pathophysiology of the development and progression of infections. The shift from the yeast to the hyphal form is a crucial transformation leading to the pathogenicity of *Candida*. The invasive nature of the hyphal form facilitates the penetration of cells into the host tissue through active penetration and induced endocytosis. While hyphae invasion-mediated induced endocytosis relies on host activity, fungal activity is necessary for active penetration.

Multiple signalling pathways contribute to the formation of hyphae. Studies have revealed that candidalysin, a toxin specific to the hyphal form, plays a critical role in the development of candidiasis. This 31-amino acid α-helical amphipathic peptide is produced by *C. albicans* hyphae and is responsible for damaging host cells. Candidalysin is believed to be a significant contributor to the establishment of systemic infection and mortality. Its mechanism of action involves direct damage to the epithelial membrane through intercalation, permeabilization, and the formation of pores that weaken the cytoplasmic contents [46].

Antifungal drugs used to treat *Candida* spp. can be grouped into four main classes based on their mechanism of action. The first class alters membrane function, as is the case with amphotericin B. The second class inhibits DNA or RNA synthesis, flucytosine. The third class inhibits ergosterol biosynthesis, which includes azoles such as fluconazole, itraconazole, and newer agents like voriconazole, posaconazole, and ravuconazole. Lastly, the fourth class inhibits glucan synthesis, echinocandins such as caspofungin, micafungin, and anidulafungin [53]. Over the last decade, there has been a notable rise in the occurrence of newly emerging drug-resistant fungal infections among hospitalized patients. This resistance is particularly evident against first and second-line antifungal medications, including fluconazole, miconazole, clotrimazole, tioconazole, amphotericin B, and echinocandins [54].

Escherichia coli is rod-shaped, non-spore-forming, gram-negative, usually motile by peritrichous flagella, and with regular dimensions of about 1.5 μm long and 0.5 μm wide. Theodor Escherich first described this microorganism in 1885. While most strains of *E. coli* are considered normal flora and do not pose a threat to humans or animals, certain strains have become pathogenic due to the acquisition of virulence factors through mechanisms such as plasmids, transposons, and bacteriophages [55]. Pathogenic *E. coli* is known to cause a wide range of human illnesses including watery diarrhoea, haemorrhagic colitis, haemolytic-uremic syndrome, UTIs, neonatal meningitis, sepsis, and infections in various other parts of the body [56]. Diarrhoea is one of the top ten leading causes of death worldwide and the second in low-income countries in children under 5 years old. Diarrheagenic *E. coli* is the main cause of paediatric diarrhoea affecting mainly children under 5 years old, particularly in developing countries [57]. In India, 0.212 million deaths were recorded due diarrheagenic *E. coli* in the year 2010 [6]. Moreover, in recent years, *E. coli* has emerged as the predominant cause of various UTIs in India, affecting individuals of all ages and genders. Particularly, infections attributed to uropathogenic *E. coli* have become a significant reason for hospital visits, with women more susceptible due to anatomical factors. Furthermore, there has been a concerning increase in multidrug-resistant strains of uropathogenic *E. coli*, which can either be inherited or spread through transmission. India has experienced a substantial rise in antibiotic resistance, encompassing various groups of antibiotics such

as penicillin, cephalosporins, aminoglycosides, quinolones/fluoroquinolones, and sulphonamides over the past decade. These resistant strains have become a leading cause of mortality in India [43].

The virulence-attributing factors of *E. coli* are encoded on genetic elements that can be mobilized into different strains to create novel combinations of virulence factors, or on genetic elements that might once have been mobile but have now evolved to become ‘‘lock” into the genome. Only the most successful combinations of virulence factors have persisted to become specific ‘‘pathotype” of *E. coli* that can cause disease in healthy individuals. Among the intestinal pathogens, six distinct categories have been identified: enteropathogenic, enterohaemorrhagic, enterotoxigenic, enteroaggregative, enteroinvasive, and diffusely adherent *E. coli*. UTIs are the most common extraintestinal *E. coli* infections and are caused by the uropathogenic type [58].

Distinct pathogenicity mechanisms and a unique virulence factor profile are associated with each *E. coli* pathotype, and these are encoded by specific gene clusters. Pathogenicity-associated genes may encode various activities, including adhesion, attachment, invasion, iron acquisition, motility, toxin activity, and more. The four main virulence classes of *E. coli* pathotypes are colonization and attachment on the surfaces of intestinal epithelial cells, secretion of enterotoxin (e.g., Shiga toxin, heat-stable enterotoxins, heat-labile enterotoxin, cytotoxin, and effectors), invasion of the cell wall of the intestinal epithelium, suppression of the host’s immune response, and direct cell-to-cell spread [59,60].

Generally, antibiotics are not recommended as first-line treatment for diarrheal illness caused by *E. coli*. However, in the case of severe disease (e.g., more than six stools per day, fever, dehydration necessitating hospitalization, or bloody diarrhoea) antibiotics like rifaximin, azithromycin, ciprofloxacin, and levofloxacin are recommended [61]. Whereas, for both community and nosocomial UTIs caused by *E. coli,* co-trimoxazole (trimethoprim/sulfamethoxazole), nitrofurantoin, ciprofloxacin, and ampicillin are suggested [62]. Recently, due to high rates of emerging resistance to antimicrobial agents, WHO has added *E. coli* to as global critical pathogen priority list for research, discovery, and new antibiotics development [63].

Enterococcus faecalis is a gram-positive, spherical (0.5–1 μm in diameter), non-spore-forming facultative anaerobic bacterium and is a commensal member of the gut microbiota. It is responsible for over 90% of enterococcal infections, which include bacteraemia, endocarditis, sepsis, surgical wound infections, and UTIs. Although the *Enterococcus* genus comprises more than 40 ecologically diverse species, this species is the most isolated healthcare pathogens, making it the third most prevalent [64,65]. Globally, many studies have revealed that *E. faecalis* tends to be one of the leading causes of several nosocomial infections, with the emergence and spread of multi-drug resistance among isolates. In general, *E. faecalis* considered harmless to gastrointestinal flora but has emerged as a significant contributor to hospital-acquired infections. These infections encompass a range of conditions, including

surgical site infections, UTIs, and bacteraemia. While enterococci were once primarily associated with endogenous sources of infection, recent reports highlight their transmission among hospitalized patients. *E. faecalis* ranks as a frequently isolated pathogen in various wound infections and surgical site infections. It also plays a substantial role in bacteraemia cases worldwide [66].

The virulence of *enterococci* has been linked to various factors, such as the production of gelatinase, *Enterococcus* surface protein (Esp), aggregation substance, and the formation of biofilm. Gelatinase is a zinc metalloprotease that is encoded by the gelE gene and has hydrolytic properties. Aggregation substance, facilitates binding to the host epithelium and bacterial aggregation during conjugation, promoting plasmid exchange. The Esp protein contributes to the colonisation and persistence of *E. faecalis* strains in ascending UTIs. Furthermore, Esp also contributes to biofilm formation, which significantly enhances bacterial survival in biopolymers and may also play a role in antimicrobial resistance [67]. The virulence factors produced by *E. faecalis* are known to weaken host immunity and degrade host tissue through the secretion of extracellular enzymes like cytolysin, gelatinase, and serine protease [68].

Treatment of *E faecalis* infections is difficult because they have intrinsic and acquired resistance to many antimicrobials. The common antibiotics recommended include ampicillin, penicillin, vancomycin (used for more severe infections), linezolid, daptomycin, and nitrofurantoin (used for urinary tract infections). *E. faecalis* exhibits inherent resistance to various antimicrobial agents, including aminoglycosides and β-lactams, attributable to the presence of multiple resistance genes. Moreover, it can acquire resistance to several antibiotics, such as macrolides, vancomycin, cephalosporin, tetracycline, and fluoroquinolones, either through genetic mutation or the acquisition of new resistance genes via gene transfer mechanisms. Hospital strains of *E. faecalis* commonly demonstrate resistance to a wide spectrum of antibiotics, including macrolides and vancomycin, and some even produce β-lactamases, which confer resistance to penicillin and cephalosporins [66].

Haemophilus influenzae, are small (1 × 0.3 μm), nonmotile, non-sporing, gram-negative rods that occur in both encapsulated and nonencapsulated forms [69]. This species most often associated with systemic disease in man, is an obligate human commensal found principally in the upper respiratory tract [70]. They are responsible for a variety of local upper respiratory and systemic infections, including sinusitis, otitis media, conjunctivitis, dental abscess, LTRI, peritonitis, biliary tract infection, brain abscess, osteomyelitis, and wound infections [71]. The virulence factor associated with *H. influenzae* is its capsular polysaccharide. The presence of this structure divides the species into non-encapsulated, non-typable strains (NTHi) and serotype b [72]. Based on the strain the infection caused by *H. influenzae* can be categorised into invasive and non-invasive. The invasive *H. influenzae* type b (Hib) infection occurs commonly in children under 5 years of age and mainly includes pneumonia and

meningitis. Less common diseases caused by this pathogen are endophthalmitis, UTIs, abscesses, osteomyelitis, and endocarditis. NTHi is also accountable for invasive infections like bacteraemia without an identifiable source, bacteraemic pneumonia, and meningitis. Considering the non-invasive infections that affect mucosal surfaces are mainly caused by NTHi across all age groups. These encompass otitis media in infants and children, sinusitis in both children and adults, non- bacteraemic pneumonia in the elderly, and acute exacerbations of chronic obstructive pulmonary disease in adults and the elderly [73,74]. While the overall prevalence of Hib has significantly reduced worldwide, India remains the country with the highest number of Hib cases and related fatalities. In 2015, an estimated 337,270 Hib cases and 15,634 deaths were recorded among Indian children aged 1 to 59 months, with pneumonia and meningitis accounting for 99% of these cases [75].

The initial step in the pathogenesis of disease due to NTHi involves the establishment of bacteria on the respiratory mucosa. To gain access to the mucosal surface, organisms need to overcome local immune mechanisms, including the mucociliary escalator and the presence of secretory immunoglobulin A (IgA) [76]. To achieve this goal, the species has evolved mechanisms to paralyze cilia and inactivate IgA_1. Furthermore, produces a variety of adhesive molecules. These molecules aid in identifying host cell receptor structures and enhancing the stability of interactions with the epithelial surface. After entering the respiratory tract, bacteria initially engage with the mucus layer and over time form microcolonies that produce lipooligosaccharide substance that leads to the disruption of ciliary orientation and ciliostasis [77]. To establish colonization within the human respiratory mucosa, *H. influenzae* needs to counteract the protective effects of IgA. Both non-typable and type b strains achieve this by producing an extracellular endopeptidase called IgA_1 protease. This enzyme is designed to specifically cleave the serum and secretory forms of IgA_1 [78]. The cleavage event takes place in the hinge region of IgA_1, resulting in the separation of the antigen-binding domains from the fragment crystallizable portion of the molecule. This process eliminates the agglutination activity both free and antigen bound IgA_1 and enables the bacteria to gain access to the respiratory epithelium [79]. Subsequently, several adhesins, including high-molecular weight, non-pilus adhesins (HMW1 and HMW2) in most strains, mediate direct binding to non-ciliated epithelial cells. In the presence of a secretory leukocyte protease inhibitor, the Hap (*H. influenzae* autotransporter protein) protein of *H. influenzae* encourages interactions between bacteria, leading to the formation of bacterial clusters. These aggregates are likely vital for persistent infection. Additionally, Hap, along with potentially other factors, supports the entry of the bacteria into epithelial cells. This allows the organism to establish a protected environment, again contributing to its persistence. When levels of secretory leukocyte protease inhibitor decrease, Hap undergoes autoproteolysis, resulting in the dispersal of organisms to more distant sites, including the middle ear, sinuses, and lungs [76].

The first-line treatment for *H. influenzae*-induced childhood pneumonia includes high-dose amoxicillin with clavulanic acid. For older children, azithromycin or erythromycin are recommended for atypical

pathogens, while vancomycin is for severe pulmonary infections involving methicillin-resistant *S. aureus*. In the case of community-acquired pneumonia, antibiotics like azithromycin, clarithromycin, or doxycycline are prescribed if no prior antibiotic therapy was administered in the past 3 months. In cases of suspected aspiration pneumonia, amoxicillin-clavulanate or clindamycin is recommended [80]. However, since the 1970s, *H. influenzae* has exhibited a significant increase in resistance to ampicillin, chloramphenicol, and trimethoprim. This resistance is primarily due to the production of beta-lactamase and the presence of transferable antibiotic resistance mechanisms. Therefore, underscore the urgent need for the development of new treatment strategies [81].

Pseudomonas aeruginosa is a gram-negative, mono-flagellated, asporogenous, pearlescent, and grape or tortilla-like odour-emitting rod-shaped bacterium about 1–5 μm long and 0.5–1.0 μm wide [82]. *P. aeruginosa* is an opportunistic pathogen that exploits vulnerabilities in the host's defence mechanisms to cause infections. The bacterium rarely infects healthy tissues, but it can invade any compromised tissue, especially in individuals with weakened immune systems. *P. aeruginosa* is responsible for high morbidity and mortality causing cystic fibrosis, pneumonia, urinary tract, surgical site, bloodstream, bone, and joint, and gastrointestinal infections, particularly in patients with severe burns, tuberculosis, cancer, and AIDS. Notably, it poses a significant challenge in patients hospitalized with cancer, cystic fibrosis, and burns, with a 50% fatality rate [83]. Also, in intensive care unit patients, *P. aeruginosa* is responsible for an even higher percentage of healthcare-associated infections *P. aeruginosa* accounts for approximately 10% of all nosocomial UTIs up to 16% of UTIs in ICU patients. In India, prevalence rate of *P. aeruginosa* infection varies from 10.5% to 30% [84,85].

Due to its ubiquitous dissemination in the environment, particularly in hospitals, *P. aeruginosa* has become a significant threat to human health as drug-resistant strains continue to emerge. The species utilizes several resistance mechanisms, which often lead to chronic infections that are challenging to eliminate. Diverse virulence factors responsible for bacterial adhesion and colonization, host immune suppression, and immune escape, play important roles in the pathogenic process. The virulence factors of *P. aeruginosa* are divided into three main categories: namely bacterial surface structures (play an important role in attachment to host cells, bacterial twitching and swarming motility, biofilm formation), secreted factors (toxins such as exotoxins, proteases, and exoenzymes, can cause extensive host tissue damage by disrupting normal cytoskeletal structure) and bacterial cell-to-cell interaction (regulates the production of virulence factors, integration of the environmental stress, modulating production of biofilm and swarming and twitching motilities) [86].

Common treatment for *P. aeruginosa* infections includes aminoglycosides (gentamicin, tobramycin, amikacin, netilmicin), carbapenems (imipenem, meropenem), cephalosporins (ceftazidime, cefepime), fluoroquinolones (ciprofloxacin, levofloxacin), penicillin with β-lactamase inhibitors (ticarcillin and piperacillin in combination with clavulanic acid or tazobactam), monobactams (aztreonam), fosfomycin

and polymyxins (colistin, polymyxin B) [87]. Recently, treatment of *P. aeruginosa* infections has become a great challenge due to the ability of this bacterium to resist many of the currently available antibiotics. The WHO has recently listed carbapenem-resistant *P. aeruginosa* as one of three bacterial species in which there is a critical need for the development of new antibiotics to treat infections [88].

Staphylococcus aureus is gram-positive, no capsulated, non-motile, non-spore-forming, coccus (spherical to ovoid in shape), with diameters of 0.5 - 1.5 μm. It is facultative anaerobe that grows by aerobic respiration or by fermentation [89,90]. The cell wall of *S. aureus* resembles the typical features of gram-positive bacterial cell walls, relatively thick (about 20 to 40 nm). It is composed of multiple layers of peptidoglycan and other polymers like wall teichoic acids, lipoteichoic acids, and attached proteins. The presence of a pentaglycine interpeptide bridge is a unique and essential component of the *S. aureus* peptidoglycan, which permits the generation of a three-dimensional mesh that allows for cell plasticity. Also, it provides the cell wall with sufficient strength and rigidity needed under different environmental and infectious conditions [91]. *S. aureus* is both a commensal bacterium and a human pathogen. Approximately 30 % of individuals carry the bacterial species in the skin, skin glands, anterior nares, nasal passage, oropharynx, and axillae of humans [92]. It can cause a wide range of diseases, ranging from mild skin infections to life-threatening conditions such as community/hospital-acquired pneumonia, endocarditis, bacteraemia, skin, and soft tissue infections (impetigo, folliculitis, and cellulitis), bone and joint infections (osteomyelitis and septic arthritis), etc [93]. Moreover, nowadays methicillin-resistant *S. aureus* contributes to nosocomial and community-acquired infections worldwide. Many studies showed the prevalence trend of MRSA across India ranging from 26.14% to 43% [94].

The pathogenesis of *S. aureus* infections depends on the production of surface proteins that mediate bacterial adherence to host tissues, secretion of a series of extracellular toxins, and enzymes that destruct host cells and tissues, avoidance of, or incapacitate, the host immune defence, and growth and spread of bacteria in host cells. During the post-exponential and early stationary phases, *S. aureus* secretes proteins known as toxins into the extracellular matrix. These toxins play a key role in enabling the bacteria to invade host tissues by promoting tissue penetration. They also have cytolytic properties, which aid bacterial growth by facilitating the acquisition of nutrients from lysed host cells, such as iron. *S. aureus* produces several toxins, including hemolysin, leukotoxin, exfoliative toxin, enterotoxin, and toxic-shock syndrome toxin-1. Additionally, staphylococcal virulence factors also encompass enzymes and surface proteins. Enzymes such as coagulase, proteases, and staphylokinase, evade host defences and promote tissue invasion and penetration. These enzymes typically operate by breaking down host molecules or disrupting signalling cascades and metabolic pathways within the host. Moreover, *S. aureus* surface proteins, such as clumping factors, fibronectin proteins, protein A, and collagen adhesin, also contribute to bacterial adhesion, tissue invasion, and evasion of host defences [95,96].

Commonly used antibiotics for *S. aureus* infections include methicillin and its derivatives, such as oxacillin, dicloxacillin, and nafcillin, these were historically effective against *S. aureus*. However, many *S. aureus* strains have developed resistance to these antibiotics, leading to the emergence of MRSA [97]. Historically it was associated with hospitals and other healthcare settings, but in recent years MRSA has emerged as a widespread cause of community infections [98]. The clinical manifestations of MRSA consist of skin and soft tissue infection to bacteraemia, septicaemia, toxic shock, and scalded skin syndrome. Moreover, due to the increasing resistance of MRSA to several antibiotics, there is a need to approach alternative ways to overcome economic as well as human losses [99].

Streptococcus pneumoniae is a non-motile, non-spore-forming, gram-positive, α-haemolytic, catalase-negative, and facultatively anaerobic organism that grows as a single coccus (about 0.5–1.25 μm in diameter) or as diplococci often identifiable because of their lancet shape, and in chains [100,101]. It is the leading cause of pneumonia and bacterial meningitis in children younger than 5 years and older adults worldwide. Apart from this, the spectrum of infection ranges from asymptomatic pharyngeal colonization to mucosal disease (otitis media, sinusitis, pneumonia) to invasive disease (bacteria in a normally sterile site; bacteraemia, meningitis, empyema, endocarditis, arthritis). Those at a higher risk of developing severe pneumococcal disease include individuals at the extremes of age (particularly those under 2 and over 65 years old), individuals with underlying organ dysfunction (such as asplenia, splenic dysfunction, chronic heart, lung, liver, and kidney disease), and individuals with compromised immune systems (especially those with antibody defects, complement deficiencies, neutropenia, and malignancies) [102]. Considering the prevalence of pneumonia caused by *S. pneumoniae*, the burden is high on developing countries responsible for three million deaths a year in young children. India bears the highest burden of pneumococcal-related fatalities as compared to other developing countries. There were 1.6 million estimated cases of severe pneumococcal pneumonia in 2015, accounting for more than 97% of all severe pneumococcal diseases [103].

The ability of *S. pneumoniae* to attach to the epithelial cells of the nasopharyngeal mucosa is a crucial step in the pathogenesis process. Surface proteins, such as pneumococcal surface adhesin A (PsaA), choline-binding protein A (CbpA), pneumococcal surface protein (PspC), and *S. pneumoniae* secretory IgA binding protein (SpsA), have been identified as pneumococcal factors involved in host cell attachment. PsaA specifically binds to host E-cadherin, while CbpA/PspC/SpsA binds to sialic acid, lacto-N-neotetraose, the polymeric immunoglobulin receptor, and vitronectin. Following the initial attachment to host cells, the subsequent step in pneumococcal pathogenesis involves the establishment of the bacteria in various sites such as the lungs, blood, middle ear, central nervous system, or other locations. The specific mode of establishment is influenced by factors such as host susceptibility, regulation of bacterial gene expression, and opportunities for interactions between *S. pneumoniae* and

host components. In the case of pneumococcal pneumonia, a critical element is the presence of pneumococcal neuraminidase, which plays a vital role in cleaving sialic acid from host cell glycoprotein receptors. This enzymatic activity facilitates the attachment of the bacterium to airway epithelial cells [104,105].

The mainstay of treatment for *S. pneumoniae* infections includes β -lactam antibiotics (e.g., penicillin, third generation cephalosporins), macrolides (e.g., erythromycin), fluoroquinolones (e.g., levofloxacin), lincosamides (e.g., clindamycin) and vancomycin can be used for management of β -lactam non-susceptible pneumococcal isolates and for individuals who cannot tolerate β -lactams. The emergence of antimicrobial drug resistance in *S. pneumoniae* has become one of the greatest challenges to global public health today. Resistance is developed for frequently employed antimicrobial agents, such as penicillin, cephalosporins, macrolides, and trimethoprim-sulfamethoxazole [106,107].

Streptococcus pyogenes is an anaerobic, β-haemolytic, gram-positive coccus with a diameter of 0.5–1.0 μm [108]. It is a non-motile and non-spore-forming bacterium, which usually occurs in chains or pairs, and typically has a capsule made of hyaluronic acid. It causes a wide variety of acute infections, such as soft tissue infections and pharyngitis to severe life-threatening infections including scarlet fever, bacteraemia, pneumonia, necrotizing fasciitis, myonecrosis, and streptococcal toxic shock syndrome [109]. Typically, *S. pyogenes* infections are more prevalent in developing countries and marginalized populations within developed countries. The infections may be observed in persons of any age, although the prevalence of infection is higher in children, presumably because of the combination of multiple exposures (in schools or nurseries, for example) and host immunity. The prevalence of pharyngeal infection is highest in children older than three years and has been described as a 'hazard' in school-aged children. *S. pyogenes* infections are more prevalent in areas of socioeconomic disadvantage, including developing countries and the marginalized populations within developed countries [110]. In India, the disease burden of streptococcal infections is considerably high. The incidence of *S. pyogenes* pharyngitis ranges from 0.3 to 5.4 per 1,000 children [111].

The process of human infection by *S. pyogenes* is complex and multifactorial, involving both host and bacterial factors that contribute to the pathogenesis of infection. The bacterium produces many cell wall-associated and other virulence factors that have various effects on tissues, cells, and components of the immune response. The key virulence factors that are important for the colonization of epithelial tissues and the progression of invasive disease include surface-expressed M protein and the capsule facilitates attachment of the pathogen to epithelial cells. Secretion of the toxins like streptococcal pyrogenic exotoxin B and streptolysin S at the epithelial surface destabilizes the junctional proteins of epithelial cells, resulting in the loss of cell–cell adhesion and translocation of *S. pyogenes* across the host epithelial barrier. Following bacterial invasion into epithelial cells, the joint action of streptolysin

O and NAD glycohydrolase leads to the disruption of the Golgi network, which further impairs epithelial barrier integrity. Invasion of deeper underlying tissue may also occur through streptolysin and NADase-induced cell death, or via gasdermin A-dependent pyroptotic cell death of epithelial cells [112].

Commonly used antibiotics for the treatment of *S. pyogenes* infection include penicillin (e.g., penicillin V or amoxicillin), amoxicillin, cephalosporins (e.g., cephalexin), macrolides (e.g., erythromycin, clarithromycin, or azithromycin), clindamycin and levofloxacin (for those allergic to penicillin). However, an increase in antimicrobial resistance of *S. pyogenes* has been observed during the last decade in Europe and worldwide. There have been reports of an increasing incidence of multiple drug resistance among streptococci strains, which hamper customary empirical antimicrobial therapy for these infections [113].

2.2 Plant-derived products

Throughout evolution, plants have developed effective mechanisms to protect themselves from biotic factors such as pests and diseases, notably through the synthesis of secondary metabolites (SM)—small antimicrobial molecules. In parallel, humans and animals have adopted similar defensive strategies, utilizing plants prophylactically and therapeutically against microbial and parasitic infections. The historical use of herbal medicines as antibiotics represents some of the earliest instances of healing in human history. Across diverse regions, plants have been systematically employed for treating a wide array of human infections through traditional folk medicines, including sophisticated remedies like Ayurvedic and Chinese herbal preparations. In modern drug development, plant-derived products play a pivotal role by offering fresh chemical frameworks for anti-infective drugs, serving as starting points for chemical modifications and the development of antimicrobial agents [15]. Focusing on the area of antibacterial and antifungal plant-derived preparations, the broad spectrum of phytochemicals and complex mixtures (e.g., extracts and EOs) are nowadays used in various forms of pharmaceutical, food, and cosmetic products to treat or to reduce the risk of various infectious diseases, for instance (*Glycyrrhiza glabra* extract, *Melaleuca alternifolia* EO, and *Pistacia lentiscus* resin), pure compounds (benzoic acid, berberine, eucalyptol, salicylic acid, and thymol) as well as their derivatives and complexes (bismuth subsalicylate and zinc pyrithione) [114,115,15].

SM exhibit a remarkable diversity in their chemical structures. In the current study, their classification is based on extraction method and chemical nature of the product obtained into EOs and plant extracts. EOs can be considered as a specific type of plant extract consisting more of volatile compounds (terpenoids) whereas plant extracts are more complex mixtures containing a broader spectrum of compounds, including alkaloids, phenolic compounds, steroids, etc [116]. In the following section, the antimicrobial properties, chemistry, and taxonomical distribution, as well as different extraction and analytical methods in the context of EOs and plant extracts are described. Furthermore, the text provides

background for understanding the significance of EOs and plant extracts in both traditional and modern medicinal applications.

2.2.1 Plant extracts

Plant extracts can be defined as the complex, multi-component mixture obtained after using a solvent to dissolve components of the plant material. These are recognized as the primary reservoirs of biomolecules that can be isolated from different parts of plants such as leaves, flowers, stems, roots, seeds, and fruits [117]. Extracting these valuable molecules from medicinal plants involves employing a variety of solvents and extraction techniques [118]. These serve as sources of beneficial chemical compounds with promising prospects for use in medicine, food, cosmetics, and the agriculture sector and hence are studied intensively [119]. The subsequent sections provide a detailed explanation of the chemistry and antimicrobial properties of plant extracts.

2.2.1.1 Chemistry

The effectiveness of plant extracts is attributed to their chemical constituents, which can be roughly divided into a few functional groups as follows: a) alkaloids, b) phenolics, and c) terpenoids and steroids [120].

Alkaloids

Alkaloids are organic compounds containing at least one nitrogen atom (amino or amido) in their structure. These are primarily biosynthesized from amino acids, and constitute about 20% of the secondary metabolites in plants. They play a crucial role in plant defence against predators and in the regulation of plant growth [121]. Alkaloids are widespread among numerous plant families, with some exhibiting a higher prevalence of alkaloid-containing taxa. Notably, in the Papaveraceae family, every genus contains at least one alkaloid. Other families include Amaryllidaceae, Apocynaceae, Asteraceae, Berberidaceae, Boraginaceae, Fabaceae, Leguminosae, Liliaceae, Ranunculaceae, Rubiaceae, Rutaceae, and Solanaceae [122]. Based on their origin, they are mainly classified into basic sub-classes true alkaloids and pseudoalkaloids. True alkaloids are derived from amino acids and contain nitrogen atoms in a heterocyclic ring, which can have different structures including among many others piperidines, with piperine and chavicine as representatives from *Piper nigrum* L. and other *Piper* spp. Whereas, Pseudoalkaloids are not derived from amino acids, but from terpenes or steroids and contain nitrogen in their ring structures. The representatives of this sub-class are stimulants caffeine and theobromine [120,123]. Humans have used alkaloid-containing plants since immemorial times as poisons, stimulants, narcotics, and medicines due to their useful pharmacological effects, and nowadays, alkaloids are still ubiquitous in food chains, stimulant drugs, medicine chemistry, insecticides, and many other industries. Some common applications of alkaloids include their use as antimalarials (quinine),

antiasthmatics (ephedrine), anticancer agents (homoharringtonine), cholinomimetics (galantamine), vasodilators (vincamine), analgesics (morphine), antibacterials (chelerythrine), and antihyperglycemics (berberine) [122,124,125,126].

Phenolics compounds

Phenolic compounds form a large, chemically diverse group of plant SMs, which are produced by the shikimate pathway. These secondary metabolites, found across a diverse range of higher plant taxa, are notable for their potent antioxidant, antimicrobial, anticancer, and anti-inflammatory properties. They contain at least one carboxyl group and phenolic hydroxyl ring and depending on the number of phenol units in the molecule, they can be classified as simple phenols or polyphenols. This subgroup of plant SMs can range from simple phenolic molecules to highly polymerized compounds. Phenolic substances are usually related to the defence response of the plant, however, they can be also employed in other processes, for example incorporating attractive substances to hasten pollination and colouring for camouflage against herbivores. Moreover, they contribute to flavour (astringency, bitterness), appearance (colour), and oxidative stability (proven anti-oxidative activity) of plant products [127,128]. The most beneficial impacts linked with a plant-based diet are associated with the well-established antioxidant activity of phenolic compounds, that are abundantly represented in fruits, vegetables, tea, coffee, herbs, and spices. However, besides the well-established free radical scavenging effects, some phenolic compounds also exhibit substantial antimicrobial activity. The exact mechanism of the antimicrobial effect is not fully elucidated yet, but these compounds are known to manifest many actions on a cellular level, that disrupt the cell wall and subsequently disrupt the cell membrane permeability of microbial cells [129,130]. This structurally divergent class can be further subdivided into anthocyanins, coumarins, lignans, flavonoids, phenolic acids, quinones, and tannins [120].

Terpenoids and steroids

Terpenoids and steroids comprise a vast group of natural compounds characterized by a carbon framework consisting of (C_5) isoprene units. These units are derived from isopentenyl diphosphate, which can be obtained from either mevalonic acid or methylerythritol phosphate. The subclasses of these organic compounds are distinguished by the number of isoprene units they contain. Initially, these units are linked together in a chain. Upon cyclization of these chains, the presence of methyl substituents results in the formation of quaternary centres in the polycyclic terpenoids. The chemistry of terpenoids and steroids is significantly influenced by the presence and stereochemistry of these structures, along with the isoprenoid alkenes, leading to a range of intriguing stereochemical interactions and molecular rearrangements [131,120].

Plant steroids are systematically classified into distinct categories based on their chemical structures, pharmacological activities, and the sources from which they are derived. One such class is the brassinosteroids, characterized by a cholestane structure featuring two vicinal diols and various substituents at position C-24. Examples of brassinosteroids include the growth-promoting phytohormones brassinolide and catasterone. Another class, bufadienolides, exhibits a polyhydroxy C-24 steroid with a six-membered pyrone ring at C-17, imparting cardiotonic effects and increasing the force of heart contraction. Notable examples within this group include selleborin, scillaridine, physodine, and ouabain. Cardenolides, another category, consists of C-24 steroids that possess a five-membered lactone ring located at C-17. This class exhibits cardiotonic properties, functions as an arrow poison, and demonstrates anti-bacterial activity. Well-known members include digoxin, digitoxin, and digitoxigenin are used in the treatment of cardiovascular diseases. Cucurbitacins, categorized as oxygenated C-30 triterpenoids with a methyl group at C-4, showcase antitumor, antimicrobial, hepatoprotective, and anti-inflammatory activities. Examples include cucurbitacin D, cucurbitacin C, and arvenin 1. Ecdysteroids, a distinct group of C-27 steroids with a 7-en-6-one chromophore and a methyl group at C-24, play a crucial role in protecting plants against insects, acting as moulting hormones. Prominent members include ecdysone and oogonial. Sapogenins/steroid saponins, characterized by oxygenated C-27 steroids with a hydroxyl group at C-3, exhibit antifungal, hepatitis, sex hormone, and antitussive properties. Representative examples encompass Spirostanol, including diosgenin, sarsepogenin, and glycyrrhetinic acid. Steroidal alkaloids, featuring a steroidal skeleton with a nitrogen atom integrated into a ring or as a substituent, function as anti-inflammatory agents and sex hormones. Notable members within this class include solasodine, solasonine, solamargine, solanidine, and guggulsterone. Lastly, withasteroids/withanolides, comprising C-28 steroids with a δ-lactone, showcase diverse pharmacological activities such as treating diarrhoea, rheumatic fever, and exerting anti-tumour, immunosuppressive, and hepatoprotective effects. Examples include withaferin A, iochromolide, and withanolide [132].

Terpenoids, also known as isoprenoids, constitute a large and diverse class of naturally occurring compounds derived from five-carbon isoprene units. These compounds are distinguished from one another by their basic skeleton and functional groups. The general formula for most natural terpenoid hydrocarbons is $(C_5H_8)_n$. Based on their chemical skeleton, terpenoids are categorized as monoterpenoids $(C_{10}H_{16})_n$, sesquiterpenoids $(C_{15}H_{24})_n$, diterpenoids $(C_{20}H_{32})_n$, sesterpenoids $(C_{25}H_{40})_n$, triterpenoids $(C_{30}H_{48})_n$, tetraterpenoids $(C_{40}H_{64})_n$, and polyterpenoids $(C_5H_8)_n$. The simpler mono and sesquiterpenes serve as the chief constituents of EOs obtained from the sap and tissues of certain plants and trees, as discussed in the upcoming chapter on EO chemistry. di- and triterpenoids, on the other hand, are not steam volatile and are derived from plant and tree gums and resins. Tetraterpenoids form a distinct group known as carotenoids [133]. These compounds exhibit a range of pharmacological activities, including anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, hypoglycaemic, and

anti-cancer activities. As an example, paclitaxel, a tricyclic diterpenoid isolated from the bark and wood of the *Taxus brevifolia* Nutt., has been utilized as a microtubule-stabilizing agent in the treatment of various tumours, such as breast cancer, ovarian cancer, and non-small-cell lung cancer [134].

2.2.1.2 **Extraction methods**

Extraction is an essential feature in natural product research. The method chosen for extracting compounds from plants plays a pivotal role in determining both the quality and quantity of plant extracts. Various methods are used for extracting such as maceration, solvent extraction (water, ethanol, methanol, and other solvents), Soxhlet extraction, and modern green extraction methods (supercritical fluid, ultrasound, accelerated solvent, microwave, enzyme-assisted extraction) [116,135]. Among all the methods solvent and Soxhlet extraction is the most widely used one especially in the laboratory scale. The extraction of natural products involves a sequential process encompassing several key stages: (1) infiltration of the solvent into the solid matrix; (2) dissolution of the solute in the solvent; (3) diffusion of the solute out of the solid matrix; and (4) collection of the extracted solutes. The effectiveness of the extraction can be significantly influenced by factors that enhance diffusivity and solubility in each of these stages. Noteworthy parameters impacting extraction efficiency include the properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ratio, extraction temperature, and the duration of the extraction process. Optimizing these variables is essential for achieving efficient and high-quality extraction of natural products [136]. Solvents commonly used in the extraction of medicinal plants are polar solvents (e.g., water, alcohols), intermediate polar (e.g., acetone, dichloromethane), and nonpolar (e.g., n-hexane, ether, chloroform), depending on the desired product suitable solvent is chosen [137]. However, ethanol has emerged as a popular and increasingly preferred solvent in various extraction processes as it is safe for infusing edibles. It has traditionally been used as a solvent in various traditional medicine systems, including British, Chinese, Indian, and Tibetan pharmacopoeias [138].

2.2.1.3 **Analytical methods**

The chemical analysis of plant extracts is of supreme importance, as it aids in comprehending the chemical composition of the plant material. This analytical process is indispensable for evaluating the potential therapeutic or nutritional properties inherent in the extracts and for ensuring the quality and consistency of herbal products. Various analytical methods are employed which allows researchers to identify and quantify specific compounds. The choice of method depends on the nature of the compounds of interest and the characteristics of the plant material. For instance, chromatographic technique like high-performance liquid chromatography (HPLC) is a versatile and robust technique

commonly employed over the years for separating constituents of herbal extracts, for identification and quantification of compounds, and for establishing the fingerprint profiles of mixtures and crude extracts. Various detection tools such as ultraviolet detectors, diode array detectors, MS (Mass spectrometry), and fluorescence detectors are coupled to the HPLC to perform its function efficiently. Presently, it is becoming increasingly popular as the primary choice for fingerprinting studies, particularly in the quality control of herbal plants [139,140]. HPLC can be categorized into two main objectives: analytical HPLC and preparative HPLC. Analytical HPLC is primarily focused on qualitatively and quantitatively determining the presence of a compound. On the other hand, preparative HPLC is designed for isolating and purifying valuable products [141]. Preparative HPLC is extensively used in the search for biologically active substances.

Following the separation of compounds, the subsequent step involves identifying the purified compounds, typically accomplished through the utilization of techniques such as MS or nuclear magnetic resonance (NMR). MS is a powerful analytical technique used to identify and quantify analytes using the mass-to-charge ratio (m/z) of ions generated from a sample. A mass spectrum is generated, plotting the ion signal against the mass-to-charge ratio. This spectrum is utilized to ascertain the elemental or isotopic signature of a sample, determine the masses of particles and molecules, and elucidate the chemical structures of compounds [142]. Considering, NMR spectroscopy is a powerful tool for unveiling the structure of compounds. It provides comprehensive insights into a molecule's arrangement of atoms, revealing the overall structure. NMR determines the count of carbon and proton atoms, fundamental for understanding molecular composition, and assists in establishing connectivity between atoms, offering crucial information about the molecule's framework and functional groups. The technique allows a glimpse into the three-dimensional arrangement of atoms, aiding in understanding spatial orientation, particularly valuable for molecules with flexible conformations [143].

2.2.1.4 **Antimicrobial activity**

Extracts isolated from medicinal plants have been reported to demonstrate diverse biological activities, including antiviral, antibacterial, antifungal, and antiparasitic properties. The efficacy of these plant extracts in inhibiting microbial growth is intricately linked to the synergistic effects arising from the active compounds within them. This synergism results from various effects, including the emergence of multi-target mechanisms, the presence of compounds capable of suppressing microbial growth, and pharmacokinetic or physicochemical effects leading to improved bioavailability, solubility, and absorption rates. Additionally, synergism plays a role in neutralizing adverse effects and reducing toxicity, collectively contributing to the enhanced effectiveness of these plant extracts against bacterial growth [144].

Phytochemical studies have identified various compounds derived responsible for conferring biological activities, such as berberine—a benzylisoquinoline alkaloid present in diverse plants like *Coptis* spp., *Berberis* spp., and *Hydrastis canadensis*, which imparts anti-diarrheal effects. *In vitro* studies have showcased berberine's growth-inhibitory potential against intestinal diarrheagenic bacteria, including *Bacillus cereus* and *E. coli* [145,146]. Clinical trials involving berberine and its derivatives (berberine-tannate, -hydrochloride, and -sulphate) in children have demonstrated efficacy in treating diarrhoea [147]. Notably, due to its promising activity, several pharmaceutical companies' market berberine as a pharmaceutical drug for treating intestinal infection diseases and diarrhoea, either in pure form (Berberine Hydrochloride Tablets, Northeast Pharmaceutical Group, Shenyang, China) or as extracts (e.g., Huang Lian Su Tablets, containing *Coptis chinensis* extract).

Among phenolic compounds, specifically, salicylic acid derived from *Salix* spp. and benzoic acid from *Myroxylon balsamum* and *Styrax* spp., exhibiting *in vitro* growth inhibitory activity against pathogens responsible for causing hair and skin infections, including *Candida* spp. and *Corynebacterium minutissimum*. Additionally, both compounds demonstrate potent antifungal potential against *Epidermophyton floccosum*, *Microsporum* spp., and *Trichophyton* spp. Various commercial products based on these compounds, such as Whitfield's ointment (e.g. Gold Cross, Barton, Australia), composed of 3% salicylic acid, and 6% benzoic acid with a suitable base (e.g. lanolin or vaseline), are prescribed for the treatment of athlete's foot (tinea pedis) and other fungal infections of the skin. Also, tincture of benzoin (benzoin, aloe, storax, and tolu balsam) is used applied as an antiseptic and wound protecting preparation [148,149].

Furthermore, extracts from fruits of *Vaccinium macrocarpon* and *Vaccinium oxycoccus* have been reported by various studies to have antimicrobial effects against urinary tract infections causing pathogens like *E. coli* and *P. aeruginosa* [150,151]. Plant-based products containing *Vaccinium* spp. dominates the market and are usually prescribed for the treatment and prevention of urinary infections. Several companies sell them as dietary supplements, functional foods, and herbal medicines in the form of capsules, drinks, herbal teas, and tinctures worldwide (e.g. Cranberry Fruit Capsules, Nature's Way Products, Green Bay, USA) [152].

2.2.2 Essential oils

According to the International Standards Organization, EOs are defined by their production method as products obtained from vegetable raw material either by distillation with water or steam, or in the case of epicarp from citrus fruits by mechanical process or dry distillation [153]. EOs are complex and highly variable mixtures of constituents characterized by distinct biogenetic origins: the group of terpenoids and the group of aromatic compounds derived from phenylpropane. The production of EO is distributed

among a limited number of plant families, such as Myrtaceae, Lauraceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae, and Piperaceae [120]. EOs have found extensive use due to their antimicrobial and insecticidal attributes, and they are commonly applied for their therapeutic, aromatic, and flavour qualities in a broad array of products, including cosmetics, foods, and medicines. The subsequent sections provide a detailed explanation of the chemistry, antimicrobial properties, and taxonomical distribution of EO.

2.2.2.1 Taxonomical distribution

EOs-bearing plants, also called aromatic plants, belong to a distinct botanical group that possesses specialized biological features allowing them to biosynthesize, store, and secrete larger quantities of EOs. Considering the plant families responsible for producing the majority of economically significant EOs are spread across various plant taxa. Currently, more than 1,500 aromatic species, spanning over 90 botanical families, have been recognized. These plants have the potential to yield around 3,000 EOs, but only around 300 of them hold significant commercial importance, particularly in the flavour and fragrance sector [154]. Within gymnosperms, the Cupressaceae family encompasses 27–30 genera and 130–140 species worldwide, such as *Cyprus* spp, *Juniperus* spp, and *Sequoia* spp, with EOs extracted mainly from their bark, leaves, and resins [155]. Similarly, the Pinaceae family, comprises about 262 species in 11 genera, including *Abies* spp, *Larix* spp, *Pinus* spp, and *Cedrus* spp, with EOs commonly found in both the needles and resin of these species [156].

In the clade of angiosperms, known as flowering plants, several noteworthy families house EO-bearing plants. Apiaceae, a significant family consisting of 3780 species distributed in 434 genera, includes cultivated plants like *Cuminum cyminum* L., *Carum carvi* L., *Foeniculum vulgare* Mill., *Coriandrum sativum* L., *Pimpinella anisum* L., *Anethum graveolens* L., and *Petroselinum crispum* L. EOs can be extracted from all parts of Apiaceae plants, but the highest concentrations are typically found in their seeds, *Foeniculum vulgare* Mill., and roots. Asteraceae (or Compositae), with nearly 1600 genera and over 23,000 species, features common species such as *Matricaria recutita* L., *Echinacea purpurea* L., *Achillea millefolium* L., and *Artemisia absinthium* L., storing EOs in their flowers. Lamiaceae, known as the mint family, with 6000 species in 236 genera, includes *Lavandula* spp., *Melissa* spp., *Mentha* spp., *Ocimum* spp., *Origanum* spp., *Perilla* spp., *Rosmarinus* spp., *Salvia* spp., *Satureja* spp., and *Thymus* spp., many of which possess high-quality EOs in all aerial parts, especially leaves and flowers. Lauraceae, the laurel family, comprises around 50 genera and over 2,500 evergreen shrubs and trees like *Cinnamomum* spp., *Camphora* spp., and *Litsea* spp., storing EOs in leaves and stems. Myrtaceae, the Myrtle family, includes approximately 6000 species of aromatic shrubs and trees, with vital aromatic species like *Corymbia* spp., *Eucalyptus* spp., *Myrtus* spp., *Melaleuca* spp., *Syzygium* spp., and *Eugenia* spp., obtaining EOs from leaves, fruits, stems, etc. Poaceae, mainly consisting of herbaceous plants or grasses in tropical and subtropical regions globally, features commercially important aromatic species

like *Cymbopogon* spp. and *Vetiver* spp. Rutaceae, commonly known as the citrus family, with approximately 160 genera, includes economically important genera such as *Citrus* (e.g., *C. sinensis*, *C. limon*, *C. paradisi*, and *C. aurantifolia*) and *Zanthoxylum*, with EOs usually stored in the fruit's skin and leaves. Zingiberaceae, the ginger family, comprises four subfamilies, six tribes, and 57 genera, with over 1960 species dispersed across tropical and subtropical regions worldwide, including *Alpinia* spp., *Curcuma* spp., *Elettaria* spp., *Kaempferia* spp., and *Zingiber* spp., with EOs mainly obtained from the rhizomes. Additionally, other families like Acoraceae (*Acorus calamus* L.), Geraniaceae (*Pelargonium* spp), Illiciaceae (*Illicium* spp.), Myristicaceae (*Myristica* spp.), Oleaceae (*Jasminum* spp.), Rosaceae (*Rosa* spp.), and Santalaceae (*Santalum* spp.) contribute to the diverse array of EOs. Their aromatic compounds are produced by glandular trichomes and other secretory structures, specialized tissues mainly diffused onto the surface of plant organs, particularly bark, buds, flowers, fruits, leaves, roots, seeds, stems, twigs, and wood, and stored in specialized structures such as secretory cells, cavities, canals, and epidermal cells [154,157,158].

2.2.2.2 Chemistry

The chemical components of EOs can be categorized into four groups based on their structural composition: terpenes, terpenoids, phenylpropanoids, and miscellaneous constituents. Terpenes or isoprenoids are the major constituents found in EOs with molecular structures containing carbon backbones of 2-methylbuta-1,3-diene (C_5H_8) i.e., isoprene units. Terpenes can be classified into different groups based on the number of isoprene units (C_5H_8) in their molecules: hemiterpenes (C_5H_8), monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{48}$), tetraterpenes ($C_{40}H_{64}$), and polyterpenes $(C_5H_8)_n$. Hemiterpenes are the simplest form of terpenes and are typically present in minor fractions in EOs. Common hemiterpenes include angelic acid, tiglic acid, isovaleric acid, and senecioic acid [154]. Monoterpenes are derived from two isoprene units and are predominant components of EOs (90%). They are partially responsible for the essence or odour of plants and are the major odoriferous compounds of many flowers and fruits. They are lipophilic liquids with high volatility, which are isolated by hydro distillation or solvent extraction. Examples of some common monoterpenes used in flavour and fragrance industries are menthol, d-carvone, limonene, citral, and 1,8-cineole, which can be found in *Mentha* spp., *Carum* spp., *Citrus* spp., *Cymbopogon* spp., and *Eucalyptus* spp., respectively [159,160]. Sesquiterpenes consist of three isoprene units and are the second important group of active compounds after monoterpenes in EOs of plants. These are unsaturated compounds with less volatility, that can be linear, branched, or cyclic. Cyclic sesquiterpenes can be further classified into monocyclic, bicyclic, or tricyclic. Common examples include zingiberene, humulene, caryophyllene, and cadinene [161]. Diterpenes are formed by the head-to-tail rearrangements of four isoprene units. These compounds are generally found in EOs of some resins. Since they are heavier than monoterpenes and sesquiterpenes, longer distillations are required. Examples of diterpenes include camphorene (monocyclic), labdane (bicyclic-like), kaur-15-ene, phyllocladene, pimaradiene,

and sandaracopimaradiene (tricyclic). Eventually, triterpenes and tetraterpenes may also be detected in small amounts [162].

Terpenoids represent a distinct class of terpenes characterized by the presence of oxygen molecules, and they are formed through biochemical alterations, including the removal or addition of methyl groups. They are synthesized through the mevalonate pathway and are present in plants in various subgroups, including alcohols, aldehydes, esters, ethers, epoxides, ketones, and phenols. Similarly to terpenes, terpenoids can also be categorized into monoterpenoids (e.g., α-terpineol, carvacrol, carvone, eucalyptol, geraniol, menthol, perillaldehyde, piperitone, and thymol), sesquiterpenoids (e.g., farnesol, nootkatone, and patchoulol), diterpenoids (ginkgolides, paclitaxel, retinol, salvinorin A, sclareol, steviol), sesterterpenoids (andrastin A, manoalide), triterpenoids (limonoids, oleanolic acid, sterols, squalene, ursolic acid), etc [163]. Phenylpropanoids are synthesized by the shikimic acid pathway, and these compounds contain a characteristic benzene nucleus to which one or more functional groups are attached (e.g., allyl, hydroxyl, methoxy, aldehyde, and many others). Common phenylpropanoids include anethole, eugenol, safrole, elemicin, or cinnamaldehyde [164]. Besides above-mentioned compounds, EOs may also occasionally include miscellaneous and less typical components like polyketides, fatty acids, and sulphur derivatives. Examples of such compounds encompass jasmonic acid, methyl jasmonate, cis-jasmone, (Z)-3-hexenal, and allicin [159].

2.2.2.3 Extraction methods

The chemical composition of EOs is influenced by various factors ranging from physiological variations to environmental conditions, whereas the choice of extraction method plays a pivotal role. Consequently, it is important to opt for the most suitable and efficient technique to capture the desired active compounds within the EOs. Various methods ranging from conventional extraction techniques such as cold expression, hydro and steam distillation to non-conventional extraction techniques (e.g. solvent-free microwave extraction and supercritical fluid extraction) are commonly used [165].

Distillation is the ancient and simplest method that has been long used for the extraction of EOs from plant materials. At first, the plant material is packed into a compartment, then subjected to boiling water (hydro distillation) or exposed to steam (steam distillation). Hot water and steam act as the main influential factors in freeing bioactive compounds of plant tissue. Indirect cooling by water condenses the system, resulting in two separate phases: the EO and the hydrosol. The hydrosol is composed of water that contains some of the more polar volatile compounds. EOs are typically less dense than water, so they float atop the distillate as the upper layer. The yield of EOs from plants varies widely, and the broad range is 0.05–18.0%. This yield can vary based on a range of factors, including the quality of the plant material, the duration of the distillation process, and the temperature and operating pressure

employed [166]. Generally, the process of steam distillation is the most widely accepted method for the production of EOs on a large scale, whereas hydro distillation is for laboratory-scale setup. But both methods suffer from some disadvantages like high time and energy consumption and due to exposure to high temperatures for long extraction periods, can cause chemical modification of the oil components and often a loss of the most volatile molecules [167].

The use of new green or non-conventional techniques for EO extraction, can shorten the extraction time, improve extraction yield, enhance the quality of the extract, prevent pollution, and reduce sample preparation costs. These techniques include supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted extraction [168]. Especially, supercritical fluid extraction has emerged as the predominant method for extracting and isolating EOs from aromatic plants. This method offers efficient and rapid extraction, necessitates only moderate temperatures, eliminates the need for cleanup procedures, and sidesteps the use of harmful organic solvents. Moreover, carbon dioxide proves to be an ideal solvent, as it is non-explosive, non-toxic, readily accessible, and easily removed from the extracted products [169].

2.2.2.4. Analytical methods

Over the years, there has been emergent attention to the production and implementation of EOs in the fields of aromatherapy, folk medicines, quality control, pharmaceuticals, flavouring, and fragrance. Therefore, analysing the chemical composition of EOs is fundamental for providing valuable insights into the unique properties and potential uses of EOs [170]. However, unravelling the chemistry of EOs has consistently been a difficult task as most of the compounds are present in minor quantities. Moreover, the extensive class of monoterpenes encompasses numerous compounds sharing nearly similar molecular formulas and distinct types of structure, as well as a great number of isomers. Consequently, the analysis of these compounds necessitates the use of methods with highly sensitive detection capabilities. Furthermore, owing to the diverse physical and chemical characteristics of EOs and their volatile components, the efficacy of extracting these compounds from the sample varies significantly. Subsequently, the chemical profiles obtained are strongly influenced by the chosen extraction method. Hence, no singular analytical technique can comprehensively capture the entire spectrum of these volatile components. It is evident that a combination of broad-spectrum profiling methods and targeted approaches for analysing the key volatiles, particularly those present at low concentrations, will remain indispensable in research and analysis [171]. The most common exclusive technique for the qualitative analysis of the volatiles is gas chromatography (GC). The first widely noticed introduction of GC was made in 1951-52 by Anthony T. James and Archer J. P. Martin of the National Institute for Medical Research, in London. Following its introduction, GC experienced remarkable development, evolving from a basic research tool into a highly sophisticated instrument [154]. The fundamental principle of GC relies on the differing affinities of vapour components for

surfaces. In GC, a mixture is first vapourised and picked up by an inert gas (He, H_2, or N_2). This carrier gas is then pushed into the column packed with small, solid particles. The volatiles/vapours which will have strong interactions with the stationary phase will be more actively retained, and eluted later, while components that have weaker interactions, will travel faster through the column, and elute earlier. The partitioning quality depends on several factors, including the VC's (volatile compound) chemical properties (e.g., molecular weight, vapour pressure, and polarity), the column properties (e.g., temperature, length, and diameter) as well as the stationary phase polarity and the mobile phase flow rate. At the end of the column is a specialized detector that produces a signal as each compound exits the column, with the signal intensity corresponding roughly to the relative amount of each component. Plotting the signal on a computer screen gives a peak for each component in the mix. The pattern of peaks, or "chromatogram," is reproducible for any given sample, assuming it is run through the column in the same way [172].

In GC, columns often sort compounds based on their boiling points. Substances that have lower boiling points move through the column more quickly and have shorter retention times than those with higher. Yet, because boiling points are not unique, different chemicals can end up with the same retention time. This indicates that relying solely on the retention time in chromatography is not sufficient for confidently identifying a component in a mixture. Therefore, to enhance the analytical power of GC, it is coupled with MS. When combined, the mass spectra and chromatographic peaks enable a clear and definite identification of each component. In the case of an unfamiliar mixture, examining the mass spectrum corresponding to each peak can help narrow down the potential identity of each component. To validate these identifications, known standards can be employed, confirming a match when both retention time and mass spectra align [171,173]. Additionally, to attain more accurate and high-quality separation of volatile compounds and to avoid overlapping of peaks, analysis can be carried out using two columns of different polarities. As a non-polar column primarily separates based on boiling points, there is a potential issue when dealing with samples containing both oxygenated and purely hydrocarbon components that share similar boiling points. In such cases, the use of a non-polar column may cause the analytical elution range to become compressed, resulting in a narrower retention time range for the sample. This compression increases the likelihood of overlap between different components. Introducing a more polar column, however, offers the advantage of expanding the elution range by retaining the more polar components (such as oxygenated compounds) for a longer duration. Consequently, this separation technique effectively pulls apart different classes of compounds, creating a more extensive separation space for the sample [174].

For the quantitative analysis of EOs, raw peak percentages or peak areas are often used alongside an internal standard (IS). An ideal IS should have similar physical and chemical properties to the analyte and must be inert [175]. However, this quantification has practical limitations and often results in poorer accuracy because it assumes all response factors are equal to unity. To address this, the International

Organization of the Flavour Industry has recommended a computational practice for the rapid quantification of volatile compounds using GC-FID. This method involves calculating predictive relative response factors based on the chemical group and molecular weight of the compounds [176,177].

Coming over to the identification of the VCs, as mentioned earlier, the MS detector is the most prevalent analytical instrument coupled with GC for detecting and identifying. Considering the working, following the elution process, volatile compounds undergo ionization and fragmentation. The resulting ions are then separated based on their mass-to-charge ratios (m/z) as they traverse through an electromagnetic field. Subsequently, they reach a detector that produces a signal proportional to their abundance. Consequently, a mass spectrum is generated for each substance, illustrating a graphical representation of the correlation between the relative abundance of ions and their m/z ratios. Furthermore, the simple and standard method for compound identification is a traditional library search, where the submitted (user) spectra are compared with the compounds in a reference library (The National Institute of Standards and Technology – NIST). However, compounds like isomers may be inaccurately identified, particularly in the analysis of EOs. As previously discussed, EOs predominantly consist of terpenes/terpenoids, which are prone to isomerism, leading to comparable mass spectra. To improve confidence in identifying volatile analytes, a more effective approach involves combining MS library search with retention indices (RI). This component provides a measure of a compound's retention time, on a determined chromatographic column and experimental conditions, relative to a set of reference standards (i.e., C_8 to C_{40} aliphatic hydrocarbons). For instance, Adams reported RIs data using a DB-5MS column with a programmed temperature ranging from 60 to 240 °C at 3 °C/min. The incorporation of RIs in GC-MS studies is already a well-established practice, with analysts routinely adopting these procedures to validate the identities of VCs [174,178].

Headspace analysis

In the context of EO vapour, the initial step in the analytical process involves the extraction of VCs from the gaseous sample. Given the substantial variation in physicochemical characteristics among different compounds in EOs, the efficiency of volatile extraction is closely tied to the specific sampling techniques employed. The most common and effective sampling technique employed is headspace analysis. The technique has existed since the late 1950s and is still actively used. While there are numerous methods available for collecting and concentrating volatile metabolites from a sample, there are two basic approaches: direct sampling of volatiles from the air (headspace) and solvent-based volatile extraction methods [171]. Headspace (HS) sampling is a non-destructive technique that captures volatile compounds, offering a more authentic representation of the volatile profile of living plants compared to conventional methods such as solvent extraction from plant tissues or steam distillation. HS sampling is generally classified as static (S-HS) or dynamic (D-HS). In S-HS, there is

an establishment of equilibrium between VCs present in the liquid EO sample and the headspace above it. This analysis is performed in a closed vessel and once this equilibrium is attained, a portion of the vapour phase is taken from the container and analysed directly using a chromatographic system. In dynamic systems an inert carrier gas is swept over the sample and the volatiles are trapped onto a support [179].

In recent years, there has been the development of the SPME technique for sampling volatiles. This method is based on utilizing a stationary phase coated onto a fiber, which captures the volatiles upon contact with the surface. The fiber can be positioned in the headspace above the sample or immersed into a liquid sample. Following a predetermined duration, the fiber is removed and directly inserted into the GC injection port. Commonly employed phases include polydimethylsiloxane, polyacrylate, and the more traditional octadecyl silyl C_{18}. SPME sampling proves to be a sensitive technique, attributed to the concentration achieved by the fiber, and it also demonstrates selectivity due to the availability of diverse coating materials. The simplicity and cost-effectiveness of this technique have resulted in its extensive application for flavour analysis. However, limitations arise from the use of fiber coating. Notably, the sensitivity of the coating material is often non-uniform, leading to competition among VCs due to the limited number of active sites. Moreover, selectivity varies depending on the coating polymer, influencing the chromatographic peak areas, which may not accurately represent the composition and proportion of compounds in the headspace. Consequently, alternative approaches, such as headspace–gas-tight syringe (HS-GTS) sampling, can be considered for analysing the EO vapour profile. This method stands out as the most convenient and cost-effective approach for sampling VCs from the headspace of a sealed vial. The gas syringe has a pressure-lock valve which is introduced into the headspace for extracting a portion of its volume. The gas sample, securely contained within the syringe, is subsequently transferred, and injected into the chromatographic system. In comparison with SPME, the HS-GTS technique is non-selective but provides a precise and, perhaps, closer assessment of the real distribution of volatile compounds in the given vial [180].

2.2.2.5 Antimicrobial activity

EOs exhibit broad-spectrum antimicrobial properties against a wide range of microorganisms, including bacteria, fungi, and viruses. The use of EOs for the development of novel plant-derived agents is indeed advantageous for several reasons, for instance, they are biologically active in both liquid and vapour phases. In liquid form, they can be applied topically or in pharmaceutical formulations while in vapour form, they can be used in inhalation therapy for treating various respiratory disorders [181]. Most EOs have powerful effects on both gram-positive and gram-negative bacterial species [182]. The common documented antibacterial mechanism involves disrupting the cell wall and membrane, resulting in the leakage of intracellular contents and bacterial lysis. Additional suggested mechanisms of antibacterial

action encompass inhibiting efflux pumps, which are associated with antibiotic resistance, disrupting ATP balance, affecting energy-mediated cellular activities, modifying protein synthesis and quorum sensing, as well as inducing pH disturbances [183]. Furthermore, the ability of EOs to inhibit the growth of microorganisms is dependent on their chemical composition and the amount of the predominant components. For instance, aromatic monoterpenoids such as carvacrol, carveol, cymene, eugenol, limonene, menthol, and thymol are known for their strong antimicrobial activity, primarily due to the presence of phenolic groups in their structures. The primary mechanism of their antibacterial action is their ability to disrupt bacterial cell membranes and inhibit enzymes essential for bacterial metabolism [184,185].

Moreover, coming on the *in vitro* antimicrobial effects of EOs and their VCs in the vapour phase, various studies have reported their potent activity against various pathogens. For example, the initial study conducted by Inouye *et al*. [186] explored the potential role of 14 different EOs in inhalation therapy. The study aimed to determine their antibacterial activity against five pathogens, namely *H. influenzae*, penicillin-resistant *S. pneumoniae*, *S. pyogenes*, and *S. aureus,* via gaseous contact within a specially designed airtight box. The measure of vapour activity was presented using a newly introduced term, minimal inhibitory dose. Among the 14 EOs tested, cinnamon bark, lemongrass, and thyme oils exhibited the highest activity. The study concluded that the antibacterial properties of EOs have been mainly attributed to their predominant bioactive constituents. For instance, the compounds responsible for antimicrobial activity in the study were cinnamaldehyde, geranial, and thymol. Research has consistently shown the antimicrobial properties of individual VCs, with specific compounds demonstrating significant activity, especially in vapour phase. For example, Houdkova *et al.* [187] found that plant-derived VCs like carvacrol, cinnamaldehyde, eugenol, 8-hydroxyquinoline, thymol, and thymoquinone were potent against pneumonia-causing bacteria in both liquid and vapour phases, using a broth microdilution volatilisation method.

Owing to their antimicrobial properties and high volatility, which allows them to vaporize spontaneously at room temperature, EOs, and their components show significant potential for inhalation therapy. This is attributed to their ability to readily reach both the upper and lower regions of the respiratory tract. Currently, the European Pharmacopoeia allows more than 25 EOs for the treatment of respiratory tract diseases. For example, *Eucalyptus globulus* Labill., *E. polybractea* subsp. polybractea*, E. smithii* R.T.Baker*, Foeniculum vulgare* Mill., *Illicium verum* Hook.f., *Melaleuca alternifolia* (Maiden & Betche) Cheel*, Mentha×piperita, Pimpinella anisum* L.*,* and *Thymus vulgaris* L. are frequently used [188]. Presently, a variety of phytomedicinal products, incorporating combinations of EOs and their volatile constituents, are accessible in the market. These herbal medicinal products are commonly available in the form of capsules, lozenges, tablets, tinctures, syrups, nasal drops, inhalers, and sprays, categorized as non-prescription drugs, dietary supplements, and confectionery. Specifically for the treatment of respiratory diseases, inhalers, sprays, and nasal drops are advantageous as their active

components are directly delivered to the infection site in the airways [189]. For example, the Pinimenthol inhaler consisting of EOs from *Eucalyptus* spp. and *Pinus* spp. is prescribed to patients suffering from cold, chronic bronchitis, bronchial catarrh, or hoarseness [190]. Bresol-NS (Himalaya, Bengaluru, India), a nasal spray with a combination of *Coleus aromaticus* Benth., *E. globulus*, and *Glycyrrhiza glabra* L. extracts, helps relieve nasal congestion caused by upper respiratory tract infections. Recently, several inhalation devices combining different EOs have been developed and patented. For instance, adhesive inhalation antiviral patches (Axogen, Alachua, FL, USA) contain safe and effective amounts of EOs from plant species such as *Cinnamomum verum* J.Presl., *C. limon*, *Gaultheria procumbens* L., *Matricaria recutita* L., *M. × piperita*, *Salvia sclarea* L., *Syzygium aromaticum* (L.) Merr. & L.M.Perry, and *Zingiber officinale* Roscoe. These patches are typically placed near the nasal pathway with an appropriate mask, preventing the entry of various respiratory infection-causing pathogens [188]

2.3 Indian herbal medicine

India has 2.4% of the world's area with 8% of global biodiversity and is one of the 12 mega diversity countries of the world. Among the world's 35 global biodiversity hotspots, four (Himalayas, Indo-Burma, Sundaland, and Western Ghats) fall in the Indian geo-political territory. The richness of phyto-diversity in India is due to the existence of 15 agro-climatic zones with varied ecological habitats, resulting in approximately 10.45% of the global floral diversity. Furthermore, the country is renowned for hosting over 40 sites known for their exceptional endemism and genetic diversity. The intricate interplay of climatic and altitudinal variances, alongside diverse ecological habitats, has fostered the evolution of an exceedingly rich vegetation in India. Considering the biodiversity among medicinal plants, India has been bestowed with an enormous wealth, due to which the country is often referred as 'medicinal garden' or 'botanical garden' of the world. Of 48,655 plant species documented 9,500 species have ethno-botanical importance and 7,500 species are in medicinal use for indigenous health practices as well as modern system of medicines [191]. These resources play a pivotal role in traditional medicine systems and contribute significantly to the pharmaceutical industries, both within the country and internationally [192].

As previously mentioned, the Indian subcontinent has a vast repository of medicinal plants that are used in traditional medical treatments. These plants are not only used in various alternative treatments within traditional medical systems, where herbs, minerals, and organic substances play crucial roles, but they also form the basis for preparing herbal drugs. The utilization of plants as medicinal sources is deeply rooted in ancient practices and represents a substantial component of India's healthcare system. In rural India, approximately 70 % of the population depends on the traditional Ayurvedic medicine system, which underscores the enduring significance of plant-based remedies. Within these traditional systems,

healers and practitioners often take a personalized approach by formulating and administering medicinal blends based on their own unique recipes, further emphasizing the close connection between traditional practices and individualized healthcare [193].

Presently, there are approximately 250,000 registered medical practitioners in the Ayurvedic system. Until now, around 20,000 medicinal plants have been recorded in India, where traditional practitioners exclusively utilize 7,000–7,500 plants for treating various diseases. The distribution of plant usage is evident across various Indian medicine systems. The Siddha system of medicine, similar to Ayurveda, considers the human body to be constituted from the five elements of the universe and utilizes numerous plant and mineral preparations in powder form, produced through procedures including calcination. The Unani system of medicine, originating in Greece and introduced by Hippocrates, was brought to India by the Arabs. This system relies on plant formulations such as oils, tinctures, powders, and ointments. Naturopathy, which combines the curative power of nature with traditional and modern techniques to restore good health, also utilizes plant formulations. Lastly, traditional, and folk medicine in India utilize approximately 25,000 effective plant-based formulations. Over 1.5 million practitioners rely on these traditional medicinal systems for healthcare, underscoring their extensive reach and influence [194,195,196].

2.3.1. Medicinal plants of Ayurveda

The term "Ayurveda" finds its roots in the Sanskrit words 'ayu,' signifying life, and 'veda,' denoting knowledge. It embodies a holistic approach to health and personalized medicine. With a history tracing back approximately 3000 years, Ayurvedic practice illustrates a comprehensive understanding of disease management. Derived from the five elements (Prithvi - earth, Jal - water, Agni - fire, Vayu – air, and Aakasha – space) of Indian philosophy, the three fundamental principles in Ayurveda, known as doshas (vata, pitta, and kapha), serve as regulatory control factors for fundamental physiological processes in living systems. These doshas maintain their identity throughout biological history: vata and its subdoshas regulate input/output processes and motion; pitta and its subdoshas regulate throughput, turnover, and hence energy; and kapha and its subdoshas regulate storage, structure, and lubrication. Consequently, Ayurveda can be acknowledged as a complete medical system, encompassing aspects of physical, psychological, philosophical, ethical, and spiritual health [197,198].

Plant-based preparations play a major role in the Ayurvedic healing process. Within Indian folk medicine, a plethora of herbal formulations is employed to address diverse infectious diseases, including wound healing, inflammation resulting from infections, skin lesions, leprosy, diarrhoea, respiratory infections, scabies, venereal diseases, and ulcers [199]. In Ayurvedic therapeutics, these plant-based materials are utilized in both dried form or as EOs in various formulations, as documented in classical texts like the Charaka Samhita, Sushruta Samhita, and Ashtanga Samgraha. Medicinal plants

are primarily utilized in five traditional forms known as 'Pancavidha Kasaaya,' from which all other formulations are derived. These five basic forms are: 'Swarasa,' the expressed juice; 'Kalka,' a fine paste made by grinding fresh or soaked dried plant material; 'Kwaatha,' a decoction; 'Sheeta' or 'Hima,' a cold water infusion; and 'Faanta,' a hot water infusion. The first two forms, 'Swarasa' and 'Kalka,' are prepared using freshly collected plant material and administered directly, while the last three, 'Kwaatha,' 'Sheeta,' and 'Faanta' are aqueous extracts derived from dried plant material. In addition to these preparations, Ayurveda also documents the use of EOs, referred to as Arka. Arka is a liquid obtained by distilling certain plant materials or substances soaked in water using the Arkayantra (a traditional steam distillation apparatus) or modern distillation equipment. This preparation results in a suspension of the distillate in water, with slight turbidity and colour, reflecting the properties of the plant material used, and bearing the scent of the dominant ingredient [200]. Additionally, in Ayurveda, great significance is placed on the appropriate season for collecting plant samples, as this practice is believed to influence their medicinal properties. This is well-documented in ancient texts such as the Charaka Samhita. These texts emphasize various factors to consider when selecting ingredients for formulations, including the plant's habitat, growth season, harvesting conditions, storage methods, and pharmaceutical processing techniques. Guidelines such as Aushadha Sangrahana Sthana (site selection for collection) and Aushadha Sangrahana Kala (time of collection) are provided, alongside field collection practices based on the harvesting season for different plant parts. Classical literature specifically advises collecting different plant parts at precise times for maximum therapeutic efficacy: leaves and branches during the rainy season (vasanta ritu), roots in winter (shishira ritu), and bark or the entire plant in autumn (sharad ritu) [201].

Below are descriptions of some commonly cited traditional medicinal plants known for treating various infectious diseases and utilized in marketed formulations. *Adhatoda vasica* Nees, commonly referred to as Adusa, is a widely distributed species across the plains of India and the lower Himalayan ranges. In Ayurveda, its leaf extract is recommended to address respiratory issues such as asthma, bronchitis, and tuberculosis. Notable formulations include Vasavaleha (Dabur, Ghaziabad, India), Kasamrit Herbal (Baidyanath, New Delhi, India), and Vasaka capsule (Himalaya, Bengaluru, India). *Achyranthes aspera* Linn., also known as Apamarga, is a prevalent weed in India with reported astringent and diuretic properties. It finds use in treating pneumonia, renal dropsy, ophthalmia, dysentery, and it is an important ingredient of Cystone tablet (Himalaya, Bengaluru, India). *Emblica officinalis* Gaertn., recognized as Amla, is a deciduous tree with diverse medicinal properties. It plays a key role in the preparation of marketed products like Triphala (Zandu, Kolkata, India) and Chyavanprash (Dabur, Ghaziabad, India). *Embelia ribes* Burm., identified as Vayavidanga, is known for its anthelmintic and carminative properties and is utilized in various Himalaya Drug Company preparations (e.g. Gasex, Diakof, Herbolax, and Koflet). *Nigella sativa* Linn., or Kalounji, is employed for upper respiratory conditions and is the key ingredient in products like Antidandruff shampoo (Himalaya, Bengaluru, India) and

Kankayan Gutika (Dabur, Ghaziabad, India). *Ocimum sanctum* Linn., commonly known as Tulasi, is a versatile herb with numerous properties and is included in various preparations such as Respinova (Lupin Herbal Lab, Mumbai, India), Abana, Diabecon, and Koflet (Himalaya, Bengaluru, India). *Solanum surattense* Burm., or Kateli, is a spiny herb with anti-asthmatic properties, utilized in Ayurvedic medicines such as Dasamula, Diakof, Koflet, and Chyawanprash (Himalaya, Bengaluru, India). Despite the existence of several Ayurvedic medicinal plants used in therapeutic applications with verified pharmacological properties, the chemistry and biological activity of many species used in traditional Indian medicine have not been fully explored yet [133].

2.3.2 Botanical, phytochemical, and pharmacological properties of studied plants

The subsequent section presents information on the antimicrobial properties, chemistry, and traditional medicinal uses of various plant species examined in this research.

***Alysicarpus monilifer* (L.) DC.**, commonly known as samervo or juhi-ghaas, is a low-growing, annual herb, belonging to the Fabaceae family and ranging from 5 to 50 cm in height. Its leaves are simple, ovate, elliptical, or lanceolate, cordate at the base, 2.5-7.5 cm long, prominently nerved, and either glabrous or sparsely pubescent beneath. The flowers are bisexual, pea-shaped, and closely arranged in axillary racemes. The pedicel is up to 0.2 cm long, with lanceolate, ciliate calyx lobes that are 0.5–0.6 cm long and non-imbricate. The corolla is pink to violet in colour, with yellow to orange hues that rarely turn purple upon opening. The blade is obovate, rounded, and features two laminal callosities, with both sides being glabrous. Stamens are 0.6–0.7 cm long, with a free part of filaments measuring 0.05–0.1 cm, all glabrous. The ovary is 0.3–0.35 cm long, covered with hooked and thin straight hairs, and at the apex, longer appressed straight hairs. It contains 5–8 ovules, and the style is 0.3–0.4 cm long. The pods are moniliform, 2- to 8-jointed, 1.2–2.5 cm long, and turgid, either glabrous or sparsely pubescent. The loment is finely downy with minute hooked hairs, and the surface ranges from obscurely to distinctly veined. The seeds are sub-globose or oblong, 0.1- 0.3 cm long, and brown to yellow in colour [202].

It occupies a significant place in the Indian indigenous system of medicine. The plant demonstrates remarkable pharmacological versatility, functioning as an anti-inflammatory agent, relieving stomach-aches, serving as an antidote for snakebites, and aiding in the treatment of various ailments, including fever, jaundice, leukoderma, diarrhoea, skin diseases, and kidney stones [203,204].

Although *A. monilifer* is acknowledged for its anti-infective effects in Ayurveda, direct investigations into its *in vitro* growth inhibitory activity are lacking. Most of the research conducted so far has focused on the antimicrobial effects of nanoparticles synthesized using *A. monilifer* extract. For instance,

Kasithevar *et al.* [205] and Ratika *et al.* [206] reported the antibacterial efficacy of silver nanoparticles synthesized using *A. monilifer* extract against MRSA with minimum inhibitory concentration (MIC) of 100 µg/mL, *S. aureus*, *E. coli* (MIC = 9 µg/mL), and *P. aeruginosa* (MIC = 8 µg/mL). Furthermore, studies on its chemical composition are also limited.

***Anethum graveolens* L.,** commonly referred to as sowa, soya, dill, garden dill, anet, and sata pushpa, is an annual herb of the Apiaceae family. The plant can grow up to 90 cm tall, with erect and slender stems. The leaves are alternate, decompound, and sheathed, measuring around 1-3 cm long, and are divided into three or four pinnate sections. The numerous umbels are terminal, 5–15 cm long, and are either equal to or longer than the long-peduncled laterals. The flowers are bisexual, rudimentary, and protandrous. The calyx is vestigial, and the corolla consists of five distinct, subovate petals that are strongly inflexed and notched, and yellow in colour. There are five stamens with filaments approximately 0.15 cm long. The ovary is inferior, bilocular, fleshy, and features a conical stylopodium bearing two spreading styles, each about 0.05 cm long. The fruit is 0.3-0.4 cm long with distinct dorsal and intermediate ridges and narrowly winged laterals. The mature seeds are dark yellow, compact dorsally, and measure 0.4 cm long, featuring a longitudinally ridged surface that is straight to slightly curved [207,208].

The fruits derived from this plant hold significant importance in Ayurveda and are employed to address various health conditions such as agalactia, amenorrhea, arthralgia, asthma, bronchitis, cardiac debility, colic, cough, dysmenorrhea, dyspepsia, flatulence, fever, gleet, halitosis, hemorrhoids, hepatopathy, hiccough, inflammation, intestinal worms, odontalgia, skin diseases, spermatorrhea, strangury, ulcers, splenopathy, syphilis and uropathy [207].

A. graveolens is recognized for its antimicrobial potential and several studies have documented its growth inhibitory activity against a broad spectrum of human pathogenic bacteria. For instance, Saleh-E-In *et al.* [205] reported the antibacterial activity of *A. graveolens* ethanolic root extracts against *Acetobacter aceti*, *B. subtilis*, *E. coli*, and *S. enteritidis*, with a MIC of 62.5 µg/mL and against *E. faecalis* and *P. aeruginosa* with MIC of 125 µg/mL. Another study demonstrated the antibacterial potential of the root EO against *E. faecalis, E. coli* and *P. aeruginosa* (MIC = 62.5 µg/mL) [208].

The study on the chemical composition of *A. graveolens* has revealed the presence of compounds that can be linked with the antimicrobial effect like coumarins (graveolone), furancoumarins (bergapten), flavonoids (kaempferol, persicarin), monoterpenes (limonene, carvone), phenylpropanoids (apiol, dillapiole, myristicin) and plant steroids (β-sitosterol) [209].

***Barleria prionitis* L.,** commonly known as koranti, saireyaka, vajradanti**,** is a perennial, acanthaceous, prickly, and bushy medicinal plant, belonging to the Acanthaceae family. The plant grows up to 150 cm in height with a cylindrical and rigid stem. The leaves feature elongated, pale-coloured spines ranging from 3-10 cm in length, with a cuneate base dotted with glands beneath. The flowers are bisexual, large, often sessile, and solitary on the lower leaf axils or in short terminal spikes. The calyx lobes are spine-tipped and oblanceolate. The corolla is golden-yellow, with oblong-ovate and obtuse lobes. There are two stamens with exserted filaments, approximately 0.2-0.6 cm long. The ovary is inferior, ovoid, and 2-loculed, with 2 or 1 ovule(s) per locule, often with one ovule reduced or aborted. The style can be up to 4 cm long. The fruits are capsular and ovoid, about 0.6 cm long, containing two seeds. These seeds are relatively long, about 0.8 cm, flattened, and densely covered with matted hairs.

In Ayurveda, the entire plant is employed for various medicinal purposes, with its pharmacological activities highlighted as a health tonic, anti dontalgic, anti-inflammatory, antipyretic, anti-flatulent, anti-asthmatic, and antipruritic agent. Moreover, due to its anti-dontalgic properties, it is popularly used as an ingredient in toothpastes such as Vicco vajradanti (Vicco Laboratories, Nagpur, India). The dried bark and root are utilized as an expectorant for managing whooping cough. In certain instances, pills formulated from the plant's shoots, combined with honey, are administered to provide a soothing effect in cases of whooping cough [210].

The plant is renowned for its antimicrobial effect. A study by Aneja *et al.* [211] reported the *in vitro* growth-inhibitory effect of the methanolic bark extracts of *B. prionitis* against oral infection-causing microbes such as *Bacillus* spp., *Pseudomonas* spp., *S. aureus*, *S. mutans*, and two oral fungi, *C. albicans* and *S. cerevisiae*, however at high concentrations (MICs > 1000 μg/mL) [212,213]. Moreover, studies on its chemical composition contributing to its antimicrobial property are limited

***Cocculus hirsutus* (L.) Diels,** commonly known as chilahinta, patalgarudi, kattu kodi, is a perennial plant belonging to the family Menispermaceae family. The plant grows up to 200-300 cm in height with a slender stem. The leaves are 5-7 cm long, simple, alternate, ovate, sub-deltoid, or three-lobed, with obtuse and mucronate tips. The flowers are minute and unisexual. The male flowers are 0.1 cm in size and form axillary cymose panicles with six free stamens embraced by the petals; the anthers are sub-globose. Female flowers, also 0.1 cm, occur in axillary clusters of 1-3, with six staminodes and usually cylindrical styles. Calyx and corolla are obovate and with 6 lobes. The corolla is yellow to white-green in colour. The ovary is superior and unilocular, with a cylindrical style and a terminal stigma. The ovules are anatropous, bitegmic, and crassinucellate. The fruits are 0.4–0.8 cm, drupes resembling small peas with a dark purple endocarp. The seeds are 0.4-0.6 cm, curved and fleshy, containing an annular embryo [214].

In Ayurveda, the plant is renowned for its pharmacological properties, including diuretic, hypolipidemic, laxative, and spermatogenic activities. The roots and leaves are applied to address various health issues such as abdominal disorders, aphrodisiac, blood disorders, dysuria, cough, eczema, fevers, leucorrhoea polyuria, piles, rheumatoid arthritis, and syphilis [215,216].

Numerous studies have highlighted the antibacterial potential of *C. hirsutus* plant extracts. For example, Gupta *et al*. [217] observed the growth-inhibitory properties of ethanolic leaf extract against *Mycobacterium tuberculosis* at the MIC of 500 µg/mL. Another study investigated the antibacterial activity of *C. hirsutus* leaf extracts against *S. aureus* and *E. coli* using the disk diffusion method [218].

Various studies have identified chemical constituents associated with the antimicrobial activity of *C. hirsutus*, including alkaloids (e.g. coclaurine, isotrilobine, magnoflorine, trilobine), flavonoids (liquiritin and rutin), and triterpenoids (hirsudiol) [219,220,221].

***Crotalaria juncea* L.,** commonly referred to as sunn hemp or madras hemp, is an erect shrubby annual plant belongs to the Fabaceae family. The plant typically reaches a height of 1000-4000 cm with a ridged and pubescent stem. The leaves are alternate and simple, with slender stipules measuring 0.1–0.2 cm in length, a petiole of 3–5 cm, and oblong-lanceolate leaflets. The inflorescence features a terminal open raceme, up to 25 cm long, bearing showy papilionaceous flowers. The flowers are bisexual and 5-merous, with a calyx measuring 1.5–2 cm long and covered in short brown hairs. The corolla is bright yellow, with an elliptical standard that is faintly reddish-marked or tinged. The anthers are dimorphic, featuring five stamens with short filaments and five with long filaments. The ovary is superior and one-celled, with a curved style and a small stigma. The fruit is a cylindrical pod measuring 3.0–5.5 cm, short and velvety hairy, containing 6–12 seeds. The seeds are oblique-cordiform, 0.6–0.7 cm long, and range in colour from dark brown to black [222].

C. juncea holds a strong traditional background in Ayurveda, exhibiting diverse pharmacological activities, including abortifacient effects, astringency, blood purification, demulcence, emesis, and purgation, and treatment for conditions like anaemia, impetigo, menorrhagia, and psoriasis [223].

Several studies have reported the growth inhibitory potential of *C. juncea* extract. For instance, Mahasawat *et al.* [224] observed strong antibacterial effect of the ethanolic flower extract of *C. juncea* against gram-positive bacteria such as *S. aureus* (128 µg/ml), *S. epidermidis* (64 µg/ml), *S. pyogenes* (16 µg/ml), and *S. mutans* (64 µg/ml). However, studies focused on identification of major compounds responsible for the antibacterial effect are missing.

***Cymbopogon citratus* (DC.) Stapf**, commonly referred to as lemongrass or gandhatrina, is a perennial herb classified within the Poaceae family. This tropical grass typically grows up to 180 cm in height with short underground stems. Leaves are coriaceous, terete, tightly embracing the stem, glabrous, smooth, striate with approximately 0.9 cm in length. Leaf blade linear, long attenuate at both ends, with an acuminate, drooping apex. It is glabrous, glaucous-green, with a prominent midrib below and white above, smooth on both surfaces, but the top part and margins are often scabrid. The inflorescence is a large, loose, decompound, nodding panicle, up to 60 cm long, with 4-9 nodes, repeatedly branched. Racemes are 1.5-2.5 cm long, with villous rachis having 0.2-0.3 cm long hairs, bearing 4-7 pairs of spikelets. Each pair consists of one sessile and one pedicellate spikelet, terminated by one sessile and two pedicellate spikelets. Sessile spikelets are 0.5-0.6 cm bearing 2 florets. The lower glume is flat or shallowly concave on the back, matching the size and shape of the spikelet, 2-keeled, glabrous, and veinless. The upper glume is boat-shaped, 1-keeled on the back. The lower floret is reduced to an empty lemma, while the upper floret is hermaphrodite with a hyaline, 2-lobed lemma, mostly lacking palea. It has 2 lodicules, cuneate or truncate, 3 stamens, and 2 styles with plumose stigmas. The pedicellate spikelet is 4.5 mm long, male or reduced to empty glumes, with a 7-9-veined lower glume and a 3-veined upper glume. The fruit is simple, indehiscent dry and brown in colour 1.5 – 3. The seeds are 3.15 cm long, cylindrical to subglobose caryopsis with a basal hilum [158].

The pharmacological activities of *C. citratus* have a distinguished history in folk and Ayurvedic medicine, where it is commonly used for its analgesic, anticonvulsant, antiemetic, antirheumatic, antiseptic, antispasmodic, antitussive, and hypotensive, properties. It is also utilized in the treatment of nervous and gastrointestinal disorders, as well as fevers [20]. Additionally, some studies have highlighted its efficacy in regulating platelet composition, alleviating anxiety, and depression, and addressing conditions such as malaria and pneumonia. Industrially, lemongrass is used as an additive, flavouring agent, insecticide, and preservative in beverages, baked goods, and various cuisines [225,226].

C. citratus EO exhibits a broad spectrum of antimicrobial activity against a diverse range of microorganisms. This includes gram-positive bacteria such as *E. faecalis*, *S. aureus*, *S. pneumoniae*, and *S. pyogenes*, gram-negative bacteria like *Aeromonas veronii, E. coli*, *Klebsiella* spp., and *Salmonella enterica*, as well as various fungi [227,228,229]. Additionally, the *in vitro* antimicrobial activity of EOs, particularly against respiratory tract pathogens, has been extensively investigated [188,230]. For instance, a study by Singh *et al*. reported its EO activity against *Klebsiella pneumoniae* and *S. aureus* at the MIC = 64 µg/mL respectively [231]. Interestingly, the antibacterial property of EO is not limited to the liquid phase, as various studies have reported bioactivity in the vapour/gaseous phase as well. For instance, Inouye *et al.* [186] reported moderate antibacterial efficacy against *S. pyogenes* with MIC of 400 µg/mL, *H. influenzae*, and *S. pneumoniae* (MIC = 800 µg/mL) using a modified agar dilution method.

Hydro-distillation of *C. citratus* aerial parts yields yellow or amber-coloured viscous EO with a strong, fresh-grassy lemon-like odour. The major chemical constituents of the EO responsible for antimicrobial activity are stereoisomers of monoterpene aldehyde citral (geranial and neral) and other monoterpene hydrocarbons like limonene and myrcene [232,233].

***Cyperus scariosus* R.Br.,** commonly referred to as nagarmotha, nagar mustaka, or nut grass, is a perennial herb belonging to the Cyperaceae family. The plant typically reaches a height of 45-75 cm, with slender stolon, covered by elliptic, acute, lax, and striate concolorous scales. Leaves are usually shorter than the stem, sharp, and variable, measuring 0.3-0.85 cm in width and up to 7.62 cm in length. The umbels are slender and contracted, with rays that can extend up to 12.7 cm long. The spikelets, ranging from 5 to 17.5 cm in length, are characterized by their linear shape and pale straw coloration, bearing between 1 to 50 flowers. These flowers are bisexual, lacking bristles or scales. Stamens can vary in number, appearing as 3, 2, or 1, with connectives often protruding beyond the thecae. Ovaries typically feature a single style, which may be divided into 3 or 2 parts, occasionally nearly undivided. The fruit is a dark brown, 3-angled, one-seeded nut, 0.13-0.15 cm long. The seeds are green and 0.5 cm long. The rhizomes are very short and woody, producing stolons with lateral shoots that ascend immediately from the base of the stem [234].

In Indian traditional medicine, it is commonly used to treat diarrhoea, fever, thirst, and a pervasive burning sensation throughout the body. Especially the plant root is highly valued in folk medicine for its pharmacological properties, serving as a cordial, desiccant, diaphoretic, emmenagogue, tonic and vermifuge. The EO is a key component in various Ayurvedic formulations designed to address a range of ailments, including epilepsy, gonorrhoea, liver damage, and syphilis [235,236].

The EO steam distilled from the rhizome of *C. scariosus* has been recognized for its potential antimicrobial properties [234]. For instance, a recent antibacterial study has reported the growth-inhibitory effect of EO against carbapenem-resistant *K. pneumoniae* and methicillin-resistant *S. aureus* (MICs = 125 µg/ml) [237]. Additionally, the major constituent of the EO, Longiverbenone, exhibited potent results against various human pathogenic bacterial species like *Vibrio cholerae* (MIC = 20 µg/ml), *B. subtilis*, *B. cereus*, *B. megaterium*, *S. dysenteriae* (MIC = 40 µg/ml) and *E. coli* (80 = µg/ml) [238]. However, the data on the bioactivity in the EO vapours is limited.

The EO extracted through hydro-distillation of *C. scariosus* rhizomes is commercially known as cyperiol oil. It is characterized by a light amber to dark brown colour and a distinctive woody, amber odour [234]. The primary chemical components of *C. scariosus* essential oil that are known to exhibit antimicrobial activity include sesquiterpene hydrocarbons such as cyperene, and sesquiterpenoids like cyperotundone and longiverbenone [239,240,241].

***Jasminum angustifolium* (L.) Willd.,** commonly referred as wild jasmine, banmallika, vanamallika is a small and scandent perennial shrub, belonging to the Oleaceae family. The plant reaches 600 cm in height with smooth and pubescent stem. Leaves are simple, 1-3 cm long, elliptic-ovate, and acute with an obtuse or nearly rounded base. The flowers typically exhibit bisexual characteristics, being fragrant, nectar-rich, and distylous, and often appearing either solitary or in clusters of three. The calyx tube, measuring 0.3 cm in length, is oblong and smooth. Meanwhile, the corolla presents as white and funnel-shaped, boasting 6-9 slender, pointed lobes. Stamens, numbering 2, feature short filaments and dorsifixed introrse anthers, positioned epipetalously. The bicarpellary, bilocular ovary contains 2 ovules per locule. Lastly, the style is slender and culminates in a capitate stigma. The fruit is a berry, 0.8 cm in length, didymous, and oblong. Seeds are small, black, and round, measuring 0.2 cm and lacking endosperm [242].

In Ayurveda, the plant's leaves and roots hold substantial recognition for their pharmacological activity in treating snake and scorpion bites, along with addressing various skin diseases. The bitter and acrid roots of *J. angustifolium* are utilized externally to treat conditions such as herpes and ringworm. Additionally, they are recommended for managing ophthalmopathy, ulcerative stomatitis, leprosy, pruritus, and wounds. In contrast, the leaves are employed as an emetic in cases of poisoning [23].

J. angustifolium have been well-documented for their antimicrobial activity against both gram-positive and gram-negative bacteria, as well as against fungi. For example, a study conducted by Balkrishna *et al.* [243] reported the growth inhibitory potential of the methanolic flower extract against representatives of various bacterial genera such as *Lactobacillus*, *Yersinia*, *Enterococcus*, and *Pseudomonas* (MICs ranging from 8 to 6 µg/mL). Data regarding the chemical constituents of the plant that contribute to its antimicrobial activity are still lacking.

***Lygodium flexuosum* (L.) Sw.,** commonly known as vallipanna, is a climbing perennial fern belonging to the family Schizaceae. The fern reaches a height of 60 cm with dwarfed branches exhibiting a slight pubescence, substerete form, and a flattened structure on one side, appearing narrowly winged or tetragonal. Fronds are held on a long stipe, to 50 cm or more, narrowly winged on the upper part. The primary rachis branches do not elongate, while the secondary rachis branches bear alternate pinnately arranged leaflets, usually 3 or 4 on each side, accompanied by a simple or forked terminal leaflet, resulting in an overall length of 15-30 cm. The fertile pinnae produce sorophores measuring 0.3–0.5 cm, located at the apices of small triangular lobes. The indusia are either glabrous or bear a few hairs similar to those on the abaxial surface of the lamina. The spores are finely and evenly verrucose [244].

The plant holds great significance in Ayurveda, with all parts exhibiting medicinal properties. Pharmacologically, the rhizomes are utilized for treating various health problems such as carbuncles, cut wounds, eczema, jaundice, rheumatism, and sprains [245].

Numerous studies have documented the antimicrobial properties of *L. flexuosum*. For instance, Nayak *et al.* [246] reported the growth-inhibitory activity of the aqueous extract of *L. flexuosum* against MRSA, *P. mirabilis*, and *E. coli*, however at relatively high concentrations (MIC > 1000 μg/mL). Another study by Borkotoky and Borah [247] assessed the antibacterial potential of *L. flexuosum* leaf extracts against *K. pneumoniae* and *P. aeruginosa* again at high concentration (MIC > 1000 μg/mL)

Studies on the chemical composition of *L. flexuosum* extract have documented a unique compound lygodinolide, which is reported to have prominent application in wound healing [244].

***Mucuna pruriens* (L.) DC**., commonly referred to as gonca, kauncha, or kavachused, is an annual climbing shrub belonging to the Fabaceae family. The plant reaches a height of 1500 cm with slender and slightly pubescent stem. The trifoliate leaves are grey-silky beneath, with long and silky petioles measuring 6-11 cm. The leaflets, membranous in nature, exhibit uneven sides, with terminal leaflets being smaller than the lateral ones. The flowers are bisexual and fascicled, typically appearing in clusters of 1-3 per brachyblast, with pedicels measuring 0.4–0.6 cm in length. The campanulate calyx is densely covered with white hairs and has longer, yellowish-brown irritant bristles, particularly near the base. The corolla is purple and broadly elliptic, with a basal claw measuring 0.2 cm long and hairy margins at the base, along with a keel formed by two partially fused petals. There are 10 diadelphous stamens with a staminal tube 3 cm in length, and staminodes are present. The pistil is 3.5 cm long, with an ovary approximately 0.7 cm long and 0.15 cm wide, covered in hairs. Its fruits are dehiscent, 5-6.3 cm long, curved, longitudinally ribbed and is densely covered with loose orange hairs that can cause a severe itch upon skin contact. The seeds are around 1.5cm long and are either shiny black or brown in colour [248].

The seeds of *M. pruriens* have been shown to hold significant medicinal importance. In the traditional Indian medical system, *M. pruriens* has historically been used to treat aches, diabetes, diarrhoea, dysmenorrhea, gonorrhoea, gout, ineptitude, Parkinson's disease, rheumatic issue, snakebite, sterility, tuberculosis, tumour, worms and wooziness [248,249]. Additionally, all parts of *M. pruriens* possess valuable pharmacological properties, including aphrodisiac, anti-diabetic, anti-epileptic, antimicrobial and anti-neoplastic activities [250].

Only a limited number of studies have documented antimicrobial effects of the plant. For instance, Stanley *et al.* [251] reported growth-inhibitory effect of ethanolic seed extract against *S. aureus* and *E. coli*, however at very high concentration (MIC = 2500 μg/mL). Similarly, study led by Salau *et al.* [252]

reported the weak growth-inhibitory activity of *M. pruriens* leaf extract against *E. coli* and *P. aeruginosa* (MIC = 1600 µg/mL).

Numerous studies have documented the chemical composition of *M. pruriens*, particularly highlighting the presence of the unusual non-protein amino acid 3-(3,4 dihydroxyphenyl)-L-alanine (L-Dopa), a potent neurotransmitter precursor believed to contribute to its toxicity. However, research on the specific chemical constituents responsible for its antimicrobial effects remains limited [248].

***Psoralea corylifolia* L.,** commonly known as bemchi, aindavi, chanderlekha, kushthahantri, sitavari, vejani, sugandhakantak, krishnaphala, kalameshi, sasankarekha, babchi, and bawachi, is an erect annual herb belonging to the Leguminosae family. The plant has pubescent stem reaching a height of 60–150 cm. The leaves have obtuse or acute tips, round, 4.5–9 cm long, cordate base, irregularly serrated margins, and a tough texture with black glandular dots on both sides. The plant bears an axillary, dense raceme, or tiny capitate inflorescence with 10–30 blooms. The bract is membranous and lanceolate with tomentose and glandular dots. The flowers are axillary, bisexual, and prominently stalked. The calyx is pale yellow brown, campanulate, and forms a tube at the base. The corolla has a butterfly shape, light purple or yellow, with an inverted broad ovoid vexillum and broad linear petals, and an oblong keel. There are 10 stamens grouped into a bundle with small anthers. The single pistil has a superior ovary that is either obovate or linear, and a filiform style. The pod is 0.5 cm long, ovoid-oblong, mucronate, black and has an irregularly reticulate surface. The seed is 0.1 cm long, reniform, slightly flattened, brownish-black, and fragrant [23,253].

In Ayurvedic medicinal systems, the seeds of *P. corylifolia* play a crucial role because they are exhibiting aphrodisiac, anti-helminthic, diuretic, and laxative properties. The seeds are used both externally and internally, in the form of paste, to treat various conditions, including alopecia, eczema, inflammation, leukoderma, leprosy and psoriasis [254]. The plant is also an essential component in various polyherbal Ayurvedic formulations. For instance, when combined with *Terminalia chebula* and *Tribulus terrestris*, it is used for urinary disorders. In the mixture with *Sida cordifolia* and *Withania somnifera*, it treats reproductive diseases and cough. Additionally, it is combined with *Myristica fragrans* and *T. chebula* to address cold symptoms and chronic diarrhoea [24].

P. corylifolia is well recognized for its antimicrobial effects; for instance, a study by Baig [255] observed the strong growth-inhibitory effects of the seed extracts against bacterial species such as *E. coli, K. pneumoniae, Proteus mirabilis, P. vulgaris, P. aeruginosa, S. aureus* and *S. epidermidis* with a MIC of 2.5 µg/ml. Furthermore, the seed extract also exhibits significant antifungal effects against *Epidermophyton floccosum, Microsporum gypseum* (MIC = 125 µg/mL)*, Trichophyton rubrum*, and *T. mentagrophytes* (MIC = 62.5 µg/mL) [256].

Considering the chemical constituents of *P. corylifolia* seed extract and EO, several compounds have been reported to possess potent antimicrobial activities and have been studied individually. For instance, polyphenolic compounds such as bakuchalcone, bakuchicin, bavachalcone, bavachin, bavachinin, bavachromanol, corylifolinin, corylin, isobavachin, isobavachalcone, psoralen, and bakuchiol a phenolic monoterpene [257,258,259,260,261,262,263,264]. Notably, bakuchiol is used as a major ingredient in various cosmetic products and has gained popularity due to its anti-acne, anti-aging, and retinol-like skin regeneration properties. [265,266].

Terminalia crenulata **Roth**, commonly known as black murdah, asaina, or kauha, is a perennial deciduous tree belonging to the family Combretaceae. It can grow up to a height of 300 cm and has dark grey, rough, and pubescent bark. The upper leaves are arranged alternately, while the lower ones are subopposite and variable in shape (elliptic, oblong, or ovate). They are 12.5-22.5 cm, long, glabrous on the upper surface, and pubescent below, with edges that are either entire or serrulate. The inflorescence is terminal or in a paniculate spike arrangement. The flowers are bisexual, dull, yellow, and accompanied by linear lanceolate bracts. The hypanthium is approximately 0.25 cm long, cup-shaped, and can be either glabrous or pubescent, with triangular teeth measuring around 0.15 cm. The calyx is tubular and angled, with a pubescent base that is constricted above the ovary. It has 5 ovate, yellow lobes that are densely villous on the inside. Petals are absent. The stamens are 10, arranged in 2 rows, with filaments measuring 0.4-0.5 cm. The ovary is inferior and densely villous, measures 0.25 cm in length and is 1-celled, containing 2 or 3 pendulous ovules. The style is subulate and 0.4 cm long, with a small stigma. The drupe, measuring 3.5-5 cm in length, is brownish, glabrous, and features five broad wings with horizontal striations. Seeds are typically 1.5 cm long, usually solitary, and reddish-brown in colour [267].

In the practice of Ayurveda, *T. crenulata* is utilized to address imbalances in pitta, cardiopathy, cough, bronchitis, diarrhoea, dysentery, gonorrhoea, haemorrhages, haemoptysis, leucorrhoea, sensations of burning, strangury, wounds, ulcers and verminosis. The bark displays a diverse array of pharmacological properties, encompassing anthelmintic, astringent, diuretic, cooling, cardiotonic, constipating, expectorant, styptic, uterine tonic and vulnerary effect [23].

Only a few studies have delved into the antimicrobial potential of *T. crenulata*. A study conducted by Jain *et al.* [268] reported the antibacterial activity of the ethanolic bark extract against *B. subtilis*, *S. epidermis*, and *S. dysenteriae* (MICs = 130 µg/ml), *E. coli* (MIC = 263 µg/ml), *M. luteus* (MIC = 263 µg/ml), and *S. typhi* (MIC = 520 µg/ml). However, data regarding the chemical constituents of the plant extract responsible for antimicrobial activity are still lacking.

***Trachyspermum ammi* (L.) Sprague,** commonly referred to as ajwain, is an annual aromatic and herbaceous plant that belongs to the Apiaceae family. It is a profusely branched herb, reaching a height of 60-90 cm with striated stems. The leaves are pinnate, 2–8 cm long, featuring a terminal leaflet and 7 pairs of lateral leaflets. The stem is striated, and the inflorescence forms a compound umbel with 16 umbellets, each containing up to 16 flowers. The flowers are white and occur as both male and bisexual; the corolla has 5 bilobed petals, and there are 5 stamens alternating with the petals. The ovary is inferior, and the stigma is knob-like. The fruit is ovoid, achene with two mericarps. Seed is single, 0.6 cm long, compressed laterally, and tapered toward both ends [21].

T. ammi is a highly valued plant in the Indian medicinal system and is commonly recommended for stomach disorders. The paste of crushed fruits is applied externally to relieve colic pains, and a hot, dry fomentation of the fruits is applied to the chest for asthma treatment. The primary parts of the plant used are its fruits and seeds. The decoction of *T. ammi* seeds is employed as a first-line treatment in Ayurveda for abdominal discomfort, loose bowels, cough, and stomach issues. The seeds are reported to have carminative, diuretic, antimicrobial, antiviral, anti-ulcer, antiplatelet, and hepatoprotective properties [269].

T. ammi seed EO has been reported to possess strong antimicrobial potential, with various studies documenting its activity against food spoilage pathogens. For instance, it has been reported effective against *B. subtilis, E. aerogenes,* and *P. aeruginosa* (MICs = 12.5 µg/mL)*, Salmonella typhimurium* (MIC = 225 µg/mL), and *S. aureus* (162.5 µg/mL) [270,271]. Furthermore, a study led by Gradinaru *et al*. [272] has reported the antibacterial activity against LTRI causing bacterial species like *P. aeruginosa* (MIC = 70 µg/mL), *S. aureus* (30 µg/mL) and *S. pneumoniae* (125 µg/mL).

Hydro-distillation of *T. ammi* seeds yields a light yellowish EO characterized by a warm, spicy, pungent, and typical thyme like odour. The major components of *T. ammi* EO responsible for attributing the antimicrobial property includes monoterpene hydrocarbons (p-cymene and γ-terpinene) and monoterpenoid thymol, which together constitute up to 90% of the total EO content [273,274,21].

The above-mentioned plants are used in various forms in Ayurveda, such as pastes, decoctions, and tinctures. In Ayurveda, tinctures are commonly referred to as asavas (alcoholic medicaments prepared from powdered herbal drugs) and arishtas (alcoholic medicaments prepared from decoctions of herbal drugs) [275]. For instance, tinctures of *A. graveolens*, *C. juncea*, *J. angustifolium*, *L. flexuosum*, and *P. corylifolia* are commercially available and used for treating various infections and ailments. In our laboratory, we followed a similar approach for preparing tinctures or Ayurvedic Asavas, where powdered herbs are dissolved in alcoholic solutions, closely resembling classical ethanol extraction [276,277,278,279,280]. Additionally, for *T. ammi*, we used hydrodistillation techniques, as these aromatic plants are traditionally processed for their volatile oils in the form of arka in Ayurveda. The preparation of arka involves soaking and boiling the plant materials in water using a traditional

apparatus known as the Arkayantra [281,282]. Although replicating the precise Ayurvedic methods is challenging, we made efforts to approximate these traditional practices as closely as possible.

3. RESEARCH QUESTIONS

1. Which species of medicinal plants used in Ayurveda for treatment of infectious diseases will demonstrate antimicrobial activity against infection-causing bacterial and fungal pathogens?
2. Which medicinal plants used in Ayurvedic medicine for treatment of respiratory infections will produce antibacterial activity against pneumonia causing pathogens in vapour phase and, in the same time, will be safe to lung cells?
3. What constituents are present in antibacterial EOs and their vapours? Is the chemical analysis of antibacterial EO vapours affected by HS sampling technique used?

4. HYPOTHESES

1. Medicinal plants used in Ayurveda for treatment of infectious diseases will inhibit growth of infection-causing bacterial and fungal pathogens *in vitro*.
2. Essential oil-bearing plants used in Ayurvedic medicine for treatment of respiratory infections will produce *in vitro* growth-inhibitory effects against pneumonia causing bacteria in vapour phase and will be safe to lung fibroblasts.
3. Each antibacterial EO will contain characteristic constituents, however, the use of different HS sampling techniques can result in distinct chemical profiles of their vapours.

5. OBJECTIVE

The main goal of the study was to investigate the *in vitro* antimicrobial activity, toxicity, and chemical composition of EOs and extracts from plants used in traditional Indian medicine for treatment of infectious diseases.

The specific aims of the study were:

1. Evaluation of the growth-inhibitory effect of ethanolic extracts against representatives of gram-positive and -negative bacteria and yeast causing infection diseases.
2. Determination of growth-inhibitory effect of EOs against bacterial pathogens causing pneumonia in liquid and vapour phase using broth macrodilution volatilization method and their cytotoxicity to the human lung fibroblast cell lines.
3. Characterizing the chemical composition of the EO and its vapours using GC-MS and two distinct HS sampling techniques.

6. MATERIAL AND METHODS

6.1 Plant materials

The selection criteria of the plant samples were rooted in their extensively documented traditional application in Ayurvedic medicine for addressing ailments commonly linked to infections triggered by pathogenic microorganisms. For EOs, dried aerial parts of *C. citratus*, rhizomes of *C. scariosus*, and seeds of *T. ammi* were purchased from Bhagyashree Herbal Farms in Chhattisgarh, India. Additionally, for plant extracts, leaves of *A. monilifer*, seeds of *A. graveolens*, leaves of *B. prionitis*, aerial parts of *C. hirsutus*, fruits of *C. juncea*, flowers of *J. angustifolium*, leaves and roots of *L. flexuosum*, seeds of *M. pruriens*, flowers, leaves and seeds of *P. corylifolia*, and bark and leaves of *T. crenulate* were collected from various districts of the state of Chhattisgarh, India, in December 2019 and 2021. The freshly collected samples were subsequently air-dried for several days and then transported to the Czech Republic for further processing and bioactivity testing. Prof. Mukund Lal Naik, a local expert, authenticated the species, and voucher specimens have been deposited in the herbarium of the Department of Botany and Plant Physiology at the Faculty of Agrobiology, Food, and Natural Resources of the Czech University of Life Sciences in Prague (Czechia).

6.2 Chemicals

The chemicals used for various assays were purchased from Sigma-Aldrich (Prague, Czech Republic), as detailed below. In biological assays, dimethyl sulfoxide (DMSO, CAS 67-68-5) was used as the solvent, and thiazolyl blue tetrazolium bromide (MTT dye, CAS 298-93-1) served as an indicator of cell viability. Positive antibiotic controls included amoxicillin (90%, CAS 26787-78-0), ampicillin (84.5%, CAS 69-52-3), oxacillin (86.3%, CAS 7240-38-2), and tetracycline (98–102%, CAS 60-54-8). Vinorelbine (≥98%, CAS 125317-39-7) was used as a positive control in cytotoxicity testing. Chemical standards of EO constituents included 3-carene (99%, CAS: 498-15-7), camphene (97.5%, CAS: 79-92-5), caryophyllene oxide (99%, CAS: 1139-30-6), citral (95%, CAS: 5392-40-5), linalool (97%, CAS: 78-70-6), m-cymene (99%, CAS: 535-77-3), p-cymene (99%, CAS: 99-87-6), thymol (99%, CAS: 89-83-8), α-pinene (99%, CAS: 7785-70-8), β-pinene (99.0%, CAS: 18172-67-3), γ-terpinene (97%, CAS: 99-85-4), and the analytical standard methyl octanoate (99.8%, CAS: 111-11-5). Additionally, n-hexane (CAS: 110-54-3) was obtained from Merck KGaA (Darmstadt, Germany).

6.3 Microorganisms and media

In this study, the following standard strains from the American Type Culture Collection (ATCC) were employed: *C. albicans* ATCC 10231, *H. influenzae* ATCC 49247, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, and ATCC 19615. The cultivation and assay media (broth/agar) consisted of Mueller–Hinton broth (MHB), supplemented with 1% glucose for *E. faecalis* and Haemophilus test medium for *H. influenzae*. For *S.*

pneumoniae and *S. pyogenes*, brain–heart infusion was used. The pH of the broths was adjusted to a final value of 7.6 using Trizma base (Sigma-Aldrich, Prague, Czech Republic). All microbial strains and cultivation media were procured from Oxoid (Basingstoke, UK). Stock cultures of bacterial strains were cultivated in broth medium at 37 °C for 24 hours before testing in the incubator (Memmert, Buchenbach, Germany).

6.4 Cell Cultures

Lung fibroblast cell line MRC-5, obtained from ATCC (Manassas, VA, USA), was propagated in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 10 µL/mL nonessential amino acids, and 1% penicillin–streptomycin solution (10,000 units/mL of penicillin and 10 mg/mL of streptomycin); all these components were purchased from Sigma-Aldrich. The cells were pre-incubated in 96-well microtiter plates at a density of 2.5×10^3 cells per well for 24 h at 37 °C in a humidified incubator (Sanyo Electric, Osaka, Japan) in an atmosphere of 5% CO_2 in air.

6.5 Preparation of the plant extracts

Plant materials were dried and homogenized into powder using a Grindomix mill (Retsch, Haan, Germany). Subsequently, 15 g of the dry matter was subjected to extraction in 450 ml of 80% ethanol (Penta, Prague, Czechia) for 24 hours at room temperature, employing a laboratory shaker (GFL3005, GFL, Burgwedel, Germany). Extracts were subsequently filtered and concentrated using a rotary vacuum evaporator (R-200, Buchi Labortechnik, Flawil, Switzerland) under vacuum conditions at 40 °C. Following the protocol recommended by Cos *et al.* [283], the dried residue was ultimately diluted in 100% DMSO (Penta, Prague, Czech Republic) to produce stock solutions with a final concentration of 51.2 mg/ml. These solutions were stored at −20°C until use. For all assayed species, the scientific names, families, local names, collection site/GPS coordinates, voucher specimen codes, collected parts (plant samples), and their uses in folk medicine are presented in Table 1.

Table 1. Ethnobotanical data on Indian medicinal plants.

Plant species	Family	Local name	GPS[a] or commercial provider (state)[b]	Plant part	Ethnomedicinal use	Yield (%)	
						Extract	EO
Alysicarpus monilifer (L.) DC.	Fabaceae	Samervo	21.2311° N, 81.6014° E (MahadevGhat)	Leaves	Diarrhoea, skin infections [203,204]	16.85	-[c]
Anethum graveolens L.	Apiaceae	Sowa	20.1857°N 81.9362°E (Sitanadi Wildlife Sanctuary)	Seeds	Bronchitis, skin infections [207]	9.18	-
Barleria prionitis L**.**	Acanthaceae	Koranti	21.2311° N, 81.6014° E (MahadevGhat)	Leaves	Skin infections [210]	12.84	-
Cocculus hirsutus (L.) Diels	Menispermaceae	Patalgarudi	20.9674° N, 81.8815° E (Rajim)	Aerial part	Oral infections [215]	20.11	-
Crotalaria juncea L.	Fabaceae	Madrashemp	20.3481° N, 81.9590° E (Nagari)	Fruits	Skin infections [223]	11.82	-
Cymbopogon citratus (DC.) Stapf	Poaceae	Gandhatrina	Bhagyashree Herbal Farms (Raipur)	Aerial part	Pneumonia [225,226]	-	0.7
Cyperus scariosus R.Br.	Cyperaceae	Nagarmotha	Bhagyashree Herbal Farms (Raipur)	Rhizome	Respiratory infections [235]	-	0.1

Jasminum angustifolium (L.) Willd.	Oleaceae	Vanamallika	20.3060° N, 81.8765° E (Birgudi)	Flowers	Skin infections [23]	11.16	-
Lygodium flexuosum (L.) Sw.	Schizaceae	Vallipanna	Sitanadi Wildlife Sanctuary 20.1857°N 81.9362°E	Leaves Roots	Skin infections [245]	25.86 13.79	- -
Mucuna pruriens (L.) DC.	Fabaceae	Kauncha	20.5646° N, 81.7059° E (Keregaon)	Seeds	Diarrhoea, tuberculosis [248]	31.39	-
Psoralea corylifolia L.	Leguminosae	Babchi	20.4476° N, 81.8257° E (Gattasill)	Leaves Flowers Stem	Diarrhoea, skin infections [24,256]	17.84 15.33 18.90	- - -
Terminalia crenulata Roth	Combretaceae	Asaina	20.4974° N, 81.8846° E (Dugli)	Bark	Diarrhoea, bronchitis [23]	28.08	-
Trachyspermum ammi (L.) Sprague	Apiaceae	Ajwain	Bhagyashree Herbal Farms (Raipur)	Seeds	Diarrhoea, respiratory infections [269]	-	2

[a] GPS- Global Positioning System coordinates of plant material collection; [b]State- Chhattisgarh; [c] - not determined

6.6 Essential oil isolation

Dried plant samples were grinded using a Grindomix apparatus (GM 100 Retsch, Haan, Germany). The residual moisture contents of the samples were determined gravimetrically at 130 °C for 1 h by a Scaltec SMO 01 analyzer (Scaltec Instruments, Gottingen, Germany) in triplicate according to the Official Methods of Analysis of the Association of Official Agricultural Chemists [284]. EOs were extracted by hydro distillation of 100 g of ground plant materials in 1 L of distilled water for 3 h using a Clevenger-type apparatus (Merci, Brno, Czech Republic) according to the procedure described in the European Pharmacopeia [285] and stored in sealed glass vials at +4 °C. Detail about plant species and EO yield is presented in the Table 1.

6.7 Broth microdilution assay

The antimicrobial efficacy of the plant extracts was assessed in 96-well microplates using the broth microdilution method according to the Clinical Laboratory Standard Institute [286] modified in accordance with previous recommendations for effectively evaluating the anti-infective potential of natural products [283]. The assay microplate preparation and serial dilution procedures were executed by the automated pipetting platform Freedom EVO 100 (Tecan, Mannedorf, CH). Serial dilutions of each extract were dispensed into the plate and diluted in MHB (final well volume 100 µL), resulting in concentrations ranging from 4 to 512 µg/mL. After optimizing bacterial cultures to an inoculum density of 1.5×10^8 CFU/ml for bacteria and 1.5×10^3 CFU/mL for yeast, achieved using a 0.5 McFarland standard with Densi-La-Meter II (Lachema, Brno, Czechia), the cultures were introduced into plate wells (5 µl/well). The plates were then incubated at 37°C for 24 hours (48 hours for *C. albicans*). Microorganism growth was assessed by measuring turbidity recorded at OD_{450nm} [283] using a Cytation 3 microplate reader (Biotech, Winooski, USA).

The MIC was defined as the lowest concentration that exhibited ≥80% inhibition of microbial growth compared to an extract-free growth control. Tetracycline and tioconazole antibiotics, dissolved in ethanol, were employed as positive controls. Notably, DMSO at a concentration of 1% did not inhibit any of the tested strains. Results, expressed as the median/mode of MICs, were obtained from three independent experiments conducted in triplicate. The widely accepted norm in MIC testing was followed, using the mode and median for final value calculation when triplicate endpoints fell within the two- and three-dilution range, respectively.

6.8 Broth macrodilution volatilization method

The *in vitro* antibacterial efficacy of the EOs was assessed using the broth macrodilution volatilization method, which enables the simultaneous evaluation of the growth inhibitory activities of EOs in both liquid and vapour phases. This method combines the principles of broth microdilution volatilization [187] and standard macrodilution techniques [287].

The experimentation utilized commercially available 2 mL microtubes with snap covers (Eppendorf, Hamburg, Germany) to prevent the loss of active agents through evaporation. In the initial step, each EO sample was dissolved in DMSO at a maximum concentration of 1% and then diluted in the appropriate broth medium. To generate sufficient stock solutions, six two-fold serially diluted concentrations of samples were prepared in 15 mL test tubes, sealed with plugs to prevent the evaporation of active compounds (Gama Group, Ceske Budejovice, Czech Republic). The starting concentration of tested EOs was 1024 µg/mL. Subsequently, 90 µL of melted agar was pipetted into the caps of the microtubes. After solidification, 5 µL of bacterial suspension was added. The proper concentrations of each sample, prepared in test tubes, were pipetted into microtubes, resulting in a final volume of 1500 µL. The microtubes were sealed after injecting 10 µL of bacterial solution. Growth and purity controls were established with microtubes containing inoculated and non-inoculated medium, respectively. The experiment was incubated at 37 °C for 24 hours.

MICs were determined by visually assessing bacterial growth after staining metabolically active colonies with MTT dye. Volumes of 30 and 375 µL of MTT at a concentration of 600 µg/mL were pipetted into the caps and microtubes, respectively, when a colour change from yellow to purple (relative to control wells) was observed in broth and agar (Figure 1 – Schematic representation of broth macrodilution volatilization assay). A black and white scheme of a cross-sectional view of a microtube filled with broth and agar shows the effective flow of sample vapours in the closed testing system (Figure 2). MIC values were defined as the lowest concentrations inhibiting bacterial growth compared to the compound-free control, expressed in µg/mL. DMSO, serving as the negative control, did not inhibit any strains at the concentrations used in the assay (≤1%). Positive antibiotic controls (ampicillin, oxacillin, amoxicillin, and tetracycline) were employed to assess the susceptibilities of *H. influenzae*, *S. aureus*, *S. pneumoniae*, and *S. pyogenes* [286]. All tests were conducted in triplicate as three independent experiments, and the results were presented as median/modal values. The mode and median were used for final value calculation when triplicate endpoints fell within the two- and three-dilution range, respectively.

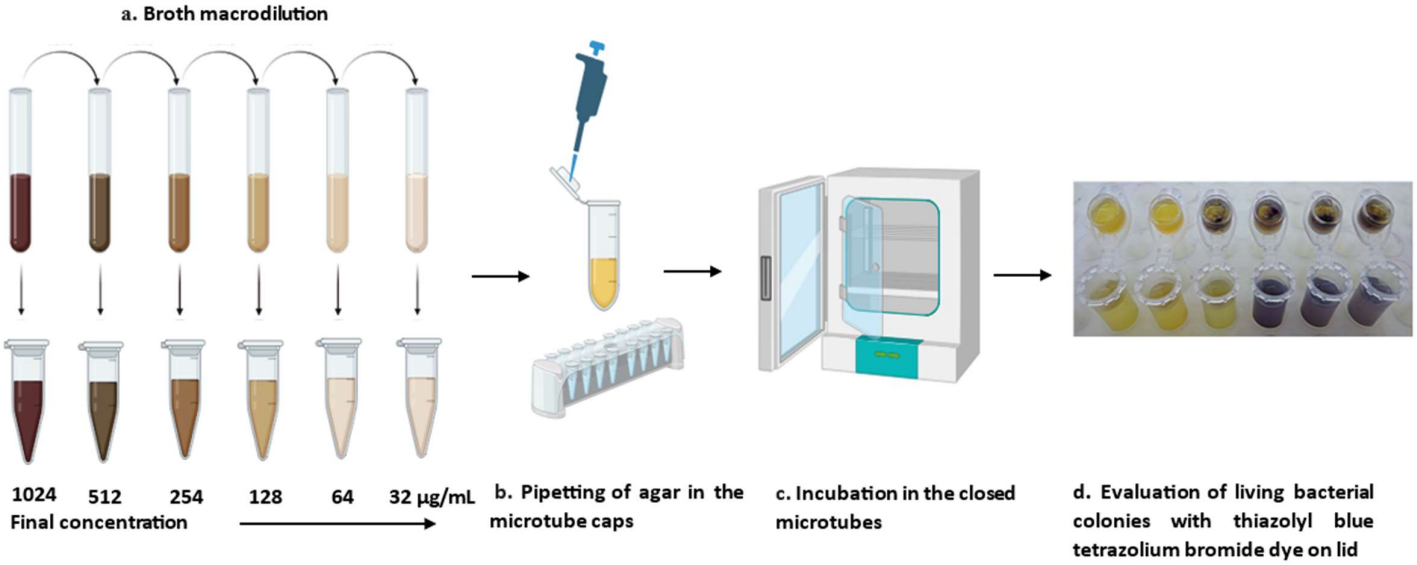


Figure 1. Broth macrodilution volatilization assay; (a) six two-fold serial dilution of the EOs samples were prepared in 15 mL test tubes, with concentrations ranging from 1024 to 32 µg/mL, followed by subsequent transfer to microtubes (b) pipetting of agar in the microtube caps: 90 µL of agar is pipetted into rim of every cap; (c) incubation: after inoculation, microtubes containing liquid medium with serially diluted samples of the EOs and their caps containing solid medium are properly closed together to prevent the losses of active compounds; (d) MIC determination: the results are evaluated visually after colouring of living bacterial colonies with MTT dye.

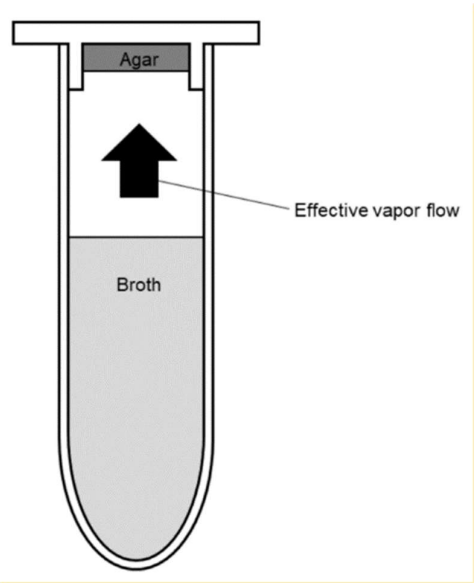


Figure 2. Detail of the cross-sectional view of the closed microtube with snap cap containing broth and agar media.

6.9 Cytotoxicity Assay

The modified MTT test, which is based on the metabolism of MTT to blue formazan by mitochondrial dehydrogenases in living lung cells, was used in the current investigation [288]. The evaluated EOs samples were applied to MRC-5 lung fibroblast cells grown in 96-well plates for 72 h. EOs were diluted in EMEM medium supplemented with 10% FBS after being dissolved in DMSO at a maximum concentration of 1%. The samples were then serially diluted eight times, yielding concentrations ranging from 256 to 2 µg/mL. The microtiter plates were incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2 in air with ethylene vinyl acetate EVA Capmats (USA Scientific Inc., Ocala, FL, USA). The use of EVA Capmat protects microtiter plates against vapour transition and provides more reliable results [289]. After that, MTT reagent (1 mg/mL) in EMEM solution was added to each well and the plates were incubated for an additional 2 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. Once the medium had been removed from the incubation, the intracellular formazan product had been dissolved in 100 µL of DMSO. The viability of the lung cells at the tested concentration (1%) was unaffected by the solvent utilized. The absorbance was measured at 555 nm using a Tecan Infinite M200 spectrometer (Tecan Group, Mannedorf, Switzerland), and the viability was computed in comparison to the untreated control. For each test, three independent experiments, each with two replicates, were conducted. Using the GraphPad Prism program (GraphPad Software, La Jolla, CA, USA), the results of the cytotoxicity effect were calculated and expressed as average half maximal inhibitory concentration (IC_{50}) with standard deviation in µg/mL. According to the Special Programme for

Research and Training in Tropical Diseases [290], the levels of cytotoxic effects were categorized as cytotoxic (IC_{50} < 2 µg/mL), moderately cytotoxic (IC_{50} 2–89 µg/mL), and nontoxic (IC_{50} > 90 µg/mL). Furthermore, for the purpose of comparing microbiological and toxicological data, IC_{80} (inhibitory concentration of proliferation) was determined to be equal to the MIC endpoint [291]. To compare the quantity of toxic antibacterial agents with the amount of effective antibacterial agents, therapeutic indices were defined as the ratio of IC_{80} and $\bar{x}$ -MIC values [292].

6.10 Chemical Analysis of EOs

The dual-column/dual-detector gas chromatograph Agilent GC-7890B system (Agilent Technologies, Santa Clara, CA, USA) equipped with autosampler Agilent 7693, two columns, a fused-silica HP-5MS (30 m × 0.25 mm, film thick-ness 0.25 µm, Agilent 19091s-433), a DB-Heavy WAX (30 m × 0.25 mm, film thickness 0.25 µm, Agilent 122–7132), and a flame ionization detector (FID) coupled with single quadrupole mass selective detector Agilent MSD-5977B were used to characterize the chemical composition of the targeted EOs. Helium was used as the carrier gas at a rate of 1 mL/min, and the injector temperature for both columns was 250 °C. After 3 min, the oven temperature for both columns increased from 50 to 280 °C. Initially, the heating velocity was 3 °C/min until the system reached a temperature of 120 °C. Subsequently, the velocity increased to 5 °C/min until a temperature of 250 °C, and after 5 min holding time, the heating speed reached 15 °C/min until obtaining a temperature of 280 °C. Heating was followed by an isothermic period of 20 min. The EO samples were diluted in *n*-hexane for GC/MS at a concentration of 20 µL/mL. One microliter of the solution was injected in split mode in a split ratio of 1:30. The mass detector was set to the following conditions: ionization energy 70 eV, ion source temperature 230 °C, scan time 1 s, and mass range 40–600 m/z. Identification of constituents was based on a comparison of their retention indices (RI) and retention times (RT) and spectra with the National Institute of Standards and Technology Library ver. 2.0.f (NIST, Gaithersburg, MD, USA), as well as with authentic standards. The RIs were calculated for compounds separated by the HP-5MS column using the retention times of *n*-alkanes series ranging from C_8 to C_{40}. Methyl octanoate is used as IS, for each EO analysed, the final number of compounds was calculated as the sum of components simultaneously identified using both columns and the remaining constituents identified by individual columns only. Quantitative data were expressed as relative percentage content of constituents determined by the FID.

6.11 Chemical Analysis of EOs' Vapours

To analyse the vapours of *C. citratus* and *T. ammi* EOs, two distinct sampling techniques (HS-SPME and HS-GTS), were employed. The analysis focused on the headspace above the solution of MHB and EOs at a concentration of 256 µg/mL for both *C. citratus* and *T. ammi*. This concentration was observed as the lowest MIC value for these EOs in the vapour phase. Except for the first sample (t = 0 h), all EO samples were placed into an oven set at a temperature of 37 °C for incubation until their analysis at 3,

6, 9, and 12 h (Figure 3). A 2 mL volume of the mixture was placed in a 4 mL glass vial for analysis. In the HS-SPME method, a fiber assembly coated with a 50/30 µm mixed layer of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, SUPELCO, Bellefonte, PA, USA) was utilized. The coated fiber was exposed to the headspace for 15 minutes to enable the adsorption of volatile chemicals above the EO and MHB mixture until equilibrium was reached (Figure 4a). The fiber remained in the injector for the analysis duration, and the injection method was set to spitless mode with an injector temperature of 250 °C. After each measurement, the needle was removed and placed into the GC injector port. For the HS-GTS sampling technique, a 2.5 mL SampleLock gas-tight syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) was employed, featuring a twist valve lock and a positive rear plunger stop to prevent sample loss. The needle was inserted through the vial septum until reaching the middle of the headspace (Figure 4b). After collecting the headspace, the syringe valve was closed, and the syringe was removed for insertion into the GC injector, maintaining a similar temperature of 250 °C but with the injection mode set to spitless. Measurements were repeated every 3 hours during the 12-hour incubation period for both sampling methods. The analysis was conducted on the HP-5MS column using operational parameters consistent with the previously described GC/MS analysis [293]. The chemical analysis of the EO sample was performed in triplicate, including the chromatographic analysis of its liquid phase and the headspace analysis using both extraction techniques (HS-SPME and HS-GTS). Relative peak area percentages were expressed as mean average of these three measurements ± standard deviation.

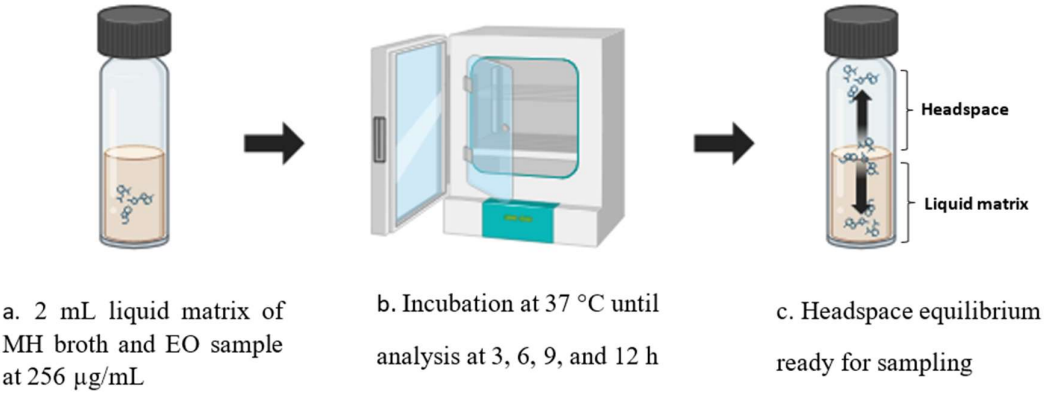
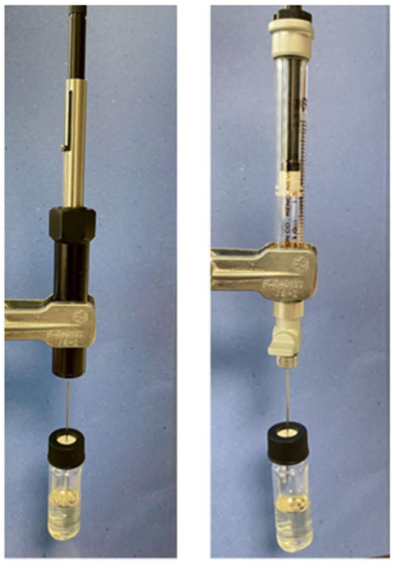


Figure 3. Schematic representation of the preparation process for headspace analysis.



a b

Figure 4. Headspace analysis using SPME and GTS above the EO and MHB mixture (a) HS-SPME sampling technique using a fiber assembly coated with a 50/30 µm mixed layer of divinylbenzene/carboxen/polydimethylsiloxane; (b) HS-GTS sampling technique carried out using a 2.5 mL SampleLock gas tight syringe.

6 RESULTS

7.1 Antimicrobial activity of plant extracts

Among the 10 plant extracts tested, the *P. corylifolia* leaf, flower, and stem extracts exhibited the strongest antimicrobial effects, inhibiting the growth of *S. aureus* with MICs of 64 and 128 µg/mL, respectively. The leaf extract produced also weak anticandidal effect (MIC = 512 µg/mL). With exception of the extracts of *M. pruriens* seed and *C. hirsutus* stem inhibiting growth of *S. aureus* at MIC of 512 µg/mL, all remaining plant samples did not show any antimicrobial effect at the concentrations tested. *S. aureus* was the most susceptible microorganism to the ethanolic extracts obtained from Indian medicinal plants. Table 2 shows the MIC values of ethanol extracts from Indian medicinal plants determined for panel of representatives of the potentially pathogenic gram-positive and gram-negative bacteria and yeast.

Table 2. Antimicrobial activity of ethanol extracts from Indian medicinal plants

Plant samples	Part used	Microorganisms[a]/MIC[b] (µg/mL)				
		Ca	***Ef***	***Ec***	***Pa***	***Sa***
Alysicarpus monilifer	Leaves	-[c]	-	-	-	-
Anethum graveolens	Seeds	-	-	-	-	-
Barleria prionitis	Leaves	-	-	-	-	-
Cocculus hirsutus	Aerial part	-	-	-	-	512
Crotalaria juncea	Fruits	-	-	-	-	-
Jasminum angustifolium	Flowers	-	-	-	-	-
Lygodium flexuosum	Leaves	-	-	-	-	-
	Roots	-	-	-	-	-
Mucuna pruriens	Seeds	-	-	-	-	512
Psoralea corylifolia	Leaves	512	-	-	-	64
	Flowers	-	-	-	-	64
	Stem	-	-	-	-	128
Terminalia crenulata	Bark	-	-	-	-	-
Antibiotics[c]		4	4	1	2	4

[a]Ca: *Candida albicans,* Ef*: Enterococcus faecalis,* Ec: *Escherichia coli,* Pa: *Pseudomonas aeruginosa,* Sa: *Staphylococcus aureus*; MIC[b]: minimum inhibitory concentration; -[c] not active (MIC >512 µg/mL); [c] tetracycline and tioconazole were used as positive controls for bacteria and yeast, respectively.

7.2 Antibacterial effect of EOs and their vapours against pneumonia causing pathogens

The current study revealed that all selected EOs exhibited a certain level of growth-inhibitory activity against the targeted bacteria- *H. influenzae*, *S. aureus*, *S. pneumoniae*, and *S. pyogenes*. The effectiveness of the EOs varied significantly, with MIC values ranging from 128 to 1024 µg/mL in the liquid phase and equal to or greater than 256 µg/mL in the vapour phase. Among the tested samples, *T. ammi* EO demonstrated the highest activity, with respective MICs of 128 and 256 µg/mL in the broth and agar phases against *H. influenzae*. Followed by *S. aureus*, with the MIC of 512 µg/mL, and for *S. pneumoniae* and *S. pyogenes*, the MICs of 512 and 1024 µg/mL were recorded, independently, in both the phases.

C. citratus EO also exhibited effective antibacterial effects, with the highest activity observed against *H. influenzae* (MIC 256 µg/mL in both phases), followed by *S. aureus*, *S. pneumoniae*, and *S. pyogenes*

with MICs of 512 and 1024 µg/mL, respectively, in the liquid and agar phases. On the other hand, *C. scariosus* EO demonstrated weak growth inhibitory effects, with an MIC of 1024 µg/mL in the liquid phase against all tested bacteria. In the vapour phase, this EO was active only against *H. influenzae* (MIC 1024 µg/mL).

Overall, a higher activity was observed in the liquid phase compared to the vapour phase, and *H. influenzae* was the most susceptible bacterium to the EOs assayed. Complete results on the antibacterial activity of Indian EOs against respiratory pathogens in both liquid and vapour phases are presented in the Table 3.

Table 3. *In vitro* growth-inhibitory effect of *C. citratus*, *C. scariosus*, and *T. ammi* essential oils in the liquid and vapour phases against respiratory pathogens.

Plant sample	**Bacterium[a]/Growth Medium[b]/MIC[c]**								
	HI		***Sa***		***Sp***		***Spy***		**x̄**
Essential oil	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**MIC[c]**
Cymbopogon citratus	256	256	512	1024	512	1024	512	1024	448
Cyperus scariosus	1024	1024	1024	>1024	1024	>1024	1024	>1024	1024
Trachyspermum ammi	128	256	512	512	512	1024	512	1024	416
Antibiotics	1[d]	n.d.	0.5[e]	n.d.	0.25[f]	n.d.	0.25[g]	n.d.	-

[a]HI: *Haemophilus influenzae,* Sa: *Staphylococcus aureus,* Sp: *Streptococcus pneumoniae,* Spy: *Streptococcus pyogenes*; [b]B: broth, A: agar; x̄-MIC[c]: mean value of minimal inhibitory concentrations in broth medium; positive antibiotic control: [d] ampicillin, [e] oxacillin, [f] amoxicillin, [g] tetracycline; n.d.: not detected

7.3 Cytotoxicity of essential oils

The cytotoxic effects of EOs extracted from *C. citratus*, *C. scariosus*, and *T. ammi* on lung fibroblast cells (MRC-5) were evaluated using a modified MTT assay in microtiter plates sealed with an EVA Capmat vapour barrier. The IC_{50} values, representing the concentration at which 50% inhibition occurred, exhibited significant variations among the tested EOs. *C. scariosus* EO demonstrated non-toxicity (IC_{50} > 258 µg/mL), while both *C. citratus* and *T. ammi* EOs were moderately toxic, with IC_{50} values of 19.63 and 82.04 µg/mL, respectively. Similarly, *C. scariosus* exhibited the highest IC_{80} values (>258 µg/mL). Adhering to the WHO guidelines on Tropical Diseases [290], the EOs were categorized

as non-toxic (*C. scariosus*) and moderately toxic (*C. citratus* and *T. ammi*). The therapeutic index (TI), which was calculated to assess safety by comparing antibacterial and cytotoxic effects, indicated toxicity for all the EO samples tested. The observed TI values of 0.065, >0.252, and 0.376 for *C. citratus*, *C. scariosus*, and *T. ammi* EOs, respectively, suggest a very narrow safety margin, where the toxic dose is close to the therapeutic dose [294]. The detailed results of the cytotoxicity of EOs to lung fibroblasts are shown in Table 2 with a graphical presentation in Figure 5.

Table 4. Cytotoxicity of Indian essential oils to the normal lung fibroblast cell line MRC-5.

Sample	**IC_{50} ± SD (µg/mL)**	**IC_{80} ± SD (µg/mL)**	**TI**
Essential oil			
Cymbopogon citratus	19.63 ± 1.02	29.54 ± 2.18	0.065
Cyperus scariosus	> 258	> 258	>0.252
Trachyspermum ammi	82.04 ± 3.39	156.57 ± 13.88	0.376
Positive control			
vinorelbine	0.54 ± 0.26	>10	n.a.

IC_{50}: half maximal inhibitory concentration of proliferation in µg/mL; IC_{80}: 80% inhibitory concentration of proliferation in µg/mL; SD: standard deviation; TI: therapeutic index (TI = $IC_{80}/\bar{x}$-MIC); n.a: not applicable.

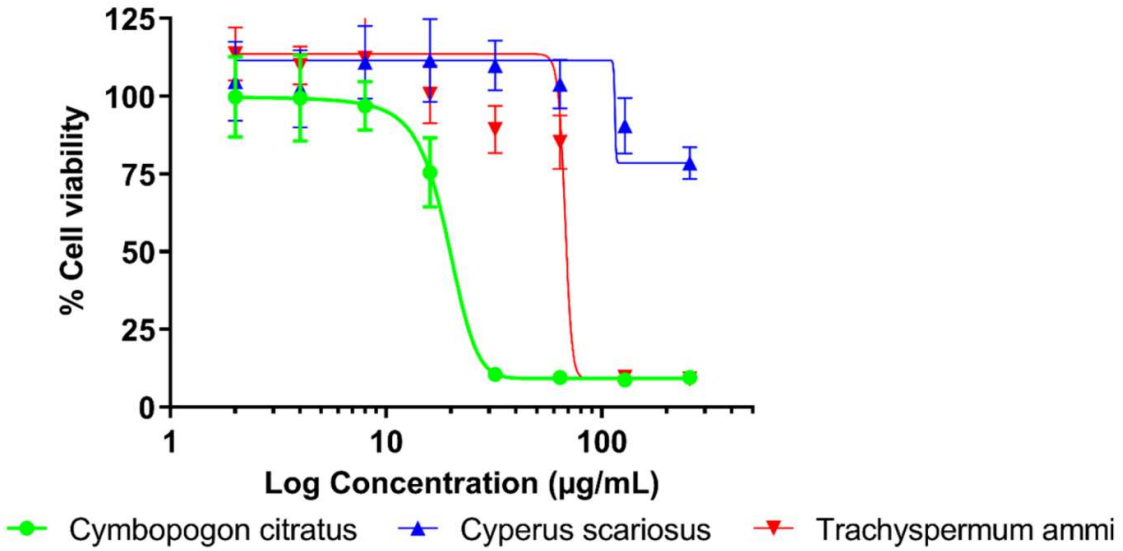


Figure 5. Cytotoxic activity of eight twofold serially diluted concentrations (2–256 µg/mL) of *Cymbopogon citratus*, *Cyperus scariosus* and *Trachyspermum ammi* EOs to lung fibroblast cells MRC-5 tested by MTT assay performed in microtiter plates sealed with vapor barrier EVA Capmat.

7.4 Chemical composition of Indian essential oils

Through GC/MS analysis of EOs obtained from the aerial parts of *C. citratus*, the rhizomes of *C. scariosus*, and the seeds of *T. ammi*, the HP-5MS column identified a total of 17, 28, and 9 components, making up 99.62%, 91.48%, and 99.47% of their respective overall content. The use of the DB-HeavyWAX column revealed 15, 36, and 13 components, comprising 68.77%, 77.17%, and 84.26% of the EOs, respectively. In the analysis of *C. citratus* EO, the primary chemical profile consists mainly of monoterpenene hydrocarbons and oxygenated monoterpenoids, followed by sesquiterpene hydrocarbons. In both columns, α – and β-citral emerged as the most abundant compounds, with peak areas of 48.9% and 35.8% in HP-5MS, and 24.3% and 33.2% in DB-HeavyWAX, respectively. The third most prevalent compound, caryophyllene oxide, exhibited peak areas of 3% and 2.88% in HP-5MS and DB-HeavyWAX columns. For *C. scariosus* EO, cyperotundone was the primary component, with peak areas of 29.1% and 28.9%, followed by caryophyllene oxide at 19.8% and 17.54%, and cyperene at 9.9% and 8.5%, when measured using HP-5MS and DB-HeavyWAX columns, respectively. Moreover, for *T. ammi* EO, thymol was identified as the most predominant substance for both columns, accounting for 51.2% and 45.8%, followed by p-cymene (22.6% and 17.1%) and γ-terpinene (21.5% and 17.6%) with HP-5MS/DB-HeavyWAX columns, respectively. Comprehensive chemical profiles and chromatograms for *C. citratus*, *C. scariosus*, and *T. ammi* EOs can be found in table 5, 6, 7 and figure 8,9 10, respectively.

Table 5. Chemical composition of *Cymbopogon citratus* essential oil.

RI[a]		Compound	Cl.[b]	Column			
				Content[c] (%)		Identification[f]	
Obs.	Lit.			HP-5MS	DB-WAX	HP-5MS	DB-WAX
915	926	Tricyclene	MH	0.26 ± 0.12	tr.[d]	RI, MS, Std	MS
927	939	α-Pinene	MH	0.20 ± 0.04	-[e]	RI, MS, Std	-
942	953	Camphene	MH	2.42 ± 0.79	1.62 ± 0.34	RI, MS, Std	MS
979	985	Sulcatone	MO	0.35 ± 0.18	-	RI, MS	-
1023	1029	Limonene	MH	0.23 ± 0.03	-	RI, MS	-
1029	1032 [295]	*trans*-β-Ocimene	MH	0.31 ± 0.09	-	RI, MS	-
1039	1011	3-Carene	MH	0.13 ± 0.02	-	RI, MS, Std	-
1063	1030	4-Nonanone	MO	1.41 ± 0.38	1.55 ± 0.97	RI, MS	MS
1098	1098	Linalool	MO	0.46 ± 0.24	0.31 ± 0.07	RI, MS, Std	MS
1178	1184.7	Isogeranial	MO	0.70 ± 0.01	0.55 ± 0.05	RI, MS	MS
1190	1189	α-Terpineol	MO	0.39 ± 0.03	-	RI, MS	-
1238	1240	β-Citral	MO	**35.8 ± 0.61**	**24.3 ± 8.82**	RI, MS, Std	MS
1268	1270	α-Citral	MO	**48.9 ± 0.55**	**33.2 ± 11.2**	RI, MS, Std	MS
1376	1381	Geranyl acetate	MO	2.02 ± 0.20	tr.	RI, MS	MS
1412	1418	Caryophyllene	SH	0.45 ± 0.31	0.75 ± 0.05	RI, MS	MS
1510	1513	γ-Cadinene	SH	1.19 ± 0.33	1.20 ± 0.23	RI, MS	MS
[g]	1797	Geraniol	MO	-	0.77 ± 0.07	-	MS
1582	1581	Caryophyllene oxide	SO	**3.0 ± 1.34**	**2.88 ± 1.40**	RI, MS, Std	MS
[g]	1430	α-Cyclocitral	MO	-	0.45 ± 0.04	-	MS
[g]	NA	Isoneral	MO	-	0.36 ± 0.02	-	MS
[g]	1669	Isoborneol	MO	-	0.83 ± 0.01	-	MS
Total content (%)				**99.62**	**68.77**		

[a]RI: retention indices; Obs. = retention indices determined relative to a homologous series of *n*-alkanes (C_8–C_{40}) on an HP-5MS column; Lit. = literature RI values [296, 297], NA = RI values not available in the literature; [b]Cl = class; MH - Monoterpene hydrocarbons, MO - Oxygenated monoterpenoids, SH - Sesquiterpene hydrocarbons, SO – Oxygenated sesquiterpenoids; [c]Relative peak area percentage as mean of three measurements ± standard deviation; [d]tr. = traces, relative peak area < 0.05%; [e]- = not detected; [f]Identification method: MS = Mass spectrum was identical to that of National Institute of Standards and Technology Library (ver. 2.0.f), RI = the retention index was matching literature database; Std = constituent identity confirmed by co-injection of authentic standards; [g] Retention indices were not calculated for compounds determined by DB-WAX column.

Table 6. Chemical composition of *Cyperus scariosus* essential oil.

RI[a]		Compound	Cl.[b]	Column Content[c] (%)		Identification[f]	
Obs.	Lit.			HP-5MS	DB-WAX	HP-5MS	DB-WAX
927	939	α-Pinene	MH	1.34 ± 0.10	0.50 ± 0.01	RI, MS, Std	MS
970	980	β-Pinene	MH	1.88 ± 0.33	0.06 ± 0.00	RI, MS, Std	MS
1025	1032	Eucalyptol	MO	0.24 ± 0.16	-[d]	RI, MS	-
1137	1137	Pinocarveol	MO	1.85 ± 0.23	0.60 ± 0.01	RI, MS	MS
1158	1165	Pinocarvone	MO	0.37 ± 0.32	0.09 ± 0.00	RI, MS	MS
1168	1176 [298]	Myrtenal	MO	0.41 ± 0.27	0.13 ± 0.30	RI, MS	MS
1314	1327	Cyprotene	SH	0.16 ± 0.05	tr.[e]	RI, MS	MS
1344	1349	α-Terpinyl acetate	MO	1.65 ± 0.18	tr.	RI, MS	MS
1371	1376	Copaene	SH	1.46 ± 0.47	tr.	RI, MS	MS
1394	**1398**	**Cyperene**	SH	**9.87 ± 0.59**	**8.5 ± 0.04**	**RI, MS**	MS
1446	1450 [299]	α-Muurolene	SH	0.16 ± 0.04	tr.	RI, MS	MS
1456	1461	Rotundene	SH	1.94 ± 0.07	1.25 ± 0.01	RI, MS	MS
1483	1473.7	γ-Patchoulene	SH	0.19 ± 0.05	tr.	RI, MS	MS
1489	1491	Valencene	SH	0.63 ± 0.08	0.57 ± 0.60	RI, MS	MS
1518	1518	β-Cadinene	SH	0.31 ± 0.18	0.08 ± 0.40	RI, MS	MS
1528	1532	Cyperene epoxide	SO	2.65 ± 0.26	1.50 ± 0.00	RI, MS	MS
1541	1542	α-Calacorene	SH	0.13 ± 0.05	-	RI, MS	-
1565	1579	Isoaromadendrene epoxide	SO	0.62 ± 0.07	1.03 ± 0.00	RI, MS	MS
1572	1627	Longiverbenone	SO	1.33 ± 0.15	1.20 ± 0.08	RI, MS	MS
1582	1581	**Caryophyllene oxide**	SO	**19.7 ± 0.58**	**17.5 ± 0.12**	RI, MS, Std	MS
1591	NA	β-Santalol	SO	0.38 ± 0.12	-	RI, MS	-
1609	1608	Humulene epoxide 2	SO	1.69 ± 0.25	2.60 ± 0.10	RI, MS	MS
1656	1604	Globulol	SO	0.23 ± 0.04	0.39 ± 0.03	RI, MS	MS
1664	1663	Patchouli alcohol	SO	0.50 ± 0.05	-	RI, MS	-
1677	1676	Mustakone	SO	6.26 ± 0.26	3.67 ± 0.30	RI, MS	MS
1697	1694	**Cyperotundone**	SO	**29.1 ± 1.11**	**28.9 ± 0.72**	RI, MS	MS
1750	1752	Aristolone	SO	3.17 ± 0.77	3.73 ± 0.10	RI, MS	MS
1808	1807	Nootkatone	SO	2.17 ± 0.49	2.03 ± 0.40	RI, MS	MS
g	NA	β-Pinone	MO	-	tr.	-	MS
g	1586	β-Elemene	SH	-	tr.	-	MS
g	1652	*cis*-Verbenol	MO	-	tr.	-	MS
g	NA	Aristolochene	SH	-	tr.	-	MS
g	1680	α-Terpineol	MO	-	tr.	-	MS
g	NA	*α*-Maaliene	SH	-	tr.	-	MS

g	1784	Myrtenol	MO	-	tr.	-	MS
g	2063	Cubenol	SO	-	0.49 ± 0.00	-	MS
g	1978	α-Cedrene epoxide	SO	-	0.40 ± 0.10	-	MS
g	NA	Aromadendrene oxide-(1)	SO	-	1.35 ± 0.40	-	MS
g	NA	Calarene epoxide	SO	-	0.52 ± 0.02	-	MS
g	NA	Diepicedrene-1-oxide	SO	-	0.05 ± 0.00	-	MS
Total content (%)				**91.48**	**77.17**		

[a]RI = retention indices; Obs. = retention indices determined relative to a homologous series of *n*-alkanes (C_8–C_{40}) on an HP-5MS column; Lit. = literature RI values [296,297], NA = RI values not available in the literature; [b]Cl = class; MH - Monoterpene hydrocarbons, MO - Oxygenated monoterpenoids, SH - Sesquiterpene hydrocarbons, SO – Oxygenated sesquiterpenoids; [c]Relative peak area percentage as mean of three measurements ± standard deviation; [d]- = not detected; [e]tr. = traces, relative peak area < 0.05%; [f]Identification method: MS = Mass spectrum was identical to that of National Institute of Standards and Technology Library (ver. 2.0.f), RI = the retention index was matching literature database; Std = constituent identity confirmed by co-injection of authentic standards; [g] Retention indices were not calculated for compounds determined by DB-WAX column.

Table 7. Chemical composition of *Trachyspermum ammi* essential oil.

RI[a]		Compound	Cl.[b]	Column			
				Content[c] (%)		Identification[e]	
Obs.	Lit.			HP-5MS	DB-WAX	HP-5MS	DB-WAX
920	917	β-Thujene	MH	0.30 ± 0.03	0.17 ± 0.02	RI, MS	MS
964	939	*α*-Pinene	MH	0.19 ± 0.01	0.42 ± 0.13	RI, MS, Std	MS
981	971	β-Pinene	MH	1.87 ± 0.25	2.13 ± 0.62	RI, MS, Std	MS
994	984	β-Myrcene	MH	0.21 ± 0.06	0.38 ± 0.03	RI, MS	MS
1007	974	2-Carene	MH	0.20 ± 0.02	-[d]	RI, MS	MS
1050	**1026**	**p-Cymene**	**MH**	**22.6 ± 0.89**	**17.1 ± 3.99**	**RI, MS, Std**	**MS**
1079	**1065**	**γ-Terpinene**	**MH**	**21.5 ± 0.86**	**17.6 ± 0.99**	**RI, MS, Std**	**MS**
1175	1086	Isoterpinolene	MH	0.08 ± 0.06	-	RI, MS	MS
1315	**1290**	**Thymol**	**MO**	**51.2 ± 1.25**	**45.8 ± 4.41**	**RI, MS, Std**	**MS**
[f]	1172	α-Terpinene	MH	-	0.12 ± 0.01	-	MS
[f]	1244	Limonene	MH	-	0.10 ± 0.01	-	MS
[f]	1195	β-Phellandrene	MH	-	0.08 ± 0.03	-	MS
[f]	NA	*trans*-2-Caren-4-ol	MO	-	0.18 ± 0.02	-	MS
[f]	1680	Terpineol	MO	-	0.06 ± 0.01	-	MS
[f]	1635	Terpinen-4-ol	MO	-	0.20 ± 0.04	-	MS
Total content (%)				**99.47**	**84.26**		

[a]RI = retention indices; Obs. = retention indices determined relative to a homologous series of *n*-alkanes (C_8–C_{40}) on an HP-5MS column; Lit. = literature RI values [296,297], NA = RI values not available in the literature; [b]Cl = class; MH - Monoterpene hydrocarbons, MO - Oxygenated monoterpenoids, SH - Sesquiterpene hydrocarbons; [c]Relative peak area percentage as mean of three measurements ± standard deviation; [d]- = not detected; [e]Identification method: MS = Mass spectrum was identical to that of National Institute of Standards and Technology Library (ver. 2.0.f), RI = the retention index was matching literature database; Std = constituent identity confirmed by co-injection of authentic standards; [f]Retention indices were not calculated for compounds determined by DB-WAX

7.5 Chemical Composition of EO Vapours

In the current study, the composition of headspace above the mixture of *T. ammi* and *C. citratus* EOs dissolved in MHB has been carried out using HS-SPME and HS-GTS. Headspace chemical compositions were measured every 3 h during a 12-h experiment using the HP-5MS column. When employing HS-SPME extraction for *C. citratus* EO vapour analysis, a total of 18 volatile compounds were detected, constituting 84.89% of the overall constituents. However, with the HS-GTS extraction method, only 12 compounds were identified, representing 75.5% of the total content. Regardless of the extraction technique, monoterpene and monoterpenoid stood out as the dominant chemical groups, followed by sesquiterpene and sesquiterpenoids in the headspace. Notably, α-citral emerged as the primary constituent using HS-SPME extraction, exhibiting a gradual decline in concentration from 27% (0 h) to 17.75% (12 h). Similarly, β-citral decreased slightly over time, from 20.35% to 11.48%, while camphene concentration increased from 9.63% to 16.4%. In contrast, HS-GTS extraction revealed camphene, limonene, and α-citral as predominant compounds, with camphene and limonene concentrations steadily increasing (32.27% to 38.36% and 8.24% to 9.5%, respectively) and α-citral decreasing consistently from 7.65% to 3.98%. Notably, significant differences were observed in the major compounds identified by each extraction method, with camphene being the prominent component in HS-GTS extraction, accompanied by a sharp decrease in α-citral concentration. Furthermore, a smaller number of compounds were observed using HS-GTS method. Apart from this, no discrepancies were noted between sampling methods, and there were no significant alterations in the chemical composition of *C. citratus* EO vapour over time. Table 8 provides a comprehensive chemical profile of *C. citratus* EO vapours.

Considering, *T. ammi* EO vapour, using HS-SPME extraction, a total of six volatile compounds were identified in the EO sample, which represented 82.89% of their respective total constituents. When using the HS-GTS extraction method, a slightly lower number (five) of compounds was detected, which accounted for 75.5% of their total content. Regardless of the extraction method used, monoterpenes were the most predominant chemical groups of volatile compounds identified in the headspace. Using the HS-SPME extraction method, p-cymene was the most abundant constituent of the headspace of *T. ammi* EO. The content of p-cymene gradually decreased during the whole experiment with the peak area value ranging from 49% (time—0 h) to 43% (time—12 h). Similarly, a slight decrease in the concentration of γ-terpinene, the second-most abounding compound in the sample, was observed during overtime incubation from 39 to 31%. In contrast, the concentration of the thymol increased in the vapour over the time from 4.9 to 12%. In the case of HS-GTS extraction, p-cymene, γ-terpinene, and β-pinene were detected as the predominant compounds. For p-cymene and γ-terpinene, a steady decrease in the concentrations was observed during the experiment, ranging from 52 to 45% and 35 to 28%, respectively. Although the concentrations of both most-abundant compounds are nearly similar to those

obtained using the HS-SPME method, the third-most abounding compound differed for the HS-GTS extraction method, β-pinene being detected with peak area values ranging from 2 to 6.5%. Interestingly, the chemical analysis showed that content of thymol detected by HS-SPME extraction was much higher in comparison with HS-GTS extraction (nearly 10%). Apart from this, no discrepancies for either sampling method were observed, and there were no significant changes in the chemical composition in the vapour of *T. ammi* EO over time. A complete chemical profile of *T. ammi* EO vapours is provided in Table 10. To discuss and compare both techniques, the chemical profiles of *T. ammi* and *C. citratus* EO vapours, originally presented in Tables 8 to 9, were converted into original heatmaps. These visual representations are depicted in Figures 4 and 5, enhancing the interpretation of the overall results.

Table 8. Chemical composition of a headspace above a *Cymbopogon citratus* essential oil dissolved in Mueller–Hinton broth at a concentration of 256 µg/mL.

RI[a]		Compound	Cl.[b]	Extraction method/Time (h)/Content[c] (%)										Ident.[e]
				Solid phase microextraction					Gas tight syringe extraction					
Obs.	Lit.			0	3	6	9	12	0	3	6	9	12	
911	926	Tricyclene	MH	1.52	1.45	1.44	1.38	1.3	2.44	2.88	3.69	3.71	4.32	RI, MS
924	939	α-Pinene	MH	0.73	1.49	1.74	1.76	1.81	5.96	5.29	4.84	4.79	4.65	RI, MS
943	953	Camphene	MH	**9.63**	**10.19**	**10.74**	**11.07**	**16.4**	**32.27**	**33.29**	**33.98**	**36.13**	**38.36**	RI, MS
984	985	Sulcatone	MO	1.49	2.05	2.23	3.15	3.18	0.39	0.47	0.75	1.18	1.43	RI, MS
995	995	Octanal	MO	0.47	0.34	0.32	0.31	0.25	-[d]	-	-	-	-	RI, MS
1024	1029	Limonene	MH	4.37	4.43	4.54	4.73	6.56	**8.24**	**8.78**	**8.83**	**8.86**	**9.5**	RI, MS
1031	1032 [292]	trans-β-Ocimene	MH	1.18	1.18	1.14	1.11	1.02	2.79	2.01	1.77	1.61	1.58	RI, MS
1053	1069	4-Nonanone	MO	5.54	5.59	5.62	5.67	5.71	3.26	3.58	3.61	3.78	4.48	RI, MS
1085	1095	Rosefuran	F	0.44	0.52	0.59	0.96	1.02	0.59	1.05	1.66	2.12	2.43	RI, MS
1097	1098	Linalool	MO	0.78	0.85	0.89	0.92	0.92	-	-	-	-	-	RI, MS
1159	1165	Isoneral	MO	2.02	1.6	1.53	1.14	1.05	-	-	-	-	-	RI, MS
1197	1188	Decanal	MO	0.86	0.9	0.96	1.03	1.13	-	-	-	-	-	RI, MS
1245	1240	β-Citral	MO	**20.35**	**20.6**	**17.88**	**17.58**	**11.48**	7.33	5.35	5.21	4.03	3.06	RI, MS
1280	1270	α-Citral	MO	**27**	**24.99**	**21.83**	**20.74**	**17.75**	**7.65**	**6.12**	**5.68**	**4.37**	**3.98**	RI, MS
1368	1381	Geranyl acetate	MO	0.24	0.26	0.31	0.32	0.34	-	-	-	-	-	RI, MS
1414	1418	Caryophyllene	SH	4.44	4.24	3.21	2.98	2.55	0.42	0.45	0.64	0.67	1.29	RI, MS
1446	1455	Humulene	SH	0.31	0.3	0.25	0.22	0.19	-	-	-	-	-	RI, MS
1580	1581	Caryophyllene oxide	SO	3.52	2.49	2.18	2.53	1.9	2.81	1.58	1.54	0.47	0.42	RI, MS
Total content (%)				**84.89**	**83.47**	**77.4**	**77.6**	**74.56**	**74.15**	**70.85**	**72.2**	**71.72**	**75.5**	

[a]RI = retention indices; Obs. = retention indices determined relative to a homologous series of *n*-alkanes (C_8–C_{40}) on an HP-5MS column; Lit. = literature RI values [296,297]; [b]Cl = class; F- Furan, MH - Monoterpene hydrocarbons, MO - Oxygenated monoterpenoids, SH - Sesquiterpene hydrocarbons; SO – Oxygenated sesquiterpenoids; [c]Relative peak area percentage; [d]- = not detected; [e]Identification method: MS = Mass spectrum was identical to that of National Institute of Standards and Technology Library (ver. 2.0.f), RI = the retention index was matching literature database.

Table 9. Chemical composition of a headspace above a *Trachyspermum ammi* essential oil dissolved in Mueller–Hinton broth at a concentration of 256 µg/mL.

RI[a]		Compound	Extraction method/Time (h)/Content[b] (%)										Ident.[e]
			Solid phase microextraction					Gas tight syringe extraction					
Obs.	Lit.		0	3	6	9	12	0	3	6	9	12	
925	939	α-Pinene	1.27 ± 0.10	1.46 ± 0.00	1.42 ± 0.01	1.41 ± 0.00	1.25 ± 0.01	tr.[c]	tr.	tr.	tr.	tr.	RI, MS
961	1011	2-Carene	0.64 ± 0.00	0.44 ± 0.00	0.62 ± 0.01	0.65 ± 0.02	0.72. ±0.01	-[d]	-	-	-	-	RI, MS
971	980	β-Pinene	1.91 ± 0.01	2.80 ± 0.05	2.36 ± 0.00	2.31 ± 0.01	1.79 ± 0.20	**6.57 ± 0.20**	**4.93 ± 0.40**	**3.89 ± 0.10**	**4.11 ± 0.01**	**2.02 ± 0.30**	RI, MS
1031	1026	p-Cymene	**49.14 ± 1.00**	**48.00 ± 1.10**	**46.57 ± 0.80**	**45.97 ± 1.80**	**43.17 ± 0.90**	**52.00 ± 3.50**	**49.18 ± 2.90**	**48.67 ± 2.50**	**46.32 ± 1.40**	**45.60 ± 0.60**	RI, MS
1065	1062	γ-Terpinene	**39.36 ± 0.70**	**35.41 ± 0.50**	**35.11 ± 0.90**	**33.26 ± 0.60**	**31.01 ± 0.20**	**35.00 ± 2.50**	**32.23 ± 0.09**	**31.60 ± 0.50**	**32.22 ± 1.30**	**28.20 ± 2.90**	RI, MS
1306	1290	Thymol	**4.96 ± 0.01**	**5.66 ± 0.40**	**9.82 ± 0.08**	**11.90 ± 0.93**	**12.10 ± 0.80**	tr.	1.23 ± 0.10	2.21 ± 0.20	2.07 ± 0.03	tr.	RI, MS
Total content (%)			**97.25**	**94.21**	**95.9**	**95.5**	**90.66**	**93.40**	**88.57**	**87.98**	**85.72**	**79.05**	

[a]RI = retention indices; Obs. = retention indices determined relative to a homologous series of *n*-alkanes (C_8–C_{40}) on an HP-5MS column; Lit. = literature RI values [296,297]; [b]Relative peak area percentage as mean of three measurements ± standard deviation; [c] tr. = traces, relative peak area < 0.05%; [d]- = not detected; [e] Identification method: MS = Mass spectrum was identical to that of National Institute of Standards and Technology Library (ver. 2.0.f), RI = the retention index was matching literature database.

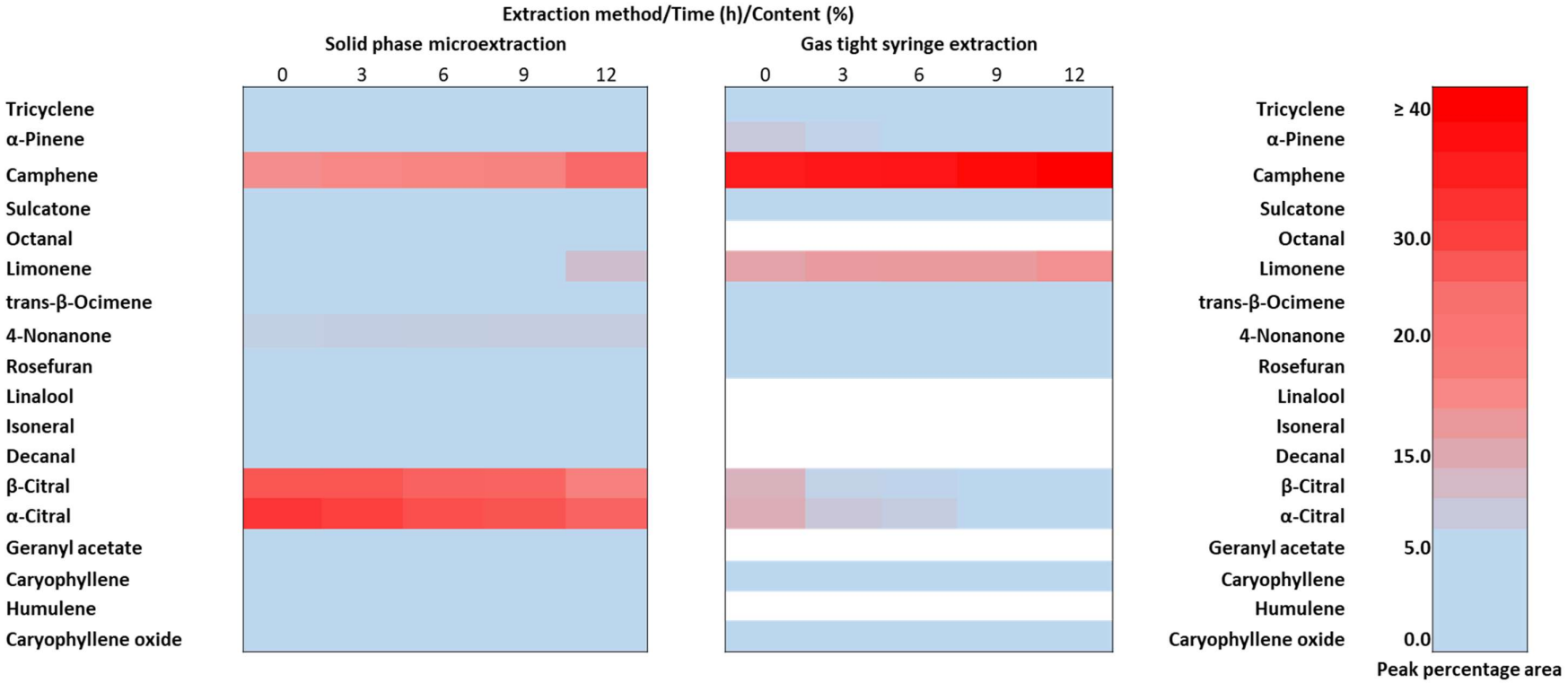


Figure 6. Heatmap comparing the headspace chemical compositions of *Cymbopogon citratus* essential oil using both Solid Phase MicroExtraction and Gas Tight Syringe sampling technique.

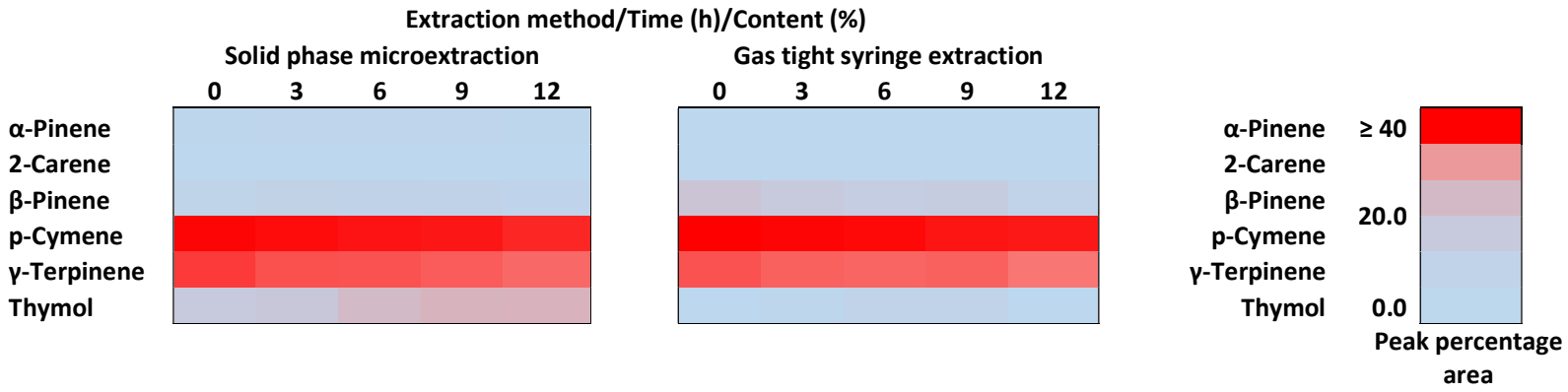


Figure 7. Heatmap comparing the headspace chemical compositions of *Trachyspermum ammi* essential oil using both Solid Phase MicroExtraction and Gas Tight Syringe sampling technique.

7 DISCUSSION

Among all the tested plant species, only *C. hirsutus*, *M. pruriens*, and *P. corylifolia* exhibited significant results. The highest growth inhibitory potency was observed for the case of *P. corylifolia* (leaf, flower, and stem) extracts against *C. albicans* and *S. aureus*. Previous studies have also documented similar results; for instance, Baig [255] investigated the antibacterial properties of seed extracts against *S. aureus*, with a MIC of 100 µg/mL. Similarly, Li *et al.* [300] reported the growth inhibitory potential of ethanolic seed extract with a comparable MIC of 100 µg/mL. Moreover, the antifungal activity of *P. corylifolia* against *C. albicans* has been reported in several studies [301,302,303]. The observed endpoints from the current research align well with a previously published study that reported the MIC of *P. corylifolia* extract against *C. albicans* at 150 µg/mL [304], falling within a three-dilution range. A variety of chemical constituents in *P. corylifolia* have been identified as major contributors to its antimicrobial activity, mostly phenolic compounds, (bakuchalcone, bakuchicin, bavachalcone, bavachin, bavachinin, bavachromanol, corylifolinin, corylin, isobavachin, isobavachalcone, psoralen) [257,258,259,260,261,262,263,264]. Additionally, bakuchiol, phenolic monoterpene extracted from the seeds or aerial parts of the plant, is known for its potent antimicrobial, anti-inflammatory, anti-osteoporotic, and antitumorigenic activities. A study led by Katsura *et al*. have reported its bactericidal effects against oral infection causing pathogens like *E. faecalis, Porphyromonas gingivalis, S. mutans* with MICs ranging from 1 to 4 µg/mL [264]. Another study reported its antifungal activity against *Candida* spp with MIC ranges from 12.5 to 100 µg/mL [304]. Moreover, it has also gained significant popularity in the cosmetic industry as a well-tolerated analogue of retinol in skin therapeutics [265].

Considering *C. hirsutus* ethanolic extract several research have been found to possess significant antimicrobial activity [305,306]. A study by Gupta *et al*. [217] observed the growth-inhibitory properties of ethanolic leaf extract against *M. tuberculosis* at the MIC of 500 µg/mL. Another study by Satish *et al*. [218] reported antibacterial activity of *C. hirsutus* petroleum ether leaf extract against *S. aureus* using disk diffusion assay. The direct comparison with reported literature is limited due to differing pathogen, extraction solvent and methodologies used for testing the antibacterial activity. Furthermore, there is a notable lack of studies utilizing standardized methods to assess the antimicrobial potential of *C. hirsutus* plant extracts. The chemical constituents of *C. hirsutus* are not well explored. Some earlier studies in 1960s and 1970s have reported the presence alkaloids and subclasses (e.g. coclaurine, isotrilobine, magnoflorine, trilobine), flavonoids (liquiritin and rutin), a triterpenoid (hirsudiol) [219,220,221].

M. pruriens has been employed in traditional medicine since ancient times and as a therapeutic agent in pharmaceutical industries due to its antioxidant, anti-inflammatory, and antimicrobial properties [307]. Previous research on the antimicrobial potential of *M. pruriens* extract has yielded promising results against both gram-positive and gram-negative bacteria, as well as some fungal species [308,

309]. A study led by Shanmugavel & Krishnamoorthy [308] reported the antibacterial potential of *M. pruriens* extract against *S. aureus* with an MIC value of 175 µg/mL, falling within a three-dilution range of the current study. Although further studies are required to isolate the bioactive components responsible for the observed anti-microbial activity [249].

Although no other plant extracts demonstrated antimicrobial effects in our experiments, some of them have exhibited antimicrobial activity in previous studies. For instance, Saleh-e-In *et al.* [209] reported moderate growth-inhibitory effect of the ethyl acetate root extract of *A. graveolens* against *E. faecalis* and *S. aureus* (MIC = 125 µg/mL for both), *E. coli* (MIC = 250 µg/mL), and *P. aeruginosa* (MIC = 125 µg/mL). Additionally, Mahasawat *et al.* [224] reported the antibacterial effect of the ethanolic flower extract of *C. juncea* against *S. aureus* (MIC = 128 µg/mL), *S. pyogenes* (MIC = 16 µg/mL) and *E. coli* (MIC = 500 µg/mL). Differences of the results observed in our and above-mentioned studies can be due to the different plant part tested and extraction solvent used. Furthermore, Aneja *et al*. [211] reported antimicrobial effect induced by methanolic bark extract of *B. prionitis* against oral pathogens including *Bacillus* spp., *Pseudomonas* spp*., S. aureus*, *S. mutans*, and two oral fungi, *C. albicans* and *S. cerevisiae*, however, at relatively higher concentrations (MICs > 1000 µg/mL). The disparity observed in the current study may be attributed to the differences in the extraction solvent, as well as the higher concentration of plant extract employed in the mentioned study.

Despite the recent progress in evaluation of biological properties of volatile agents, development of new methodologies suitable for determination of antimicrobial effects in the vapour phase remains a challenge [181]. The primary issue arises from their hydrophobic nature, which hampers their solubility in water-based media such as agar and broth, while their volatility increases the risk of losing active substances through evaporation during handling, preparation, and incubation. Moreover, the transition of vapours can impact microplate assay results, as noted in previous studies [310,311]. Unlike established methods for antimicrobial susceptibility testing on solid (agar disc diffusion) and liquid (broth dilution) media [312,313,314,315,316], there are no standardized methods for determining microbial sensitivity to volatile compounds in the vapour phase. While several methods for testing the antimicrobial effects of volatile plant-derived products in the vapour phase have been developed in recent years, many have specific limitations, such as not being designed for high-throughput screening or requiring specialized equipment not commonly available [181].

Recently, our team developed a broth macrodilution volatilization assay for evaluating the antimicrobial activity of volatile agents in both liquid and vapour phases [317]. This assay combines the principles of broth microdilution volatilization [187] and standard macrodilution methods [287]. It is conducted in commercially available microtubes, which can be tightly sealed with snap caps to prevent the loss of active agents by evaporation. Another advantage is that appropriate amounts of media suitable for cultivating a broad spectrum of microorganisms, including slow-growing fungi, can be applied in

microtubes and their caps. However, our previous study assessed only several representatives of volatile phytochemicals [317]. The current study demonstrates the validity of this new assay for testing the susceptibility of bacterial pathogens causing respiratory infections to EOs and their vapours. Below, the observed growth inhibitory activity of the respective EOs is discussed, along with their cytotoxic effects on lung cells.

T. ammi is a medicinal plant highly valued in traditional Ayurvedic medicine [21]. Its EO has been reported to have pivotal antibacterial properties against foodborne and spoilage bacteria; however, only a few studies have reported its effect against pneumonia-causing pathogens [272, 273]. The current study suggests the significant antibacterial activity of *T. ammi* EO against the targeted bacteria in both liquid and vapour phases. The observed endpoints for the liquid phase correspond well with a previously published study reporting the MIC of *T. ammi* EO against *S. aureus* at 500 µg/mL [318] and within a three-dilution MIC range for *S. pneumoniae* (MIC = 250 µg/mL) [319]. In another study led by Gradinaru *et al.* [272], the MIC of *T. ammi* EO against *S. pneumoniae* was observed to be 500 µg/mL, this corresponds well with the endpoint obtained in the current study. Although reports on the growth inhibitory potential in the vapour phase are scarce, to the best of our knowledge, the antibacterial activity of *T. ammi* EO vapours was described for the first time in this investigation. The growth inhibitory property of *T. ammi* EO is mainly due to the presence of phenolic and hydrocarbon monoterpenes such as thymol, cymene, and γ-terpinene [320]. Especially, thymol has been reported to have strong antibacterial potency against a wide range of bacteria [321,322,323]. The mechanisms underlying the inhibition and reduction of growth by this phenolic monoterpenoid compound are attributed to its effect on the bacterial cell membrane. Thymol is a potent outer membrane damaging compound [322]. Due to its hydrophobic nature, this compound can integrate into bacterial cell membranes, causing disruption and disturbance to normal membrane function [324,325]. This disruption leads to increased permeability of ATP and an increased release of other cellular components [183].

Considering the toxicity, the current study reports that *T. ammi* EO is moderately cytotoxic to lung fibroblasts. This result corresponds with an *in vivo* study published by Vazirian *et al.* [326], who observed mild oral toxicity of the EO in a rat model. Regarding inhalation toxicity, the sources of information on the safety of EO vapours are scarce. However, data on its predominant compound, thymol, might suggest its possible inhalation safety. The European Chemicals Agency reported thymol to be nontoxic to mice when they are exposed to the chemical via inhalation of its vapour for 2 h with a lethal dose 50% (LD_{50}) of 7.57 mg/L [327]. Moreover, another study published by Xie *et al.* [328] reported no evidence of chronic toxicity of thymol through inhalation in a mouse model. Nevertheless, further experiments on their *in vivo* inhalation toxicity are necessary to determine the safety of *T. ammi* EO.

C. citratus EO is extensively used in Ayurvedic medicine as a folk remedy for coughs, flu, and pneumonia [226]. Previously published studies have reported the antibacterial and anti-fungal properties of *C. citratus* EO [329,186]. As a result of the modified agar dilution method, Inouye *et al*. [186] observed a moderate growth-inhibitory effect of *C. citratus* EO against *S. pyogenes* (MIC = 400 µg/mL), *H. influenzae*, and *S. pneumoniae* (MIC = 800 µg/mL). In addition, *C. citratus* EO vapours were reported to be more efficient against *H. influenzae* as compared to *S. aureus*, *S. pneumoniae*, and *S. pyogenes*. Despite certain variances in the MIC values caused probably by different bacterial strains and antimicrobial assays used, these results correspond well with our findings. Another study led by Acs *et al.* [330] reported the MIC in the vapour phase using modified disc volatilization method, for *S. pyogenes* (125 µg/mL), *H. influenzae*, and *S. pneumoniae* (50 µg/mL). In contrast to these findings, MIC values recorded in our study were usually higher – i.e., 1024–512 µg/mL. The variation in results could be attributed to several factors. Firstly, the quality and chemical compositions of the EO samples [331,332]. Additionally, differences in the choice of solvents and bacterial strains employed in antimicrobial assays contribute to this inconsistency [283]. Moreover, the diverse methods used to explore the antimicrobial effect, and the matrix (liquid/solid) employed for EO vapour dispersion, are crucial factors. These matrices not only influence the intensity and rate of evaporation but also significantly impact in shaping the vapour chemical profile [333]. Citral is identified as the primary compound responsible for the antibacterial activity in *C. citratus* EO. It has been recognized as an effective antimicrobial agent against various gram-positive and gram-negative bacteria as well as fungi [334,335,336] The common mechanism of action for citral involves its ability to bind to amino groups in the bacterial cell wall and penetrate the cytoplasm, causing coagulation and precipitation of cellular components [337].

Regarding cytotoxicity, the current study reports that *C. citratus* EO is moderately toxic (IC_{50} = 19.63 µg/mL) to the lung fibroblasts. A previous study reported a similar mild cytotoxic effect on human fibroblast cell line WI38 with an IC_{50} value of 49.39 µg/mL [338]. The minor variance in the IC_{50} values can be attributed to the modification of the cytotoxicity assay previously recommended for evaluation of biological properties of the volatile agents. The use of EVA Capmat protects microtiter plates against vapour transition and provides more reliable results [289]. The safety of *C. citratus* EO for potential inhalation use can be supported by a previously published study reporting a nontoxic effect of vapours of its predominant compound citral on Sprague–Dawley rats at concentrations up to 34 ppm [339]. Furthermore, other published data on the *in vivo* acute oral toxicity of *C. citratus* EO in mice and rabbit models have been reported to be nontoxic with an LD_{50} value > 2000 mg/kg [340]. Although *C. citratus* EO is classified as GRAS, further toxicological evaluation is necessary to confirm its nontoxicity for practical application in inhalation therapy.

The EO from rhizomes of *C. scariosus* is used as an ingredient in several Ayurvedic formulations as an anti-infective agent [341]. Previous studies have reported the growth-inhibitory effect of *C. scariosus*

EO against carbapenem-resistant *Klebsiella pneumoniae* and methicillin-resistant *S. aureus* with (MIC = 125 μg/mL) in liquid media respectively [237]. In our study, we observed only mild antibacterial activity of the EO in both phases. However, direct comparison is limited due to differences in the bacterial strains tested. Considering cytotoxicity, the present research reports that *C. scariosus* EO is nontoxic to the human lung cells. According to our best knowledge, there are no studies reporting the toxicity of the EO from this species.

The antibacterial properties of EOs are primarily attributed to their chemical composition. Therefore, studying the composition is crucial for understanding and harnessing their therapeutic potential. The chemical composition of EO is influenced by various factors such as the time of harvesting, the type of sample used (dry or fresh), drying method employed, etc. Drying is commonly employed to ensure the safe storage of medicinal and aromatic plants over extended periods, as water promotes microbial and chemical interactions that can lead to quality degradation. It extends the shelf life of the plants by slowing down microbial activity and preventing unwanted biochemical changes that could affect their sensory properties. However, this process poses a challenge for aromatic and spice plants, as they are rich in highly volatile and heat-sensitive compounds like monoterpenes, monoterpenoids, and certain sesquiterpenes. Monoterpenes have lower molecular weights than sesquiterpenes, making them more prone to evaporation during drying. As a result, EOs extracted from dried plants often show a reduced concentration of monoterpenes compared to oils from fresh plants. Several studies have documented this difference, noting that monoterpenes are more abundant in oils obtained from fresh samples than in those extracted after drying [342,343]. In the current study, there may have been a loss of some volatile monoterpenes; however, the lack of a direct comparison limits the scope of this discussion.

Regarding the chemical composition of *T. ammi* EO, oxygenated monoterpenoid (thymol) and monoterpene hydrocarbons (p-cymene, γ-terpinene, β-pinene) have been identified as the major constituents. The chromatographic profile obtained in the current study aligns with previously published reports using similar HP-5 capillary column, identifying thymol as the main component (ranging from 54.32% to 67.4%), p-cymene as the second-most abundant compound (ranging from 17.9% to 21.74%), and γ-terpinene as the third-most abundant component (ranging from 11.3% to 19.38%) [307, 330]. Another study by Dutta *et al.* [273] observed a chemotype for *T. ammi* EO using HP-5MS capillary, with thymol at 50.43%, γ-terpinene at 24.77%, and p-cymene at 21.69%. Minor differences are attributed to variations in the timing of plant growth and the geographical locations from which the plants were collected [344].

In the case of *C. citratus* EO, GC/MS analysis revealed that the major constituents are oxygenated monoterpenoids (α- and β-citral) and monoterpene hydrocarbon (camphene, and geranyl acetate), oxygenated sesquiterpenoids (caryophyllene oxide) and sesquiterpene hydrocarbons (γ-cadinene). These findings align with several previously published studies. For instance, El-Kased and El-Kersh *et*

al. [345] and Hanaa *et al.* [346] reported α-citral (36.35% and 34.98%, respectively) and β-citral (34.99% and 40.72%, respectively) as the main components of hydrodistilled EO from this species while using an HP-5 column. Another study reviewing the chemotype of *C. citratus* reported α-citral at 48.14% and β-citral at 38.22% as the principal components, which corresponds well with the current study [347].

Furthermore, in the chemical profile of *C. scariosus* EO, oxygenated sesquiterpenoid (cyperotundone and caryophyllene oxide) and sesquiterpene hydrocarbons (cyperene) were observed to be the abundant compounds. A study conducted by Clery *et al.* [236] reported a similar chemical constituent to the current research using a polar Innowax column, cyperene (20.1%) and cyperotundone (10.30%) have previously been identified as abundant components of *C. scariosus* EO. Another study reported differences in the chemical composition, with the content of caryophyllene oxide varying by 17.14% and cyperene by 14.3% while using TR 50MS column [348]. This significant variability in chemotype is attributed to different harvesting periods. For instance, Srivastava *et al*. [239] conducted a study that revealed variations in the chemical composition of *C. scariosus* EO primarily linked to changes in harvesting times. Notably, these differences were particularly prominent in sesquiterpene hydrocarbons and oxygenated sesquiterpenoids.

There was some disparity observed between RI reported in this study and those found in the literature, particularly for compounds such as 2-carene, 3-carene, 4-nonanone, longiverbenone, and α-pinene. These differences can be explained by variations in the oven temperature gradient program. In this study, the program was specifically designed to expedite the analysis and conserve materials. However, this approach impacted the RI, causing discrepancies when compared to literature values. The rapid heating in the latter stages of the gradient likely altered the interaction times of the compounds with the stationary phase, contributing to the observed RI shifts. This phenomenon has been documented in several studies, where even slight changes in heating rates significantly affect RI values, underscoring the importance of careful temperature programming for reproducibility [349]. To address these discrepancies, it is advisable to run authentic standards to confirm compound identification. In this study, authentic standards were used to confirm the identity of α-pinene and 3-carene. For future work, adjusting the oven program to an optimal gradient is recommended for improved identification accuracy

The current study underscores the effectiveness of a dual-column GC-MS system in characterizing the chemical composition of EOs from *C citratus*, *C. scariosus*, and *T ammi*. The broad spectrum of compounds identified in all EO samples aligns well with previously published literature. Notably, in the case of *C. scariosus* EO, a greater number of compounds were identified using the DB-wax column. This variation in detection may be attributed to the different polarities and materials of the columns used. These findings suggest that combining non-polar and polar columns provides a more accurate

representation of the EO composition, allowing for the resolution of overlapping signal peaks and improving the identification of separated compounds [350].

Furthermore, for headspace analysis in this study, we employed two different sampling methods: HS-SPME and HS-GTS. Both techniques offer distinct advantages in identifying volatile compounds present in EOs. HS-SPME, known for its simplicity, speed, selectivity, and sensitivity, yields precise qualitative results. Conversely, HS-GTS may provide more accurate data by reflecting the actual headspace distribution of EO volatile agents [283]. This method offers valuable insights into the components responsible for the antimicrobial activity associated with the EO vapours. Consequently, both methods were employed in the current study to ensure comprehensive analysis

The key constituents identified in the vapours of *T. ammi* EO were p-cymene, γ-terpinene, and thymol using the HS-SPME technique. This aligns well with the findings of Liu *et al*. [351], who reported γ-terpinene (26.21%), p-cymene (23.58%), and thymol (20.02%) as the major components of *T. ammi* EO vapour analysis via HS-SPME. Variations in the concentration of γ-terpinene and thymol observed in this study's headspace analysis can be attributed to differences in experimental conditions, as the EO samples were prepared by dissolving them in microbiological growth medium. Our study, identifying p-cymene, γ-terpinene, and β-pinene as predominant components of *T. ammi* EO vapours, represents the first report on its analysis using the HS-GTS method. When comparing both sampling techniques, disparities in thymol and β-pinene concentration are noted. The analysis of samples collected by the HS-GTS technique showed a lower amount of thymol and higher of β-pinene compared to the HS-SPME method. This difference may be due to the sensitivity and affinity of SPME fibers toward certain oxygenated volatile components [352]. In contrast, the HS-GTS technique is less selective but provides a precise and, perhaps, closer assessment of the real distribution of volatile compounds in vials [180]. These findings align with data published by Antih *et al.* [293], who conducted *T. vulgaris* EO headspace analysis under similar experimental conditions The antibacterial potential of volatile monoterpene hydrocarbons (such as p-cymene and γ-terpinene) and phenolic monoterpenoid (thymol) is well-known; for instance, the bioactivity of thymol in the vapour phase against respiratory tract bacteria such as *H. influenzae*, *S. aureus*, and *S. pneumoniae* has been previously documented [187]. As previously mentioned, the antibacterial activity of *T. ammi* EO and its vapours is mostly attributed to its composition, particularly the presence of monoterpene hydrocarbon and phenolic monoterpenoid in high concentrations, which are associated with the growth-inhibitory effect. Additionally, the current study reports higher activity in the broth medium compared to the vapour phase. This finding can be related to data obtained from the headspace analysis, where thymol, considered the main antimicrobial constituent, was detected in lower amounts in the EO vapours. The moderate water solubility and low volatility of thymol, compared to other monoterpenes, could explain its lower content in the vapor phase and its reduced antimicrobial activity [186].

Regarding the vapour of *C. citratus* EO, the major components observed using HS-SPME were α-citral, β-citral, and camphene. In contrast, for HS-GTS, the main compounds were camphene, limonene, and α-citral. These current findings align with a study led by Schweitzer *et al.* [229], which reported α-citral (34.5%) and β-citral (26.1%) as the predominant components of the headspace analysis of *C. citratus* EO using SPME fiber (carboxen/polydimethylsiloxane/divinylbenzene). Another study investigating the vapour chemotype of *C. citratus* EO using the HS-GTS extraction technique reported α-citral (15.27%), γ-terpineol (12.92%), and β-citral (10.89%) as the abundant compounds, with a much lower concentration of camphene (3.49%) [353]. The significant disparity is attributed to the difference in the extraction medium used for headspace analysis. It is important to note that the volatile profile of our sample may significantly deviate from that of pure EO. Our sample likely resembles an oil-in-water microemulsion, where the EO forms droplets within the MHB mixture, aided by DMSO, a weak surfactant. This results in an uneven distribution throughout the medium, potentially affecting the dispersion of EO components into the vial's atmosphere [310]. Overall, the disparity observed in the chemical composition when comparing both sampling methods can be attributed to several factors. For SPME, key elements influencing the adsorption of volatile compounds include heating temperature, extraction time, sample volume, the nature of the sample matrix (liquid or solid), and crucially, the composition of the fiber. Certain coatings of fibers are sensitive to specific volatile compound classes and can concentrate them more in the headspace [354,355]. For instance, a study by Tian *et al.* [356] optimized conditions for the absorption of citral and its degradation products in oil-in-water emulsions. The study compared different SPME fibers coatings (PDMS/DVB, PDMS, and CAR/PDMS) and found that a mixed-layer coating of PDMS/DVB was more suitable for comprehensive adsorption of α- and β-citral. The strong interactions between the volatile compounds and the fiber coating material explain the higher concentration of citral isomers observed in the HS-SPME analysis in this study.

Furthermore, for non-sensitive GTS, the actual distribution of volatiles in the headspace is reported. The higher concentration of camphene observed in the current study can be attributed to its physicochemical property of low solubility in water [357] and high volatility. Many studies have found that the compound is unstable at room temperature and continuously dissipates from the medium during overtime analysis. [358,359,360]. Similarly, in the case of limonene low solubility in aqueous medium, instability, and volatile nature leads to faster evaporation [361]. Therefore, the matrix chosen for the dispersion of the EO vapour is an important factor. For instance, a study conducted by Antih [333] reported the influence of the matrix on *T. vulgaris* EO vapour composition, revealing significant disparities, particularly in the concentration of thymol. When extracted from a solid matrix, the abundance of thymol increased almost tenfold compared to its extraction from a liquid matrix. The headspace study of *C. citratus* EO suggests monoterpene hydrocarbon - camphene limonene and oxygenated monoterpenoids - α and β-citral as the major compounds responsible for impacting

antibacterial effect in the vapour phase. This corresponds well with the previously published studies reporting the growth inhibitory property of these compounds in the vapour phase [346,330,352].

8 CONCLUSION

In conclusion, this study reports the *in vitro* antimicrobial activity of EOs and plant extracts from Indian medicinal plants against different infection causing pathogens. Among all plants tested, *P. corylifolia* ethanolic extract demonstrated notable activity against *S. aureus* and *C. albicans* and *T. ammi* EO inhibited growth of respiratory pathogens in both liquid and vapour phases. Although the antimicrobial properties of *P. corylifolia* are well described in the literature, this is the first study reporting the antibacterial potential of *T. ammi* EO vapours against pneumonia-causing pathogens. Moreover, the results of the study demonstrate the validity of broth microdilution volatilization method for testing the susceptibility of bacterial pathogens causing respiratory infections to EOs and their vapours. In addition, the cytotoxicity assessments of the targeted EO revealed that *C. scariosus* EO is relatively safe for normal lung fibroblasts whereas *C. citratus* and *T. ammi* EOs were mild toxic. Based on above mentioned findings, *T. ammi* EO can be suggested for further research focused on development of volatile antibacterial agents for inhalation therapies. However, experiments confirming it's *in vivo* efficacy and safety connected with the volatile components will be necessary before considering it for clinical trials and possible practical use in clinical practice. Additionally, to accurately estimate the potential toxicity of the EO vapour components, it is important to determine the absolute amounts of the volatile compounds, rather than relying solely on the relative proportions measured in the current study. This requires rigorous calibration studies to quantify the main volatile compounds using internal or external standards and to develop calibration curves within appropriate concentration ranges. Such precise quantification is crucial for a comprehensive understanding of the potential toxicity and safety of the vapours and should be a key focus in future studies.

The chemical analysis conducted using a dual column/dual detector GC-MS system revealed that oxygenated monoterpenoids and monoterpene hydrocarbons (thymol, p-cymene, and γ-terpinene) were the most abundant compounds of *T. ammi* EO. In the vapour phase, both headspace extraction techniques (HS-GTS and HS-SPME) identified p-cymene, γ-terpinene, thymol, and β-pinene as the major compounds with slight differences in the concentrations of thymol and β-pinene. Monoterpene aldehydes (α-citral and β-citral) were main compounds of *C. citratus* EO. In case for the headspace analysis, α-citral, β-citral and camphene were identified as major components by HS-SPME technique. In contrast, camphene, limonene, and α-citral were reported to be the predominant compounds using HS-GTS technique. Oxygenated sesquiterpenoids and sesquiterpene hydrocarbons (cyperotundone, caryophyllene oxide, and cyperene) were dominated compounds in *C. scariosus* EO. In general, the GC-MS analysis of EO contributed to a deeper understanding of phytochemistry of Indian medicinal plants and provided important chemical data for their possible future pharmacological use. The study also demonstrated that employing dual columns (non-polar and polar) provides a more accurate characterization of EOs. This approach enables the resolution of overlapping signal peaks and enhances

the identification of separated compounds. Considering the headspace analysis, the variations observed in predominant compounds highlight the critical importance of selecting the appropriate HS technique. As previously noted, HS-SPME, known for its selectivity and sensitivity, produced qualitative results, while HS-GTS offered more precise data by accurately reflecting the headspace distribution of EO volatile agents. Utilizing both extraction techniques proves advantageous, providing valuable insights into the components responsible for the antimicrobial activity of EO vapours. The study concludes that both HS techniques complement each other and are suitable for studying the qualitative and quantitative aspects of the volatile profiles of EOs.

Overall, the current study highlights the promising potential of EOs and extracts derived from Indian medicinal plants for the development of new anti-infective agents. However, future comprehensive research on the chemical composition and toxicology of the active plant extracts will be essential to ascertain their safety and potential applications. Furthermore, concerning the antimicrobial testing of EO vapours, a significant challenge is posed by their physical properties, characterized by high volatility and low solubility in water. In future studies, the use of solid volatilization matrices (e.g. cellulose-based disks) and appropriate modification of broth macrodilution volatilization method could be important steps in research of the antimicrobial efficacy of EOs in the vapour phase. Moreover, regarding the application of EOs in inhalation therapy, a significant challenge involves achieving a stable and controlled release of EO volatiles to enhance their prolonged effectiveness against microbes. Future studies on encapsulation of EOs with polymeric complexes can offer a promising approach to address this challenge and improve their efficacy and stability. Additionally, regarding the cytotoxicity of EOs, existing studies primarily investigate their effects in the liquid phase. Further research on evaluating their cytotoxicity in the vapour phase is crucial for advancing their potential applications in inhalation therapies. Addressing these challenges through continued research could pave the way for innovative patents and significant progress in the development of new pharmaceutical products utilizing EO and plant extracts to treat infectious diseases.

10. REFERENCES

1. World Health Organization (WHO) 2020. Geneva. The top 10 causes of death. Available from https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death (accessed July 2023).
2. Odoki M, Almustapha Aliero A, Tibyangye J, Nyabayo MJ, Wampande E, Drago KC, Agwu E, Bazira J. 2019. Prevalence of bacterial urinary tract infections and associated factors among patients attending hospitals in Bushenyi district, Uganda. International Journal of Microbiology **17**: 4246780.
3. Boutayeb A. 2010. The burden of communicable and non-communicable diseases in developing countries. Handbook of Disease Burdens and Quality of Life Measures 531-546.
4. Bitew A, Zena N, Abdeta A. 2022. Bacterial and fungal profile, antibiotic susceptibility patterns of bacterial pathogens and associated risk factors of urinary tract infection among symptomatic pediatrics patients attending St. paul's hospital millennium medical college: a cross-sectional study. Infection and Drug Resistance **15**: 1613–1624.
5. Ram B, Thakur R. 2022. Epidemiology and economic burden of continuing challenge of infectious diseases in India: analysis of socio-demographic differentials. Front Public Health **10**: 901276.
6. Dutta S, Guin S, Ghosh S, Pazhani GP, Rajendran K, Bhattacharya MK, Takeda Y, Nair GB, Ramamurthy T. 2013. Trends in the prevalence of diarrheagenic *Escherichia coli* among hospitalized diarrheal patients in Kolkata, India. PLoS One **8**: e56068.
7. Ray A, Aayilliath K A, Banerjee S, Chakrabarti A, Denning DW. 2022. Burden of serious fungal infections in India. Open Forum Infectious Diseases **9**: ofac603.
8. Ikuta KS, Swetschinski LR, Aguilar GR, Sharara F, Mestrovic T, Gray AP, Weaver ND, Wool EE, Han C, Hayoon AG, Aali A. 2022. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the global burden of disease study 2019. The Lancet **400**: 2221-2248.
9. Denning DW. 2024. Global incidence and mortality of severe fungal disease. The Lancet Infectious Diseases 1-11.
10. Majumder MAA, Rahman S, Cohall D, Bharatha A, Singh K, Haque M, Gittens-St Hilaire M. 2020. Antimicrobial stewardship: fighting antimicrobial resistance and protecting global public health. Infection and Drug Resistance **13**: 4713–4738.
11. Borghardt JM, Kloft C, Sharma A. 2018. Inhaled therapy in respiratory disease: the complex interplay of pulmonary kinetic processes. Canadian Respiratory Journal **2018**: 1-8.
12. Tiddens HA, Bos AC, Mouton JW, Devadason S, Janssens HM. 2014. Inhaled antibiotics: dry or wet? European Respiratory Journal **44**: 1308-1318.

13. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool, MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF, Baloch Z. 2018. Antibiotic resistance: a rundown of a global crisis. Infection and Drug resistance **11**: 1645–1658.
14. Dias DA, Urban S, Roessner U. 2012. A historical overview of natural products in drug discovery. Metabolites **2**: 303–336.
15. Kokoska L, Kloucek P, Leuner O, Novy P. 2019. Plant-derived products as antibacterial and antifungal agents in human health care. Current Medicinal Chemistry **26**: 5501-5541.
16. Leigh-de Rapper S, Van Vuuren SF. 2020. Odoriferous therapy: a review identifying essential oils against pathogens of the respiratory tract. Chemistry & Biodiversity **6**: e2000062.
17. Prasathkumar M, Anisha S, Dhrisya C, Becky R, Sadhasivam S. 2021. Therapeutic and pharmacological efficacy of selective Indian medicinal plants – a review. Phytomedicine Plus **1**: 100029.
18. Vaidya VN, Tatiya AU, Elango A, Kukkupuni SK, Vishnuprasad CN. 2018. Need for comprehensive standardization strategies for marketed Ayurveda formulations. Journal of Ayurveda and Integrative Medicine **9**: 312-5.
19. Ningthoujam SS, Talukdar AD, Potsangbam KS, Choudhury MD. 2013. Traditional uses of herbal vapour therapy in Manipur, North East India: an ethnobotanical survey. Journal of Ethnopharmacology **147**: 136-47.
20. Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. 2011. Scientific basis for the therapeutic use of *Cymbopogon citratus* stapf (Lemon grass). Journal of Advanced Pharmaceutical Technology & Research **2**: 3–8.
21. Bairwa R, Sodha RS, Rajawat BS. 2012. *Trachyspermum ammi*. Pharmacognosy Reviews **11**: 56–60.
22. Singh V, Ali M, Negi A, Sultana S. 2018. Analysis and antimicrobial activity of the essential oil of *Cyperus rotundus* L. rhizomes. Journal of Medicinal Plants Studies **6**: 101-5.
23. Warrier PK. 1993. Indian medicinal plants: a compendium of 500 species (Vol. 5). Orient Blackswan, Chennai.
24. Alam F, Khan GN, Asad MHHB. 2018. *Psoralea corylifolia* L: Ethnobotanical, biological, and chemical aspects: a review. Phytotherapy Research **32**: 597–615.
25. Casadevall A, Pirofski LA. 1999. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infection and Immunity **67**: 3703–3713.
26. Baron S. 1996. Medical Microbiology 4th edition. University of Texas Medical Branch at Galveston, Texas.
27. Kesavelu D, Jog P. 2023. Current understanding of antibiotic-associated dysbiosis and approaches for its management. Therapeutic Advances in Infectious Disease **10**: 20499361231154443.

28. Ehrmann S, Chastre J, Diot P, Lu Q. 2017. Nebulized antibiotics in mechanically ventilated patients: a challenge for translational research from technology to clinical care. Annals of Intensive Care **7**: 1-2.
29. Caeiro JP, Garzon MI. 2018. Controlling infectious disease outbreaks in low-income and middle-income countries. Current Treatment Options in Infectious Diseases **10**: 55–64.
30. Ismahene Y. 2022. Infectious diseases, trade, and economic growth: a panel analysis of developed and developing countries. Journal of the Knowledge Economy **13**: 2547-2583.
31. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, Han C, Bisignano C, Rao P, Wool E, Johnson SC.2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet **12**:629-55.
32. Rayens E, Norris KA. 2022. Prevalence and healthcare burden of fungal infections in the United States, 2018. In Open Forum Infectious Diseases **9**: ofab593.
33. Singh R, Chakrabarti A. 2017. Invasive candidiasis in the southeast-Asian region. *Candida albicans*: cellular and molecular biology. Springer, Cham.
34. Mave V, Chandanwale A, Kagal A, Khadse S, Kadam D, Bharadwaj R, Dohe V, Robinson ML, Kinikar A, Joshi S, Raichur P. 2017. High burden of antimicrobial resistance and mortality among adults and children with community-onset bacterial infections in India. The Journal of Infectious Diseases **215**: 1312-20.
35. Torres A, Cilloniz C, Niederman MS, Menendez R, Chalmers JD, Wunderink RG, van der Poll T. 2021. Pneumonia. Nature Reviews Disease Primers **7**: 25.
36. Huang G, Guo F. 2022. Loss of life expectancy due to respiratory infectious diseases: Findings from the global burden of disease study in 195 countries and territories 1990–2017. Journal of Population Research **39**: 1–43.
37. Baptista EA, Dey S, Pal S. 2021. Chronic respiratory disease mortality and its associated factors in selected Asian countries: evidence from panel error correction model. BMC Public Health **21**: 1-1.
38. Yadav, K. K., & Awasthi, S. 2023. Childhood pneumonia: what's unchanged, and what's new?. Indian Journal of Pediatrics **7**: 693–699.
39. Aston SJ. 2017. Pneumonia in the developing world: characteristic features and approach to management. Respirology **22**: 1276– 1287.
40. United Nations International Children's Emergency Fund. 2018. Available from http://www.data.unicef.org/topic/child-health/diarrhoeal-disease/ (accessed July 2023)
41. Ugboko HU, Nwinyi OC, Oranusi SU, Oyewale JO. 2020. Childhood diarrhoeal diseases in developing countries. Heliyon **6**: e03690.
42. Mohanty A, Saxena, A. Diarrheal disease, sanitation, and culture in India. 2023. Social Science & Medicine **317**: 115541.

43. Ghosh K, Chakraborty AS, Mog M. 2021. Prevalence of diarrhoea among under five children in India and its contextual determinants: A geo-spatial analysis. Clinical Epidemiology and Global Health **12**: 100813.
44. Terreni M, Taccani M, Pregnolato M. 2021. New antibiotics for multidrug-resistant bacterial strains: Latest research developments and future perspectives. Molecules **26**: 2671.
45. Whiteway M, Bachewich C. 2007. Morphogenesis in *Candida albicans*. Annual Review of Microbiology **61**: 529–553.
46. Talapko J, Juzbasic M, Matijevi T, Pustijanac E, Bekic S, Kotris I, Skrlec I. 2021. *Candida albicans*-The virulence factors and clinical manifestations of infection. Journal of Fungi **7**: 79.
47. Mayer FL, Wilson D, Hube B. 2013. *Candida albicans* pathogenicity mechanisms. Virulence **4**: 119–128.
48. Ray A, Aayilliath KA, Banerjee S, Chakrabarti A, Denning DW. 2022. Burden of serious fungal infections in India. Open Forum Infectious Diseases **9**: ofac603.
49. Habibzadeh A, Lankarani KB, Farjam M, Akbari M, Kashani SMA, Karimimoghadam Z, Wang K, Imanieh MH, Tabrizi R, Ahmadizar F. 2022. Prevalence of fungal drug resistance in COVID-19 infection: a global meta-analysis. Current Fungal Infection Reports **16**: 154–164.
50. Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rizk MA. 2020. Oral candidiasis: a disease of opportunity. Journal of Fungi **6**: 15.
51. Willems HME, Ahmed SS, Liu J, Xu Z, Peters BM. 2020. Vulvovaginal candidiasis: a current understanding and burning questions. Journal of Fungi **6**: 27.
52. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. 2018. Invasive candidiasis. Nature Reviews Disease Primers **4**: 1-20.
53. Chen SC, Sorrell TC. 2007. Antifungal agents. Medical Journal of Australia **187**: 404.
54. Perlin DS. 2015. Echinocandin resistance in Candida. Clinical Infectious Diseases **6**: 612-7.
55. Percival SL, Williams DW. 2014. Microbiology of Waterborne Diseases. Academic Press, London.
56. Poolman JT. 2017. International Encyclopedia of Public Health. Academic Press, Oxford.
57. Khairy RM, Fathy ZA, Mahrous DM, Mohamed ES, Abdelrahim SS. 2020. Prevalence, phylogeny, and antimicrobial resistance of *Escherichia coli* pathotypes isolated from children less than 5 years old with community acquired diarrhoea in upper Egypt. BMC Infectious Diseases **20**: 1-9.
58. Kaper JB, Nataro JP, Mobley HL. 2004. Pathogenic *Escherichia coli*. Nature Reviews Microbiology **2**: 123-40.
59. Pakbin B, Bruck WM, Rossen JW. 2021.Virulence factors of enteric pathogenic *Escherichia coli*: A review. International Journal of Molecular Sciences **22**: 9922.
60. Farfan MJ, Torres AG. 2012. Molecular mechanisms that mediate colonization of Shiga toxin-producing *Escherichia coli* strains. Infection and Immunity **80**: 903-13.

61. Mueller M, Tainter CR. 2023. *Escherichia coli* infection. StatPearls Publishing, Florida.
62. Alanazi MQ, Alqahtani FY, Aleanizy FS. 2018. An evaluation of *E. coli* in urinary tract infection in emergency department at KAMC in Riyadh, Saudi Arabia: retrospective study. Annals of Clinical Microbiology and Antimicrobials **17**: 1-7.
63. Nji E, Kazibwe J, Hambridge T, Joko CA, Larbi AA, Damptey LA, Nkansa-Gyamfi NA, Stalsby Lundborg C, Lien LT. 2021. High prevalence of antibiotic resistance in commensal *Escherichia coli* from healthy human sources in community settings. Scientific Reports **11**: 3372.
64. Van Tyne D, Martin MJ, Gilmore MS. 2013. Structure, function, and biology of the *Enterococcus faecalis* cytolysin. Toxins **5**: 895-911.
65. Ramos S, Silva V, Dapkevicius MD, Igrejas G, Poeta P. 2020. *Enterococci*, from harmless bacteria to a pathogen. Microorganisms **8**: 1118.
66. Esmail MAM, Abdulghany HM, Khairy RM. 2019. Prevalence of multidrug-resistant *Enterococcus faecalis* in hospital-acquired surgical wound infections and bacteremia: concomitant analysis of antimicrobial resistance genes. Infectious Diseases **12**: 1178633719882929.
67. Comerlato CB, Resende MC, Caierao J, d'Azevedo PA. 2013. Presence of virulence factors in *Enterococcus faecalis* and *Enterococcus faecium* susceptible and resistant to vancomycin. Memorias do Instituto Oswaldo Cruz **108**: 590-5.
68. Fiore E, Van Tyne D, Gilmore MS. 2019. Pathogenicity of Enterococci. Microbiology Spectrum **7**: 10-128.
69. Parija SC. Haemophilus and Bordetella. 2023. In Textbook of Microbiology and Immunology. Springer Nature Singapore, Singapore.
70. Tang CM, Hood DW, Moxon ER. 2001. Principles of Bacterial Pathogenesis. Academic Press, San Diego.
71. Murphy TF. 2015. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition). W.B. Saunders, Philadelphia.
72. Hendrixson DR, Geme JW. 1998. The *Haemophilus influenzae* Hap serine protease promotes adherence and microcolony formation, potentiated by a soluble host protein. Molecular Cell **2**: 841-50.
73. Khattak ZE, Anjum F. 2023. *Haemophilus influenzae* infection, StatPearls Publishing, Florida.
74. Slack MP. 2015. A review of the role of *Haemophilus influenzae* in community-acquired pneumonia. Pneumonia **6**: 26-43.
75. Nandi A, Deolalikar AB, Bloom DE, Laxminarayan R. 2019. *Haemophilus influenzae* type b vaccination and anthropometric, cognitive, and schooling outcomes among Indian children. Annals of the New York Academy of Sciences **1449**: 70–82.

76. Rao VK, Krasan GP, Hendrixson DR, Dawid S, St. Geme III JW. 1999. Molecular determinants of the pathogenesis of disease due to non-typable *Haemophilus influenzae*. FEMS Microbiology Reviews **23**: 99-129.
77. Johnson AP, Inzana TJ. 1986. Loss of ciliary activity in organ cultures of rat trachea treated with lipo-oligosaccharide from *Haemophilus influenzae*. Journal of Medical Microbiology **22**: 265-8.
78. Kilian M, Mestecky J, Schrohenloher RE. 1979. Pathogenic species of the genus *Haemophilus* and *Streptococcus pneumoniae* produce immunoglobulin A1 protease. Infection and Immunity **26**: 143-9.
79. Plaut AG, Qiu J, Grundy F, Wright A. 1992. Growth of *Haemophilus influenzae* in human milk: synthesis, distribution, and activity of IgA protease as determined by study of iga+ and mutant iga− cells. Journal of Infectious Diseases **166**: 43-52.
80. Tristram S, Jacobs MR, Appelbaum PC. 2007. Antimicrobial resistance in *Haemophilus influenzae*. Clinical Microbiology Reviews **20**: 368-89.
81. Bae S, Lee J, Lee J, Kim E, Lee S, Yu J, Kang Y. 2010. Antimicrobial resistance in *Haemophilus influenzae* respiratory tract isolates in Korea: results of a nationwide acute respiratory infections surveillance. Antimicrobial Agents and Chemotherapy **54**: 65-71.
82. Wu W, Jin Y, Bai F, Jin, S. 2015. Molecular Medical Microbiology (Second Edition). Academic Press, Boston.
83. Wu M, Li, X. 2015. Molecular Medical Microbiology (Second Edition). Academic Press, Boston.
84. Bhagirath AY, Li Y, Somayajula D, Dadashi M, Badr S, Duan K. 2016. Cystic fibrosis lung environment and *Pseudomonas aeruginosa* infection. BMC Pulmonary Medicine **16**: 174.
85. Reynolds D, Kollef, M. 2021. The epidemiology and pathogenesis and treatment of *Pseudomonas aeruginosa* infections: an update. Drugs **81**: 2117–2131.
86. Liao C, Huang X, Wang Q, Yao D, Lu W. 2022. Virulence factors of *Pseudomonas aeruginosa* and antivirulence strategies to combat its drug resistance. Frontiers in Cellular and Infection Microbiology, **12**: 926758.
87. Bassetti M, Vena A, Croxatto A, Righi E, Guery B. 2018. How to manage *Pseudomonas aeruginosa* infections. Drugs in Context **7**: 212527.
88. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. 2019. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. Biotechnology Advances **37**: 177-92.
89. Erkmen, O. 2022. Microbiological Analysis of Foods and Food Processing Environments. Elsevier Science, Amsterdam.
90. Gotz F, Bannerman T, Schleifer KH. 2006. The Prokaryotes. Springer, USA.

91. Wang M, Buist G, van Dijl JM. 2022. *Staphylococcus aureus* cell wall maintenance – the multifaceted roles of peptidoglycan hydrolases in bacterial growth, fitness, and virulence. FEMS Microbiology Reviews **46**: fuac025.
92. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews **28**: 603–661.
93. Foster T. 1996. Medical Microbiology. University of Texas Medical Branch at Galveston, Galveston.
94. Lohan K, Sangwan J, Mane P, Lathwal S. 2021. Prevalence pattern of MRSA from a rural medical college of North India: A cause of concern. Journal of Family Medicine and Primary Care **10**: 752–757.
95. Oogai Y, Matsuo M, Hashimoto M, Kato F, Sugai M, Komatsuzawa H. 2011. Expression of virulence factors by *Staphylococcus aureus* grown in serum. Applied and Environmental Microbiology **77**: 8097-105.
96. Kong C, Neoh H-m, Nathan S. 2016. Targeting *Staphylococcus aureus* Toxins: A potential form of anti-virulence therapy. Toxins **8**: 72.
97. Esposito S, Blasi F, Curtis N, Kaplan S, Lazzarotto T, Meschiari M, Mussini C, Peghin M, Rodrigo C, Vena A, Principi N. 2023. New antibiotics for *Staphylococcus aureus* infection: an update from the world association of infectious diseases and immunological disorders (WAidid) and the Italian society of anti-infective therapy (SITA). Antibiotics **12**: 742.
98. Chambers HF, Deleo FR. 2009. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nature reviews. Microbiology **7**: 629–641.
99. Shoaib M, Aqib AI, Muzammil I, Majeed N, Bhutta ZA, Kulyar MF, Fatima M, Zaheer CF, Muneer A, Murtaza M, Kashif M, Shafqat F, Pu W. 2023. MRSA compendium of epidemiology, transmission, pathophysiology, treatment, and prevention within one health framework. Frontiers in Microbiology **13**: 1067284.
100. Peter G, Klein JO. 2008. Principles and Practice of Paediatric Infectious Disease (Third Edition). W.B. Saunders, Edinburgh.
101. Ahmed J, Malik F. 2022. Encyclopedia of Infection and Immunity. Elsevier, Oxford.
102. Janoff EN, Musher DM. 2015. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition). W.B. Saunders, Philadelphia.
103. Nagaraj G, Govindan V, Ganaie F, Venkatesha VT, Hawkins PA, Gladstone RA, McGee L, Breiman RF, Bentley SD, Klugman KP, Lo SW. 2021. *Streptococcus pneumoni*ae genomic datasets from an Indian population describing pre-vaccine evolutionary epidemiology using a whole genome sequencing approach. Microbial Genomics **7**: 000645.
104. Marquart ME. 2021. Pathogenicity and virulence of *Streptococcus pneumoniae*: Cutting to the chase on proteases. Virulence **12**: 766–787.

105. Zomer A, Hermans PWM, Bootsma HJ. 2015. *Streptococcus Pneumoniae*. Academic Press, Amsterdam.
106. Sharew B, Moges F, Yismaw G, Abebe W, Fentaw S, Vestrheim D, Tessema B. 2021. Antimicrobial Resistance Profile and Multidrug Resistance Patterns of *Streptococcus Pneumoniae* Isolates from Patients Suspected of Pneumococcal Infections in Ethiopia. Annals of Clinical Microbiology and Antimicrobials **20**: 26.
107. Sallam M, Abbadi J, Natsheh A, Ababneh NA, Mahafzah A, Ozkaya Sahin G. 2019. Trends in antimicrobial drug resistance of *Streptococcus pneumoniae* isolates at Jordan University Hospital (2000–2018). Antibiotics **8**: 41.
108. Zhu L, Olsen RJ, Nasser W, de la Riva Morales I, Musser JM. 2015. Trading capsule for increased cytotoxin production: contribution to virulence of a newly emerged clade of emm89 *Streptococcus pyogenes*. MBio **6**: 10-128.
109. Stevens DL, Bryant AE. 2016. *Streptococcus pyogenes*: Basic Biology to Clinical Manifestations. University of Oklahoma Health Sciences Center, Oklahoma City.
110. Efstratiou A, Lamagni T. 2022. *Streptococcus pyogenes*: Basic Biology to Clinical Manifestations. University of Oklahoma Health Sciences Center, Oklahoma City.
111. Haggar A, Nerlich A, Kumar R, Abraham VJ, Brahmadathan KN, Ray P, Dhanda V, Joshua JM, Mehra N, Bergmann R, Chhatwal GS, Norrby-Teglund A. 2012. Clinical and microbiologic characteristics of invasive *Streptococcus pyogenes* infections in north and south India. Journal of Clinical Microbiology **50**: 1626–1631.
112. Brouwer S, Rivera-Hernandez T, Curren BF, Harbison-Price N, De Oliveira DMP, Jespersen MG, Davies MR, Walker MJ. 2023. Pathogenesis, epidemiology, and control of group A *Streptococcus* Infection. Nature Reviews Microbiology **21**: 431–447.
113. Doumith M, Mushtaq S, Martin V, Chaudhry A, Adkin R, Coelho J, Chalker V, MacGowan A, Woodford N, Livermore DM, BSAC Resistance Surveillance Standing Committee. 2017. Genomic sequences of *Streptococcus agalactiae* with high-level gentamicin resistance, collected in the BSAC bacteraemia surveillance. Journal of Antimicrobial Chemotherapy **72**: 2704-7.
114. Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, Dzobo K. 2018. Natural products for drug discovery in the 21st century: innovations for novel drug discovery. International Journal of Molecular Sciences **19**: 1578.
115. Kebede B, Shibeshi W. 2022. *In vitro* antibacterial and antifungal activities of extracts and fractions of leaves of *Ricinus communis* Linn against selected pathogens. Veterinary Medicine and Science **8**: 1802-15.
116. Rashmi HB, Negi PS. 2022. Plant extracts: applications in the food industry. Elsevier Inc, London.

117. Monagas M, Brendler T, Brinckmann J, Dentali S, Gafner S, Giancaspro G, Johnson H, Kababick J, Ma C, Oketch-Rabah H, Pais P. 2022. Understanding plant to extract ratios in botanical extracts. Frontiers in Pharmacology **13**: 981978.
118. Abdullahi A, Tijjani A, Abubakar A, Khairulmazmi A, Ismail M. 2022. Herbal biomolecules in healthcare applications. Elsevier, Amsterdam.
119. Bolouri P, Salami R, Kouhi S, Kordi M, Asgari Lajayer B, Hadian J, Astatkie T. 2022. Applications of essential oils and plant extracts in different industries. Molecules **27**: 8999.
120. Bruneton J. 1999. Pharmacognosy: phytochemistry, medicinal plants. Intercept Limited, Andover.
121. Heinrich, M, Mah J, Amirkia V. 2021. Alkaloids used as medicines: structural phytochemistry meets biodiversity- an update and forward look. Molecules **7**: 1836.
122. Wansi JD, Devkota KP, Tshikalange E, Kuete V. 2013. Alkaloids from the medicinal plants of Africa. Medicinal Plant Research in Africa, Elsevier Amsterdam.
123. Bennett RN, Wallsgrove RM. 1994. Secondary metabolites in plant defence mechanisms. New Phytologist **4**: 617-33.
124. Rajput A, Sharma R, Bharti R. Pharmacological activities, and toxicities of alkaloids on human health. Materials Today: Proceedings **48**: 1407-15.
125. Cowan MM. 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews **12** :564–582.
126. Vuddanda PR, Chakraborty S, Singh S. 2010. Berberine: a potential phytochemical with multispectrum therapeutic activities. Expert Opinion on Investigational Drugs **19**: 1297-307.
127. Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X, Kong M, Li L, Zhang Q, Liu Y, Chen H. 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules **21**: 1374.
128. Luna-Guevara ML, Luna-Guevara JJ, Hernandez-Carranza P, Ruiz-Espinosa H, Ochoa-Velasco CE. 2018. Phenolic compounds: A good choice against chronic degenerative diseases. Studies in Natural Products Chemistry **59**: 79-108.
129. Beya MM, Netzel ME, Sultanbawa Y, Smyth H, Hoffman LC. 2021. Plant-based phenolic molecules as natural preservatives in comminuted meats: a review. Antioxidants **10**: 263.
130. Bouarab Chibane L, Degraeve P, Ferhout H, Bouajila J, Oulahal N. 2019. Plant antimicrobial polyphenols as potential natural food preservatives. Journal of the Science of Food and Agriculture **99**: 1457-74.
131. Pearlman WH. 1970. Steroids & Terpenoids – BioScience. Academic Press, New York.
132. Patel SS, Savjani JK. 2015. Systematic review of plant steroids as potential anti-inflammatory agents: current status and future perspectives. The Journal of Phytopharmacology **4**: 121-5.
133. Shah B, Seth AK. 2010. Textbook of Pharmacognosy and Phytochemistry. Elsevier, New Delhi.

134. Adedokun KA, Imodoye SO, Bello IO, Lanihun A. 2023. Therapeutic potentials of medicinal plants and significance of computational tools in anti-cancer drug discovery- Phytochemistry, computational tools and databases in drug discovery. Elsevier, Amsterdam.
135. Bucar F, Wube A, Schmid M. 2013. Natural product isolation--how to get from biological material to pure compounds. Natural Product Reports **30**: 525-45.
136. Zhang QW, Lin LG, Ye WC. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine **13**: 20.
137. Abubakar AR, Haque M. 2020. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy & Bio allied Sciences **12**: 1–10.
138. Sikder L, Khan MR, Smrity SZ, Islam MT, Khan SA. 2022. Phytochemical and pharmacological investigation of the ethanol extract of *Byttneria pilosa* Roxb. Clinical Phytoscience **8**: 1.
139. Salam AM, Lyles JT, Quave CL. 2019. Methods in the extraction and chemical analysis of medicinal plants. Springer Protocols Handbooks. Humana Press, New York.
140. Heinrich M, Jalil B, Abdel-Tawab M, Echeverria J, Kulic Z, McGaw LJ, Pezzuto JM, Potterat O, Wang JB. 2022. Best Practice in the chemical characterisation of extracts used in pharmacological and toxicological research—The ConPhyMP—Guidelines12. Frontiers in Pharmacology **13**: 953205.
141. Hubber U, Majors RE. 2007. Principles in preparative HPLC. Agilent, Germany.
142. Rockwood AL, Kushnir MM, Clarke NJ. 2018. Principles and applications of clinical mass spectrometry. Elsevier, Amsterdam.
143. Cynthia KM. 1999. Structural chemistry using NMR spectroscopy, organic molecules - Encyclopedia of spectroscopy and spectrometry. Elsevier, London.
144. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. 2021. Towards advances in medicinal plant antimicrobial activity: a review study on challenges and future perspectives. Microorganisms **9**: 2041.
145. Cernakova M, Kostalova D. 2002. Antimicrobial activity of berberine-a constituent of *Mahonia aquifolium*. Folia Microbiologica **47**: 375–378.
146. Ali S, Igoli J, Clements C, Semaan D, Almazeb M, Rashid MU, Shah SQ, Ferro V, Gray A, Khan MR. 2013. Antidiabetic and antimicrobial activities of fractions and compounds isolated from *Berberis brevissima* Jafri and *Berberis parkeriana* Schneid. Bangladesh Journal of Pharmacology **8**: 336-42.
147. Yu M, Jin X, Liang C, Bu F, Pan D, He Q, Ming Y, Little P, Du H, Liang S, Hu R. 2020. Berberine for diarrhoea in children and adults: a systematic review and meta-analysis. Therapeutic Advances in Gastroenterology **13**:1756284820961299.

148. Chowdhury B, Adak M, Bose SK. Flurbiprofen, a unique non-steroidal anti-inflammatory drug with antimicrobial activity against Trichophyton, Microsporum and Epidermophyton species. Letters in Applied Microbiology **37**: 158-61.
149. Holti G, Ingram JT. 1960. Some recent trends and developments in dermatology. Postgraduate Medical Journal **36**: 250.
150. Cote J, Caillet S, Doyon G, Dussault D, Sylvain JF, Lacroix M. 2011. Antimicrobial effect of cranberry juice and extracts. Food Control **22**: 1413-1418.
151. Caillet S, Cote J, Sylvain JF, Lacroix M. 2012. Antimicrobial effects of fractions from cranberry products on the growth of seven pathogenic bacteria. Food Control **23**: 419-428.
152. Kenee PRM, Christie AL, Zimmern PE. 2022. Cranberry supplement, D-mannose, and other OTC modalities for prevention of recurrent UTI in women post-electrofulguration. International Journal of Women's Health **14**: 643–653.
153. Baser KH, Buchbauer G. 2009. Handbook of essential oils: science, technology, and applications. CRC press, Boca Raton.
154. Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M, Tundis R, Sharifi-Rad M, Loizzo MR, Ademiluyi AO, Sharifi-Rad R, Ayatollahi SA, Iriti M. 2017. Biological activities of essential oils: from plant chemoecology to traditional healing systems. Molecules **22**: 70.
155. Simpson MG. 2010. Evolution and diversity of woody and seed plants. In Plant Systematics. Academic Press, San Diego.
156. Lee WG. 2001. Introduced Plants, Negative Effects of - Encyclopaedia of Biodiversity. Elsevier Ltd, Waltham.
157. Raut JS, Karuppayil SM. 2014. A status review on the medicinal properties of essential oils. Industrial Crops and Products **62**: 250-64.
158. Oyen LPA. 1999. Plant Resources of South-East Asia No 19: Essential-oil plants. PROSEA Foundation, Bogor, Indonesia.
159. Masyita A, Sari RM, Astuti AD, Yasir B, Rumata NR, Emran TB, Nainu F, Simal-Gandara J. 2022. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chemistry **13**: 100217.
160. Abdelgaleil SA, Gad HA, Ramadan GR, El-Bakry AM, El-Sabrout AM. 2021.Monoterpenes: chemistry, insecticidal activity against stored product insects and modes of action—a review. International Journal of Pest Management 1-23.
161. Shinde SS, Sarkate AP, Nirmal NP, Sakhale BK. 2023. Bioactivity, medicinal applications, and chemical compositions of essential oils: detailed perspectives - Recent Frontiers of Phytochemicals. Elsevier, Amsterdam.

162. Zuzarte M, Salgueiro L. 2015. Essential oils chemistry - Bioactive essential oils and cancer. Springer, Cham.
163. Ludwiczuk A, Skalicka-Wozniak K, Georgiev MI. 2017. Terpenoids - Pharmacognosy. Academic Press, Boston.
164. Sadgrove NJ, Padilla-Gonzalez GF, Phumthum M. 2022. Fundamental chemistry of essential oils and volatile organic compounds, methods of analysis and authentication. Plants **11**: 789.
165. Kant R, Kumar A. 2022. Review on essential oil extraction from aromatic and medicinal plants: techniques, performance, and economic analysis. Sustainable Chemistry and Pharmacy **30**: 100829.
166. Oreopoulou A, Tsimogiannis D, Oreopoulou V. 2019. Extraction of polyphenols from aromatic and medicinal plants: an overview of the methods and the effect of extraction parameters - Polyphenols in Plants. Academic Press, Watson.
167. Mohamadi M, Shamspur T, Mostafavi A. 2013. Comparison of microwave-assisted distillation and conventional hydrodistillation in the essential oil extraction of flowers *Rosa damascena* Mill. Journal of Essential Oil Research **25**: 55–61.
168. Putra NR, Yustisia Y, Heryanto RB, Asmaliyah A, Miswarti M, Rizkiyah DN, Yunus MAC, Irianto I, Qomariyah L, Rohman GAN. 2023. Advancements and challenges in green extraction techniques for Indonesian natural products: a review. South African Journal of Chemical Engineering **46**: 88–98.
169. Yousefi M, Rahimi-Nasrabadi M, Pourmortazavi SM, Wysokowski M, Jesionowski T, Ehrlich H, Mirsadeghi S. 2019. Supercritical fluid extraction of essential oils. TrAC Trends in Analytical Chemistry **118**: 182-93.
170. Waseem R, Low KH. 2015. Advanced analytical techniques for the extraction and characterization of plant-derived essential oils by gas chromatography with mass spectrometry. Journal of Separation Science **38**: 483-501.
171. Rowan DD. 2011. Volatile metabolites. Metabolites **1**: 41-63.
172. Malhotra P. 2023. High performance liquid chromatography - Analytical chemistry: basic techniques and methods. Springer International Publishing, Cham.
173. Sparkman OD, Penton Z, Kitson FG. 2011. Gas chromatography and mass spectrometry: a practical guide. Academic press, Oxford.
174. Marriott PJ, Shellie R, Cornwell C. 2001. Gas chromatographic technologies for the analysis of essential oils. Journal of Chromatography A **936**: 1–22.
175. Wilschefski SC, Baxter MR. 2019. Inductively coupled plasma mass spectrometry: introduction to analytical aspects. The Clinical Biochemist Reviews **40**: 115–133.
176. Bicchi C, Liberto E, Matteodo M, Sgorbini B, Mondello L, Zellner B, d'Acampora Costa R, Rubiolo P. 2008. Quantitative analysis of essential oils: a complex task. Flavour and Fragrance Journal **23**: 382–39.

177. Cachet T, Brevard H, Chaintreau A, Demyttenaere J, French L, Gassenmeier K, Joulain D, Koenig T, Leijs H, Liddle P, Loesing G, Marchant M, Merle PH, Saito K, Schippa C, Sekiya F, Smith T. 2016. IOFI recommended practice for the use of predicted relative-response factors for the rapid quantification of volatile flavouring compounds by GC-FID. Flavour and Fragrance Journal **31**: 191–194.

178. Stein SE. 1999. An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data. Journal of the American Society for Mass Spectrometry **10**: 770–781.

179. Scanlan FP. 2000. Flavours: gas chromatography - Encyclopaedia of Separation Science. Academic Press, Oxford.

180. Chialva F, Gabri G, Liddle PA, Ulian F. 1982. Qualitative evaluation of aromatic herbs by direct headspace GC analysis. Applications of the method and comparison with the traditional analysis of essential oils. Journal of High-Resolution Chromatography **5**: 182-8.

181. Houdkova M, Kokoska L. 2020. Volatile antimicrobial agents and *in vitro* methods for evaluating their activity in the vapour phase: A review. Planta Medica **86**: 822-57.

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

183. Kachur K, Suntres Z. 2020. The antibacterial properties of phenolic isomers, carvacrol and thymol. Critical Reviews in Food Science and Nutrition **60**: 3042–3053.

184. Da Silva WP, Lopes GV, Ramires T, Kleinubing NR. 2023. May phenolics mitigate the antimicrobial resistance in foodborne pathogens?. Current Opinion in Food Science **23**: 101107.

185. Erguden B. 2021. Phenol group of terpenoids is crucial for antibacterial activity upon ion leakage. Letters in Applied Microbiology **73**: 438-45.

186. Inouye S, Yamaguchi H, Takizawa T. 2001. Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. Journal of Infection and Chemotherapy **7**: 251-4.

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

188. Horvath G, Acs K. 2015. Essential oils in the treatment of respiratory tract diseases highlighting their role in bacterial infections and their anti-inflammatory action: a review. Flavour and Fragrance Journal, **30**: 331–341.

189. Houdkova M, Urbanova K, Doskocil I, Rondevaldova J, Novy P, Nguon S, Chrun R, Kokoska L. 2018. *In vitro* growth-inhibitory effect of Cambodian essential oils against pneumonia causing bacteria in liquid and vapour phase and their toxicity to lung fibroblasts. South African Journal of Botany **118**: 85-97.

190. Kamin W, Kieser M. 2007. Pinimenthol® ointment in patients suffering from upper respiratory tract infections – a post-marketing observational study. Phytomedicine **14**: 787–791.
191. Gowthami R, Sharma N, Pandey R, Agrawal A. 2021. Status and consolidated list of threatened medicinal plants of India. Genetic Resources and Crop Evolution **68**: 2235–2263.
192. Bhattacharyya R, Bhattacharya S, Chaudhuri S. 2006. Conservation and documentation of the medicinal plant resources of India. Biodiversity & Conservation **15**: 2705–2717.
193. Pandey MM, Rastogi S, Rawat AK. 2013. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evidence-based Complementary and Alternative Medicine **2013**: 1-12.
194. Jaiswal YS, Williams LL. 2016. A glimpse of Ayurveda - The forgotten history and principles of Indian traditional medicine. Journal of Traditional and Complementary Medicine *7*: 50–53.
195. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. 2005. Ayurveda and traditional Chinese medicine: a comparative overview. Evidence-Based Complementary and Alternative Medicine **2**: 465–473.
196. Pandey MM, Rastogi S, Rawat AKS. 2008. Indian herbal drug for general healthcare: an overview. The Internet Journal of Alternative Medicine **6**: 3.
197. Chauhan A, Semwal DK, Mishra SP, Semwal RB. 2015. Ayurvedic research and methodology: present status and future strategies. AYU (An International Quarterly Journal of Research in Ayurveda) **36**: 364-9.
198. Kumar, S, Dobos GJ, Rampp T. 2017. The significance of ayurvedic medicinal plants. Journal of Evidence-based Complementary & Alternative Medicine **22**: 494–501.
199. Perumal Samy R, Ignacimuthu S, Sen A. 1998. Screening of 34 Indian medicinal plants for antibacterial properties. Journal of Ethnopharmacology **62**: 173–181.
200. Kumar S, Dobos GJ, Rampp T. 2017. The significance of ayurvedic medicinal plants. Journal of evidence-based complementary & alternative medicine **22**: 494–501.
201. Chincholikar MB, Mahajon B, Tripathi AK. 2021. Ayurveda-based seasonal collection practices for selected medicinal plants: A scientific appraisal—book review. Journal of Drug Research in Ayurvedic Sciences **6**: 195-196.
202. Karthikeyan K, Dhanapal CK. 2016. GC-MS analysis of ethyl acetate extract of *Alysicarpus monilifer*-whole plant. Der Pharmacia Lettre **8**: 106-14.
203. Gholami A. 2015. Biology of *Alysicarpus monilifer* (L.) DC.: an important medicinal plant. Medicinal Plants **7**: 248–251.
204. Bashir M, Uzair M, Ahmad B. 2018. Ethnobotanical, phytochemical and pharmacological aspects of genus *Alysicarpus*. International Journal of Pharmacy **8**: 1-15.
205. Kasithevar M, Saravanan M, Prakash P, Kumar H, Ovais M, Barabadi H, Shinwari ZK. 2017. Green synthesis of silver nanoparticles using *Alysicarpus monilifer* leaf extract and its

antibacterial activity against MRSA and CoNS isolates in HIV patients. Journal of Interdisciplinary Nanomedicine **2**: 131-41.

206. Rathika K, Murali S, Arockiaraj SP, Angel P. 2018. Green synthesis of silver nanoparticle by using *Alysicarpus monilifer* plant extract; characherization of the particles and study of the antibacterial activity. International Journal of Research and Analytical Reviews **3**: 555-558.
207. Saleh-e-In MM, Sultana N, Hossain MN, Hasan S, Islam MR. 2016. Pharmacological effects of the phytochemicals of *Anethum sowa* L. root extracts. BMC Complementary and Alternative Medicine **16**: 1-4.
208. Jana S, Shekhawat GS. 2010. *Anethum graveolens*: An Indian traditional medicinal herb and spice. Pharmacognosy Reviews **4**: 179–184.
209. Saleh-E-In MM, Choi YE. 2021. *Anethum sowa* Roxb. ex fleming: A review on traditional uses, phytochemistry, pharmacological and toxicological activities. Journal of Ethnopharmacology **280**: 113967.
210. Banerjee S, Banerjee S, Jha GK, Bose S. 2021. *Barleria prionitis* L.: an illustrative traditional, phytochemical and pharmacological review. The Natural Products Journal **11**: 258-74.
211. Aneja KR, Joshi R, Sharma C. 2010. Potency of *Barleria prionitis* L. bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin. New York Science Journal **3**: 5-12.
212. Gangaram S, Naidoo Y, Dewir YH, El-Hendawy S. 2021. Phytochemicals and Biological Activities of *Barleria* (Acanthaceae). Plants **11**: 82.
213. Singh KD, Sharma D, Gupta RS. 2017. A comphrenensive review on *Barleria prionitis* (L). Asian Journal of Pharmaceutical and Clinical Research **10**: 22-29.
214. Das A, Bondya SL. 2016. *Cocculus hirsutus* (L.) Diels: diverse uses by the tribal population of Dumka. The Biobrio **3**: 190-3
215. Madhavan V, Ullah MS, Gurudeva MR, Yoganarasimhan SN. 2010. Pharmacognostical studies on the leaves of *Cocculus hirsutus* (Linn.) Diels–Chilahinta, an Ayurvedic drug. International Journal of Natural Products and Research **1**: 38-43.
216. Abiramasundari P, Priya V, Jeyanthi GP, Devi SG. 2012. Evaluation of the antibacterial activity of *Cocculus hirsutus*. Hygeia - Journal for Drugs and Medicine **3**: 26-31.
217. Gupta VK, Kaushik A, Chauhan DS, Ahirwar RK, Sharma S, Bisht D. 2018. Anti-mycobacterial activity of some medicinal plants used traditionally by tribes from Madhya Pradesh, India for treating tuberculosis related symptoms. Journal of Ethnopharmacology **227**: 113-20.
218. Satish V, Ravichandran VD, Usha G, Paarakh MP. 2010. Antimicrobial studies on the extracts of *Cocculus hirsutus* Linn. and *Hyptis suaveolens* Poit. Indian Journal of Natural Products and Resources **1**: 49-52.

219. Logesh R, Das N, Adhikari-Devkota A, Devkota HP. 2020. *Cocculus hirsutus* (L.) W.Theob. (Menispermaceae): a review on traditional uses. Phytochemistry and Pharmacological Activities. Medicines **7**: 69.

220. Patil V, Angadi S, Devdhe S, Wakte P. 2015. Recent progress in simultaneous estimation of Rutin, Quercetin and Liquiritin in *Cocculus hirsutus* by HPTLC. Research Journal of Pharmacognosy **2**: 49–55.

221. Ahmad VU, Mohammad FV, Rasheed T. 1987. Hirsudiol a triterpenoid from *Cocculus hirsutus*. Phytochemistry **26**: 793–794.

222. Al-Snafi AE. 2016. The contents and pharmacology of *Crotalaria juncea*- a review. IOSR Journal of Pharmacy **6**: 77-86.

223. Dinakaran SK, Banji D, Godala P, Harani A. 2011. Pharmacognostical evaluation study on *Crotalaria juncea* Linn. American-Eurasian Journal of Scientific Research **6**: 139-145.

224. Mahasawat P, Boukaew S, Prasertsan P. 2023. Exploring the potential of *Crotalaria juncea* flower extracts as a source of antioxidants, antimicrobials, and cytoprotective agents for biomedical applications. Biotechnologia **104**: 359–370.

225. Oladeji OS, Adelowo FE, Ayodele DT, Odelade KA. 2019. Phytochemistry and pharmacological activities of *Cymbopogon citratus*: a review. Scientific African **6**: e00137.

226. Karkala M, Bhushan B. 2014. Review on pharmacological activity of *Cymbopogon citratus*. International Journal of Herbal Medicine **1**: 5-7.

227. Edward MJ. 1997. American herbal products association's botanical safety handbook. CRC Press, Boca Raton.

228. Pereira RS, Sumita TC, Furlan MR, Jorge AOC, Ueno M. 2004. Antibacterial activity of essential oils on microorganisms isolated from urinary tract infections. Revista de Saude Publica **38**: 326–328.

229. Schweitzer B, Balazs VL, Molnar S, Szogi-Tatar B, Boszormenyi A, Palkovics T, Horvath G, Schneider G. 2022. Antibacterial effect of lemongrass (*Cymbopogon citratus*) against the aetiological agents of pitted keratolyis. Molecules **27**: 1423.

230. Naik MI, Fomda BA, Jaykumar E, Bhat JA. 2010. Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacteria. Asian Pacific Journal of Tropical Medicine **3**: 535–538.

231. Singh BR, Singh V, Singh RK, Ebibeni N. 2011. Antimicrobial activity of lemongrass (*Cymbopogon citratus*) oil against microbes of environmental, clinical and food origin. International Research Journal of Pharmacy and Pharmacology **9**: 228-36.

232. Premathilake UG, Wathugala DL, Dharmadasa RM. 2018. Evaluation of chemical composition and assessment of antimicrobial activities of essential oil of lemongrass (*Cymbopogon citratus* (DC.) Stapf). International Journal of Minor Fruits, Medicinal and Aromatic Plants **4**: 13-19.

233. Dangol S, Poudel DK, Ojha PK, Maharjan S, Poudel A, Satyal R, Rokaya A, Timsina S, Dosoky NS, Satyal P, Setzer WN. 2023. Essential oil composition analysis of *Cymbopogon* species from eastern Nepal by GC-MS and chiral GC-MS, and antimicrobial activity of some major compounds. Molecules **28**: 543.

234. Kumar A, Chahal KK, Kataria D. 2017. A review on phytochemistry and pharmacological activities of *Cyperus scariosus*. Journal of Pharmacognosy and Phytochemistry **6**: 510-7.

235. Nagarajan M, Kuruvilla GR, Kumar KS, Venkatasubramanian P. 2015. Pharmacology of Ativisha, Musta and their substitutes. Journal of Ayurveda and Integrative Medicine **2**: 121–133.

236. Clery RA, Cason JR, Zelenay V. 2016. Constituents of cypriol oil (*Cyperus scariosus* R.Br.): N-containing molecules and key aroma components. Journal of Agricultural and Food Chemistry **64**: 4566–4573.

237. Jha V, Patel R, Devkar S, Shaikh MA, Rai D, Walunj S, Koli J, Jain T, Jadhav N, Narvekar S, Shinde R. 2022. Chemical composition, bioactive potential, and thermal behaviour of *Cyperus scariosus* essential oil. Chemical Science International Journal **31**: 1-14.

238. Rahman MS, Anwar MN. 2008. Antibacterial and cytotoxic activity of longiverbenone isolated from the rhizome of *Cyperus scariosus.* Bangladesh Journal of Microbiology **25**: 82-84.

239. Srivastava RK, Singh A, Srivastava GP, Lehri A, Niranjan A, Tewari SK, Kumari K, Kumari S. 2014. Chemical constituents and biological activities of promising aromatic plant nagarmotha (*Cyperus scariosus* R. Br.): a review. Proceedings of the Indian National Science Academy **80**: 525-536.

240. Zhang LL, Zhang LF, Hu QP, Hao DL, Xu JG. 2017. Chemical composition, antibacterial activity of *Cyperus rotundus* rhizomes essential oil against *Staphylococcus aureus* via membrane disruption and apoptosis pathway. Food Control **80**: 290-6.

241. Siroua K, El Ghallab Y, Mouss RA, Kadiri F, Belamine H, El Kouali M, Kenz A. 2022. Chemical composition of essential oil from invasive Moroccan *Cyperus rotundus* L., in vitro antimicrobial and antiradical activities, and in silico molecular docking of major compounds on drug efflux pumps. South African Journal of Botany **147**: 782-9.

242. Reshma D, Anitha CT, Tharakan S. 2021. Phytochemical and pharmacological properties of five different species of *Jasminum*. Plant Archives **21**: 126-136.

243. Balkrishna A, Rohela A, Kumar A, Kumar A, Arya V, Thakur P, Oleksak P, Krejcar O, Verma R, Kumar D, Kuca K. 2021. Mechanistic insight into antimicrobial and antioxidant potential of *Jasminum* species: a herbal approach for disease management. Plants **10**: 1089.

244. Yadav E, Mani M, Chandra P, Sachan N, Ghosh AK. 2012. A review on therapeutic potential of *Lygodium flexuosum* Linn. Pharmacognosy Reviews **6**: 107–114.

245. Wills PJ, Asha VV. 2006. Protective effect of *Lygodium flexuosum* (L.) Sw. (Lygodiaceae) against d-galactosamine induced liver injury in rats. Journal of Ethnopharmacology **108**: 116–123.

246. Nayak N, Rath S, Mishra MP, Ghosh G, Padhy RN. 2013. Antibacterial activity of the terrestrial fern *Lygodium flexuosum* (L.) Sw. against multidrug resistant enteric- and uro-pathogenic bacteria. Journal of Acute Disease **2**: 270–276.

247. Borkotoky S, Borah VV. 2023. Antimicrobial potential of crude extract and fatty acid fractions of four pteridophyte species from Assam, Northeast India, and their identification by GC-MS. International Journal of Pharmaceutical Sciences and Research **9**: 4489-4508.

248. Pathania R, Chawla P, Khan H, Kaushik R, Khan MA. 2020. An assessment of potential nutritive and medicinal properties of *Mucuna pruriens*: a natural food legume. 3 Biotech **10**: 261.

249. Lampariello LR, Cortelazzo A, Guerranti R, Sticozzi C, Valacchi G. 2012. The magic velvet bean of *Mucuna pruriens*. Journal of traditional and complementary medicine **2**: 331–339.

250. Sathiyanarayanan L, Arulmozhi S, 2007. *Mucuna pruriens*. A comprehensive review. Pharmacognosy Reviews **1**: 157-162.

251. Stanley MC, Ifeanyi OE, Chinedum OK, Chinwe IA, Emmanuel O, Nwamaka ON. 2014. Antimicrobial activities of *Mucuna pruriens* (Agbara) on some human pathogens. IOSR Journal of Pharmacy and Biological Sciences 2278-3008.

252. Salau AO, Odeleye OM. 2007. Antimicrobial activity of *Mucuna pruriens* on selected bacteria. African Journal of Biotechnology **6**: 2092.

253. Chen L, Chen S, Sun P, Liu X, Zhan Z, Wang J. 2023. *Psoralea corylifolia* L.: a comprehensive review of its botany, traditional uses, phytochemistry, pharmacology, toxicology, quality control and pharmacokinetics. Chinese Medicine **18**: 4.

254. SJc B. 2015. Systema Naturae The Netherlands, Amsterdam.

255. Baig MMV. 2022. Phytochemical and antimicrobial activity screening of seeds of *Psoralea corylifolia* L. Phytomedicine Plus **2**: 100278.

256. Prasad NR, Anandi C, Balasubramanian S, Pugalendi KV. 2004. Antidermatophytic activity of extracts from *Psoralea corylifolia* (Fabaceae) correlated with the presence of a flavonoid compound. Journal of Ethnopharmacology **91**: 21-4.

257. Cui Y, Taniguchi S, Kuroda T, Hatano T. 2015. Constituents of *Psoralea corylifolia* fruits and their effects on methicillin-resistant *Staphylococcus aureus*. Molecules **7**: 12500-12511.

258. Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhtar S, Alsharif MA, Parveen H, Zakaria ZA. 2022. Antibacterial effects of flavonoids and their structure-activity relationship study: a comparative interpretation. Molecules **4**: 1149.

259. Lim JS, Kim JY, Lee S, Choi JK, Kim EN, Choi YA, Jang YH, Jeong GS, Kim SH. 2020. Bakuchicin attenuates atopic skin inflammation. Biomedicine & Pharmacotherapy **129**: 110466.

260. Sun L, Tang Z, Wang M, Shi J, Lin Y, Sun T, Zou Z, Weng Z. 2022. Exploration of antimicrobial ingredients in *Psoralea corylifolia* L. seed and related mechanism against methicillin-resistant *Staphylococcus aureus*. Molecules **20**: 6952.

261. He N, Zhou J, Hu M, Ma C, Kang W. 2018. The mechanism of antibacterial activity of corylifolinin against three clinical bacteria from *Psoralen corylifolia* L. Open Chemistry **1**: 882-889.

262. Assis LR, Theodoro RDS, Costa MBS, Nascentes JAS, Rocha MDD, Bessa MAS, Menezes RP, Dilarri G, Hypolito GB, Santos VRD, Duque C, Ferreira H, Martins CHG, Regasini LO. 2022. Antibacterial activity of Isobavachalcone (IBC) is associated with membrane disruption. Membranes **3**: 269.

263. Ren Y, Song X, Tan L, Guo C, Wang M, Liu H, Cao Z, Li Y, Peng C. 2020. A review of the pharmacological properties of psoralen. Frontiers in Pharmacology **11**: 571535.

264. Katsura H, Tsukiyama RI, Suzuki A, Kobayashi M. 2001. *In vitro* antimicrobial activities of bakuchiol against oral microorganisms. Antimicrobial Agents and Chemotherapy **11**: 3009–3013.

265. Nizam NN, Mahmud S, Ark SA, Kamruzzaman M, Hasan MK. 2023. Bakuchiol, a natural constituent and its pharmacological benefits. F1000Research **12**: 1-24.

266. Zhang X, Zhao W, Wang Y, Lu J, Chen X. 2016. The chemical constituents and bioactivities of *Psoralea corylifolia* Linn.: a review. The American Journal of Chinese Medicine **44**: 35-60.

267. Chakrabarty T, Krishna G, Rasingam L. 2019. Taxonomic notes on Indian Terminalia (Combretaceae). Plant Science Today **6**: 281-6.

268. Jain VC, Patel NM, Shah DP, Patel PK, Joshi BH. 2010. Antioxidant and antimicrobial activities of *Terminalia crenulata* Roth bark. Pharmacology **2**: 204-17.

269. Goyal S, Chaturvedi V, Dhingra G, Tawar S, Sharma K, Singh S. 2022. *Trachyspermum ammi*: a review on traditional and modern pharmacological aspects. Biological Sciences **2**: 324–37.

270. Paul S, Dubey RC, Maheswari DK, Kang SC. 2011. *Trachyspermum ammi* (L.) fruit essential oil influencing on membrane permeability and surface characteristics in inhibiting food-borne pathogens. Food Control **22**: 725-731.

271. Javan AJ, Salimiraad S, Khorshidpour B. 2019. Combined effect of *Trachyspermum ammi* essential oil and propolis ethanolic extract on some foodborne pathogenic bacteria. In Veterinary Research Forum **10**: 235.

272. Gradinaru AC, Trifan A, Spac M, Brebu A, Miron AC. 2018. Aprotosoaie, Antibacterial activity of traditional spices against lower respiratory tract pathogens: combinatorial effects

of *Trachyspermum ammi* essential oil with conventional antibiotics. Letters in Applied Microbiology **67**: 449–457.

273. Dutta S, Kundu A, Saha S, Prabhakaran P, Mandal A. 2020. Characterization, antifungal properties and in silico modelling perspectives of *Trachyspermum ammi* essential oil. LWT **131**: 109786.
274. Moein MR, Zomorodian K, Pakshir K, Yavari F, Motamedi M, Zarshenas MM. 2015. *Trachyspermum ammi* (L.) sprague: chemical composition of essential oil and antimicrobial activities of respective fractions. Journal of Evidence-based Complementary & Alternative Medicine **20**: 50-6.
275. Sabu A, Haridas M. 2015. Fermentation in ancient Ayurveda: Its present implications. Frontiers in Life Science **8**: 324-31.
276. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Bampidis V, Azimonti G, Bastos MD, Christensen H, Fasmon Durjava M, Kouba M, Lopez-Alonso M, Lopez Puente S, Marcon F, Mayo B. 2023. Safety and efficacy of a feed additive consisting of a tincture derived from the fruit of *Anethum graveolens* L. (dill tincture) for use in all animal species (FEFANA asbl). EFSA Journal **21**: e07691.
277. Chandrasekar R, Sivagami B. 2016. Alternative treatment for psoriasis-A review. International Journal of Research and Development in Pharmacy & Life Sciences **5**: 2188-97.
278. Gupta N, Jain UK. 2011. An update-prominent wound healing property of indigenous medicines. Research Journal of Pharmacy and Technology **2**: 203-13.
279. Puri HS. 1970. Indian pteridophytes used in folk remedies. American Fern Journal **60**: 137-43.
280. Khushboo PS, Jadhav VM, Kadam VJ, Sathe NS. 2010. *Psoralea corylifolia* Linn.—"Kushtanashini". Pharmacognosy reviews **4**: 69.
281. Chincholikar MB, Mahajon B, Tripathi AK. 2021. Ayurveda-based seasonal collection practices for selected medicinal plants: A scientific appraisal—book review. Journal of Drug Research in Ayurvedic Sciences **6**: 195-196.
282. Palekar S, Joshi A. 2019. Standardization of ova Ark: An effort to standardise formulation from grandma's pouch. Journal of Pharmacognosy and Phytochemistry **8**: 4801-6.
283. Cos P, Vlietinck AJ, Berghe DV, Maes L. 2006. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. Journal of Ethnopharmacology **106**: 290–302.
284. AOAC International. 2012. Official Methods of Analysis, Official Method 925.10 - Association of Official Analytical Chemists, Gaithersburg.
285. European Pharmacopoeia. 2013. Convention on the Elaboration of a European Pharmacopoeia (7th ed.), Strasbourg.
286. Clinical and Laboratory Standards Institute (CLSI). 2015. Performance Standards for Antimicrobial Susceptibility Testing. 25th Informational Supplement M100-S25, Wayne.

287. Clinical and Laboratory Standards Institute (CLSI). 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests (11th ed.). Approved Standard, CLSI Document M02-A11, Wayne.

288. Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods **65**: 56–63.

289. Houdkova M, Albarico G, Doskocil I, Tauchen J, Urbanova K, Tulin EE, Kokoska L. 2020. Vapors of volatile plant-derived products significantly affect the results of antimicrobial, antioxidative and cytotoxicity microplate-based assays. Molecules **25**: 6004.

290. Special Programme for Research and Training in Tropical Diseases. Available online: **http://www.who.int/tdr/grants/workplans/en/cytotoxicity_invitro.pdf** (accessed on 21 December 2022).

291. Kokjohn K, Bradley M, Griffiths B, Ghannoum, M. 2003. Evaluation of *in vitro* activity of ciclopirox olamine, butenafine HCl and econazole nitrate against dermatophytes, yeasts and bacteria. International Journal of Dermatology **42**: 11–17.

292. Trevor AJ, Katzung BG, Kruidering-Hall M. 2015. Katzung and Trevor's pharmacology examination and board review (11th ed.). McGraw-Hill Education, New York.

293. Antih J, Houdkova M, Urbanova K, Kokoska L. 2021. Antibacterial activity of *Thymus vulgaris* L. essential oil vapours and their GC/MS analysis using solid-phase microextraction and syringe headspace sampling techniques. Molecules **26**: 6553.

294. Muller P, Milton M. 2012. The determination and interpretation of the therapeutic index in drug development. Nature Reviews Drug Discovery **11**: 751–761.

295. Couladis M, Chinou IB, Tzakou O, Petrakis PV. 2003. Composition and antimicrobial activity of the essential oil of *Hypericum rumeliacum* subsp. apollinis (Boiss. Heldr.), Phytotherapy Research **2**: 152-154.

296. Adams RP. 2007. Identification of essential oil components by gas chromatography/mass spectorscopy, 4th ed.; Allured Publishing Corp: Carol Stream, IL, USA.

297. National Institute of Standards and Technology. Available online: https://www.nist.gov/ (accessed on 27 March 2023).

298. Gkinis G, Tzakou O, Iliopoulou D, Roussis V. 2003. Chemical Composition and Biological Activity of *Nepeta parnassica* Oils and Isolated Nepetalactones, Zeitschrift fur Naturforschung **58**: 681-686.

299. Gudzic B, Dordevic S, Palic R, Stojanovic G. 2001. Essential oils of *Hypericum olympicum* L. and Hypericum perforatum L., Flavour and Fragrance Journal **3**: 201-203.

300. Li HN, Wang CY, Wang CL, Chou CH, Leu YL, Chen BY. 2019. Antimicrobial effects and mechanisms of ethanol extracts of *Psoralea corylifolia* seeds against *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus*. Foodborne Pathogens and Disease **16**: 573-80.

301. Sharath K, Kowmudi V, Suresh N, Rao CSR. 2016. Evaluation of antibacterial and anti-fungal activity of hexane and methanol extracts of *Psoralea Corylifolea* Seed. International Journal of Applied Pharmaceutical Sciences and Research **1**: 25-30.
302. Lee HS, Kim Y. 2017. *Paeonia lactiflora* inhibits cell wall synthesis and triggers membrane depolarization in *Candida albicans*. Journal of Microbiology and Biotechnology **27**: 395-404.
303. Putri NM, Putri JR, Elya B, Adawiyah R. 2020. Antifungal activity of *Polyscias scutellaria* Fosberg leaves against *Candida albicans*. Pharmaceutical Sciences and Research **7**: 7.
304. Nordin MA, Abdul Razak F, Himratul-Aznita WH. 2015. Assessment of antifungal activity of bakuchiol on oral-associated *Candida* spp. Evidence-Based Complementary and Alternative Medicine **2015**: 918624.
305. Nayak S, Singhai AK. 2003. Antimicrobial activity of the roots of *Cocculus hirsutus*. Ancient science of life **22**: 101–105.
306. Jeyachandran R, Mahesh A, Cindrella L, Baskaran X. 2008. Screening antibacterial activity of root extract of *Cocculus hirsutus* (L.). Journal of Plant Sciences **3**: 194-198.
307. Sowdhanya D, Singh J, Rasane P, Kaur S, Kaur J, Ercisli S, Verma H. 2023. Nutritional significance of velvet bean (*Mucuna pruriens*) and opportunities for its processing into value-added products. Journal of Agriculture and Food Research **15**: 100921.
308. Shanmugavel G, Krishnamoorthy G. *In vitro* evaluation of the antibacterial activity of alcoholic extract from *Mucuna pruriens* seed. International Journal of Herbal Medicine **2**: 7-9.
309. Ogunremi O, Ishola O, Ogunedina H. 2018. Phytochemical screening and *in vitro* antimicrobial activity of aqueous and ethanol extracts from *Mucuna pruriens* husks against some foodborne microorganisms. Food and Environment Safety Journal **17:** 233-240.
310. Reyes-Jurado F, Franco-Vega A, Ramirez-Corona N, Palou E, Lopez-Malo A. 2015. Essential oils: Antimicrobial activities, extraction methods and their modeling. Food Engineering Reviews **7**: 275–297.
311. Novy P, Kloucek P, Rondevaldova J, Havlik J, Kourimska L, Kokoska L. 2014. Thymoquinone vapor significantly affects the results of *Staphylococcus aureus* sensitivity tests using the standard broth microdilution method. Fitoterapia **94**: 102–107.
312. European Committee on Antimicrobial Susceptibility Testing. Antimicrobial Susceptibility Testing EUCAST Disk Diffusion Method, Version 9. Available online: **https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2021_manuals/Manual_v_9.0_EUCAST_Disk_Test_2021.pdf**
313. Clinical and Laboratory Standards Institute (CLSI). 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (10th ed.). CLSI document M07-A10, Wayne.

314. National Committee for Clinical Laboratory Standards (NCCLS). 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that growth aerobically - Approved standard (6th ed.). NCCLS document M7-A6, Wayne.

315. Food and Drug Administration (FDA). 2009. Guidance for industry and FDA. Class II special controls guidance document: antimicrobial susceptibility test (AST) systems. Center for devices and radiological health, Rockville.

316. International Organization for Standardization (ISO). 2019. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility devices - Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases (2nd ed.), Geneva.

317. Houdkova M, Chaure A, Doskocil I, Havlik J, Kokoska L. 2021. New broth macrodilution volatilization method for antibacterial susceptibility testing of volatile agents and evaluation of their toxicity using modified MTT assay *in vitro*. Molecules **26**: 4179.

318. Vitali LA, Beghelli D, Nya PC, Bistoni O, Cappellacci L, Damiano S, Lupidi G, Maggi F, Orsomando G, Papa F, Petrelli D. 2016. Diverse biological effects of the essential oil from Iranian *Trachyspermum ammi*. Arabian Journal of Chemistry **9**: 775-86.

319. Gardener AC, Trifan A, Spac A, Brebu M, Miron A, Aprotosoaie AC. 2018. Antibacterial activity of traditional spices against lower respiratory tract pathogens: Combinatorial effects of *Trachyspermum ammi* essential oil with conventional antibiotics. Letters in Applied Microbiology **67**: 449–457.

320. Modareskia M, Fattahi M, Mirjalili MH. 2022. Thymol screening, phenolic contents, antioxidant and antibacterial activities of Iranian populations of *Trachyspermum ammi* (L.) Sprague (Apiaceae). Scientific Reports **12**: 15645.

321. Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LGM, Wright VA. 1998. Characterization of the action of selected essential oil components on gram-negative bacteria. Journal of Agricultural and Food Chemistry **46**: 3590–5.

322. Walsh SE, Maillard JY, Russell AD, Catrenich CE, Charbonneau DL, Bartolo RG. 2003. Activity and mechanisms of action of selected biocidal agents on gram-positive and -negative bacteria. Journal of Applied Microbiology **94**: 240–7.

323. Oussalah M, Caillet S, Saucier L, Lacroix M. 2007. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157: H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control **18**: 414–20.

324. Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology **91**: 453–62.

325. Gill AO, Holley RA. 2006. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. International Journal of Food Microbiology **108**: 1–9.

326. Vazirian M, Hekmati D, Ostad S, Manayi A. 2019.Toxicity evaluation of essential oil of *Trachyspermum ammi* in acute and sub-chronic toxicity experiments. Journal of Medicinal Plants **18**: 70–77.

327. ECHA. European Chemicals Agency. Available online: **https://echa.europa.eu/** (accessed on 26 February 2023).

328. Xie K, Tashkin DP, Luo MZ, Zhang JY. 2019. Chronic toxicity of inhaled thymol in lungs and respiratory tracts in mouse model. Pharmacology Research & Perspectives **7**: e00516.

329. Valkova V, Duranova H, Galovicova L, Borotova P, Vukovic NL, Vukic M, Kacaniova M. 2022. *Cymbopogon citratus* essential oil: Its application as an antimicrobial agent in food preservation. Agronomy **12**: 155.

330. Acs K, Balazs VL, Kocsis B, Bencsik T, Boszormenyi A, Horvath G. 2018. Antibacterial activity evaluation of selected essential oils in liquid and vapor phase on respiratory tract pathogens. BMC Complementary and Alternative Medicine **18**: 1-9.

331. Madi YF, Choucry MA, Meselhy MR, El-Kashoury EA. 2021. Essential oil of *Cymbopogon citratus* cultivated in Egypt: seasonal variation in chemical composition and anticholinesterase activity. Natural Product Research **35**: 4063-4067.

332. Silva TLMD, Rosa GID, Santos MALD, Graf SL, Maia BHLDNS, Beltrame FL, Ferrari PC. 2023. Lemongrass essential oil (*Cymbopogon citratus* (DC) Stapf.) seasonal evaluation and microencapsulation by spray-drying. Brazilian Archives of Biology and Technology **66**: e23230016.

333. Antih J (2024) Chemical composition and in vitro antibacterial effects of vapours of essential oils from plants recommended by the European Medicines Agency against respiratory infections [Ph.D]. Prague: Czech University of Life Sciences Prague. 80p.

334. Gao S, Liu G, Li J, Chen J, Li L, Li Z, Zhang X, Zhang S, Thorne RF, Zhang S. 2020. Antimicrobial activity of lemongrass essential oil (*Cymbopogon flexuosus*) and its active component citral against dual-species biofilms of *Staphylococcus aureus* and *Candida* species. Frontiers in Cellular and Infection Microbiology **10**: 603858.

335. Viktorova J, Stupak M, Rehorova K, Dobiasova S, Hoang L, Hajslova J, Van Thanh T, Van Tri L, Van Tuan N, Ruml T. 2020. Lemon grass essential oil does not modulate cancer cells multidrug resistance by citral—its dominant and strongly antimicrobial compound. Foods **9**: 585.

336. Aiemsaard J, Aiumlamai S, Aromdee C, Taweechaisupapong S, Khunkitti W. 2011. The effect of lemongrass oil and its major components on clinical isolate mastitis pathogens and their

mechanisms of action on *Staphylococcus aureus* DMST 4745. Research in Veterinary Science **91**: e31-7.

337. Toukourou H, Gbaguidi F, Quetin-Leclercq J. 2019. Phytochemical composition, antibacterial activity against sore throat pathogens and toxicological evaluation of *Cymbopogon citratus* essential oil from Benin. Journal of Pharmacognosy and Phytochemistry **8**: 3258–3263.
338. Gaworski CL, Vollmuth TA, York RG, Heck JD, Aranyi C. 1992. Developmental toxicity evaluation of inhaled citral in Sprague-Dawley rats. Food and Chemical Toxicology **30**: 269–275.
339. Lulekal E, Tesfaye S, Gebrechristos S, Dires K, Zenebe T, Zegeye N, Feleke G, Kassahun A, Shiferaw Y, Mekonnen A. 2019. Phytochemical analysis and evaluation of skin irritation, acute and sub-acute toxicity of *Cymbopogon citratus* essential oil in mice and rabbits. Toxicology Reports **6**: 1289–1294.
340. Bhawna K, Sharma SK, Singh L, Mohapatra S, Singh T. 2013. *Cyperus scariosus*: A potential herb. International Research Journal of Pharmacy **4**: 17–20.
341. Beigi M, Torki-Harchegani M, Ghasemi Pirbalouti A. 2018. Quantity and chemical composition of essential oil of peppermint (*Mentha × piperita* L.) leaves under different drying methods. International Journal of Food Properties **21**: 267–276.
342. Caputo L, Amato G, de Bartolomeis P, De Martino L, Manna F, Nazzaro F, De Feo V, Barba AA. 2022. Impact of drying methods on the yield and chemistry of *Origanum vulgare* L. essential oil. Scientific Reports **12**: 3845.
343. Howyzeh MS, Noori SAS, Shariati JV, Niazian M. 2018. Essential oil chemotype of Iranian ajowan (*Trachyspermum ammi* L.). Journal of Essential Oil Bearing Plants **21**: 273–276.
344. Mirniyam G, Rahimmalek M, Arzani A, Matkowski A, Gharibi S, Szumny A. 2022. Changes in essential oil composition, polyphenolic compounds and antioxidant capacity of ajowan (*Trachyspermum ammi* L.) populations in response to water deficit. Foods **11**: 3084.
345. El-Kased RF, El-Kersh DM. 2022. GC–MS profiling of naturally extracted essential oils: antimicrobial and beverage preservative actions. Life **12**: 1587.
346. Hanaa AM, Sallam YI, El-Leithy AS, Aly SE. 2012. Lemongrass (*Cymbopogon citratus*) essential oil as affected by drying methods. Annals of Agricultural Sciences **57**: 113-6.
347. Majewska E, Kozlowska M, Gruszczynska-Sekowska E, Kowalska D, Tarnowska K. 2019. Lemongrass (*Cymbopogon citratus*) essential oil: extraction, composition, bioactivity and uses for food preservation-a review. Polish Journal of Food and Nutrition Sciences **69**: 113-116.
348. Kirchkeszner C, Petrovics N, Szeles A, Koshman Y, Szabo BS, Nyiri Z, Novak M, Rikker T, Eke Z. 2024. Comprehensive study of retention influencing gas chromatographic parameters affecting linear retention indices. Journal of Chromatography A **1729**: 465052.
349. Kumar A, Niranjan A, Lehri A, Srivastava RK, Tewari SK. 2016. Effect of geographical climatic conditions on yield, chemical composition and carbon isotope composition of

nagarmotha (*Cyperus scariosus* R. Br.) essential oil. Journal of Essential Oil Bearing Plants **19**: 368-73.

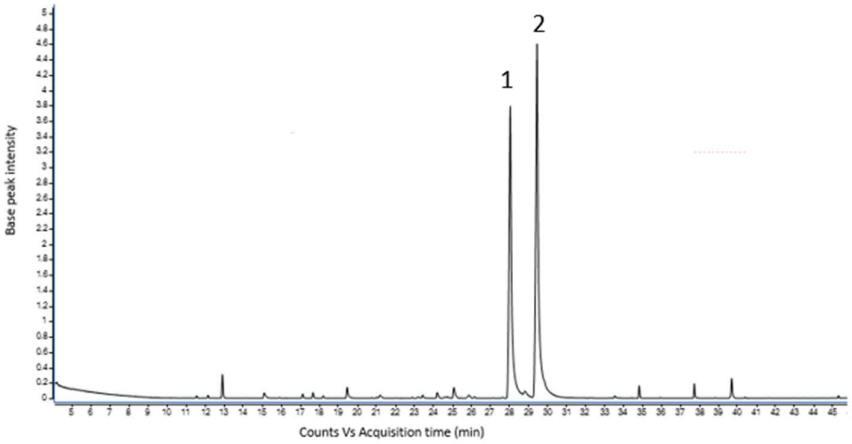
350. Netopilova M, Houdkova M, Urbanova K, Rondevaldova J, Kokoska L. 2021. Validation of qualitative broth volatilization checkerboard method for testing of essential oils: Dual-column GC–FID/MS analysis and in vitro combinatory antimicrobial effect of Origanum vulgare and Thymus vulgaris against Staphylococcus aureus in liquid and vapor phases. Plants **10**: 393.
351. Liu LL, Wang Q, Tang J, Zhang ZF. 2013. Component analysis of volatile oil from fruits of *Trachyspermum ammi* by headspace solid-phase microextraction GC-MS. Chinese Journal of Pharmaceutical Analysis **33**: 607-10.
352. Perestrelo R, Silva CL, Rodrigues F, Caldeira M, Camara JS. 2016. A powerful approach to explore the potential of medicinal plants as a natural source of odour and antioxidant compounds. Journal of Food Science and Technology **53**: 132–144.
353. Phillips CA, Gkatzionis K, Laird K, Score J, Kant A, Fielder MD. 2012. Identification and quantification of the antimicrobial components of a citrus essential oil vapor. Natural Product Communications **7**: 1934578X1200700135.
354. Guerrini A, Tacchini M, Chiocchio I, Grandini A, Radice M, Maresca I, Paganetto G, Sacchetti G. 2023. A comparative study on chemical compositions and biological activities of four amazonian Ecuador essential oils: *Curcuma longa* L. (Zingiberaceae), *Cymbopogon citratus* (DC.) Stapf, (Poaceae), *Ocimum campechianum* Mill. (Lamiaceae), and *Zingiber officinale* Roscoe (Zingiberaceae). Antibiotics **12**: 177.
355. Richter TM, Eyres GT, Silcock P, Bremer PJ. 2017. Comparison of four extraction methods for analysis of volatile hop-derived aroma compounds in beer. Journal of Separation Science **40**: 4366-4376.
356. Tian H, Yang X, Ho CT, Huang Q, Song S. 2013. Development of a solid phase microextraction protocol for the GC-MS determination of volatile off-flavour compounds from citral degradation in oil-in-water emulsions. Food Chemistry **141**: 131-138.
357. Loffler RJG, Hanczyc MM, Gorecki J. 2021. A perfect plastic material for studies on self-propelled motion on the water surface. Molecules **26**: 3116.
358. Loffler RJ, Hanczyc MM, Gorecki J. 2019. A hybrid camphor–camphene wax material for studies on self-propelled motion. Physical Chemistry Chemical Physics **21**: 24852-24856.
359. Nakata S, Doi Y, Hayashima Y. 2002. Intermittent motion of a camphene disk at the center of a cell. The Journal of Physical Chemistry B **106**: 11681-11684.
360. Wu KG, Lin YH, Chai XH, Duan XJ, Zhao XX, Chun C. 2019. Mechanisms of vapor-phase antibacterial action of essential oil from *Cinnamomum camphora* var. linaloofera Fujita against *Escherichia coli*. Food Science & Nutrition **7**: 2546–2555.

361. Ibanez MD, Sanchez-Ballester NM, Blazquez MA. 2020. Encapsulated limonene: a pleasant lemon-like aroma with promising application in the agri-food industry. a review. Molecules **11**: 2598.

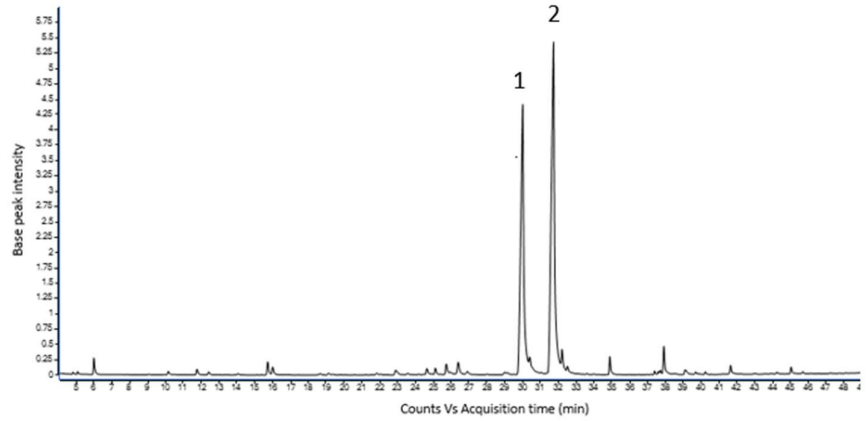
11. APPENDICES

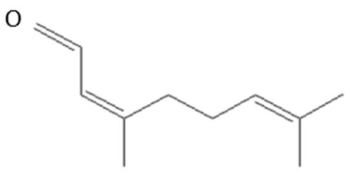
APPENDIX 1: Chromatograms and structures of the predominant compounds in the EO samples.

8a)

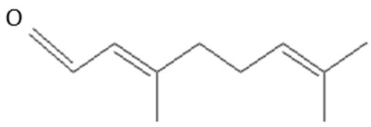


8b)





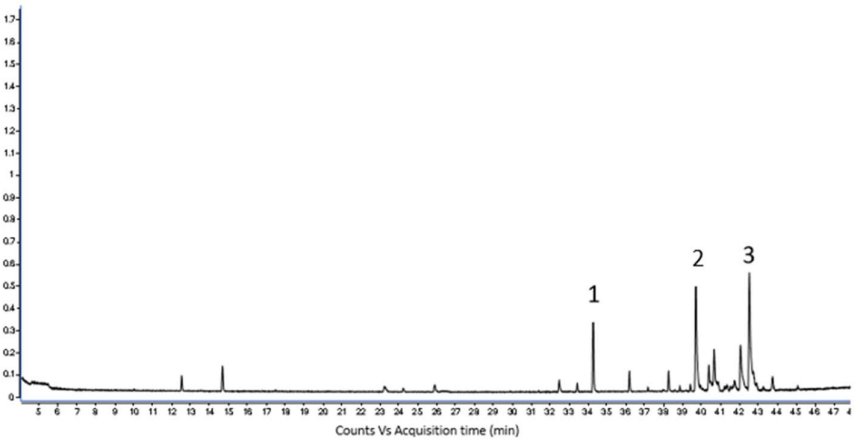
1. β-Citral

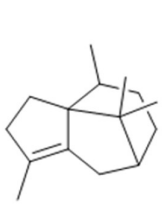


2. α-Citral

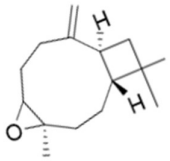
Figure 8. GC chromatograms of *Cymbopogon citratus* essential oil analysed by HP-5MS (8a) and DB-wax (8b) columns

9)

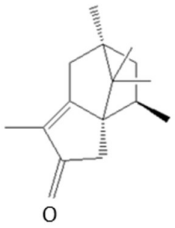




1. Cyperene



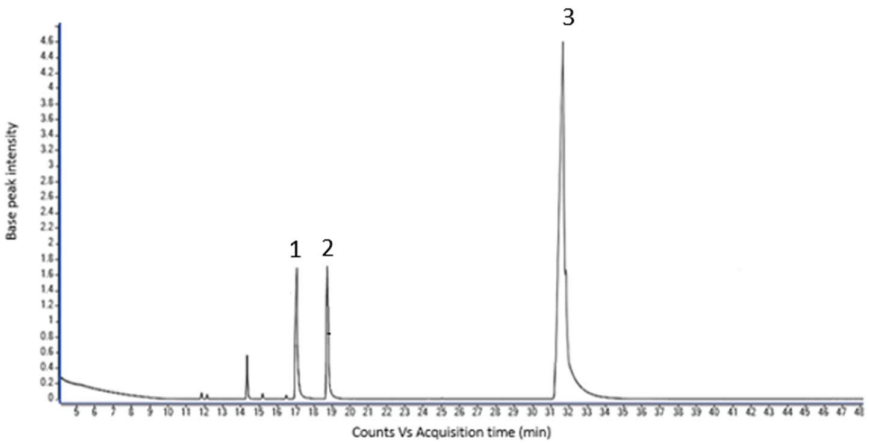
2. Caryophyllene oxide



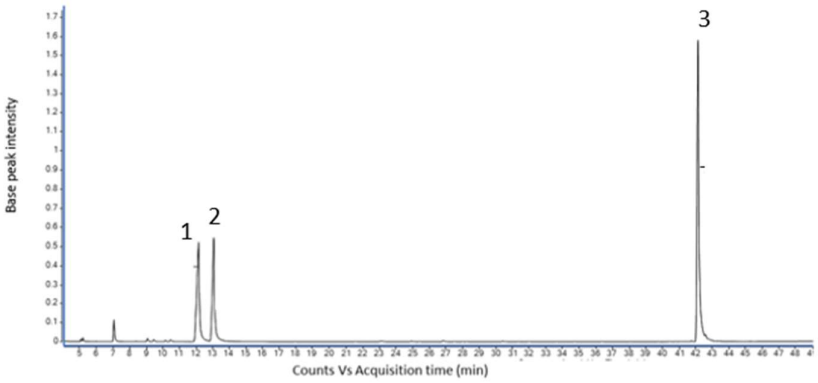
3. Cyperotundone

Figure 9. GC chromatograms of *Cyperus scariosus* essential oil analysed by HP-5MS.

10a)



10b)



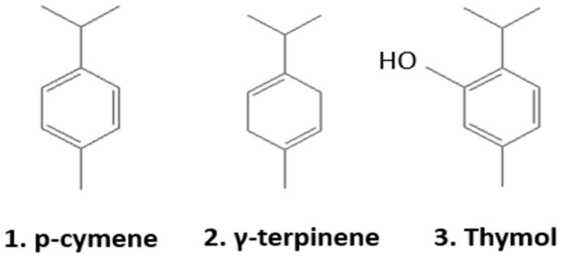
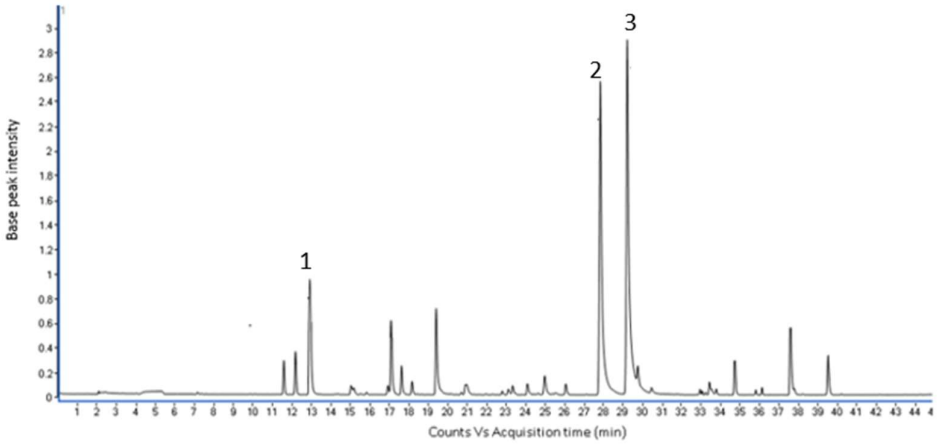
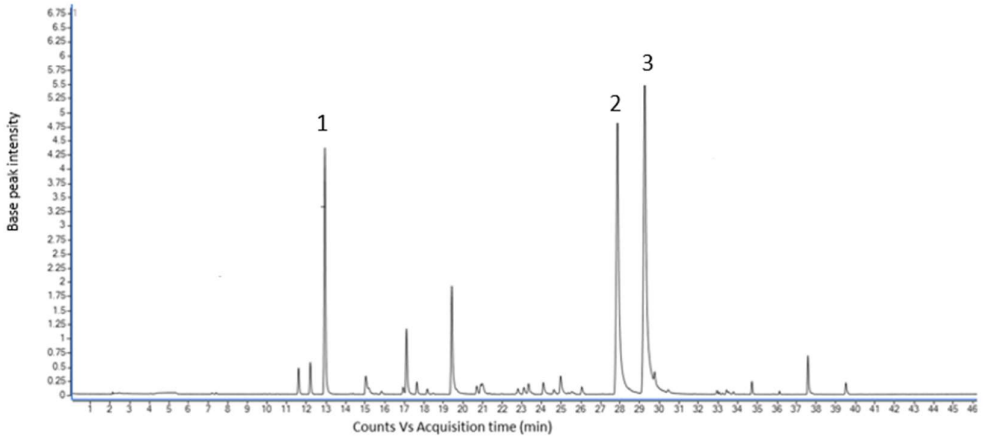


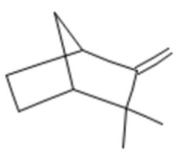
Figure 10. GC chromatograms of *Trachyspermum ammi* essential oil analysed by HP-5MS (10a) and DB-wax (10b) columns

11a)

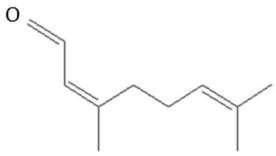


11b)

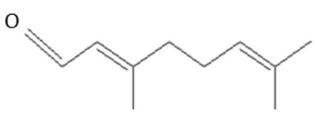




1. Camphene

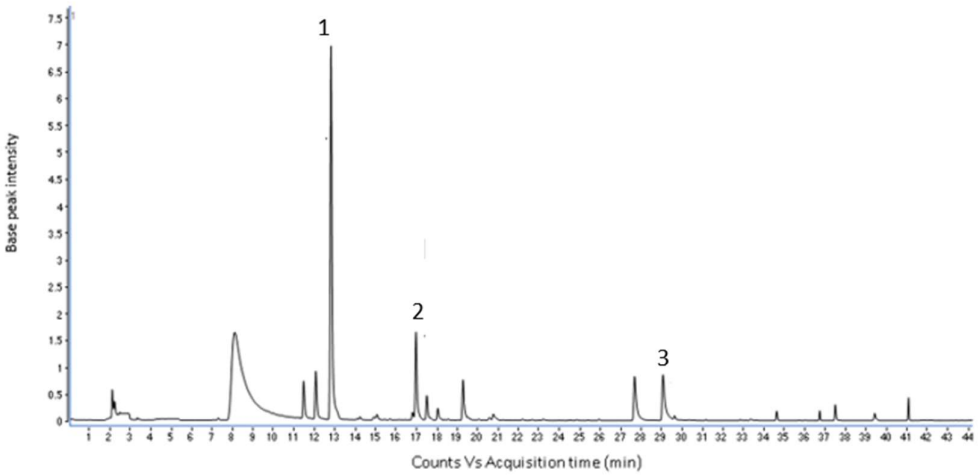


2. β-Citral

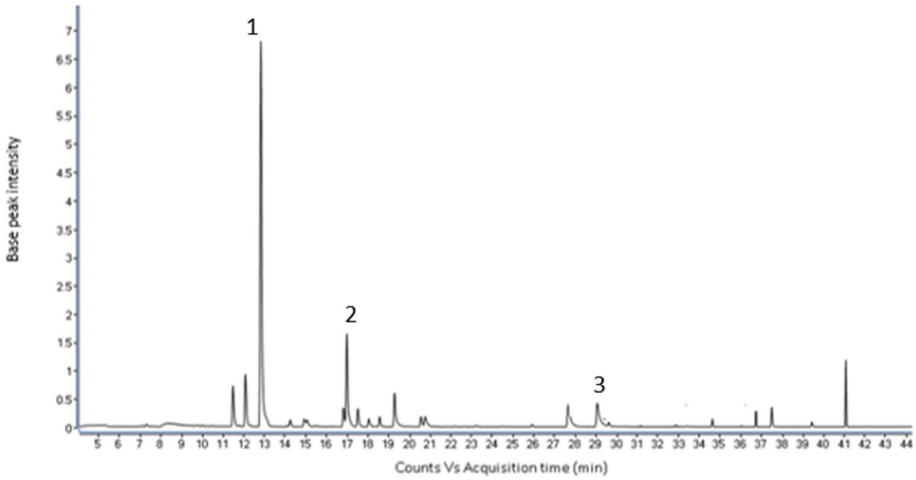


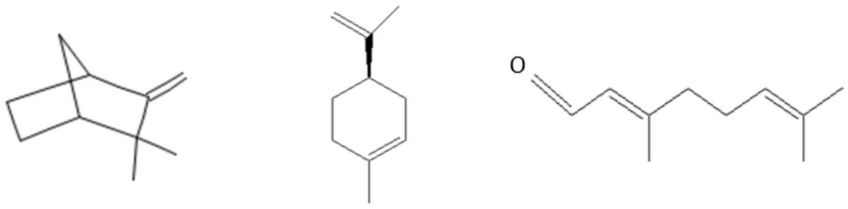
3. α-Citral

11c)



11d)

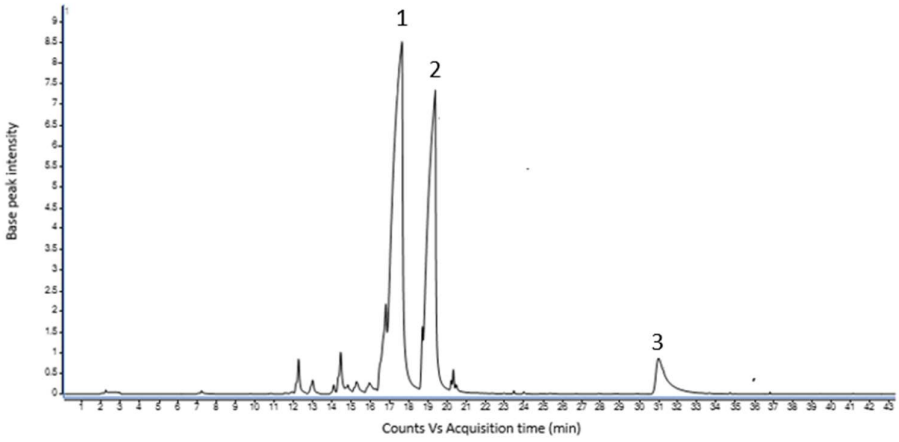




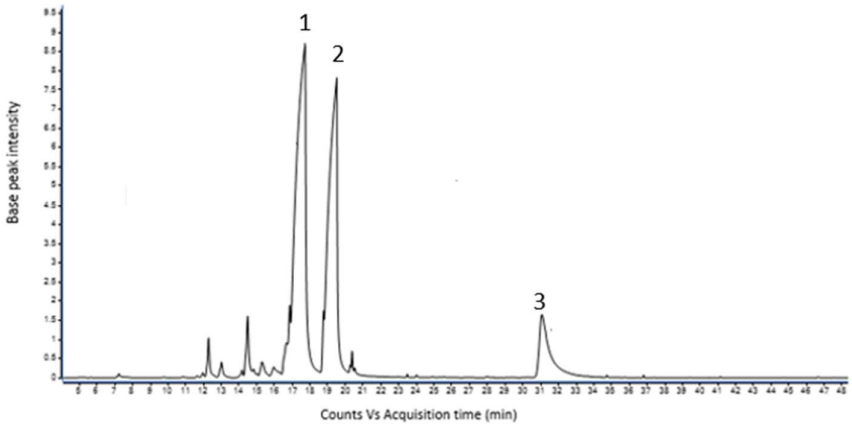
1. Camphene **2. D-Limonene** **3. α-Citral**

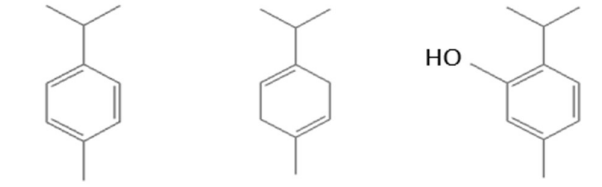
Figure 11. GC chromatogram of the headspace above a mixture MH broth and *Cymbopogon citratus* EO at a concentration of 256 µg/mL using HS-SPME at time zero (11a) and (11b) at 6-hour incubation. For HS-GTS t-0 (11c) and t-6 (11d) analysed by HP-5MS column.

12a)



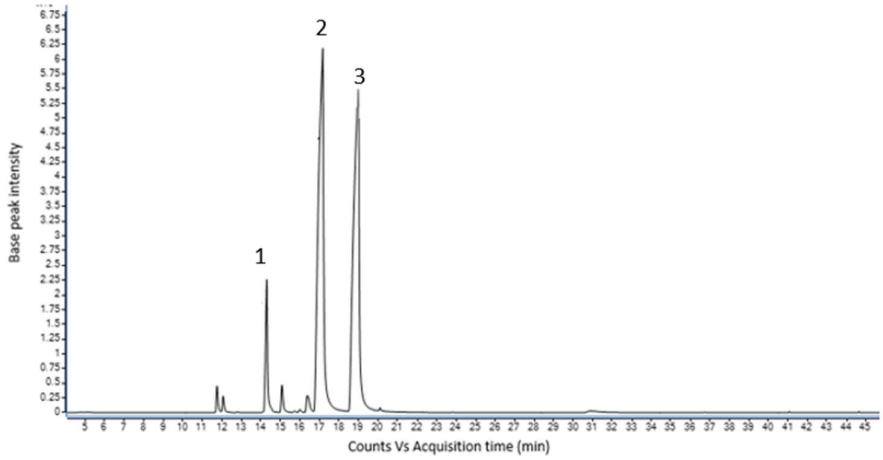
12b)



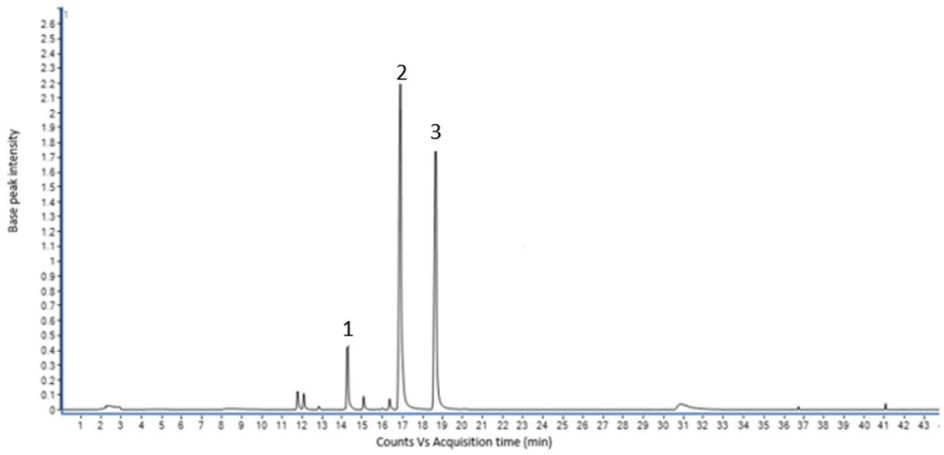


1. p-cymene **2. γ-terpinene** **3. Thymol**

12c)



12d)



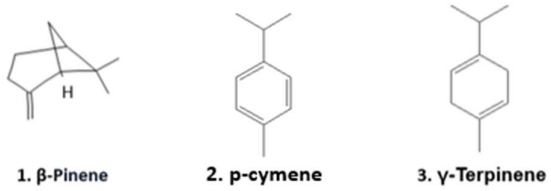


Figure 12. GC chromatogram of the headspace above a mixture MH broth and *Trachyspermum ammi* EO at a concentration of 256 µg/mL using HS-SPME at time zero (12a) and (12b) at 6-hour incubation. For HS-GTS t-0 (12c) and t-6 (12d) analysed by HP-5MS column.

APPENDIX 2: Curriculum vitae

PERSONALIA

Name	Aishwarya Chaure
Address	Raipur, India
Mobile	+420 770699695
E-mail	aishwarya.anmol@gmail.com
Date of birth	27 March 1990
Nationality	Indian

EDUCATION

2018 – present **Doctoral Study**

Czech University of Life Sciences Prague

Faculty of Tropical AgriSciences

Study Programme: Tropical Agrobiology and Bioresource Management

Thesis: Antimicrobial activity and chemical composition of essential oils and extracts from Indian medicinal plants

2011-2013 **Master's degree**

Pt. Ravishankar Shukla University

Study Programme: Biotechnology

Thesis: Antibacterial potential of silver nanoparticles

2008-2011 **Bachelor's degree**

Pt. Ravishankar Shukla University

Study Programme: Biotechnology

EXPERIENCES

2015 – 2017 **Research fellow**

National centre for natural resources, Raipur, India

Project: "Identification of medicinal plants used in polyherbal formulations for treating rheumatoid arthritis and psoriasis"

INTERNSHIP

11/11 – 11/12/2021	National centre for natural resources, Raipur, India Pt. Ravishankar Shukla University Title: "Ethnobotanical survey and collection of Indian medicinal plant"
22/01 - 02/02/2024	Institute of Pharmaceutical Sciences, University of Graz Title: "Assessing the chemical composition of a synergistic blend of essential oils demonstrating effectiveness against foodborne pathogens in the vapour phase"

COLLABRATION

01/23 – 05/23	Faculty of Pharmacy, Université Paris-Cité Title: "A clinical aromatherapy study, seeks to assess the impact of EO blends via brain-targeted nasal delivery on the consumption of benzodiazepines prescribed upon request in outpatient psychiatry"

PROJECT PARTICIPATION

2018	Chemical composition and biological activity of medicinal and edible tropical plants (IGA 20185019)
2020	Evaluation of biological effects and chemical analysis of compounds from tropical plants (IGA 20205001)
2021	Evaluation of biological effect and chemical analysis of compounds from tropical plants (IGA 20213109)

APPENDIX 3: LIST OF PUBLICATIONS

1. **Chaure A**, Houdkova M, Antih J, Urbanova K, Doskocil I, Naik ML, Patel KS, Kokoska L. Validation of Broth Macrodilution Volatilization Method for Testing of Essential Oils in Liquid and Vapor Phase: Chemical Composition, Cytotoxicity, and Antibacterial Effect of Indian Medicinal Plants against Pneumonia-Causing Pathogens. Molecules. 2023; 28(12): 4625. (JCR - Q2, IF 4.6)
2. Houdkova M, **Chaure A**, Doskocil I, Havlik J, Kokoska L. New Broth Macrodilution Volatilization Method for Antibacterial Susceptibility Testing of Volatile Agents and Evaluation of Their Toxicity Using Modified MTT Assay *In Vitro*. Molecules. 2021; 26(14):4179. (JCR - Q2, IF 4.6)

CONFERENCE CONTRIBUTION

1. **Chaure A**, Houdkova M, Kokoska L. Evaluation of antibacterial effect of essential oil from Indian medicinal plant using new broth macrodilution volatisation method. ISEO 2021: International Symposium on essential oils (poster).
2. **Chaure A**, Antih J, Houdkova M, Kokoska L. Antibacterial activity of Indian essential oil vapours against pneumonia-causing bacteria and headspace analysis of *Trachyspermum ammi* sample. 70th International Congress and Annual Meetings of the Society for Medicinal Plant and Natural Product Research, GA 2022 (poster).
3. **Chaure A**, Antih J, Houdkova M, Kokoska L. Antibacterial activity of Indian essential oil vapours against pneumonia-causing bacteria and headspace analysis of *Trachyspermum ammi* sample. PSE Trends in Natural Products - Young Scientists' Meeting, Paris 2023 (poster).
4. Antih J, Boutefnouchet S, **Chaure A**, Kokoska L. Headspace analysis of 3 essential oil blends delivered via aromastick inhaler within HECBA-Psy clinical study. PSE Trends in Natural Products - Young Scientists' Meeting, Paris 2023 (poster).
5. Houdkova M, **Chaure A**, Kokoska L. Liquid matrix volatilization methods for susceptibility testing of respiratory bacteria to volatile agents in liquid and vapour phase. PSE Trends in Natural Products - Young Scientists' Meeting, Brno 2024 (poster).

APPENDIX 4: Pictures of plant sample collection

Location – Amarkantak

Collection of plant samples from wild

Location – Chhattisgarh, India

a) ***Alysicarpus monilifer*** **(L.) DC.; b)** ***Barleria prionitis*** **L.; c)** ***Psoralea corylifolia*** **L.**