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CHAPTER

VOLATILE OILS: POTENTIAL AGENTS FOR THE TREATMENT OF RESPIRATORY INFECTIONS

16

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1 INTRODUCTION

Referring to infectious disease, respiratory tract infections engage with all surfaces in the respiratory tract. Based on the infected zone, respiratory infections can be categorized into upper tract infection (URI or URTI) and lower tract infection (LRI or LRTI). Each involves different parts of the respiratory tract infections, which vary in type and severity of microorganisms. Although there are different types of respiratory tract infections, the acute form in the upper respiratory tract infection predominates and includes several complications, such as sinusitis, pharyngitis, epiglottitis, laryngitis, and tracheitis. On the other hand, lower respiratory tract infection (LRTI) includes both acute and chorionic types, such as pneumonia and bronchitis. Based on pathogenicity, bacterial and viral pathogens are the most common microorganisms in both types (ie, LRTI and URTI). Moreover, infection distribution leads to varieties based on the patient's age; for example, acute respiratory infections pose severe problem in childhood, which mainly occur in upper respiratory tract. Although the bacterial pathogens play a significant role in intensifying LRTIs, the major acute respiratory infections occur in upper respiratory tract, in these cases viral pathogens are the primary common pathogens, including influenza A and B, parainfluenza (type 1 and 3), adenovirus, and respiratory syncytial virus. Some of the common pathogens of the respiratory tract are listed in Table 16.1. Pathogen biodiversity, complexity, and mixed infections in many cases of respiratory tract infection have generated several problems for the treatment of respiratory infections. For example, various bacterial pathogens are encountered in several cases of viral infections. Therefore, the treatment of respiratory infections is a complex therapy which consists of several chemotherapy strategies.[1–3] Antiviral (the same as antibacterial medication) is used to control the treatment and prevention of respiratory infections.

There are several restrictive factors, such as medication resistance, recurrency, and inflammation, which will guide researchers to find new effective compounds. This will be an important field in drug development for respiratory infections. Natural compounds are considered to be one of the main sources in new drug development. Historically, numerous plants have been utilized as traditional medicines

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Table 16.1 Some of the Common Pathogens Involved in Respiratory Tract Infections

Pathogen Name	Common Infected Form	Category	References
Streptococcus pneumoniae	Pneumonia/invasive pneumococcal diseases	Gram-positive	a
Haemophilus influenzae	Pneumonia, epiglottitis and sinusitis	Gram-negative	b
Chlamydophila pneumoniae	Atypical pneumonia	Obligate intracellular bacterium	c
Staphylococcus aureus	Sinusitis, pneumonia	Gram-positive	d
Pseudomonas aeruginosa	Sinusitis, pneumonia	Gram-negative	e
Legionella pneumophila	Cough with sputum or bloody sputum/pneumonia, bronchiolitis	Gram-negative	f
Moraxella catarrhalis	Bronchitis, sinusitis, laryngitis and bronchopneumonia	Gram-negative	g
Rhinoviruses	Common cold, sinusitis, pneumonia (in middle-aged adults)	Enterovirus	h
Coronaviruses	Pneumonia	Coronavirinae	i
Influenza virus	Pneumonia	Orthomyxovirus	j
Respiratory syncytial virus	Bronchiolitis, pneumonia	Pneumovirus	k
Adenovirus	Pneumonia, tonsillitis, bronchiolitis	Adenoviridae	l
Herpes simplex virus	Pneumonia, pharyngitis	Respirovirus	m
Histoplasma capsulatum	Pneumonia	Histoplasma (dimorphic fungi)	n
Cryptococcus neoformans	Pneumonia	Cryptococcus (yeast)	o
Coccidioides immitis	Pneumonia	Coccidioides (pathogenic fungus)	p
Pneumocystis jirovecii	Pneumonia	Pneumocystis (yeast-like fungus)	q

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by people in many nations.[4] Many of these plants have been investigated for their antimicrobial and antiviral properties.[5–9] With regard to massive variation among natural products, chemical structure diversity causes different antimicrobial potential in natural compounds.[10]

Besides the antimicrobial activity of the essential oils in natural products, other characteristics such as high vapor pressure, low toxicity, and antiinflammatory potential create a worthwhile theme for using of these natural compounds for new drug development in respiratory infections. Parallel to the roles of the microorganisms in the pathology of respiratory infections diseases, inflammatory process also have a considerable role in the persistence and recurrence of respiratory infectious diseases.

This chapter reviews the antibacterial, antiviral, and antiinflammation effects of essential oils as effective natural compounds. It will also discuss the use of these natural compounds as traditional remedies in treatment of respiratory infections.

2 TRADITIONAL REMEDIES IN RESPIRATORY INFECTIONS

Traditional medicines utilize natural sources for the treatment of the many diseases.[11–13] Historically, infectious diseases have been the major human ailment. Natural sources are used in a variety of forms, including water extracts, tincture or alcoholic extract and incense.[14] Based on the historical uses and effective treatments that have been based on many of these traditional remedies, extensive pharmacological research of their antibacterial, antiviral, and antiinflammation activity have been performed.[15–17] Abundant information about plants and active compounds in infectious diseases and inflammation related process is available.[18–21] Aromatic and fragrant plants are a major part of traditional therapeutic remedies, and they have shown remarkable antibacterial and antiviral activity. Furthermore, many of them also have a significant antiinflammatory activity and are used as adjuvant remedies in the treatment of infection (Table 16.2 and Fig. 16.1). Some of the most active extracts of traditional herbs which have been used as antibacterial and antiviral in the treatment of respiratory infections are summarized in Table 16.3.

The use of aromatic extracts or burning plants is a common process in traditional medicine. The resultant smoke or fragrance is inhaled to treat respiratory complaints, including cough, cold, infections, and asthma.[22,23] Inhalation administration goes back to the ancient cultures and its techniques may be considered as a progressive point in respiratory complaints treatment. The direct effect of such fragrance on the respiratory tract is an advantage of this form of treatment.

Inhalation therapy often involves the aromatic extracts or burning of plant material, and the volatile fraction liberated during the process is inhaled to aid in the healing process. Inhalation of the volatile fraction from aromatic extracts or burning plant matter is a unique method of administration and has been used traditionally to treat respiratory conditions, such as, asthma, bronchitis, and other respiratory infections including the common cold.[24] In addition, aerosol delivery of such remedies is well practiced in allopathic medicine and has the advantage of being site specific, thus enhancing the therapeutic ratio for respiratory ailments.[25]

Table 16.4 and Fig. 16.2 describe several essential oils from *Achilla* species (Asteraceae family) that have demonstrated appropriate effects on some of the major respiratory infections caused by microorganisms.

Table 16.2 Some Famous Traditional Plants That Are Used as Treatment Remedies for Respiratory Diseases

Plant Species (Family)	Plant Parts Used	Indications	References
Acacia polyacantha Willd. (Forssk.) Willd. (Mimosaceae)	Stem bark	Cough	a
Andira inermis (Wright) DC. (Fabaceae)	Leaves	Cough, respiratory diseases	a
Asparagus africanus Lam. (Asparagaceae)	Whole plant	Respiratory diseases	a
Cussonia arborea Hochst. ex A. Rich. (Araliaceae)	Leaves	Cough, respiratory diseases	a
Entada africana Guill. and Perr. (Mimosaceae)	Roots	Respiratory diseases	a
Euphorbia hirta L. (Euphorbiaceae)	Whole plant	Sore throat	a
Keetia hispida (Benth.) Bridson (Rubiaceae)	Leaves	Respiratory diseases	a
Phyllanthus muellerianus (O. Ktze) Exell (Euphorbiaceae)	Leaves	Respiratory diseases	a
Terminalia schimperiana Hochst. (Combretaceae)	Leaves	Cough, respiratory diseases	a
Sophora flaescens Ait. (Fabaceae)	Roots	Respiratory diseases	b
Scutellaria baicalensis Georgi (Lamiaceae)	Root	Respiratory diseases	b
Artemisia afra (Asteraceae)	Leaves and bark	Colds, coughs, and influenza	c
Sambucus nigra L. (Caprifoliaceae)	Leaves and bark	Bronchitis	d
Anchusa italica Retz. (Boraginaceae)	Flowers	Common colds	e
Cynodon dactylon (L.) Pers. (Gramineae)	Whole plant	Coughs	e
Thymus kotschyanus Boiss. et Hoh. (Lamiaceae)	Leaves, flowers	Common colds, bronchitis	e
Glycyrrhiza echinata L. (Leguminosae)	Roots, stolons	Coughs, bronchitis	e
Trigonella foenum-graecum L. (Leguminosae)	Seeds, leaves	Cure of inflamed throat	e
Althaea officinalis L. (Malvaceae)	Flowers, leaves, roots	Coughs, bronchitis	e
Malva sylvestris L. (Malvaceae)	Whole plant	Coughs, respiratory inflammation	e
Prunus mahaleb L. (Rosaceae)	Fruits	Emollient for upper respiratory organs	f
Adiantum capillus-veneris L. (Adiantaceae)	Leaves	Respiratory ailments, cough	g
Ferula oopoda (Boiss. & Buhse.) Boiss. (Apiaceae)	Seed, latex	Cough, asthma, respiratory disorders	g
Stachys turcomica Trautv (Lamiaceae)	Whole plant	Bronchitis, influenza	g
Acacia kempeana F. Muell. (Mimosaceae)	Bark, leaves, root bark	Chest infection, severe cold	h

(*Continued*)

Table 16.2 Some Famous Traditional Plants That Are Used as Treatment Remedies for Respiratory Diseases (*cont.*)

Plant Species (Family)	Plant Parts Used	Indications	References
Acacia ligulata Cunn. ex Benth. (Mimosaceae)	Bark, leaves	Cough, cold, chest infection	i
Eremophila alternifolia R. Br. (Myoporaceae)	Seed, leaves	Respiratory tract infection	j
Cymbopogon ambiguus (Steudel) A. Camus (Poaceae)	Leaves	Respiratory tract infection	k

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3 SCREENING OF THE ANTIBACTERIAL EFFECTS OF ESSENTIAL OILS

The antimicrobial effects of plants and their extracts have been recognized for a long time. Essential oil is one of the most important and wide spread secondary metabolite in plants and this class of phytochemical compounds and their activities needs attention. These phytochemicals are generally isolated from plant material by distillation methods, such as, hydrodistillation and steam distillation. They contain variable mixtures of several chemical classes, such as terpenoids, specifically monoterpenes and simple phenolic compounds. Some of the higher molecular structures with high molecular weight, such as sesquiterpenes and diterpenes, may be present. A variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, esters or lactones, sulfur-containing compounds and other chemical groups may also be observed. Among the phytochemical compounds, terpenes are responsible for many therapeutic effects in medicinal plants.[26–30] Most terpenes are derived from the condensation of isoprene units and are categorized according to the number of these units present in the carbon skeleton. These compounds are responsible for aromaticity and fragrance in many of the plants. The antibacterial activity of volatile oils has been assessed by many researchers.[31–34] This potential of essential oils has been used in many pharmaceutical, cosmeceutical, and nutraceutical applications and industrials. There are many differences between the antimicrobial effects of different essential oils. Essential oils and their constituents are an attractive source in new antimicrobial compounds evaluation.[35,36]

FIGURE 16.1 Some of the Famous Edible Plants That Are Used as Traditional Antibacterial and Antiinflammations Remedies

(a) *Citrus paradise* (grapefruit), (b) *Perilla frutescens* (perilla), (c) Cymbopogon citratus (lemmon grass), (d) *Origanum vulgare* (oregano), (e) *Salvia officinalis* (sage), (f) *Thymus vulgaris* (thyme), (g) *Satureja hortensis* (savory).

Many of the essential oils have been tested for bactericidal and bacteriostatic effects against a wide range of microorganisms including food spoiling organisms, pathogenic bacteria, yeasts, fungi, and many others. The major differences in antimicrobial activity have been yielded of several distinctive parameters which identify antibacterial characters of the essential oils, some of the major parameters include: (1) bacterial membrane permeability, (2) the hydrophobicity/hydrophilicity of the bacterial membrane, (3) the metabolic characteristics of the microorganism, and (4) their Gram-positive or negative pattern. Although susceptibility of the bacteria to the essential oils is not exactly predictable, many

Table 16.3 Some Active Traditional Plants Remedies Extracts With Antibacterial and Antiviral Effects

Plant Species (Family)	Antiinfection Activity	Indications	Using Form of Plants Extracts	References
Polygonum punctatum (Polygonaceae; aerial parts)	RSV	ED_{50} = 120 (mg/μL) against RSV of the assayed extracts in HEp-2 cells	Aqueous extracts	a
Lithraea molleoides (Anacardiaceae; aerial parts)	RSV	ED_{50} = 87 (mg/μL) against RSV of the assayed extracts in HEp-2 cells	Aqueous extracts	a
Myrcianthes cisplatensis (Myrtaceae; aerial parts)	RSV	ED_{50} = 78 (mg/μL) against RSV of the assayed extracts in HEp-2 cells	Aqueous extracts	a
Azadirachta indica (Meliaceae; stem bark)	S.a, P.a	$MIC_{90\%}$ = 1, $MBC_{90\%}$ = 1 (mg/mL) for S.a $MIC_{90\%}$ = 1, $MBC_{90\%}$ = 2 (mg/mL) for P.a	Methanolic extracts	b
Entada abyssinica (Leguminosae; stem bark)	S.a, P.a	$MIC_{90\%}$ = 0.5, $MBC_{90\%}$ = 2 (mg/mL) for S.a $MIC_{90\%}$ = 0.5, $MBC_{90\%}$ = 2 (mg/mL) for P.a	Methanolic extracts	b
Eremophila duttonii (Myoporaceae; leaves)	S.a	Diameters of the zones of growth inhibition in plate-hole diffusion assays (12 mm) with 0.77 mg/mL of extract	Ethanolic extracts	c
Artemisia capillaries Thunb. (Asteraceae; aerial parts)	RSV	IC_{50} = 13 (μg/mL) concentration of the sample required to inhibit virus-induced	Aqueous extracts	d
Arctium lappa L. (Asteraceae; aerial parts)	RSV	IC_{50} = 6.3 (μg/mL) concentration of the sample required to inhibit virus-induced	Aqueous extracts	d
Prunella vulgaris L. (Lamiaceae; fruit spike)	RSV	IC_{50}= 10.4 (μg/mL) concentration of the sample required to inhibit virus-induced	Aqueous extracts	d
Anemone obtusiloba (Ranunculaceae; aerial parts)	HSV	Lowest concentration of extract able to partially inhibit the virus (100 μg/mL)	Methanolic extracts	e
Centipeda minima (Asteraceae; aerial parts)	HSV	Lowest concentration of extract able to partially inhibit the virus(13 μg/mL)	Methanolic extracts	e
Byrsonima verbascifolia (Malphigiaceae; aerial parts)	HSV	Minimum concentration causing complete inhibition (MIC) of viral (2.5 μg/mL)	Methanolic extracts	f
Symphonia globulifera (Clusiaceae; aerial parts)	HSV	Minimum concentration causing complete inhibition (MIC) of viral (2.5 μg/mL)	Methanolic extracts	f

Table 16.3 Some Active Traditional Plants Remedies Extracts With Antibacterial and Antiviral Effects (*cont.*)

Plant Species (Family)	Antiinfection Activity	Indications	Using Form of Plants Extracts	References
Dracaena cinnabari (Agavaceae; aerial parts)	I.A	IC_{50} = 1.5 (μg/mL) concentration of the sample required to inhibit virus-induced	Methanol extracts	g
Exacum affine (Gentianaceae; aerial parts)	I.A	IC_{50} = 0.7 (μg/mL) concentration of the sample required to inhibit virus-induced	Methanol extracts	g
Scrophularia amplexicaulis Benth. (Scrophulariaceae; aerial parts)	S.a	Diameters of the zones of growth inhibition in well-diffusion method (13 mm) with 100 mg/mL of essential oil	Essential oil	h
Cinnamomum zeylanicum (Lauraceae; bark)	H.i, S.p, S.a	The lowest concentration of oil inhibiting the growth of each organism (MIC = 0.00625, 0.00625, 0.0125 mL/mL)	Essential oil	i
Cupressus sempervirens (Cupressaceae; aerial parts)	H.i, S.p, S.a	MIC = 0.00625, 0.00625, 0.0125 mL/mL	Essential oil	i

RSV, *respiratory syncytial virus;* ADV, *adenovirus;* HSV, *herpes simplex virus 1;* I.A, *influenza virus-A;* S.a, Staphylococcus aureus*;* P.a, Pseudomonas aeruginosa*;* S.p, Streptococcus pneumonia*;* H.i, Haemophilus influenza.

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[e]*Taylor R, Manandhar N, Hudson J, Towers G. Antiviral activities of Nepalese medicinal plants.* J Ethnopharmacol *1996;* **52***: 157–63.*

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researchers have tried to determine the relationship between the origin of the essential oils and their compounds with their antimicrobial activity. Furthermore, the delivery of medications to the respiratory tract has become an increasingly important method for respiratory disease treatment. The use of inhaler medications has become an invaluable therapeutic in the treatment of different pulmonary disorders, including bronchitis, pneumonia, and others complications.[37] Several studies have reported the clinical efficacy of inhalation therapy for the treatment of lung disorders.[38,39] Through the effective delivery of medication to the action site, the active compounds are delivered directly into the lungs and this can result in respiratory tract local treatment. This method achieves maximum therapeutic

Table 16.4 *Achilla* Species Essential Oils, Their Major Chemical Compositions and Their Effects on Some of the Microorganisms That Cause Major Respiratory Infections

Plant Name	Tested Microorganisms	Major Compounds	References
Achillea clavennae L.	*K. pneumonia*, penicillin-susceptible and penicillinresistant *S. pneumonia*, *H. influenza* and *P. aeruginosa*	Camphor, 1,8-cineole	a
A. fragrantissima (Forssk) Sch. Bip.	*K. pneumonia, P. aeruginosa, S. faecalis, S. aureus* and *C. albicans*	Terpinen-4-ol	b
A. sintenisii Hub. Mor.	*K. pneumonia, P. aeruginosa* and *S. aureus*	Camphor, 1,8-cineole	c
A. biebersteinii Afan.	*S. pneumonia* and *S. aureus*	Piperitone, 1,8-cineole, camphor	d
A. taygetea Boiss & Heldr.	*K. pneumonia, P. aeruginosa* and *S. aureus*	Borneol, 1,8-cineole	e
A. frasii Schultz Bip.	*K. pneumonia, P.aeruginosa* and *S. aureus*	1,8-cineole, α-pinene (4), β-pinene (5)	e
A. holosericea Sibth. & Sm.	*K. pneumonia, P. aeruginosa* and *S. aureus*	Borneol, camphor	f

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[b] *Barel S, Segal R, Yashphe J. The antimicrobial activity of the essential oil from* Achillea fragrantissima. J Ethnopharmacol *1991;* **33***: 187–91.*
[c] *Sökmen A, Vardar-Ünlü G, Polissiou M, Daferera D, Sökmen M, Dönmez E. Antimicrobial activity of essential oil and methanol extracts of* Achillea sintenisii *Hub. Mor. (Asteraceae).* Phytother Res *2003;* **17***: 1005–10.*
[d] *Sökmen A, Sökmen M, Daferera D, Polissiou M, Candan F, Ünlü M, et al. The in vitro antioxidant and antimicrobial activities of the essential oil and methanol extracts of* Achillea biebersteini *Afan. (Asteraceae).* Phytother Res *2004;* **18***: 451–6.*
[e] *Magiatis P, Skaltsounis A-L, Chinou I, Haroutounian SA. Chemical composition and in vitro antimicrobial activity of the essential oils of three Greek Achillea species.* Z Naturforsch *C 2002;* **57***: 287–90.*
[f] *Stojanović G, Asakawa Y, Palić R, Radulović N. Composition and antimicrobial activity of* Achillea clavennae *and* Achillea holosericea *essential oils.* Flavour Fragr J *2005;* **20***: 86–8.*

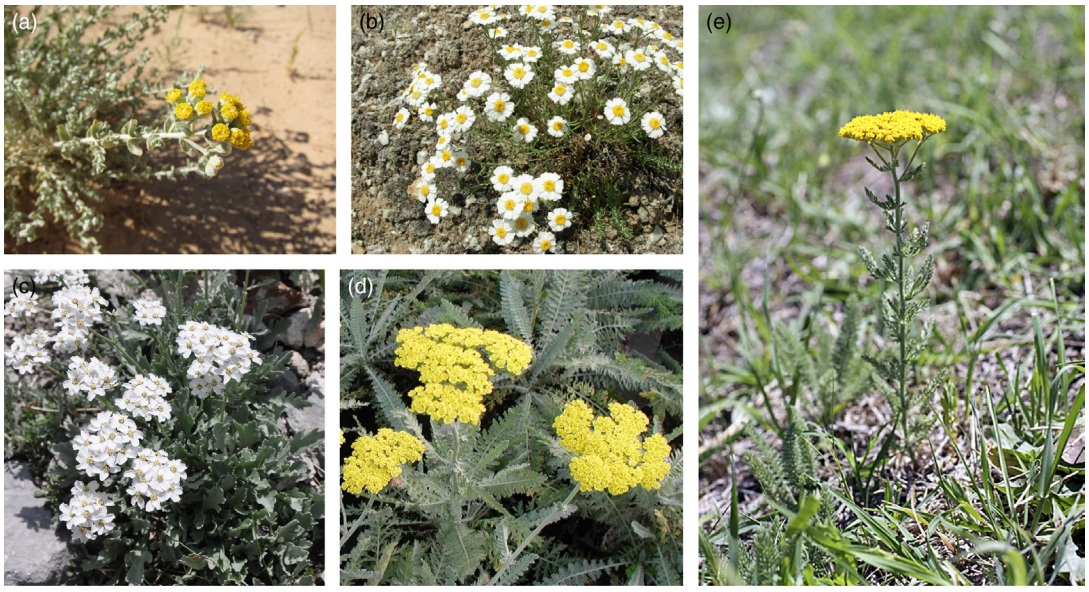

FIGURE 16.2 Some Plants of *Achillea* Species Whose Essential Oils Are Used in the Treatment of Respiratory Infections

(a) *Achillea fragrantissima*, (b) *A. sintenisii*, (c) *A. clavennae*, (d) *A. taygetea*, (e) *A. biebersteinii*.

effect, small dose usage, and has fewer side-effect risks compared with those associated with larger doses. Inhalation is a unique treatment with direct effects on respiratory disorder site and is based on the volatility potential of essential oils. Furthermore, there is a need to develop new therapeutic agents for respiratory infections.[40–42]

Research has been carried out on the wide spectrum of edible plants essential oils to determine the antibacterial potential of their essential oils. The role of these plants as therapeutic agents is remarkable in many cultures. Investigations have reported that thyme and oregano essential oils, based on the phenolic components [such as carvacrol (**1**) and thymol (**2**) (Fig. 16.3)] have shown a strong correlation with the inhibition of some of the pathogenic bacterial strains (eg, in *Escherichia coli*). The correlation between the antibacterial effect of the volatile oils and their chemical compounds, including high amount of the phenolic components such as carvacrol (**1**) or eugenol (**3**), has also been confirmed.[43] Other essential oils such as oregano, savory, clove, and nutmeg with high concentrations of volatile phenolic compounds inhibit Gram-positive more than Gram-negative pathogenic bacteria.[44] However, in some essential oils such as *Achillea* spp. (Yarrow) strong antibacterial activity was observed against the Gram-negative respiratory pathogens (*Haemophilus influenzae*, *Pseudomonas aeruginosa*) while *Streptococcus pyogenes* was the most resistant to the this oil.[45] The other essential oils such as peppermint and spearmint inhibit the methicillin-resistant type of *Staphylococcus aureus*. Previous reports have clarified that the essential oils containing aldehyde or phenol as a major component represent the highest antibacterial activity. These antibacterial potencies are lower in the essential oils that contain high amounts of terpene alcohols compared to the essential oils containing aldehyde or phenol as a major component.

Other essential oils containing terpene ketone, or ether showed much weaker activity, and oil containing terpene hydrocarbon was relatively inactive. Based on these findings, essential oils such as thyme, cinnamon, lemongrass, perilla, and peppermint have demonstrated suitable effects on respiratory tract infection.[46] The tolerance of Gram-negative bacteria to essential oils has been attributed to the presence of a hydrophilic outer membrane that blocked the penetration of hydrophobic essential oils to the target cell membrane because the Gram-positive bacteria were more exposed to the essential oils than Gram-negative bacteria, which has been reported several times.[47–50]

Lipids are one of the principal constituents for normal cell membrane function and these compounds supply many operations, such as barrier function in the bacterial cell membrane. The external capsule of some Gram-negative bacteria limits or prevents the penetration of the essential oils into the microbial cell. One of the pronounced examples of the hydrophobicity/hydrophilicity role in bacterial sensitivity to antibacterial compound is *H. influenzae*. It should be pointed out that the outer membrane of *H. influenzae* (which forms rough colonies) was more hydrophobic. Hydrophobic antibiotics, such as macrolides, are more active against *H. influenzae* than *E. coli* through their shorter oligosaccharide chains than those in *E. coli*. The effects of the cytoplasmic membrane and/or the embedded enzymes in it have demonstrated lipophilic biocide actions.[51] It is generally recognized that the antimicrobial action of essential oils depends on their hydrophilic or lipophilic character. Based on these observations, investigators are trying to indicate the relationship between structural activity of the essential oils compounds and their antibacterial activity.

Certain components of the essential oils can act as uncouplers, which interfere with proton translocation over a membrane vesicle and subsequently interrupt ADP phosphorylation pathways (primary energy metabolism). As a member of the phytochemicals, terpenoids have been observed as a model of lipid soluble agents, which have an impact on the activities of membrane catalyzed enzymes; for

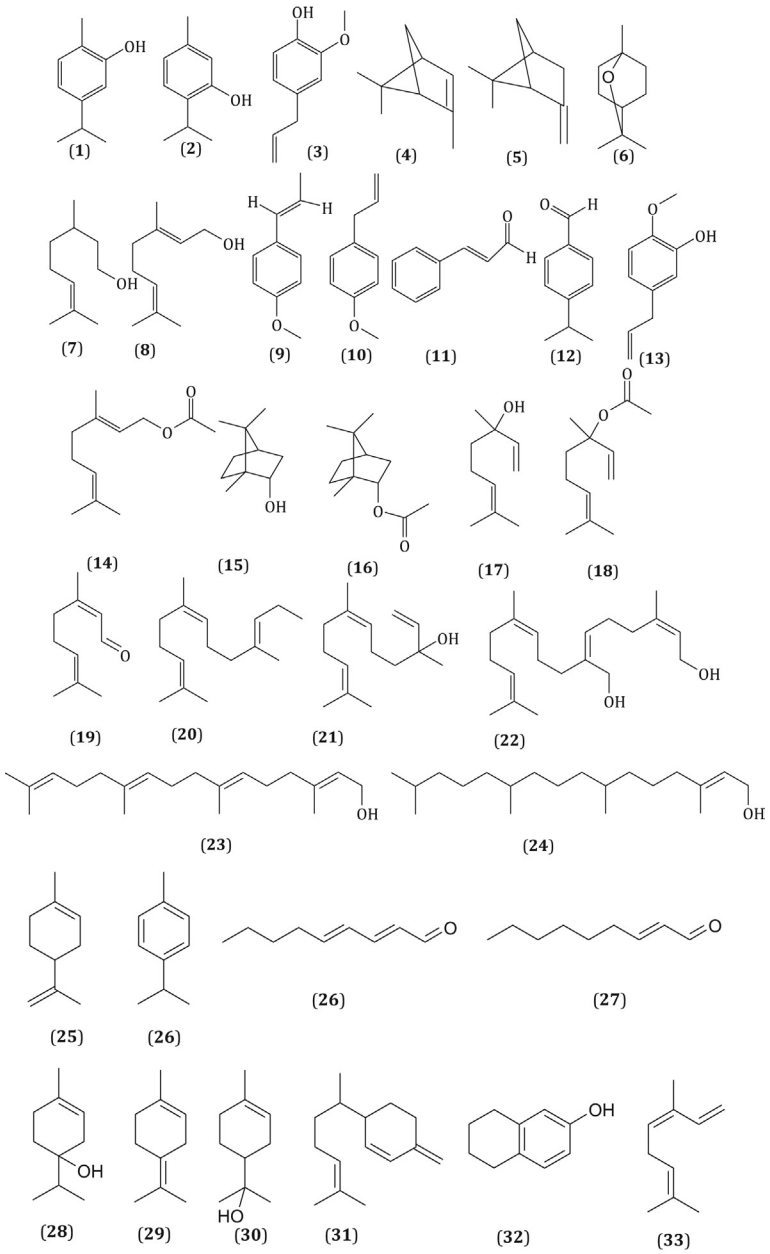

FIGURE 16.3 Structures of the Major Bioactive Chemical Compounds Isolated From Essential Oils

example, enzymes involved in respiratory pathways. Particular terpenoids with functional groups, such as phenolic alcohols or aldehydes, also interfere with membrane-integrated or associated enzyme proteins, inhibiting their production or activity. A good deal of antimicrobial compounds which act on the bacterial cytoplasmic membrane cause the loss of 260 nm absorbing material. This causes an increased susceptibility to NaCl, the lysosomes formation and loss of potassium ions, which results in inhibiting respiration and the loss of cytoplasmic material.

Investigations about the cytoplasmic membrane effects of α-pinene (**4**), β-pinene (**5**), 1,8-cineole (**6**), and electron microscopy studies have shown that the essential oils containing these compounds triggered such cytoplasmic material with losing in treated bacterial cells.[31,32] The perturbation of the lipid fraction in the plasma membrane causes antimicrobial activity of some of the phytochemicals such as α,β-unsaturated aldehydes and some of monoterpenes. Although these aldehyde compounds can elicit antibacterial effects by acting on membrane functional proteins, such antibacterial effect would be achieved with modifications of membrane permeability and intracellular materials leakage.[52–54] The membrane damage leading to whole-cell lysis has been reported by oregano and rosewood essential oils which contains major components as: carvacrol (**1**), citronellol (**7**), and geraniol (**8**).[26,55] Phenols such as carvacrol (**1**), thymol (**2**), eugenol (**3**), and other oxygenated aromatic essential oil compounds including phenol ethers [*trans*-anethole (**9**), methyl chavicol (**10**)] and aromatic aldehydes [cinnamaldehyde (**11**), cuminaldehyde (**12**)] have been reported to exert both antibacterial and antifungal activity. However, this chemical class—based on the concentration used—are known as either bactericidal or bacteriostatic agents,[56] but the phenolic component's high activity may be further explained in terms of the alkyl substitution into the phenol nucleus, which is known to increase the antimicrobial activity of phenols. The alkylation has been known to change the distribution ratio between the hydrophilic and the hydrophobic phases (including bacterial phases) by the surface tension reduction or the species selectivity mutate based on the bacteria cell wall characters.[57] This does not happen with etherified or esterified isomeric molecules, it is possible by describing their relative lack of activity.[58] As a member of these compounds carvacrol (**1**) is one of the few components that has a break apart from effect on the outer membrane of Gram-negative bacteria and causes release of lipopolysaccharide and alters cytoplasmic membrane ions transportation, Similar to carvacrol (**1**), thymol (**2**) antimicrobial activity results in structural and functional alterations in the cytoplasmic membrane.[59] Interestingly, eugenol (**3**) and isoeugenol (**13**) exhibit higher activity against Gram-negative bacteria than Gram-positive bacteria, and when cinnamaldehyde (**11**) is used against *E. coli*, its activity is similar to carvacrol (**1**) and thymol (**2**) (Fig. 16.3). These compounds alter the membrane, affect the transport of ions and ATP, and change the fatty acid profile of different bacteria.[60]

Although in some cases alcoholic form shows better potencies compared to acetate form, the presence of an acetate moiety in the structure appeared to increase the activity of the parent compound. In the case of geraniol (**8**), the geranyl acetate (**14**) demonstrated an increase in activity against the test microorganisms.[48,61,62] A similar effect was also observed in the case of borneol (**15**), bornyl acetate (**16**), linalool (**17**), and linalyl acetate (**18**) (Fig. 16.3). In addition, the effectiveness of alcoholic compounds very closely depended on the bacterial cell wall, which showed different permeability to alcohol based on chain length.[44,63] It has been suggested that an aldehyde group conjugated to a carbon double bond such as citral (**19**) is an extremely electronegative order, which may explain their activity, and an increase in electronegativity can raise up the antibacterial activity.[64] In addition, the under research of aldehydes potency seems to depend not only on the existence of the α,β-double bond but also on the

chain length from the renal group and on microorganism tested. It seems that some electronegative compounds, mainly from the cell surface, are responsible for the inhibited growth of the microorganisms, which may interfere in biological processes involving electron transfer and respond with vital nitrogen components and alteration in the operation of membrane-associated proteins. Actually, a greater electronegativity of the molecule would cause a greater encounter of intermolecular hydrogen bond formation with membrane nucleophilic groups and thus a significant irregularity in the lipidic bilayer. Some studies have recommend that carbon tail length also affects the electronegativity of the aldehyde oxygen atom and thus its interaction with the nucleophilic groups of the cell membrane.[65] Comparably, the similar antimicrobial activity was detected in the series of the long-chain alcohols which is demonstrated to be resulted from the alkyl chain length.[66,67] This structural activity relationship is notable between farnesol (**20**), nerolidol (**21**), plaunotol (**22**), geranylgeraniol (**23**), phytol (**24**), geraniol (**8**), and linalool (**17**) which act on *S. aureus* with damages of the cell membranes and losing of K+ ions, while similar mode of actions can be detected by the aminoglycosides such as kanamycin and streptomycin. Farnesol (**20**) was able to damage cell membranes most effectively than other terpene alcohols. The activities of farnesol (**20**), nerolidol (**21**) (sesquiterpenes compounds) on *S. aureus* were higher than that of plaunotol (**22**) (diterpene). The effectiveness against *S. aureus* are in order as follows: farnesol (**19**) > nerolidol (**20**)> plaunotol (**22**) > geranylgeraniol (**23**), phytol (**24**) > geraniol (**8**) and linalool (**17**) (Fig. 16.3). It has been suggested that maximum activity against *S. aureus* might depend on the number of carbon atoms in the hydrophobic chain from hydrophilic hydroxyl group, which should be less than 12 but as close to 12 as possible. Neither a shorter nor a longer aliphatic carbon chain, could increase such activity.[68,69] The increased effectiveness of sesquiterpenes as enhancers of membrane permeability may stem from their structural resemblance to membrane lipids (eg, linear molecules with internal lipophilic character and a more polar terminus).[70] The bacteriostatic potential of the terpenoids was also increased when the carbonyl groups increased in structure.[63]

The type of alkyl substituent incorporated into a nonphenolic ring structure is responsible for enhancement of antibacterial activity. Such as an alkenyl substituent (1- methylethenyl) makes an increase in antibacterial activity, as seen in limonene [1-methyl-4-(1-methylethenyl)-cyclohexene] (**25**), compared to an alkyl (1-methylethyl) substituent as in p-cymene [1-methyl-4-(1-methylethyl)-benzene] (**26**). Furthermore, principally Gram-negative were the sensitive organisms that propose alkylation control of the Gram reaction sensitivity of the bacteria. An allylic side-chain seems to raise the inhibitory role of the simple phenols mainly against Gram-negative organisms. This was suggested due to the majority of the antimicrobial activity of alkylated phenols in relation to phenol which has been earlier reported (Fig. 16.3).[56]

It was observed that α-isomers are inactive relative to β-isomers in many compounds in stereochemistry, which is also effective in antibacterial activity observed from essential oils.[44] The (*E,E*)-2,4-decadienal (**26**) appears to be more toxic to bacterial cells than the correspondent monounsaturated aldehyde (*E*)-2-decenal (**27**) as another example of stereochemistry effectiveness in activity potential observed in the unsaturated aldehyde, but it is noticeable that two double bonds in the *cis* configuration in the side-chain of 2,4-decadienal (**26**) produce more bends and shorten the length of the carbon tail, such α,β-unsaturated aldehydes might be a good choice compared to other highly toxic sterilizers (Fig. 16.3).[53]

In summary, the antimicrobial activity of essential oils depends on different amounts of specific compounds. As an example, antimicrobial properties of the essential oils with high concentrations of eugenol (**3**), cinnamaldehyde (**11**), or citral (**19**), is predictable. Remarkable antimicrobial, antifungal,

and antiviral activity of the monoterpenes and phenols relieve from essential oils present in thyme, sage, and rosemary. Due to the formation of polysaccharides that increase the resistance to essential oils there are some other essential oils, such as basil, sage, hyssop, rosemary, and oregano, which are active against *E. coli*, *S. aureus* but are less effective against *Pseudomonas* spp.[71–73] The typical characteristic of the essential oils is hydrophobicity, which is responsible for the disruption of bacterial structures and makes an increase in permeability due to a weakness to pull apart the essential oils from the bacterial cell membrane. Many cellular functions, including maintaining the energy status of the cell, membrane-coupled energy transducing processes, solute transport, and metabolic regulation result from the cell membrane permeability barrier. Actually, they are responsible for leakage of the cell contents, reducing the proton motive force, reducing the intracellular ATP pool via decreased ATP synthesis and augmented hydrolysis are the mechanisms of action of the essential oils including the degradation of the cell wall, damaging the cytoplasmic membrane, cytoplasm coagulation, damaging the membrane proteins, increased permeability that is different from the increased membrane permeability and reducing the membrane potential via increased membrane permeability.[33,74,75]

3.1 LABORATORY METHODS OF EVALUATION OF ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS

Unfortunately, scientific investigations on the antimicrobial activity of essential oils have been retarded by the lack of appropriate susceptibility testing methods for the essential oils and because no generally approved assay method has been established for the assessment of their antimicrobial activity. Many researchers have employed the disk assay method. However, the results of this method were not always in parallel with those of dilution assay methods. The differences were caused not only by differences in the solubility of the oils but also by interactions of the components in the concentrated solution used in the disk assay.[48] The dilution method could be more reliable than the disk method with regard to reproducibility and clinical relevance. When testing nonwater-soluble and highly volatile essential oils by the dilution method, it is necessary to obtain a homogeneous dispersion of the oils in the medium. Chemical emulsifiers such as Tween 80, Tween 20 and other have been used frequently for this homogenization, but it has been reported that emulsifiers reduced the bioactivity of the oils, probably because of the formation of micelles, which inhibit adequate contact between the oil and the test organism.[48] Evidence also shows that the minimum inhibitory concentration (MIC) values of the essential oils under open conditions of incubation caused a two- to eight-fold rise in the MICs of highly volatile oils, as compared with values obtained under sealed conditions. Sealed conditions are used to examine whether essential oils showed antibacterial activity against major respiratory tract pathogens (this method was authorized by the Japan Society of Chemotherapy to adjust for the physico-chemical properties of essential oils). The scientific information concerning the antimicrobial effectiveness of the essential oils in the vapor phase compared with direct contact case show that potential of this form of the essential oils is ambiguous, although some degree of inhibition by volatile components of the essential oils has been demonstrated in the vapor phase. In fact, more investigation into the antibacterial effectiveness of the vapor phase is required. It seems that the composition of the atmosphere generated by the essential oils is also potentially correlated with their antimicrobial behavior. This part mentioned the main antibacterial activity laboratory assay methods, as already noted. These methods do not show coordinated results in some case and this appearance is reasoned to the necessity of simultaneously running two or more methods for antibacterial activity determination of essential oils.

3.1.1 Solid diffusion assays

In this method, a Petri dish is commonly used as an assay chamber (5–12 cm diameter and filled with 10–20 mL of agar broth). The solidified medium in the Petri dish was inoculated with an appropriate colony of the microorganisms. This inoculation was accomplished by using a solution such as physiological saline solution containing adequate colony unit (commonly 100–200 (CFU)/mL). The essential oil is incorporated into the mediums in two ways: on a paper disc or into a well (hole) which is made in the agar medium. Diluted or undiluted essential oils based on the goals were added to a sterile blank filter disk (5 mm diameter) and placed on top of the cultured media in a Petri dish or added to the holes. After incubation under optimal conditions such as temperature and time, two different zones were considered in view of the average diameter of changes: (1) the zone where there is no growth of the microorganism, called total inhibition; and (2), the zone where growth of the microorganisms was significantly reduced in terms of amount of colonies, compared to blank assays. The growth of the microorganisms is recognizable with instruments or can be seen visually by one of the commonly used techniques, called turbidimetry, in which the optical density changes in the growing culture (OD) are measured. Some commercial systems have been produced for monitoring of the microorganisms growth as well as other methods were proposed (eg, bioautography).[76]

3.1.2 Vapor diffusion assays

An appropriate volume (100 μL physiological saline solution) of the microorganism's colony (10–20 CFU/mL) will be inoculated to the solidified medium. Each essential oil sample was diluted in suitable solvent such as ethyl ether to obtain serial dilutions (v/v). The required volume (10 μL) of each dilution was then added to sterile blank filter disks or cups and placed on the medium free cover of each Petri dish. Experiments were designed in two forms of Petri dish positions, including direct and invert placement. The Petri dishes were then sealed using sterile adhesive tape. Blanks were prepared by adding the same volume (10 μL) of samples of the solvent to the filter disks or cups, and this had no effect on the viability of any of the tested organisms. After the incubation period, the minimal inhibitory concentration (MIC), expressed as microliters of the essential oil per volume unit of atmosphere above the organism growing on the agar surface, that caused inhibition by comparison with control tests was measured.[77]

3.1.3 The dilution method (agar or liquid broth)

Although the modification with liquid broth is mostly practical for fungi, the serial dilution agar method is also common for bacteria and fungi. The difference is that agar broth cultures are grown in Petri dishes or tubes, but liquid broth cultures are cultivated in conical flasks filled with 100 mL medium or test tubes with 2.5–5 mL medium (bacteria and moulds). The inhibitory growth index is determined for the liquid broth in conical flasks (percent changes in mould's biomass comparing to the control culture). There is an inhibitory effect of essential oil which appears in the test tube cultures and is measured turbidimetrically or with the plate count method. The estimation of essential oil activity both in agar and liquid broth would be simplified through counting the tested microorganisms that can remain in the membrane. The lowest essential oil concentration in the broth that results in the lack of visible microorganism growth changes is known as the MIC. The microorganisms are then transferred from the lowest essential oil concentration medium (with no visible microorganism growth) into a new broth medium and incubation to determine the lethal activity of essential oil.[78,79]

4 SCREENING OF THE ANTIVIRAL EFFECTS OF ESSENTIAL OILS

Viruses as invasive microorganisms may cause serious respiratory illness and in some cases life-threatening conditions, such as acute pneumonia. Although acute respiratory infection rates are not very high, this condition has been steadily increasing in children and persons over 60 years of age. The rates of hospitalization and death increase substantially in these cases. Multiple factors, such as decline in respiratory and immune function, likely contribute to increased morbidity. Natural products in all forms including pure compounds or extracts provide massive opportunities for new antiviral-lead compounds.[4,80] At the moment, only a few effective antiviral drugs are available for the treatment of viral diseases, especially in respiratory viral infections. Therefore, finding new substances with antiviral properties is a required for medical systems.

Much evidence has been reported about antiviral potential of various essential oils and their constitutions on several genera of viruses.[9,81] In some investigations the essential oils with high hydrocarbons long-chain contents such as (*E,E*)-2,4-decadienal (**26**), showed activities against influenza virus.[82,83] On other spectrum of antiinfluenza virus compounds, sesquiterpenes and sesquiterpenes-rich essential oils showed clear effects as well as aromatic rich essential oils.[84–86] Among the terpenoids compounds, terpinen-4-ol (**28**), terpinolene (**29**), and α-terpineol (**30**), show inhibitory effect on influenza A/PR/8PR virus subtype H1N1. Also indicated that some sesquiterpenes such as β-sesquiphellandrene (**31**), and tetrahydronaphthalenol (**32**), showed potent antirhinoviral activity in a plaque reduction test.[87] In other researches that conducted about the antiviral essential oils and their active constitutions indicated that *Laurus nobilis* essential oil which was characterized by the presence of β-ocimene (**32**), 1,8-cineole (**6**), α-pinene (**4**), and β-pinene (**5**), as the main constituents, showed interesting activity against SARS-CoV.[88] This overview caused the development of commercial therapeutics based on monoterpens compounds for viral infections especially in respiratory viral infections (Fig. 16.3).[89,90]

Although, antiviral potential is an important factor for bioactivity of natural compounds, cytotoxicity effects that probably triggered host cell damaged cycles are important. Therefore, this is a limiting factor for presentation of the new useful compound in treatment or control of the viral infections. Viruses as invasive parasites are capable of using host cell organelles and mechanisms for reproduction, such perspicaciously reproducing process caused a creation of tenacious shield against many potent natural molecules used. Therefore selective activity is an important point, as are antiviral potencies.

5 ROLE OF INFLAMMATION IN RESPIRATORY TRACT INFECTIONS

The immune response to respiratory tract infection is a double-edged sword because many of the symptoms that accompany these infections are largely due to the microorganism's induction of cytokines and chemokines, which may result in protracted inflammatory responses. Phagocytic clearance of an infecting organism by inflammatory cells is an appropriate and necessary component of the host defense system.[91] At the same time, much of the evidence points to destructive effects spectrum made by products of these inflammatory cells.[92] These products can increase mucus secretion and impair ciliary clearance, thereby setting the stage for exacerbations and recurrences of infection. Primary inflammatory cell products can amplify the other steps of the inflammatory cycles and can paradoxically even impair the immune response. These observations suggest that modulating the inflammatory response may be an important aspect of definitive therapy for respiratory tract infection. The primary defense systems are

the lung secretions and the mucociliary escalator, which entrap organisms and sweep them away. At the same time, the lung secretions also contain a variety of proteins that inhibit microorganisms, especially bacterial adherence for example immunoglobulin A (IgA) systems, which prevents bacterial adherence to epithelial cells, inhibit bacterial growth, and attempt to neutralize bacteria. The macrophages are a secondary defense system, which not only phagocytose microorganisms but also release biochemotactic factors that recruit other defense cells such as monocytes and neutrophils that are necessary for further phagocytosis process.[93] The inflammatory response form a key part of the secondary defense system that accompanies additional proteins, such as immunoglobulins and complement factors. Although neutrophil infestation is part of the natural defenses against an invading organism, it may have destructive effects on the pulmonary systems. One of the destructive factors is the proteolytic enzyme neutrophil elastase, which is preformed and stored within the neutrophil. This proteinase is released after the neutrophil is activated and migrates into the tissues and phagocytosis invasive microorganisms. It produces a condition that histologically simulates chronic bronchitis. The potential role of neutrophil elastase in the pathogenesis of pulmonary diseases is characterized by neutrophil infiltration of the airways and increased mucus secretion.[94] It has also been reported that "secretions from patients with acute bronchial infection cause a significant reduction in ciliary beat frequency and that the addition of a neutrophil elastase inhibitor can reverse this effect."[95] The increase in the mucus secretion and the decrease in ciliary beat have been found as an important feature of chronic lung disease frequently. Besides the phagocytic cell immediate responses, stimulated epithelial cells and macrophages produce the potent neutrophil chemoattractant, such as interleukin- 8 (IL-8), which was found to be present in high concentrations in the sputum of patients with chronic inflammatory airway disease.[96] At the same time, elastase released by activated neutrophils also stimulates IL-8 production by the epithelial cells.[97] Therefore, when these events are initiated, they become a self-amplifying cycle. This phenomenon is observed in patients with acute pneumonia that has apparently been cured by appropriate antibiotic therapy.

Natural products as inflammation inhibitors have for a long time played a key role in many traditional treatment systems. Several mechanisms including interaction with prostaglandin biosynthesis, interaction with other inflammatory mediators, and corticosteroid-like effects are involved in antiinflammatory action of natural products.[98] Among the natural chemical compounds, significant antiinflammatory activities of plant-based essential oil have been reported by many researchers, which showed the basis for folk and traditional uses of these herbs for treatment of inflammatory diseases. The essential oils and aromatic plants have had a significant place in inflammation control in many folklore medications. This evidence supports the view that appropriate investigations about the potential of essential oils should be made and their active constituents should also be evaluated for inflammation control. Monoterpene alcohols such as linalool (**17**) and linalyl acetate (**18**) and other corresponding esters which are reported as one of the major volatile components of many aromatic plants essential oil were evaluated for antiinflammatory activity. These compounds have shown promising antiinflammatory potency among the many essential oil compounds.[99] For example, 1,8-cineole (**6**) the major constituent of eucalyptus oil is another active essential oil constituent that is well tolerated in inhalation administrations. This compound can also be effective for airways inflammation in clinical trials, based on such finding 1.8-cineole (**6**) is registered as a licensed medicinal product and has been available for airways inflammation for many years.[100] Similar effects were also observed in other monoterpenes especially in carvacrol (**1**) and thymol (**2**), which are main constituents in thymus, oregano, savory, clove, and nutmeg essential oils. In other reports, similar observations have confirmed that many of the other essential oils and their constitutions can play an important role in inflammation control.

Emphasizing the structure–activity relationships is necessary to define different potential of natural compounds such as essential oils compositions. For instance, aliphatic aldehydes and aromatic aldehydes are said to predominantly have antiinflammatory and antimicrobial potentials.[101] The terpenes and sesquiterpenes evaluated appeared to be good inhibitors of 5-LOX in vitro. There is a good correlation observed between antiinflammatory activity of the limonene (**25**) and the essential oils rich in limonene (**25**) like grapefruit, lime, and celery. Other similar activity has been observed on the various inflammations pathways such as cyclooxygenase (COX) and lipoxygenases (LOX) between sesquiterpenic alcohols, aliphatic aldehydes, and some phenolic esters that caused these phytochemicals considered for more investigations in respiratory inflammations during the air ways infections (Fig. 16.3).[101,102] It has been evaluated that activity of some of the essential oils such as Western red cedar (*Thuja plicata*) to inactivate several viruses implicated in respiratory infections, and to inhibit the influenza virus-induced secretion of cytokine (IL-6) in cultured human lung cells. However, the liquid essential oil phases are generally a higher irritant and possibly toxic for nasopharyngeal or oral applications, although a few reports have indicated that the vapors of some essential oils might be useful for application in inhaled vapors for respiratory infections in low concentrations.[103] Because of the noteworthy role of inflammation in respiratory infectious disease persistence and recurrence, many laboratory models have been developed for inflammation and inflammation/infection-mixed respiratory complications research. Some of these models, such as ovalbumin-induced respiratory allergic eosinophilia arise especially for inflammation process which responds to them, including eosinophils, neutrophils, and other immune cell infiltration to the inflammation sites.[104] In other models, in addition to this cellular pathogenesis, more attention is considered on the infection's correlation with inflammation mechanisms.[105] Table 16.5 shows some of the attributes of inflammation and inflammation/infection major models that are used in screening of the new antiinflammation compounds.

Table 16.5 Typical Laboratory Models of Inflammation and Inflammation/Infection Mixed Diseases

Models	Disease	Type	Rationale	Ref
Inhaled antigen-induced tracheal constriction models	Inflammation in the upper airways	Eosinophilic inflammatory	Airway inflammation	a
Ovalbumin-induced respiratory allergic eosinophilia	Inflammation in the lower airways	Eosinophilic inflammatory	Airway inflammation	b
Neutrophil elastase (NE) inhibitory activity	Pulmonary acute injury inflammation	Neutrophils released (NE) at inflammatory sites	Pulmonary inflammation	c
Chronic respiratory infection with *Mycoplasma pneumoniae*	Chronic pulmonary infection- associated chronic inflammation	Peribronchial and perivascular mononuclear infiltrates	Pulmonary infection-inflammation	d
Antirespiratory syncytial virus (RSV)	Acute and chorionic pulmonary (lower respiratory) infection-associated with inflammation	Histopathological change including: peribronchiolar, bronchial and perivascular infiltrations	Pulmonary infection-inflammation	e

(*Continued*)

Table 16.5 Typical Laboratory Models of Inflammation and Inflammation/Infection Mixed Diseases (*cont.*)

Models	Disease	Type	Rationale	Ref
Chronic rat lung model of *P. aeruginosa* infection	Chronic pulmonary infection-associated chronic inflammation	Airway neutrophilic inflammation	Pulmonary infection-inflammation	f

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6 CONCLUSIONS

Respiratory infections are one of the most prevalent human health problems and they cause major difficulties for all age ranges.[106] Research to find new and effective therapeutic compounds for the control and treatment of these ailments is a most attractive field in natural product screening. The massive biodiversity of natural sources, such as plants, means that the selection of the suitable starting source point is a critical step for achieving the best screening results. In particular, paying attention to the main chemical constitutions of these natural sources will help to clarify the results of this research. Analysis of the relationship between the chemical structures of natural compounds has led to a better overview of the rational uses of natural compounds and natural remedies for the control and treatment of disease, and has led to the development of therapeutics. The broad diversity of the chemical makeup of essential oils has caused some uncertainty over their best selection in complementary or investigative research. Therefore, a correct understanding of the relation between the potential effects and the plant's family is a useful guide for physicians and researchers. According to this finding, the volatile oils of plant families such as

Asteraceae, Lamiaceae, Scrophulariaceae, Fabaceae, Myrtaceae, Rutaceae, Cupressaceae, and Pinaceae have good potential for use in infectious and inflammatory respiratory diseases, in both research and treatment. These plant based essential oils have a good feasibility for presentation as therapeutics in many of the respiratory infections and inflammatory diseases. Although these natural compounds have been used as complementary therapeutics, scientists must still give attention to their safety and dosage.

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