Plant Essential Oils as an Alternative Antimicrobial Therapy

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ABSTRACT

Essential oils, natural volatile liquids, are characterized by strong aroma and low mol. weight. They are usually colorless, lipid-soluble, and organic solvents, synthesized by various plant parts including stems, seeds, roots, flowers, leaves, etc. They are typically extracted through processes like steam distillation, cold pressing, or solvent extraction from various plant parts. They may serve as a replacement therapy in treating microbial infection. The antibacterial effects of essential oils can either be bacteriostatic or destroy bactericidal. EO has been employed besides the restricted efficacy of antiviral drugs. Numerous studies have shown the antiviral activity of EO against DNA and RNA viruses by hindering the replication process. EOs exhibited antifungal activity that makes it a therapeutic alternative to synthetic drugs by interfering with chitin synthesis leading to abnormalities in glycoprotein synthesis, mitochondrial structure, and inhibition of sporulation, reducing fungal growth, and suppressing mycotoxin production. Plant essential oils have been employed as an alternative therapy against both endo and ectoparasites due to the increasing resistance of certain parasites to conventional drugs, leading to significant mortality and morbidity rates. Their action mechanism is their effect on the cell wall, cell membrane, respiration, hereditary material, and quorum sensing of micro-organisms. Researchers have extensively studied the antimicrobial effects of EOs and their chemical components.

KEYWORDS	Received: 07-May-2024	SCHENTIFIC ALLE	A Publication of
Essential oils, Microbes, <i>E. coli</i>	Revised: 12-July-2024		Unique Scientific
	Accepted: 05-Aug-2024	USP	Publishers

Cite this Article as: Naz G, Sarwar A, Mahmood S, Sultana S, Sarwar F, Rasheed M, Ali H, Hameed A and Zahra H, 2024. Plant essential oils as an alternative antimicrobial therapy. In: Zafar MA, Abbas RZ, Imran M, Tahir S and Qamar W (eds), Complementary and Alternative Medicine: Essential oils. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 114-122. https://doi.org/10.47278/book.CAM/2024.121

INTRODUCTION

Different aromatic medicinal plants constitute a substantial part of the natural ecosystem and have valuable applications in various sectors including pharmaceuticals, perfumery, flavor and fragrance, and cosmetics (Swamy and Sinniah, 2015). Despite the availability of modern medicine, most of the world's population is using traditional herbal remedies to cure various health issues (Sudipta et al., 2012). In the global market, herbal products are currently valued at around 62 billion USD with projections indicating a potential increase to 5 trillion USD by 2050. (Bhattacharya et al., 2014). Approximately 1500 species are identified and recorded for their aroma and flavor, while over 9000 native plants have been found and documented for their medicinal qualities. Annually, more than 250 varieties of essential oils, valued at 1.2 billion USD, are traded internationally (Swamy and Sinniah, 2016). Essential oils, natural volatile liquids are marked by strong aroma and low molecular weight. They are usually colorless, lipid and organic solvents soluble, produced by various plant parts such as buds, stems, bark, leaves, seeds, fruits, flowers, twigs, roots or wood and are stored in specialized structures such as oil ducts, secretary cells, cavities, canals, resin ducts, glands, or trichomes. They are utilized as flavor enhancers in food and beverages, as well as in perfumery, pharmaceuticals, and cosmetics, essential oils possess notable medicinal properties (Bozin et al., 2006). They are widely used in treating various chronic non-infectious diseases such as diabetes, cancer and hypertension as well as various infectious diseases. The antimicrobial properties of essential oil and their chemical composition have been extensively studied by researchers (Ali et al., 2015).

Essential oils are commonly obtained from plants using methods such as hydro distillation, dry distillation, steam distillation or mechanical cold pressing. The Clevenger steam distillation apparatus was utilized in the late 19th century (Arora et al., 2016). Methods such as fermentation, extraction, crushing, or hydrolysis are used to obtain essential oils (Baj et al., 2015). The EOs demand and usage are increasing not only in medicine but also in food,

fragrances, and cosmetics. They may serve as a replacement therapy in treating microbial infection. There's a potential for EO to replace antibiotics in the future, although their effectiveness is still under scrutiny. Different mechanisms such as cell wall disruption, interference with metabolic pathways, or lowering cellular membrane potential are involved in inhibiting microbial growth (Swamy et al., 2016).

Chemical Composition

The plant parts from which essential oils are taken determine the chemical composition of oils. These oils contain terpenoids and phenylpropanoids, alongside aromatic and aliphatic constituents. Terpenoids, naturally occurring hydrocarbons, are categorized into various types based on their structure and function, typically organized by units of five carbons. The terpenes contain monoterpenes, sesquiterpenes, hemiterpenes, diterpenes, triterpenes, and tetraterpenes. These terpenes constitute 90% of essential oils (Bakkali et al., 2008). More than 55,000 terpene molecules have been identified. Essential oils also contain aromatic compounds derived from phenylpropane but in lower concentrations than terpenes. These compounds encompass methylenedioxy compounds, methoxy derivatives, aldehydes, alcohols, and phenols (Suzuki et al., 2015). However, various terpenes and aromatic compounds are listed in Table 1.

	Functional Groups Compounds						
	Carbures	Acyclic: myrcene, ocimene.					
		monocyclic: terpinenes, phellandrenes, p-cymene. bicyclic: 3-carene, camphene, pinenes,					
		sabinene.					
	Alcohols	Acyclic: geraniol, linalol, lavandulol, citronellol, nerol.					
		Monocyclic: a-terpineol, carveol, menthol.					
Terpenoids		Bicyclic: borneol, chrysanthenol, fenchol, thuyan-3- ol.					
	Peroxides	Ascaridole, hydroperoxide, isoborneol peroxide, and d-limonene peroxide.					
	Phenols	Thymol, cinnamic acid, and phenethyl alcohol.					
	Ethers	1,8-cineole, menthofurane, estragole, safrole, myristicin, anethole					
	Esters	Linalyl acetate or propionate, citronellyl acetate, Benzyl acetate, menthyl or a-terpinyl acetate, and bornyl acetate. isobornyl acetate, methyl salicylate, and geranyl acetate.					
	Aldehydes	Geranial, neral, citronellal, citral.					
	Ketones	Tegetone, menthones, carvone, thuyone, ombellulone, pulegone, piperitone, camphor, fenchone, menthone pinocamphone, pinocarvone.					
	Aldehyde	Cinnamaldehyde, benzaldehyde.					
Aromatic	Alcohols	Cinnamic alcohol, linalool, geraniol, citronellol, and benzyl alcohol.					
Compounds	Phenols	Eugeno, chavicol.					
	Methoxy	Anethole, methyleugenols, estragole, elemicine, safrole.					
	derivatives						
	Methylenedioxy compounds	Apiole, myristicine, elemicin.					

Antimicrobial Effects of Plant Essential Oils

Plant essential oils are well known for their antibacterial, antiviral, antifungal and antiparasitic properties. Currently, there is a wide range of antibiotics utilized to cure different bacterial infections. However, due to increasing multidrug resistance, compromised immune systems in some people and bacteria's ability to form protective biofilms may leads to the death of individuals. Moreover, the administration of multiple antibacterial agents is the major cause of toxicity in humans. In current scenario, plant essential oils and their primary chemical constituent emerge as potent alternatives for antimicrobial activity (Galvão et al., 2012; Raut and Karuppayil, 2014).

Antibacterial Activity

The antibacterial effects of essential oils can be either bacteriostatic or bactericidal. However, distinguishing between these actions can be challenging. The parameters such as minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) are used to assess antimicrobial activity. The agar well diffusion and agar dilution methods are commonly employed to check antibacterial properties of essential oils (Burt, 2004; Faleiro, 2011).

A study reported that certain plants and essential oils (EOs) including rosemary, cinnamon, thyme, clove and oregano demonstrating strong inhibitory effects against different bacterial infections. The phenolic compounds found in essential oils such as carvacrol, thymol, rosmarinic acid, menthol, vanillin, and eugenol play an antimicrobial role against *Bacillus cereus, Streptococcus pneumoniae, Escherichia coli* and *Staphylococcus aureus* (Lopez-Romero et al., 2015). Furthermore, citronellol and carveol exhibit an inhibitory effect on *Escherichia coli* growth due to the penetrating ability of oils into cell wall components leading to disruption in cell wall integrity. Some chemical compounds can inhibit the growth of most Gram-positive bacteria and a few Gram-negative bacteria. (Liu et al., 2017) reported that cardamom from the Zingiberaceae family, anise from the Apiaceae family, and oregano, rosemary, and basil of the Lamiaceae mint family along

with parsley, and coriander showed notable antimicrobial properties against saprophytic microbes. This also demonstrated oregano EO's effectiveness against *Yersinia enterocolitica, Salmonella typhimurium* and *E. coli* by slowing down their growth and lactic acid production. Certain EOs have varying levels of effectiveness against both gram-positive and gram-negative bacteria. On the other hand, compounds like eugenol, thymol, citral and carvacrol EO demonstrated efficacy against both Gram-positive as well as Gram-negative bacteria (Swamy et al., 2016). However, the chemical composition of essential oils and their antimicrobial activity against various pathogens are summarized in Table 2.

Scientic Name	Common Name	Parts used	Chemical Composition Inhibited Microorganism	References
Artemisia longifolia	longleaf wormwood or Indian wormwood	Aerial part	Camphene, Camphor, 1,8- Staphlococcus epidermidis, cineole, Sabinene, Alpha- Escherichia coli, Staphlococcus pinene, aureus (Eucalyptol)	(Lopes-Lutz et al., 2008)
Daucus littoralis	seacoast carrot		Carotol, acorenone B, Daucol, S. aureus, B. cereus, S. Caryophyllene, Germacrene D typhimurium, E. coli	(Yousefbeyk et al., 2014)
Cymbopogn nardus	citronella grass	Leaves, stems	Carene,beta-citronellal, S.putrefaciens,BrochothrizGeranylacetate,β-thermosphacta,Listeria innocua,Caryophyllene, CitronellolP.putida, S.typhimurium, E.coli,L.monocytogenesL.coli,L.	
Eugenia caryophyllata	Clove	Buds, leaves, stems	Eugenylacetate, L. monocytogene, S. epidermidis,Phenylpropanoids,carvacrol, S.aureus,Salmonellathymol,Acetyleugenol, typhimurium,E.coli,P.eugenol, cinnamaldehydeaeruginosaaeruginosaAcetylAcetyl	r al., 2007)
Foeniculum vulgare	Fennel	Leaves, seeds	Phellandrene, trans-anethole, <i>E. coli, S. typhimurium</i> methyl chavicol, limonene, Camphen, pinene, fenchone fenchyl alcohol, anisaldehyde.	(Bisht et al., 2014)
Nigella sativa	black seed, black cumin, or fennel flower	Seeds	, , ,	(Singh et al., 2014)
Origanum vulgare	oregano	Leaves, Arial part	Carvacrol, thymol, γ- Escherichia coli, Salmonella terpinene, trans-sabinene typhimurium, Ampicillin- hydrate, cis-piperitol, borneol, resistant Pseudomonas terpinen-4-ol, linalool aeruginosa, Staphylococus aureus, Bacillus subtilis	. ,
Verbena officinalis	Vervain	Arial part	Borneol, 1,8-cineole, α - S. aureus, E. coli, S. typhimurium, pinene, geraniol, β -pinene, α - L. monocytogenes terpineol, citral,	(Shan et al., 2011)
Warionia saharae	desert wormwood or desert ragwort	Arial part	Eudesmol, trans-nerolidol, <i>E. coli, S. aureus, P. aeruginosa</i> , linalool, 1,8 cineole, camphor, <i>B. cerus</i> p-cymene, terpinen-4-ol	, (Sellam et al., 2014)

Antiviral Activity

Antiviral agents, also known as antivirals, are substances or medications employed for treating viral infections by targeting and impeding the processes involved in viral replication. Many antivirals have developed resistance against viral pathogens such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Alternative therapeutic compounds, like EO, have been employed besides the restricted efficacy of antiviral drugs. Numerous studies have shown the antiviral activity of EO against DNA and RNA viruses by hindering the replication process Notably, essential oils like oregano and clove were evaluated against non-enveloped viruses such as poliovirus (RNA virus), coxsackievirus B1 (RNA virus), and adenovirus type 3 (DNA virus) (Orhan et al., 2012; Tariq et al., 2019).

Certain compounds in essential oils have demonstrated effective antiviral activity against viruses by exhibiting viricidal activity preventing the viral replication and viral adsorption to host cells. Gavanji et al. (2015) have conducted a study to confirm the antiviral properties of essential oils extracted from Artemisia kermanensis, Eucalyptus caesia, Zataria multiflora, rosemary and Satureja hortensis L against herpes simplex virus-1 (HSV-1). Not all essential oils are effective including thyme EO, which did not yield an effective response against HSV and influenza viruses (Tepe et al., 2004). Furthermore, β -caryophyllene, a constituent found in essential oils showed inhibitory effects against HSV-1 significantly, while a lower

inhibitory effect was noted against parainfluenza virus (Orhan et al., 2012). The 0.5 mg/mL ajwain essential oil showed an effective antiviral response against the Japanese encephalitis virus (JEV) (Roy et al., 2015).

Essential oils exhibit various mechanisms of action to combat viral infection, typically targeting nucleic acid polymerases to inhibit replication. Furthermore, thymol and phenylpropanoids have been recognized as antiviral against herpes simplex virus and Japanese encephalitis virus. Human trials are being conducted to improve efficacy and safety conclusively (Aljaafari et al., 2021). The antiviral effects of different essential oils are summarized in Table 3.

Scientific	Common Name	Parts	Chemical Composition	Inhibited Microorganism	Reference
name		used			S
Achillea	fragrant yarrow or	Aerial	2,5,5-Trimethyl-3,6- heptadien-2-ol,	-	(Zeedan
fragrantissima	sweet yarrow	part	yomogi alcohol, eucalyptol, 1,8- cineole, artemisia alcohol, thujone, Cissabinol, Lavandulol		et al., 2014)
Fortunella margarita	kumquat	Leaves	Gurjunene, eudesmol, muuroleneterpineol, t-carveol, limonene, muurolene and cadinene		(Ibrahim et al., 2015)
Pogostemon cablin	Patchouli	Leaves	α -Pinene, Camphene, 3-octanone, Limonene, Terpinolene, Linalool, and Acetophenone		(Swamy and Sinniah, 2015)
Trachyspermm ammi	Ajwain or ajowan	Leaves, seed-like fruit	$\alpha\text{-thujene,}$ Carvacrol, Thymol, $\gamma\text{-}$ terpinen, $\alpha\text{-pinene,}$ Sabinen, $\rho\text{-}$ cymene		(Roy et al., 2015)
Melissa officinalis	Lemon balm	Leaves	Caryophyllene oxide, citronellal, β- lonone, Nerolidol, β-Ocimene, geranial, neral	Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV), avian influenza virus (AIV) subtype H9N2	yev et al., 2004)
Ocimum campechianum	Camphor basil	Leaves	Linalool, eugenol, β -Caryophyllene, α -Terpinene, Caryophyllene oxide	Herpes simplex virus type 1	(Wani et al., 2021)
Glechon spathulata	Coastal cudweed or coastal fleabane		Caryophyllene, bicyclogermacrene	Herpes simplex virus type 1	(Venturi et al., 2015)
Glechon marifolia	Coastal cudweed	Leaves	Caryophyllene, bicyclogermacrene	Herpes simplex virus type 1	(Venturi et al., 2015)

Antifungal Activity

Table 2. Antiviral Effect of Diant Eccential Oils

Plant essential oils exhibit antifungal activity that makes them a therapeutic alternative to synthetic drugs, especially considering the rising prevalence of drug-resistant fungal strains akin to bacterial resistance. Fungal infections can manifest as either superficial or invasive having treatment via oral tablets or topical creams. Treating fungal infection exhibits challenges as compared to bacteria due to the eukaryotic nature of both human and fungal cells. The chitin, a polysaccharide in fungal cell walls, is crucial in antifungal drug development to avoid potential toxicity to human cells and ensure host safety. Eugenol, terpenes, farnesol, menthol, benzoquinone and menthone have antifungal effect against *Candida albicans, Candida tropicalis, Candida neoformans, Candida glabrata* as well as *Paracoccidioides brasiliensis.* Additionally, essential oils can interfere with chitin synthesis leading to abnormalities in glycoprotein synthesis, mitochondrial structure, inhibition of sporulation, reducing fungal growth, and suppressing mycotoxin production (Hu et al., 2017; Nazzaro et al., 2017). Scalas et al. (2018) have conducted a study to demonstrate the utilization of essential oils extracted from *Origanum vulgare, Pinus sylvestris,* and *Thymus vulgaris* along with their primary constituents to augment the effectiveness of itraconazole against both azole-susceptible and azole-not-susceptible Cryptococcus neoformans strains and the findings have shown favorable results.

Ksouri et al. (2017) have conducted a study to determine an antifungal property of various essential oils (EO) against *C. albicans.* The essential oils obtained from plants such as rosemary, oregano and thyme exhibited inhibitory effects. Hu et al. (2019) observed the antifungal efficacy of essential oils from anise, peppermint, clove, cinnamon, pepper, citronella, and camphor derived from seven distinct spices was validated using an agar diffusion assay against three fungal strains. Among these Essential oils, cinnamon EO exhibited the most potent antifungal property against fungal strains. Clove EO showed the next highest level of antifungal activity following cinnamon EO. Dias Ferreira et al. (2013) found that essential oil of Curcuma longa exhibited toxicity against *Aspergillus flavus* and effectively suppressed the production of aflatoxin. Aflatoxins are harmful toxins generated by molds, capable of causing liver damage and potentially leading to liver cancer in humans. These mycotoxins occur naturally and are produced by two mold species. *A. flavus* and *A. parasiticus* are the

two mold species responsible for aflatoxin production. The potential impacts of essential oils (EOs) on inhibiting aflatoxin production are highly intriguing and warrant further investigation (da Cruz Cabral et al., 2013). However, the antifungal effects of plant essential oils are summarized in Table 4.

Scientific Name		arts used	Chemical Compounds	Inhibited Microorganism	References
	Name				
Aegle marmelos	Bael or bael Le fruit		δ -Cadinene, $δ$ -carene, $α$ -pinene, trans-2-hydroxycinnamic acid, $β$ -myrcene		(Ibrahim et al., 2015)
Cinnamomum	Ceylon Ba	ark,	Cinnamaldehyde, (E)-cinnamyl	C. auris, C. parapsilosis, C.	(Unlu et al.,
zeylancium	cinnamon or lea true cinnamon	aves		albicans, C. krusei	2010)
Daucus littoralis	fru	aves, oots,	Polyacetylenes, Flavonoids, Germacrene D, acorenone B, phenylpropanoids, Carotol, Daucene, Trans-α-bergamotene	C. albicans	(Yousefbeyk et al., 2014)
Eremanthus	Brazilian Le	eaves	germacrene D, (Z)-caryophyllene,	C. albicans, Cryptococcus,	(Santos et al.,
erythropappus	Arnica or Candeia		viridiflorol, p-cymene, γ- terpinene, carvacrol	C. tropicalis	2015)
Feoniculum vulgare	Fennel Se		(E)-anethole, fenchone, methyl chavicol	C. Albicans, A. Niger, Trichoderma, Metarizium	
Glechon marifolia	Coastal Le cudweed		β-Caryophyllene, bicyclogermacrene	T. rubrum, E. floccosum	(Venturi et al., 2015)
Glechon spathulata	Coastal Le cudweed		β-Caryophyllene, bicyclogermacrene	Trichophyton rubrum, Epidermophyton floccosum	(Venturi et al., 2015)
Momordica charantia	Bitter melon Se or bitter gourd		α-momorcharin, trans-nerolidol, Catechin, epicatechin apiole, cis- dihydrocarveol, germacrene D	C. albicans	(Braca et al., 2008)
Nigella sativa	black seed, Se black cumin, or fennel flower		Thymoquinone, p-cymene, D- limonene, Thymol, Carvacrol, α- thujene, thymohydroquinone	A. parasiticus, C. albicans	(Singh et al., 2014)
Syzygium aromaticum	Clove Le	eaves	. <u>.</u>	Fusarium moniliforme, Fusarium oxysporum, Aspergillus sp., Mucor sp., Trichophyton rubrum	

Table 4: Antifungal Effect of Plant Essential Oils

Antiparasitic Effect

Plant essential oils have been employed as an alternative therapy against both endo and ectoparasites. This is because certain parasites, like *Plasmodium falciparum* (malaria) and *Leishmania donovani* (leishmaniasis), exhibit resistance to conventional treatments, resulting in notable mortality and morbidity rates. However, studies have shown that essential oils extracted from *Lavandula angustifolia Mill*. and *Lavandula x intermedia* have demonstrated antiparasitic properties against human protozoal pathogens such as *Giardia duodenalis* and *Trichomonas vaginalis*, as well as the fish pathogen *Hexamita inflata* (Ritzefeld et al., 2018; Tariq et al., 2016).

Drawbacks of EOs

Demonstrated potential as antimicrobials is possessed by only limited EOs. In comparison to synthetic compounds (such as antibiotics), their real effect is noticeably weaker. Their high volatility limits the effective duration of action and allows for the modification of properties like encapsulation. Therefore, it is important to emphasize that EO usage for microbial stability is feasible, but each situation must be examined separately (Wińska et al., 2019).

Mechanism of Actions

The essential oils' efficacy mainly depends upon their major chemical components or the synergistic effects among these components. Various antimicrobial agents demonstrated distinct modes of action (Pellerito et al., 2018). Consequently, the antibacterial mechanism of EOs involves the combination of multiple modes rather than a single one (Ju et al., 2019). Different EOs target different sites within organisms which are elaborated in Fig.1.

Effect on Cell Envelope

The outer layer of microbes, the cell wall, plays a crucial role in reducing sensitivity to antimicrobial agents due to major components and enzymes. The bioactive constituents in essential oils can disrupt the peptidoglycan structure or inhibit its synthesis. This leads to cell wall damage and can either deform or kill the bacteria (Meychik et al., 2011; Sun et al., 2020). Moreover, essential oils affect the structure of the cell membrane by altering its permeability. For instance, cinnamaldehyde readily dissolves within the fatty acyl chain of the membrane which results in disrupting its outer layer. This creates leaks in the membrane, allowing essential components like adenosine triphosphate (ATP) to flow out, ultimately killing the bacteria. It can also interact with the phospholipids of the cell membrane, altering the proportion and structure of fatty acids within the membrane. Furthermore, essential oils inhibit the ergosterol synthesis such as eugenol which hinders ergosterol production in microorganisms, compromising cell membrane integrity. Furthermore, EOs can traverse via porins present on the cell membrane resulting in reducing the expression of porin-related genes, and disrupting amino acid transporters (Nazzaro et al., 2013).

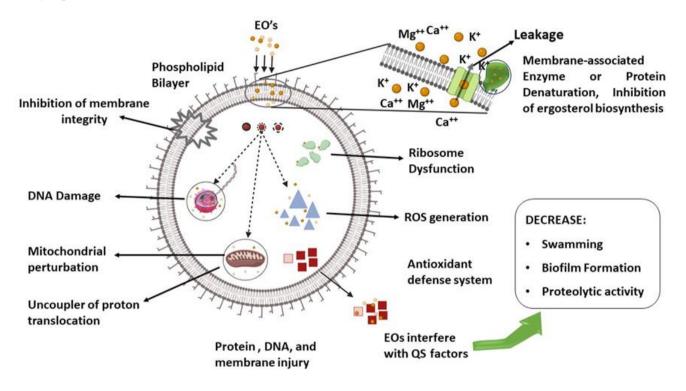


Fig. 1: Different EOs target different sites within organisms

Effect on Respiration

Microorganisms rely on respiratory metabolism to produce energy by oxidatively breaking down carbohydrates. This energy production primarily occurs through pathways such as the Entner–Doudoroff (ED) pathway, the phosphogluconate pathway, and citric acid cycle. When the oxidation and decomposition of sugars are hindered, normal metabolic processes are disrupted, potentially leading to cell death (Ju et al., 2020). Antimicrobial agents target energy production in pathogenic bacteria by interfering with nutrient absorption and transport, thereby impeding their growth and reproduction (Ulanowska et al., 2006). Di Pasqua et al. (2010) observed that thyme essential oil hinders the activity of ATP synthase within *Salmonella typhimurium*, thereby interfering with the tricarboxylic acid cycle pathway.

Effect on Heredity Material

The heredity material, whether DNA or RNA within microorganisms, plays a crucial role in their development, reproduction, mutation, and growth. The accuracy and stability of genetic material are crucial for maintaining consistent inheritance across generations. Additionally, genetic material governs protein synthesis and metabolism. Consequently, any damage to the genetic material can disrupt normal microbial reproduction and self-replication (Li et al., 2014).

Effect on Quorum sensing

Research has demonstrated that cinnamaldehyde can downregulate bcsA and luxR gene expression in *E. coli*, both of which are involved in quorum sensing. Moreover, cinnamaldehyde has shown significant inhibitory effects on quorum sensing in *P. aeruginosa* and *Streptococcus pyogenes* (Brackman et al., 2009; Brackman et al., 2011). It's important to note that different essential oils (EOs) may exhibit varying inhibitory effects on quorum sensing in different types of cells. Discovering novel approaches to inhibit quorum sensing in bacteria presents an intriguing strategy for developing new antibiotics that can mitigate bacterial pathogenicity without inducing drug resistance (Rasko et al., 2008).

Conclusion

In conclusion, EOs have the potential to replace antibiotics, antivirals, antifungals, and antiparasitic drugs in the future by inhibiting microbial growth by interference with different mechanisms such as cell wall disruption, interference with metabolic pathways, or lowering cellular membrane potential, although their effectiveness is still under scrutiny. The growing global issue of multidrug resistance has made diseases more severe caused by these pathogens so further research on EOs can help to combat this problem.

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